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Introduction to Electron Paramagnetic Resonance (EPR)
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Overview

In this video we will learn the basic principles behind Electron Paramagnetic Resonance (EPR). We will use EPR spectroscopy to study how dibutylhydroxy toluene (BHT) behaves as an antioxidant in the autoxidation of aliphatic aldehydes.

Principles

Fundamentals of EPR

EPR is a spectroscopic technique that relies on similar physical phenomena as nuclear magnetic resonance (NMR) spectroscopy. While NMR measures nuclear spin transitions, EPR measures electron spin transitions. EPR is chiefly used to study paramagnetic molecules, which are molecules with unpaired electrons. Recall that an electron has a spin quantum number, $s = \frac{1}{2}$, which has magnetic components $m_s = \frac{1}{2}$ and $m_s = -\frac{1}{2}$. In the absence of a magnetic field, the energy of the two m_s states are equivalent. However, in the presence of an applied magnetic field (B_0), the magnetic moment of the electron aligns with the applied magnetic field and, as a result, the m_s states become non-degenerate (**Figure 1**). The energy difference between the m_s state is dependent on the strength of the magnetic field (**equation 1**). This is called the Zeeman effect.

$$E = m_s g_e \mu_B B_0 \quad (1)$$

where $g_e = g$ -factor, which is 2.0023 for a free-electron

and μ_B = Bohr magneton

At a given magnetic field, B_0 , the energy difference between the two m_s states is given by **equation 2**.

$$\Delta E = E_{1/2} - E_{-1/2} = g_e \mu_B B_0 = h\nu \quad \square\square\square$$

An electron moves between the two m_s states upon emission or absorption a photon with energy $\Delta E = h\nu$. Equation 2 applies to a single, free-electron. However, similar to the way that the chemical shift in ^1H NMR depends on the chemical environment of the H atom, electrons within molecules do not behave in the same way as an isolated electron. The electric field gradient of the molecule will influence the effective magnetic field, given by **equation 3**.

$$B_{\text{eff}} = B_0 (1 - \sigma) \quad (3)$$

where σ = effect of local fields, which can be a + or – value

Plugging **equation 3** into **equation 2**, we can define the g -factor for an unpaired electron in a given molecule as $g = g_e(1 - \sigma)$, which simplifies the overall equation to:

$$h\nu = g\mu_B B_0 \quad (4)$$

During the EPR experiment, the frequency is swept, most commonly in the microwave region ranging from 9000–10000 MHz, and the field is held constant, around 0.35 T, allowing for the calculation of g . Experimentally determining g using EPR provides information about the electronic structure of a paramagnetic molecule.

Application of EPR

In this experiment, we will use EPR spectroscopy to investigate the chemistry of antioxidants. O_2 , which comprises ~21% of the earth's atmosphere, is a strong oxidant. Despite its potential to act as an oxidant, O_2 is a ground state triplet and thus only reacts quite slowly with most organic molecules. One important, though often undesired, reaction mediated by O_2 is autoxidation. In autoxidation chemistry, O_2 initiates radical chain processes, which can quickly consume organic molecules. **Figure 2** illustrates a common autoxidation, in which aldehydes are oxidized to carboxylic acids.

Preventing autoxidation chemistry is important to prevent decomposition of many common organic materials, such as plastics, and a large field has developed around identifying effective antioxidants to inhibit autoxidation. One mechanism by which antioxidants can function is by reacting with the radical intermediates to inhibit radical chain processes. Because radical species have unpaired spins, EPR is a valuable tool for understanding the chemistry of antioxidants. In this experiment, we will use EPR spectroscopy to explore the role of dibutylhydroxy toluene (BHT) as an antioxidant in the autoxidation of aliphatic aldehydes.

Procedure

1. Autoxidation of butyraldehyde.
 - 1.1. Prepare a solution of butyraldehyde (100 mg) and $CoCl_2 \cdot 6H_2O$ (1 mg) in 1,2-dichloroethane (4 mL) in a 20-mL scintillation vial. Add a magnetic stir bar and fit the vial with a rubber septum.
 - 1.2. Attach the barrel of a 1-mL plastic syringe to a short piece of rubber tubing. Insert the rubber tubing into a latex balloon and secure the balloon to the tube with a rubber band and electrical tape. Inflate a latex balloon with O_2 .
 - 1.3. Insert the needle of the O_2 balloon into the reaction vial. Insert a second needle into the septum and purge the head-space of the reaction vessel with O_2 .
 - 1.4. Using a stir plate, stir the reaction at room temperature for 4 hours under an O_2 atmosphere.
 - 1.5. Concentrate the reaction mixture using a rotary evaporator and take a 1H NMR spectrum of the resulting oily residue in $CDCl_3$.

2. Using BHT as an antioxidant for the autoxidation of butyraldehyde. Set up TWO vials as described below. One will be used to analyse the product distribution and one will be used in Step 3 for EPR spectroscopy.

2.1. Prepare a solution of butyraldehyde (100 mg) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (1 mg) in 1,2-dichloroethane (4 mL) in a 20-mL scintillation vial. Add BHT (10 mg) to the solution. Add a magnetic stir bar and fit the vial with a rubber septum.

2.2. Attach the barrel of a 1-mL plastic syringe to a short piece of rubber tubing. Insert the rubber tubing into a latex balloon and secure the balloon to the tube with a rubber band and electrical tape. Inflate a latex balloon with O_2 .

2.3. Insert the needle of the O_2 balloon into the reaction vial. Insert a second needle into the septum and purge the head-space of the reaction vessel with O_2 .

