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Capillary Electrophoresis (CE)

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Overview

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Capillary electrophoresis (CE) is a separation technique that separates molecules in an electric field according to size and charge. CE is performed in a small glass tube called a capillary that is filled with an electrolyte solution. Analytes are separated due to differences in electrophoretic mobility, which varies with charge, solvent viscosity, and size. Traditional electrophoresis in gels is limited in the amount of voltage that can be applied because Joule heating effects will ruin the gel and the separation. Capillaries have a large surface area-to-volume ratio and thus dissipate heat better. Therefore, the voltages applied for a capillary electrophoresis experiment are quite large, often 10,000–20,000 V.

Capillary electrophoresis is useful for high-performance separations. Compared to liquid chromatography, CE separations are often faster and more efficient. However, capillary electrophoresis works best to separate charged molecules, which is not a limitation of liquid chromatography. CE has a greater peak capacity than high-performance liquid chromatography (HPLC), meaning the separations are more efficient and more peaks can be detected. The instrumentation can be very simple. However, HPLC is more versatile and many stationary and mobile phases have been developed for different types of molecules.

Principles

Capillary electrophoresis separates molecules due to their electrophoretic mobilities. A molecule's electrophoretic mobility depends on its charge and how much it is attracted or repelled by the voltage as well as the frictional drag force that resists movement. Friction is proportional to the radius of the molecule. Thus, electrophoretic mobility is based on size and charge. The velocity a charged molecule travels down a capillary is the product of its electrophoretic mobility and the applied electric field. Higher voltages therefore lead to faster velocities and faster separations.

Most capillary electrophoresis instruments are set up with the negative voltage at the detector end and the positive voltage at the inlet. This means that positively-charged molecules migrate towards the cathode at the end, while negatively-charged molecules migrate the other way. All molecules are seen at the detector however, because there is a bulk fluid flow called electroosmotic flow. The migration order is thus positively-charged, neutral, and then negatively-charged molecules.

Electroosmotic flow is caused by applying a high voltage to a small glass capillary filled with a salt solution. The positively-charged ions in the salt solution form a double layer with the negatively-charged silanol groups on the walls of the glass. When a negative voltage is applied to the end of the capillary, it pulls the cations from the double layer, which also pulls the solution around it due to frictional forces. This type of flow is plug-shaped and leads to less band-broadening than the parabolic-shaped flow plugs of HPLC.

Neutral molecules all flow at the same rate as the electroosmotic flow. However, a pseudo-stationary phase can be added to the run buffer to form micelles that molecules can partition in and out of. A typical pseudo-stationary phase is sodium dodecylsulfate. The micelles are negatively-charged on the outside, so they have an electrophoretic mobility, so the time spent in the micelle determines the migration time. This form of capillary electrophoresis is called micellar electrokinetic chromatography (MEKC).

Detection in CE is similar to that for HPLC. UV-Vis is general and does not require tagging as long as the molecule has a double bond. However, the absorbance depends on the path length, which is small for a 50-µm capillary. A bubble cell or z-cell will increase the path length. Laser-induced fluorescence is a more sensitive detection method. A laser is shone through a window in the capillary and fluorescence of the product measured. While fluorescence provides very high sensitivity, it generally requires molecules to be tagged because most are not fluorescent. Electrochemical detection and electrospray mass spectrometry detection are gaining in popularity. The issue with either of these detectors is that the high voltage from the separation must be brought to ground before the detection, as electrochemistry and electrospray require the application of a voltage and the CE voltage can interfere. New methods of decoupling the CE voltage, using electrodes to drain the current or a small crack in the capillary, are overcoming these challenges.

Procedure

1. CE Instrumentation Setup

1. Turn on the CE instrument and computer. Using the computer software, turn on the light source for UV analysis to allow it to warm up. Some software has an indicator when the lamp is ready for use (lamp icon turns color).
2. Make a methods file. Set the important parameters for running the CE. In this analysis the temperatures of the cartridge and sample storage are 35 °C. The wavelength for UV detection is 214 nm.
3. Write a time program. The program generally consists of rinse steps (to clean the capillary before analysis), injection steps, and then an electrophoresis step. For the rinse step, perform 2 rinses for 1 min using 20 psi of pressure. The first rinse is with NaOH, which helps make sure the silanol groups on the capillary wall are deprotonated. The second rinse is with run buffer (0.025 M borate buffer here) to make sure the capillary is left equilibrated with buffer.
4. For the injection, pressure injection is used at 0.5 psi for 5 s.

