# Using Capillary Electrophoresis to Determine the Purity of Acetylsalicylic Acid Synthesized in the Undergraduate Laboratory

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Acetylsalicylic acid, commonly called aspirin, has been used as an analgesic for many years. Since the marketing of aspirin by the Bayer Company in 1899, this drug has been one of the most widely used over-the-counter pain relievers. Today, many students in undergraduate freshman and sophomore laboratories synthesize acetylsalicylic acid (ASA) from its precursor salicylic acid (SA) by way of an esterification reaction involving SA and acetic anhydride (*1*). Most synthesis procedures for ASA do not incorporate any product analysis beyond simple percent yield calculations based on weight percentages. Thus, students do not often account for unreacted starting material and may fail to recognize that their synthesized product is, in fact, a mixture of ASA and unreacted SA. It would be instructive to provide a means for students to analyze their synthesized product, emphasizing identification and quantitation of both product and remaining starting material.

The goal of the experiment described here is to determine the concentration of student-synthesized ASA product, along with unreacted SA, from a typical undergraduate lab. To achieve this goal, we have developed a capillary electrophoresis (CE) method capable of separating ASA and SA. Given its high efficiencies and other advantages of reduced analysis time and reduced sample and solvent requirements (*1, 2*), CE seems ideally suited for use in the undergraduate laboratory. Notably, Thompson et al. described a CE method for the analysis of commercial analgesics (*3*); and more recently, a number of publications in this *Journal* have described the incorporation of CE in the undergraduate curriculum, albeit in junior- or senior level laboratories (*4–7*). By using CE as a teaching tool to determine product purity in support of a preexisting laboratory experiment (the synthesis of ASA from SA), we hope to incorporate early exposure to instrumental analysis with minimal disruption to the regular freshman and sophomore curriculum. The fast analysis times and low sample and solvent requirements inherent to CE analysis make this an ideal instrumental method even for large laboratory classes.

In addition to simply resolving their synthesized ASA from unreacted SA, this experiment will enable the students to quantify the amount of their product by using standard curves or an internal standard. This would nicely complement the traditional melting point determination of product purity, which would indicate to students that their synthesized product was indeed impure, but would leave them with the questions "What impurity or impurities are present in my product?" and "How much impurity is present?" CE analysis can answer these important questions. Students will acquire the usual synthetic skills afforded by the traditional ASA synthesis lab, while learning important quantitative analytical skills and CE instrumental technique.

## Materials and Methods

### Chemicals

A separation buffer of 2 mM sodium tetraborate decahydrate (borax) and 4 mM sodium dodecyl sulfate (SDS) was used. Buffer pH was adjusted to 9.0 with 0.1 M NaOH. Pure acetylsalicylic acid and salicylic acid was used to prepare $1.0 \times 10^{-3}$ M and $5.0 \times 10^{-4}$ M stock solutions, respectively. In all cases, Millipore distilled and deionized water was used as the solvent. Reagents required for student preparation of ASA from SA are specified elsewhere, in numerous published procedures for the ASA undergraduate synthesis lab (*8–12*).

### Equipment

This experiment can be conducted using either a commercial CE system or a system built in-house. UV detection at 230 nm is necessary for analysis. Other conditions for analysis include 25 °C capillary temperature, 20-kV run voltage, 50-cm capillary (50 μm i.d., with detection window 45.4 cm from the inlet end) and 10 psi s injection (although electrokinetic injection at 10 kV for 10 s is also satisfactory). All electropherograms presented here were recorded on a Bio-Rad TC3000 CE system, although equivalent results were obtained with a home-built instrument employing a Spellman CZE1000R power supply and a Thermo Separation Products UV detector, operated under computer control with LabView software.

## Experimental Procedure

Before the experiment the capillary was flushed with borax buffer for 60 s at 100 psi. Additional 60-s flushes with buffer were conducted between injections. Serial dilution of the $1.0 \times 10^{-3}$ M ASA stock solution was conducted to produce five ASA standard solutions with concentrations $1.0 \times 10^{-5}$, $5.0 \times 10^{-5}$, $1.0 \times 10^{-4}$, $5.0 \times 10^{-4}$, and $1.0 \times 10^{-3}$ M. Similarly, the $5.0 \times 10^{-4}$ M SA stock solution was serially diluted to yield $5.0 \times 10^{-6}$, $1.0 \times 10^{-5}$, $5.0 \times 10^{-5}$, $1.0 \times 10^{-4}$, and $2.5 \times 10^{-4}$ M standard solutions. Student-synthesized ASA samples were prepared for CE analysis by dissolving them in water to an apparent concentration of $5.0 \times 10^{-5}$ M (assuming 100% pure product).

