Capillary Electrophoresis in the Undergraduate Instrumental Analysis Laboratory: Determination of Common Analgesic Formulations

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During the last ten years, the use of capillary electrophoresis (CE) has been rapidly increasing in analytical research (Fig. 1). It is clear that CE has been embraced by the scientific community and is here to stay. Consequently, it is important to introduce this technique to undergraduates by incorporating it into the student instrumental analysis laboratory. This paper will briefly describe the operational principles behind CE and present an example of a simple laboratory experiment involving free solution CE to determine the components of several common analgesic formulations.

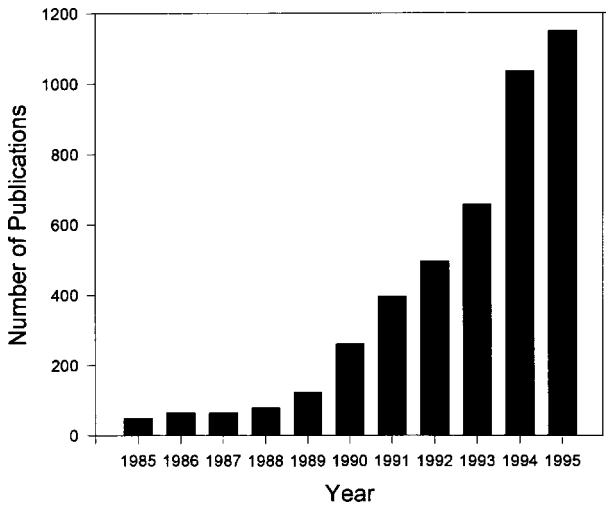

Figure 1. Illustration of dramatic increase of CE reports in the chemical literature. Data were collected via a *Chemical Abstracts* on-line search performed for the word "capillary" followed by the word "electrophoresis" for each year.

Overview of Capillary Electrophoresis

Capillary electrophoresis, like slab gel electrophoretic techniques used by biologists, is a separation scheme in which chemicals are separated by differential migration in a potential field. Unlike slab gel methods, CE separations occur in a narrow-bore capillary tube (25–100 μm i.d.). Under most operational conditions there is a strong flow, called electroosmotic flow, of solution from the anodic end of the capillary to the cathodic end. This flow sweeps all components through the capillary tube so that all analytes can be injected in one end and elute at the other end of the separation column, much as in high-performance liquid chromatography (HPLC). Consequently, the data output (called an electropherogram) looks much like a chromatogram. The superb resolving power of electrophoretic separations with high potentials, coupled with a single on-line detection scheme, has made CE a popular choice for the separation of samples from biological, chemical, and agricultural sources (*1*). In addition, CE often achieves separation efficiencies superior to those for HPLC.

A schematic of a typical CE setup is shown in Figure 2. We will focus here on the simplest mode of CE, capillary zone electrophoresis (CZE). The CZE system consists of a fused silica capillary column (50–100 cm in length, 50–100 μm i.d.) that is filled with an aqueous buffer solution and immersed in that same buffer at both ends. The "front" end of the capillary is placed into the solution of interest and a small volume (typically 1–50 nL) of the analysis solution is then injected onto the front (anodic) end of the capillary. Pressure and/or voltage is used to facilitate movement of the analysis solution onto the column. The capillary is then replaced into the front-end buffer reservoir and a large potential (10–30 kV) is applied across the capillary via the platinum electrodes at each end of the system. Electroosmotic flow (Fig. 3a) moves the entire solution toward the cathode, and separation of analytes within this moving stream occurs as a result of differential migration (or electrophoretic movement, Fig. 3b) of the charged species in the potential field. Electroosmotic flow (EOF), the "pump" in CE, is a result of the inner walls of the fused silica capillary having some level of negative charge due to deprotonated siloxyl groups. These negative charges, which are covalently fixed to the inner walls of the capillary tube, attract oppositely charged cations in the aqueous buffer. When the separation potential is applied across this sytem, the "tube" of cations adjacent to the inner wall of the capillary will migrate in the direction of the cathode, resulting in bulk flow of the solution toward the cathode. This flow, which is initiated at the capillary wall, is a plug flow. Separation of charged analytes is a result of the electrophoretic migration of the individual analyte species toward the electrode of opposite charge. As illustrated with vectors in Figure 3c, the net movement of charged species is the sum of the EOF and the migration of the individual species. Cations will elute first because their electrophoretic migration is in the same direction as the EOF. The neutrals travel at the same speed as the EOF, and the anions travel slowest owing to their electrophoretic movement in the opposite direction of the EOF (*2*). A very nice analogy to describe this system is fish swimming in a river. The flow of the river is analogous to the EOF, and the fish can swim upstream (like anions), or downstream (like cations). Dead fish and chunks of wood are unresolved from one another (like neutrals).