5. For the electrophoresis step, the conditions are separation voltage: 20 kV, time: 5 min, normal polarity. For each step, also indicate which vial is the inlet and which vial is the outlet. Save the methods file after all the parameters are entered.

2. Preparation of the Standards and Soda Samples

1. Make 500-ppm stock solutions of aspartame, caffeine, and benzoic acid in water. Make 50 mL of each, using a volumetric flask.
2. Make a standard solution of 150 ppm aspartame, 150 ppm caffeine, and 100 ppm benzoic acid in a 10-mL volumetric flask.
3. Make standard caffeine solutions of 50 ppm, 100 ppm, 150 ppm, and 200 ppm in a 10-mL volumetric flasks.

3. Run the Samples on the CE

1. Place the vials containing either standards or soda samples into the sample vial holder. Make sure to write down which sample is in which slot. Two slots have the borate run buffer and the 0.1 M NaOH rinse solution.
2. In the method file, input which slot the first sample vial is in.
3. Acquire a single run, making sure to input all the data information the program requests.
4. Continue to acquire data, changing the input vial for each sample. Run the combination standard, the 3 concentrations of caffeine, and a Pepsi and diet Pepsi sample.
5. Insert the target into the instrument and choose the appropriate instrument.
6. Analyze the data on the computer. Calculate the peak areas and overlay standards and real samples to help identify peaks. Make a calibration curve for the caffeine data.

Results

Electropherograms collected for diet Pepsi and Pepsi samples are shown in **Figures 1** and **2**, respectively. The three peaks for caffeine, aspartame, and benzoic acid are observed in diet Pepsi and have similar migration times as the standards. For regular Pepsi, the caffeine peak is present but not the aspartame and benzoic acid peaks. The CE analysis is fast as the migration times are only 3–4 min.

The calibration curve for caffeine is shown in **Figure 3**. This curve can be used to calculate the concentration of caffeine in each sample.

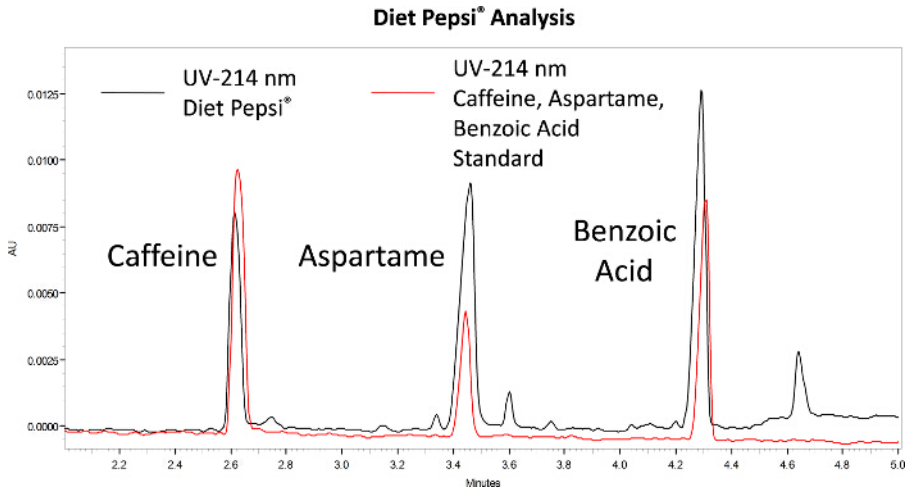

Figure 1. CE analysis of Diet Pepsi. The red are standards of caffeine, aspartame, and benzoic acid. The black is a diet Pepsi sample. Please click here to view a larger version of this figure.