Electrophoresis of these standard solutions, under the same conditions used for student samples, provided both an average migration time for ASA and SA (useful for peak identification) and the peak-area-versus-concentration data used for the construction of calibration curves (necessary for quantitation). One set of calibration curves recorded by the instructor just prior to the laboratory class can be provided for the entire class, or each student group can obtain the standard data necessary to construct its own calibration curves. The former

procedure is obviously more time efficient and therefore may be more suitable for large freshman and sophomore laboratory classes.

The CE analysis of student ASA samples should be preceded by a brief prelab lecture to introduce students to the underlying principles of CE. Unlike conventional chromatographies, CE does not rely upon the partitioning of analytes between stationary and mobile phases to effect their separation. Instead, CE relies upon the differential migration of charged analytes, in a buffer solution, in response to an applied electric field. Coverage of this relatively simple concept can be tied into other typical freshman and sophomore chemistry topics such as ionic charge and radius, buffers and pH, ionic conductivity, and basic electrochemical principles such as solute transport. Conveniently, many chemistry students (particularly sophomores) will have encountered slab gel electrophoretic techniques in one of their biology laboratory classes, and so an introduction to CE could alternatively be presented as an extension of this perhaps more commonly encountered bioanalytical method.

Ideally, the CE analysis of student ASA samples is enacted in two stages. First, the newly-prepared student samples are submitted for analysis by CE at the end of the synthetic laboratory period. If a CE instrument with an autosampler is available, the teaching assistant or technician can process samples from a freshman or sophomore laboratory section of 40–48 students (as typically encountered at our institution) in about four hours. Second, the students can reanalyze their samples the following week, to determine the effects of aging on ASA content. In this case, the teaching assistant could demonstrate the CE system to individuals or small groups of students as their samples are run. This would require students to spend no more than 10 minutes away from their current laboratory experiment, and so would not interfere with the laboratory schedule.

## Hazards

Salicylic acid and acetylsalicylic acid can cause skin irritation; they should be handled carefully, and gloves should be worn. Extreme caution must be used when conducting these separations with extremely high voltages. Instruments must possess a safety interlock to prevent accidental contact with electrodes or solutions while high voltage is applied.

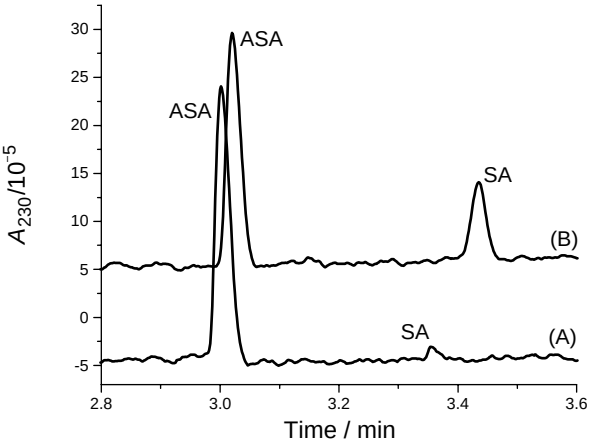


Figure 1. Electropherograms for typical student-synthesized ASA product (A) immediately after synthesis, and (B) one week later. In both cases, solutions were prepared in water to a concentration of $5.0 \times 10^{-5}$ M (assuming 100% pure product).

## Results and Discussion

For this method to be suitable for an undergraduate laboratory, separation conditions must be found that effectively resolve ASA from SA in a very short period of time using relatively inexpensive and "safe" reagents. Conditions amenable to the CE separation of ASA from various other analytes have been reported (*13–16*), but none of these were developed with the needs of the undergraduate laboratory in mind. Thompson et al. (*3*) established conditions for the resolution of ASA from SA and other analytes in commercial analgesics for an undergraduate laboratory. However, their buffer conditions, when employed in our laboratory, gave irreproducible peaks and required nearly 12 minutes to resolve ASA from SA, and hence would not be suitable for a large laboratory section. We have developed a 2 mM borax/4 mM SDS system (pH 9.0) that reproducibly separates ASA from SA in about four minutes (see Fig. 1). This buffer is stable and inexpensive, making it suitable for undergraduate laboratory use. From Figure 1A the calculated net mobility of ASA and SA is $6.3 \times 10^{-4}$ and $5.6 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$, respectively, and the electroosmotic mobility is $9.3 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$.