There are several more advanced modes of CE designed to separate the neutral components by using micellar buffer systems, optical isomers by using chiral buffer systems, or biopolymers by using gel-filled capillaries or isoelectric focusing. Each of these methods is described in one of the many fine texts now available on CE (*3–6*). As mentioned

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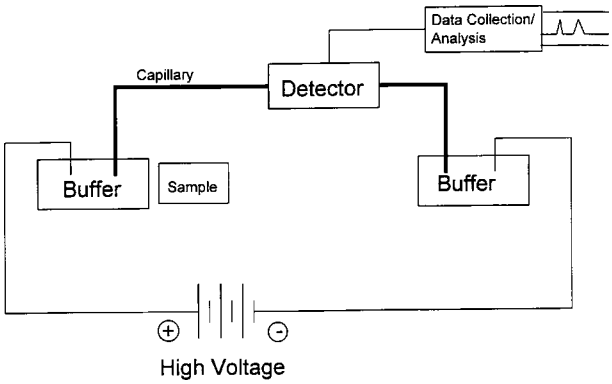

Figure 2. Schematic of CE set-up.

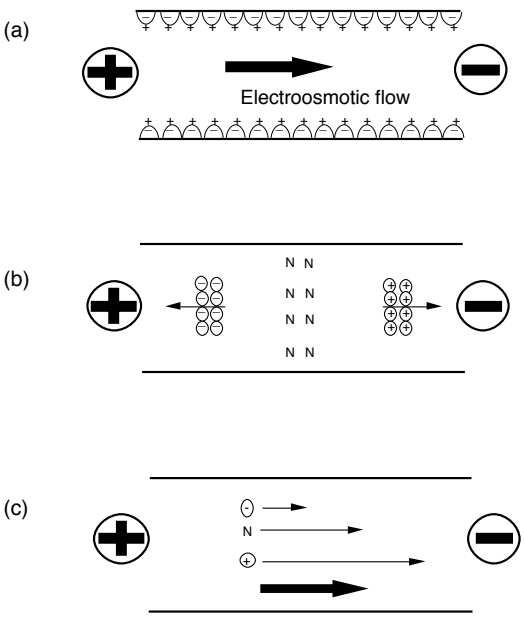

Figure 3. Graphic illustration of the flow, migration of individual analytes, and net movement of analytes within the capillary. (a) Electroosmotic flow in the capillary. Shown are the immobilized charges (negative) on the inner capillary wall due to deprotonated siloxyl groups and the counter ions (positive) that are mobile and migrate toward the cathode, pulling the bulk solution with them. (b) Migration of analyte ions in the presence of a potential field. (c) Relative magnitude of the net movement of ions and neutral species in CZE. Anions, ⊖, elute last owing to negative combination of electrophoretic and electroosmotic flows. Neutrals, N, elute at the same rate as the electroosmotic flow. Cations, ⊕, elute first owing to positive combination of electrophoretic and electroosmotic flow. The bold arrows indicate the magnitude of the electroosmotic flow.

earlier, we will confine this discussion to the CZE separation scheme, which is also known as free solution CE.