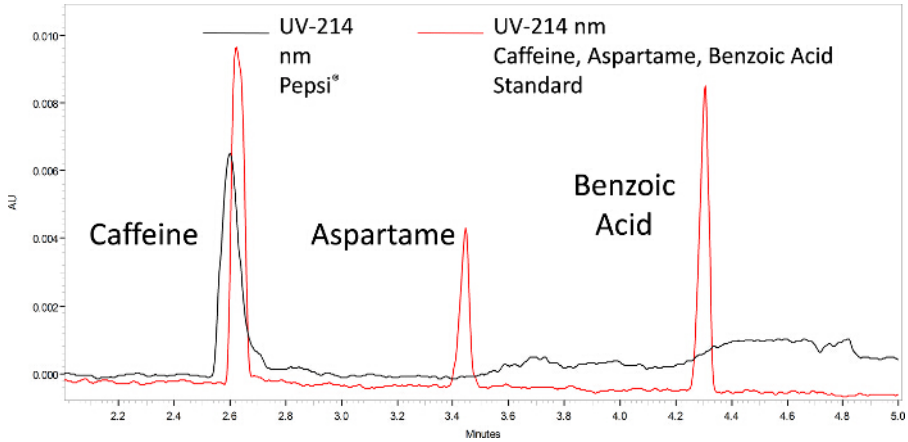

Figure 2. CE analysis of Pepsi. The black is a Pepsi sample while the red is a sample of standards of caffeine, aspartame, and benzoic acid. There is no aspartame or benzoic acid, indicating the soda is not diet. Please click here to view a larger version of this figure.

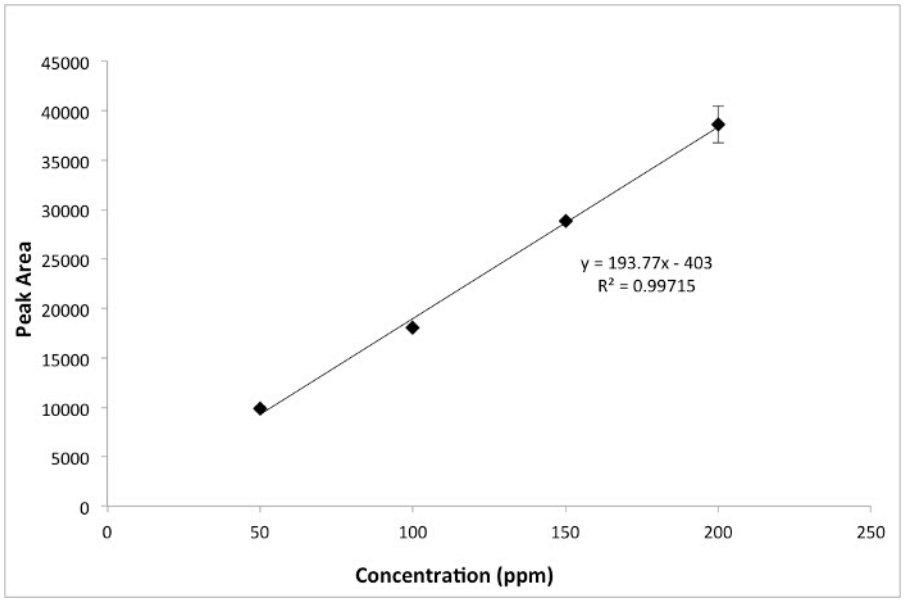

Figure 3. Caffeine calibration plot with CE. A plot of the peak area vs concentration for caffeine standards measured with CE. Please click here to view a larger version of this figure.

Applications and Summary

Capillary electrophoresis is used for many specialty separations. For example, it is used in the pharmaceutical industry for quality testing, to make sure there are no side products or interferents. CE is particularly useful for separating drugs with a basic amino group, as the walls of the capillary can be made neutral with an acidic pH and thus the drug will not stick to the capillary.

A mode of CE was also used to sequence the human genome and separate DNA. This mode of CE is capillary gel electrophoresis and for these separations, a polymer is injected into the CE capillary. The polymer gives an additional mode of separation based on size, as the smaller fragments can travel faster through the gel. This is called sieving, and along with the electrophoretic separation, it has 1 base pair resolution for DNA analysis.