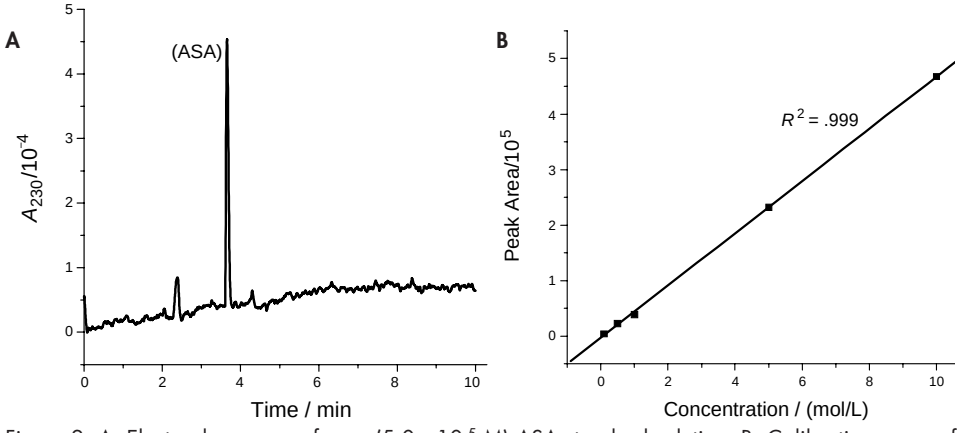


Figure 2. A: Electropherogram of pure ($5.0 \times 10^{-5}$ M) ASA standard solution. B: Calibration curve of peak area versus concentration.

Students can determine the concentrations of ASA and SA in their synthesized samples by comparing their peak areas to standard curves (see Figs. 2 and 3). Peak height should not be used owing to inferior linearity within similar concentration ranges. Limits of detection for ASA and SA determined by serial dilution are $3.0\times10^{-6}$ and $2.0\times10^{-6}$ M, respectively. Notice the appearance of "markers" of electroosmotic flow in Figures 2A and 3A (small peaks at around 2.5 min). These are commonly observed when the sample is prepared in a medium different from the running buffer, and will coincide with any neutral sample components (such as caffeine in some commercial aspirin samples). Notice also the longer migration times for ASA and SA in Figures 2A and 3A relative to those in Figure 1. This illustrates the variability in electroosmotic flow from capillary to capillary and even for a given capillary over time, since the electropherograms in Figures 2 and 3 were recorded 20 days before those in Figure 1. Electropherogram B in Figure 1 was recorded one week after electropherogram A; here, too, we see a change in electroosmotic flow as well as a change in the peak area of ASA relative to SA due to hydrolysis of the student-synthesized sample during the week. In all cases, increasing the applied voltage or decreasing the capillary length could shorten migration times.

These results demonstrate that it is entirely feasible to expose freshman- or sophomore-level chemistry students to a modern instrumental method (CE) without major alterations to existing curricula. Since many students choose not to pursue chemistry beyond the equivalent of the first four semesters (as required by most medical schools, for example), this experiment will ensure that even nonmajors become acquainted with the powerful instrumental tools available to analytical labs. As CE becomes more widely used in industrial and clinical settings, these same students may well find themselves face to face with such instrumentation in their future career, and this experiment will certainly help to demystify and stimulate interest in these powerful quantitative methods.

## Acknowledgment

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## Supplemental Material

A handout for students and notes for the instructor are available in this issue of *JCE Online*.

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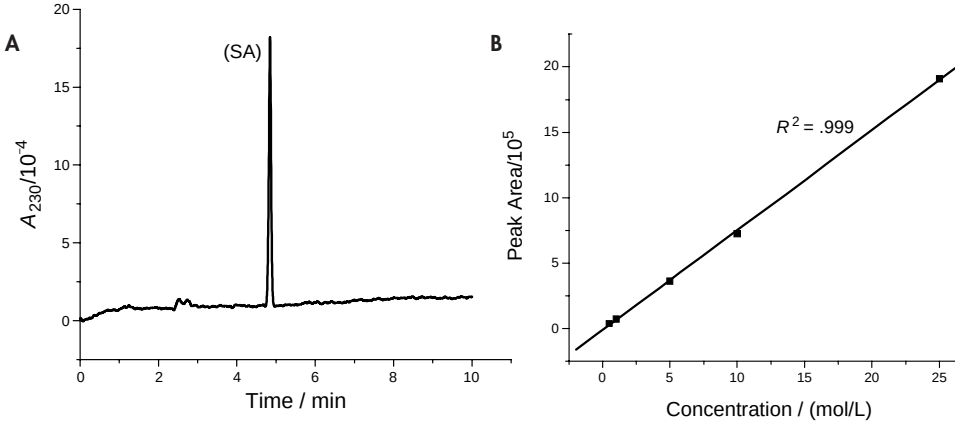


Figure 3. A: Electropherogram of pure ($5.0\times10^{-5}$ M) SA standard solution. B: Calibration curve of peak area versus concentration.