The key equation that describes the elution behavior in CZE is given by

$$t_m = \frac{L_d L_t}{\left(\mu_{eo} + \mu_{ep}\right) V}$$

In this equation, t_m is the migration time or time for an injected analyte to reach the detector (s), L_d is the length of the capillary from injection to detection (cm), L_t is the total length of the capillary (cm), μ_{eo} represents the magnitude of the EOF ($cm^2\,s^{-1}\,V^{-1}$), μ_{ep} is the magnitude of the eletrophoretic migration of the analyte ($cm^2\,s^{-1}\,V^{-1}$), and V is the voltage applied across the capillary (V). For a given separation system, L_d, L_t, μ_{eo}, and V will be constant. The factor determining t_m, the elution time of each analyte, is the electrophoretic mobility, μ_{ep}. Thus, μ_{ep} provides the means of analyte identification, similar to the role of k' in HPLC. Quantification can be accomplished via the acceptable analytical procedures (calibration curves, internal standards, or standard additions).

In this paper we present a simple laboratory experiment designed to illustrate the principles of CE to undergraduate instrumental analysis students. The analysis of common over-the-counter analgesic formulations is a well-known laboratory experiment to illustrate the separation power and utility of high-performance liquid chromatography (*7–11*). We report here an expanded, integrated HPLC/CE laboratory experiment aimed at illustrating the separation power and utility of these two important separation techniques.

Experimental Procedure

Overview

An in-house lab manual describing the basic operational aspects of the CE and HPLC instruments, along with an outline of the experimental steps to be taken, is sold to the students at the beginning of the semester. Briefly, each student (or pair of students) prepares a series of standard solutions of each of the following five compounds: caffeine, norephedrine HCl, salicylic acid, acetylsalicylic acid, and 4-acetamidophenol (acetaminophen). Separations of these standards, along with an "unknown" solution prepared by dissolving one dose of a commercially available analgesic, are then carried out using HPLC and CZE. Calibration plots are made from the standards, and the formulations of the analgesics are determined.

Chemicals

Caffeine, norephedrine HCl, 4-acetamidophenol, and salicylic acid were obtained from Aldrich Chemical Company (Milwaukee). Acetylsalicylic acid was obtained from Matheson, Coleman, and Bell (Norwood, OH), and boric acid was obtained from Fisher Scientific (Pittsburgh).

The analgesics analyzed in this experiment include Goody's Headache Powder®, Excedrin®, Bufferin®, and Anacin®. Bufferin and Excedrin are manufactured by Bristol Meyers (New York). Anacin is manufactured by Whitehall Laboratories (New York) and Goody's Headache Powder is manufactured by Goody's Manufacturing Corporation (Winston-Salem, NC). All these analgesics can be purchased at a local pharmacy.

Solutions

A 70.0 mM borate buffer solution (pH = 9.00) was prepared for CZE by dissolving the appropriate mass of boric acid in distilled water and adjusting the pH with ca. 1 M NaOH before dilution to the final volume.[1] Standard solutions of 200 ppm (concentrations should be accurately known) of each standard (acetylsalicylic acid, salicylic acid, 4-acetamidophenol [acetaminophen], norephedrine HCl, and caffeine) were prepared, each in its own 100-mL volumetric flask, using deionized water as the solvent. A sixth standard solution containing 500 ppm of each of the above reagents (except salicylic acid, which was 300 ppm owing to solubility constraints) was prepared in a 100-mL flask using deionized water as the solvent.[2] Secondary standard solutions ranging in concentration from 50 to 400 ppm were

made via dilutions of the 500-ppm standard. All solutions were refrigerated when not in use. To prepare the unknown, one dose of a commercial formulation was dissolved in a 1-L volumetric flask using deionized water as the solvent. Tablets were pulverized with a mortar and pestle, and the insoluble particulate matter was removed by filtration through a 0.45-μm disposable syringe (Nuclepore Filtration Products, Pleasanton, CA) filter prior to injection.

Capillary Electrophoresis

A Hewlett Packard ^{3D}CE was used for this experiment along with a Hewlett Packard Vectra 486/66 computer for analysis. The capillary used was obtained from Polymicro Technologies (Phoenix, AZ). The following parameters were used for the capillary electrophoresis analysis: voltage, 20 or 25 kV (resulting current across the capillary, 17–24 mA); total column length (L_t), 53.0 cm; column length, injection to detection (L_d), 44.5 cm; column i.d., 50 μm; injection by pressure, 50.0 mbar for 2–5 s; injection volume, 2.9–7.2 nL (*12*); column temperature, 40.0 °C, detection, UV at 210, 220, and 254 nm. All electropherograms presented in this paper were collected at 210 nm.