2.4. Using a stir plate, stir the reaction at room temperature for 4 hours under an O_2 atmosphere.

2.5. Concentrate the reaction mixture using a rotary evaporator and take a ^1H NMR spectrum of the resulting oily residue in CDCl_3 .

3. Measuring EPR spectra.

3.1. Turn the EPR spectrometer on and let the instrument warm up for 30 minutes. Set up an EPR acquisition with the following parameters: center field 3345 G, sweep width 100 G, sweep time 55 s, time constant 10 ms, MW power 5 mW, modulation 100 kHz and modulation amplitude 1 G.

3.2. Measure an EPR spectrum of an empty EPR tube to insure that there are no background signals from either the EPR tube or the instrument resonator.

3.3. Prepare a solution BHT in 1,2-dichloroethane (DCE) in an N_2 -filled glovebox. Transfer 0.5 mL of the solution to an EPR tube and measure EPR spectrum of BHT using the acquisition parameters set up in Step 3.1.

3.4. Transfer 0.5 mL of the BHT-added reaction solution from Step 2 to an EPR tube and acquire an EPR spectrum using the acquisition parameters set up in Step 3.1.

Representative Results

The autoxidation of butyraldehyde affords butyric acid. The ^1H NMR spectrum obtained from the reaction carried out in Step 1 shows the lack of an aldehydic C-H resonance and the presence of the resonances expected of butyric acid. In contrast, the NMR obtained from the reaction mixture from Step 2 (with added BHT) displays signals consistent with

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butyraldehyde, with no butyric acid present. From these data, we observe the butyraldehyde has served as an antioxidant in aldehyde autoxidation.

The role of BHT in inhibiting aldehyde autoxidation is illuminated by the EPR spectra obtained of BHT and of BHT added to the aldehyde autoxidation reaction. BHT is a diamagnetic organic molecule, meaning that there are no unpaired electrons. Accordingly, the EPR spectrum of BHT displays no signals. In contrast, the EPR spectrum of the autoxidation reaction in which BHT was added displays a strong three-lined pattern, consistent with an organic radical. This spectrum arises because the O–H bond of BHT is weak and in the presence of radicals generated during autoxidation, H-atom transfer from BHT quenches the radical chain mechanism and generates a stable O-centered radical.

Summary

In this experiment, we explored the role of antioxidants in inhibiting autoxidation chemistry. We probed the mechanism of inhibition using EPR spectroscopy, which revealed that BHT serves as an antioxidant by quenching reactive radical intermediates via H-atom transfer.

Applications

Molecules with unpaired electrons can be challenging to characterize by NMR and thus EPR spectroscopy frequently provides useful and complementary information regarding these species. EPR spectroscopy is an experimental technique that is frequently used to detect and characterize organic radicals. In addition, paramagnetic inorganic complexes also frequently display EPR spectra that can be instructive for characterization. Experimental EPR spectra delineate the *g*-factor of the unpaired electron, which provides information about the electronic structure of the paramagnetic center. In addition, the nuclear spins of the nuclei with the unpaired electron as well as neighboring nuclei also influence the magnetic moment of an electron, giving rise to additional splitting of the m_s states and multiple lines in the EPR spectrum. The resulting hyperfine and super-hyperfine coupling provides further information about the electronic structure of the molecule.

In addition to characterizing open-shell organic and inorganic species, the exquisite sensitivity of EPR spectroscopy is critical to application to bioinorganic systems, where the concentration of metal cofactors is low. EPR spectra are routinely used in bioinorganic chemistry to provide direct information about the structures and oxidation states of metal ions at the heart of enzymes.

Legend

Figure 1. Splitting of magnetic moment states, m_s , in the presence of a magnetic field.

Figure 2. Aldehyde autoxidation proceeds via a radical chain mechanism.

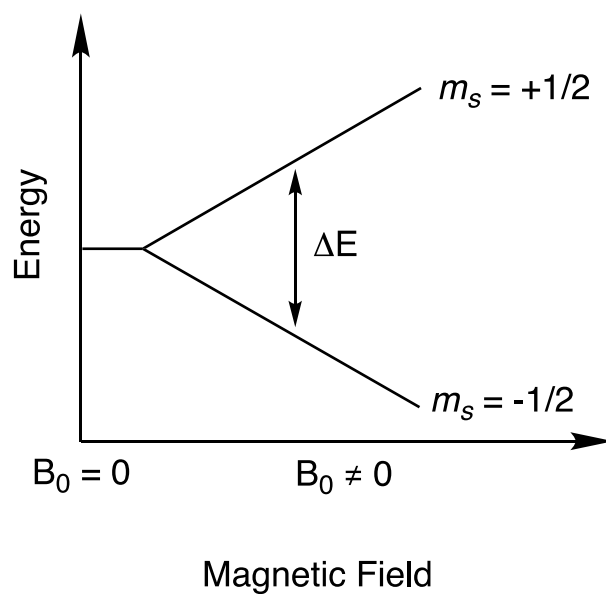


Figure 1.

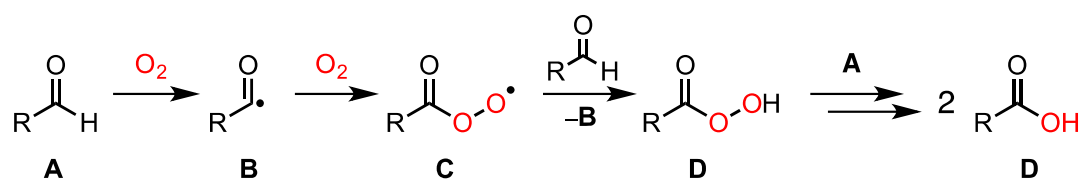


Figure 2.