EDULAB FOR STUDENTS

Brewing Secrets: Power Saturation in Coffee Unveiled by EPR

EPR of Coffee

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Experiment Hashtag: #Coffee, #Antioxidants, #Roasting, #Educate2Resonate

Keywords:

Coffee, Roasting, Free Radicals, Antioxidants, Food Analysis, Microwave Power Optimization, Saturation

Target group:

Advanced Undergraduate or Graduate, General Chemistry, Analytical Chemistry, Food Chemistry, Food Safety and Control Laboratory, General Life Sciences

Objectives:

Electron Paramagnetic Resonance (EPR) spectroscopy is a sensitive and versatile technique for analyzing molecules that contain unpaired electrons, such as paramagnetic metal ions and free radicals. The formation of free radicals in foods, including coffee, is an indication of coffee oxidation mainly due to redox chemistry reactions. In this exercise, students will learn what information can be extracted from coffee samples using EPR. The goal of this exercise is to teach students how to detect free radical signals generated during coffee roasting and how to correlate the radical concentration with the roasting level. Additionally, students will learn how to run EPR experiments on a bench-top Magnettech ESR5000 spectrometer, optimize experimental parameters, and create power saturation curves using the ESRStudio software. Optimizing microwave power in EPR spectroscopy is essential for obtaining accurate and reliable data. The intensity of the EPR signal rises with the square root of the microwave power when there are no saturation effects, making it crucial to operate within the linear regime (Figure 1). When saturation sets in, the signals broaden, and the peak-to-peak amplitude decreases. Proper adjustment of microwave power ensures that the EPR signal is strong enough to be detected without causing saturation, which can distort the spectrum and reduce resolution. Additionally, optimizing microwave power protects sensitive samples from potential damage caused by excessive power levels. Overall, careful control of microwave power is crucial for precise and meaningful EPR measurements.

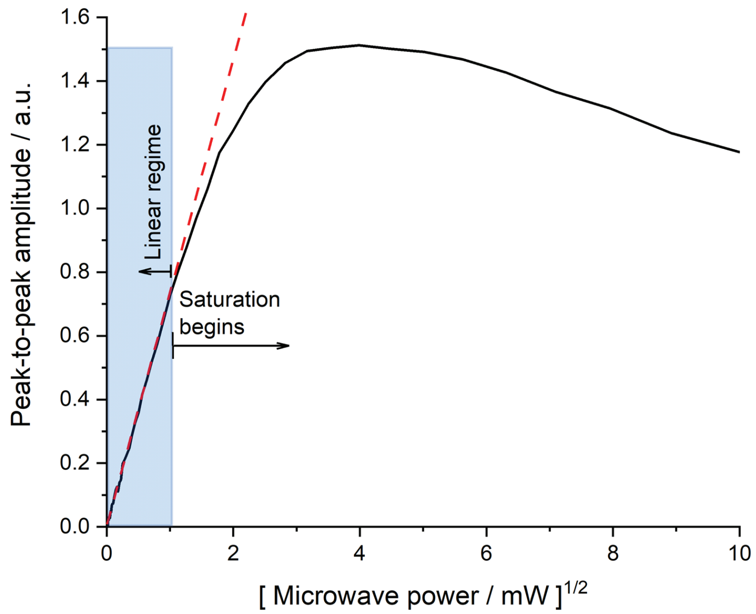

Figure 1: An example of a power saturation curve illustrating the linear regime that is ideal for microwave power.

Students will use the $P_{1/2}$ value to characterize the saturation behavior of the EPR signal. Various definitions of $P_{1/2}$ are available in the literature. In ESRStudio, the term $P_{1/2}$ refers to the power at which the signal intensity is half of its maximum value. The half-saturation power is used to determine the optimal microwave power for EPR experiments, ensuring that the signal is strong enough to be detected without causing saturation effects that can distort the spectrum. The higher the $P_{1/2}$ value, the higher the microwave power that can be used before the signal is saturated.

Background of the Experiment:

Coffee and tea are among the most widely consumed beverages globally. Both have historical associations with medicinal properties, though neither is typically classified as traditional medicine. Traditionally, the quality of coffee and tea is evaluated based on their sensory attributes by expert tasting panels, as these attributes are directly relevant to consumers' immediate experiences. However, recent research has revealed that both beverages contain compounds beneficial to long-term health and well-being, a quality increasingly recognized and valued by consumers. Consequently, our understanding of coffee and tea quality is gradually expanding to include long-term health benefits alongside immediate sensory impact.

Glossary

EPR (Electron Paramagnetic Resonance): Also known as Electron Spin Resonance (ESR) Spectroscopy, is a technique used to study materials with unpaired electrons. The fundamental principles of EPR are similar to those of nuclear magnetic resonance (NMR), but instead of exciting the spins of atomic nuclei, EPR focuses on the spins of electrons

Free radicals: An atom, molecule, or ion that possesses at least one unpaired valence electron.

Antioxidants: Molecules that help protect your body from damage caused by free radicals, which are unstable molecules that can harm cells. Common antioxidants include vitamins C and E, selenium, and beta-carotene. These can be found in many fruits, vegetables, nuts, and grains.