High Performance Liquid Chromatography

A Hewlett Packard 1090 HPLC system equipped with a ternary pumping system and data acquisition workstation was used for all HPLC separations. The aqueous portion of the mobile phase for HPLC separations was prepared by dissolving 3% (by vol) glacial acetic acid in ultrapure water. An HP LiChrospher 100 RP-18 5 μm, 125 × 4-mm column was used with a combination of aqueous 3% acetic acid and methanol as the mobile phase.

Results and Discussion (with Typical Student Results)

An electropherogram of a mixture of the five analgesic analytes is shown in Figure 4a, and for reference, an isocratic HPLC separation of the same mixture is shown in Figure 4b. The CZE separation exhibits considerably higher efficiency (average of 90,000 theoretical plates) than the HPLC separation (average of 5,600 theoretical plates). By injecting individual standards, students are able to identify each compound positively in the CE separation of the mixture by calculating the electrophoretic mobility for each compound.[3] They are then asked to briefly describe the separation mechanism involved in both the HPLC and the CE separations and to rationalize the elution order observed with each technique. To do this effectively, they must determine the polarity and charge on each structure under acidic conditions for the HPLC separation and under basic conditions for the CE separation. Briefly, all the analytes except norephedrine are neutral under acidic conditions, and the elution order of the four neutrals in the HPLC separation can be explained by the relative polarities of the compounds. The elution order of the five compounds in the CE separation can be rationalized by determining the charge on each analyte at pH 9.00. Norephedrine will be the only cationic species of the five, and will therefore elute first. Caffeine will be neutral, and the other three analytes will carry, on average, a partial negative charge. Finally, the students are instructed to calculate k' (for the HPLC separations) and μ_{ep} (for the CZE separations) for each component, and to compare the separation efficiency observed with each technique. A list of average values of μ_{ep} and k' under the separation conditions described is given in Table 1.

Norephedrine is not found in analgesic tablets, but is a common decongestant found in other pharmaceutical formulations. It is used here to vividly illustrate how a cation moves ahead of the flow in CE. One very interesting twist to the use of norephedrine is that its UV absorption spectrum demonstates significant absorption only below 230 nm. One can (and does, in this experiment) work with such low wavelengths with CE, but the mobile phase used with the HPLC separation prohibits using a detection wavelength below 230 nm. Consequently, a peak for norephedrine is observed in the CE separation, but not with HPLC. This causes some interesting discussions between students and the instructor during the laboratory period.

Once students have identified each peak and characterized the separation of the five components, they inject mixtures of varying concentrations of the components and construct calibration plots of peak area versus concentration for each one. Finally, the unknown mixture is analyzed with each technique (at least three injections each) to determine the concentrations of the analytes in a solution prepared by dissolving a commercial analgesic formulation. Representative electropherograms of the separations of Anacin, Goody's, Bufferin, and Excedrin are shown in Figure 5. The final "result" is then obtained from a simple back-calculation for the number of milligrams of each analyte in the commercial tablet.

The amount of each analyte reported by the students to be present in the commercial products in terms of milligrams per tablet is summarized in Table 2. Comparing these numbers with what is reported by the products' manufacturers (Table 3), one can see that this experiment works quite well. Similar results are obtained with HPLC as long as the analysis solutions are prepared and analyzed in the same laboratory period. The values listed in Table 2 represent an average of values reported by three years of instrumental analysis students, and the uncertainties reported are the standard deviations. A graphic illustration of the "spread" of individual results for the analysis of Excedrin is given as a set of histograms in Figure 6.

