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**Determination of caffeine in Peruvian coffee at different levels of roast as measured through Capillary Electrophoresis**

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Determination of caffeine in Peruvian coffee at different levels of roast as measured through Capillary Electrophoresis
Jaspreet Kaur Rishi*, Kashif Shaikh, Dr. Joshua Sebree

**Background**

- Caffeine from natural sources has been consumed and enjoyed by people throughout the world for centuries, dating back to perhaps as early as Paleolithic period (1).
- Caffeine, 1,3,7-trimethylxanthine, is the world's most widely consumed drug with its main source found in coffee (2).
- The amount of caffeine intake by individuals should be monitored depending on body types because it produces central nervous system stimulation and has been found to positively influence human performance (2).
- An average cup of coffee has about 95 mg of caffeine and on average, an American consumes 3.1 kg of coffee in a year (3).

![Caffeine molecule](image)

**Objective**

For this project, the amount of caffeine per gram of Peruvian coffee at different levels of roast was determined in 14 samples using capillary electrophoresis.

![14 different samples of coffee beans collected during the coffee roasting process.](image)

**Sample Prep**

- 3g coffee soaked in 50 mL water
- 4mL coffee + 2mL dichloromethane – centrifuged
- Organic layer containing caffeine collected, dried and diluted.

![Brewed coffee samples, before the caffeine extraction process.](image)

**Instrumentation**

- Capillary Electrophoresis – a technique in which molecular species are separated based on charge and size by their different rates of migration in an electric field.
- A single micro-capillary filled with an electrolyte is used that connects reservoirs at either end. Running buffer is added to the capillary.
- The sample is thus run through, the small cations are collected first through the detector which is also the cathode end, followed by the bigger cations, then by small anions and lastly by big anions, separating the molecules by charge and size.
- Since caffeine molecule is a polar molecule, without any formal charge, a basic buffer was used to protonate the nitrogen and acquire positive charges (pKa 14).

![A schematic of the capillary electrophoresis instrument being used. a.) enlarged inside view of the capillary. b.) Cross section of the capillary.](image)

**Results**

![graph showing the content of caffeine as a function of roasting time](image)

**Discussion**

- A general trend of concentration of coffee as a function of roasting time was established.
- The amount of caffeine first decreases per gram of bean as the roasting time (and temperature) increases, but then starts to go up again as the density of coffee starts becoming lower.
- Significant matrix effect had to be accounted for.
- In another experiment performed earlier in class, caffeine concentration was measured by using the quenching property of caffeine on aspirin’s fluorescence. This technique eliminates the matrix effect.

**Conclusions**

- This study proved the concept of being able to determine the concentration of caffeine using a capillary electrophoresis.
- Fluorescence (quenched aspirin) is a better technique to accommodate for the matrix effect in coffee.

**Future directions**

This study can be repeated while keeping certain factors in mind such as:
- consistency in brewing time and
- consistency and exact measurement of distilled water at every step of the extraction process.
This will ensure better reproducibility of results.

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**References**