The primary beneficial compounds in these beverages are antioxidants, which protect body cells from oxidative damage in three ways: 1) by preventing the formation of oxidizing agents, 2) by selectively scavenging oxidizing agents, and 3) by scavenging the products of reactions involving oxidizing agents, which can also cause cellular damage. Mechanism 1 typically involves antioxidant enzymes, while mechanisms 2 and 3 can be carried out by both small and large molecules. A common characteristic of biological antioxidants is their ability to cycle between reduced and oxidized forms, both of which are generally stable.

Coffee brews are rich in polyphenols, known for their antioxidant properties. During the roasting of coffee beans, antioxidants in green beans, such as chlorogenic acids, are partially decomposed or incorporated into melanoidins. Melanoidins are a diverse group of polymers with varying molecular weights, formed through the Maillard reaction between reducing sugars and amino acids during roasting (non-enzymatic browning). Although there is substantial evidence that the biological activity of coffee melanoidins and flavonoids in vivo may arise from non-antioxidant mechanisms, numerous studies have focused on the antioxidant activity of coffee brews and extracts. It is well established that roasting coffee beans produces stable radicals associated with Maillard reaction products rather than phenolic compounds.

In this exercise, students will examine light, medium, and dark roast coffee. They will search for a free-radical EPR signal originating from Maillard reaction products formed during roasting. Students will then characterize this signal by conducting a power sweep experiment and obtaining saturation curves for each sample.

Preparation:

To ensure the experiments are completed efficiently, it is recommended to form groups of no more than three students. Sample preparation is estimated to take about 10 minutes. The EPR experiments, including power sweep experiments, are expected to take approximately 2 hours in total. After completing the experiments, an additional 1 to 1.5 hours will be needed to write a report. It is assumed that students have already covered introductory concepts of EPR and have a basic understanding of instrumental parameters.

For detailed information on EPR basics and optimizing instrumental parameters, students can refer to the Magnettech ESR5000 educational kit, which is provided on a USB drive along with the bench-top EPR spectrometer. An installed Magnettech ESR5000 spectrometer is required to perform these experiments.

Experimental Setup:

Materials:

- 3 samples of light roast, medium roast, and dark roast ground coffee (1 g each)
- 5 mm OD tubes – 3 pieces (tubes can either be made from borosilicate or from quartz)

Glossary

Power saturation:
In EPR spectroscopy, saturation occurs when the microwave power used to excite the electron spins becomes too high, causing the populations of the spin states to equalize. To avoid saturation and obtain accurate measurements, it's important to use an appropriate microwave power level. This ensures that the spin relaxation processes can restore the population difference between the spin states, maintaining a detectable EPR signal.

Abbreviations

EPR: Electron Paramagnetic Resonance

$P_{1/2}$: In ESRStudio software, the term $P_{1/2}$ refers to the power at which the signal intensity is half of its maximum value

Sample Preparation:

Fill each 5 mm tube with your coffee material to a height of about 3 cm and insert it into the spectrometer resonator. You can easily scoop the material through the open end of the tube. A 3 cm filling height is recommended because it ensures the entire cavity is filled with the sample. This results in consistent outcomes and increased signal intensity, as the EPR signal is directly proportional to the number of radicals in the sample. More volume means stronger signals.

Experimental Procedure:

1. Turn on the EPR spectrometer by switching on the power located at the back of the unit. Launch the ESRStudio software and connect to the spectrometer by clicking the Initialize button. Carefully insert one of the coffee samples using the appropriate sample holder. Use the positioning tool (cavity template) to ensure the sample is properly centered inside the resonator.

2. In the recipe editor, create a recipe named 'Coffee' using the specified parameters:

   - B0 - 337 mT (optimize B0 after acquiring the first spectrum)
   - Sweep – 10 mT
   - Sweep Time – 30 s
   - Modulation – 0.5 mT
   - Accumulations – 1
   - Power – 1 mW

3. In ESRStudio, create a new container and name it 'Coffee'. Select the folder and start collecting the spectra from the coffee samples in this container.

4. For the power sweep experiment, select the 'Power sweep' option and set the power range from 0.001 mW to 100 mW with a step count of 20. The rest of the parameters remain the same.

Data Processing:

1. To determine the g-factors of the peaks in each spectrum in ESRStudio, switch the X-axis to display the g-factor.

2. In ESRStudio, the peak-to-peak amplitude for each spectrum is automatically calculated. Highlight the spectrum of interest and note the amplitude value displayed in arbitrary units above the spectrum window. Record these values.

3. In 'Evaluation View,' examine the saturation curves for the power sweep data. From the drop-down menu, choose 'Power saturation level' and fit the curve. In the saturation curves, the peak-to-peak amplitude is presented as a function of the square root of the microwave power. The $P_{1/2}$ value will also be displayed.

Notes

Questions for the Students:

1. How would you account for the variations in peak-to-peak amplitudes among the samples?

2. In your opinion, what types of radicals did you detect?

3. What conclusions can be drawn from the power saturation curves and $P_{1/2}$ value?

Notes

Key Take Home Messages:

- There are several types of radicals in roasted coffee.
- The roasting process increases the antioxidant capacity of coffee.
- Learn how to perform power sweep measurements and generate saturation curves.
- Gain an understanding of power saturation and the significance of the $P_{1/2}$ value.

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