In terms of the unknowns, Goody's Powder and Excedrin both contain three different analgesics, and therefore constitute the most popular choices for the students and the instructor. Salicylic acid is not listed on the label of any of the four formulations, but is uniformly detected by CZE. This fact often results in students prematurely questioning the validity of the manufacturer's label, and jumping to the conclusion that there are "impurities" in the formulation—when, in fact, acetylsalicylic acid (aspirin) degrades into salicylic acid in aqueous solutions. Indeed, if the analyses are not performed on the same day as the solu-

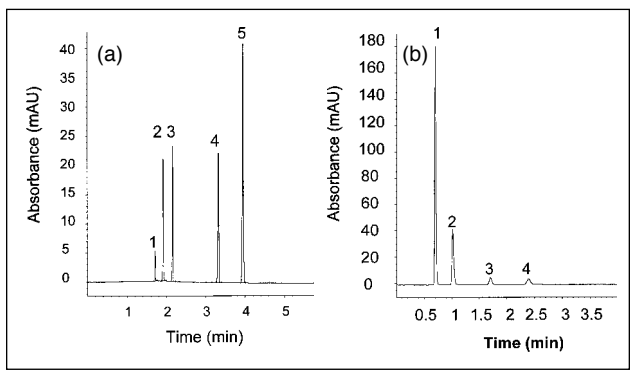

Figure 4. Separation of mixture of five analgesic analytes using (a) CZE and (b) HPLC. Key for CZE separation (a): 1 = norephedrine HCl; 2 = caffeine; 3 = acetominophen; 4 = acetylsalicylic acid; 5 = salicylic acid. Key for HPLC separation (b): 1 = salicylic acid; 2 = caffeine; 3 = acetominophen; 4 = acetylsalicylic acid. Separation conditions are given in the text.

Table 1. Experimental Values of μ_{ep} and k' Determined from Students' Data

Analyte	μ_{ep} ($cm^2/V \cdot s$)[a,b]	k'[b]
Acetylsalicylic acid	$(-3.5 \pm 0.2) \times 10^{-4}$	3.53 ± 0.09
Salicylic acid	$(-4.2 \pm 0.3) \times 10^{-4}$	7.0 ± 0.4
4-Acetamidophenol	$(-8.2 \pm 0.9) \times 10^{-5}$	0.484 ± 0.008
Norephedrine HCl	$(1.16 \pm 0.08) \times 10^{-4}$	1.53 ± 0.05

[a] $\mu_{eo} = (8.1 \pm 0.9) \times 10^{-4}$.
[b] Uncertainties represent standard deviations ($n = 27$).

Table 3. Manufacturer's Report of Ingredients for Analgesic Formulations Studied[a]

Analgesic	Caffeine	Acetamino-phen	Acetylsalicylic Acid	Salicylic Acid
Excedrin®	65	250	250	0
Goody's®	32.5	260	520	0
Anacin®	32	0	400	0
Bufferin®	0	0	325	0

[a] Results presented as milligrams per dose.

Table 2. CZE Results for Amount of Some Compounds in Analgesic Formulations[a,b]

Analgesic	Caffeine	Acetaminophen	Acetylsalicylic Acid	Salicylic Acid	*n*
Excedrin®	62 ± 9	240 ± 40	230 ± 50	17 ± 7	15
Goody's®	40 ± 10	270 ± 40	500 ± 100	17 ± 5	6
Anacin®	28	—	369	6	1
Bufferin®	—	—	270 ± 70	40 ± 20	5

[a] Results presented as milligrams per dose.
[b] Uncertanties represent standard deviations.

tions are prepared, acetylsalicylic acid determinations are not reliable. Minimization of acetysalicylic acid degradation (*13*) was not attempted in this experiment.

Overall, we have found the use of both CE and HPLC to be reasonably accurate in determining the level of analgesics in these over-the-counter formulations. This exercise, which often requires two laboratory periods to complete, provides students hands-on experience with both CE and HPLC and gives them a fundamental understanding of the mechanisms of the two techniques as well as of the limitations of UV detection and sample preparation and degradation.

Acknowledgments

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Notes

1. Sodium borate is often sold as sodium tetraborate (NaB_4O_7) or sodium metaborate ($NaBO_2 \cdot xH_2O$), so the use of boric acid (H_3BO_3) is strongly recommended to avoid confusion of buffer solution concentration.

2. Dissolving the standards in basic solution aids the solubility of salicylic and acetylsalicylic acid, but is not recommended because it can result in accelerated degradation of acetylsalicylic acid.

3. To calculate μ_{ep} for the charged species, one must first calculate the value of μ_{eo} by using the time of the neutral peak (caffeine), assuming a μ_{ep} of zero for this substance.

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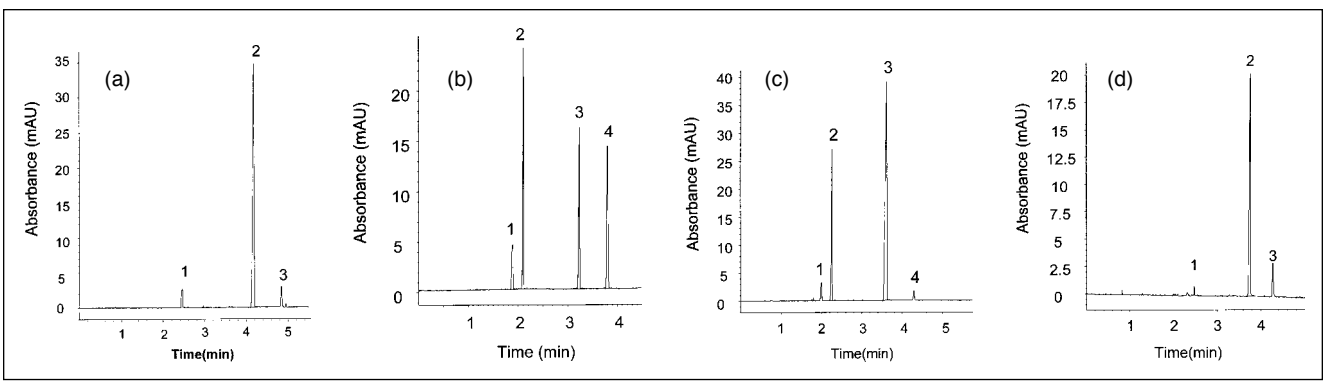

Figure 5. CZE separation of components of several commercial analgesic formulations. (a) Anacin®. Key: 1 = caffeine; 2 = acetylsalicylic acid; 3 = salicylic acid. (b) Excedrin®. Key: 1 = caffeine; 2 = acetominophen; 3 = acetylsalicylic acid; 4 = salicylic acid. (c) Goody's®. Key: 1 = caffeine, 2 = acetominophen; 3 = acetylsalicylic acid; 4 = salicylic acid. (d) Bufferin®. Key: 1 = caffeine; 2 = acetylsalicylic acid; 3 = salicylic acid. Separation conditions are given in the text.

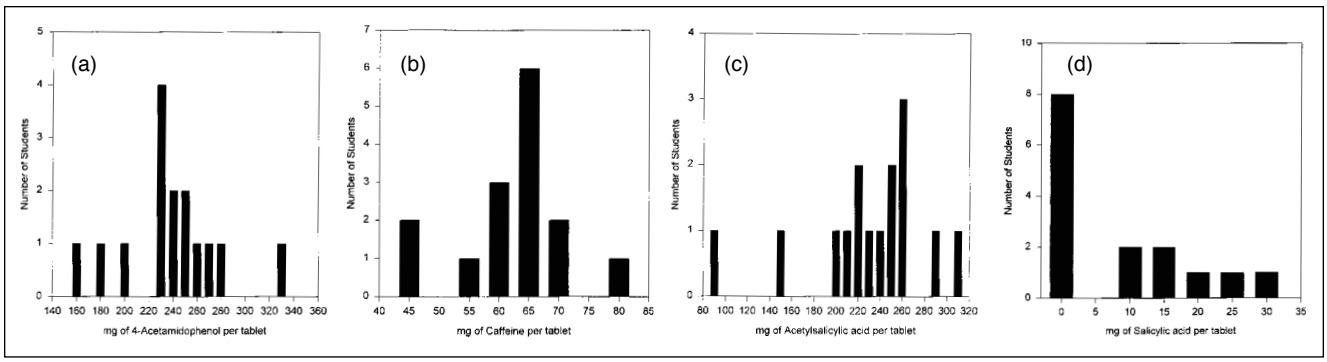

Figure 6. Histograms of students' results for (a) 4-acetamidophenol (label claims 250 mg), (b) caffeine (label claims 65 mg), (c) acetylsalicylic acid (label claims 250 mg), and (d) salicylic acid (not listed on label) in Excedrin®.

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