Magnetization of Yeast by Labeling with Iron Complexes: An Undergraduate Laboratory Experiment

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Abstract

This undergraduate laboratory experiment is designed for first-year general chemistry students with the goal of introducing the brilliant colors and magnetic properties of transition metal complexes within a bioinorganic/cell biology context. In the first laboratory period, a coordination complex formed from ferric chloride and maltol is prepared ($Fe(maltol)_3$). The intense red color of the complex is noted, and the UV-Vis spectra of the ligand and complex are compared. Yeast cells are incubated with ferric chloride and with $Fe(maltol)_3$. In the second laboratory period, the yeast cells treated with the ferric chloride or $Fe(maltol)_3$ are isolated and placed over ring magnets to study whether the yeast have paramagnetic properties. Yeast viability studies are done to compare the toxicity of $Fe(maltol)_3$ and $FeCl_3$. Students are asked to predict the magnetic properties of iron complexes of different spin and oxidation states and to consider the basis for iron uptake into yeast cells.

Introduction

Bioinorganic chemistry is most likely a topic of interest to undergraduates taking lower-level chemistry courses. Many of these students express an interest in biological, biomedical, or environmental science and bioinorganic chemistry is of fundamental importance to each of these areas. However, first year science students do not encounter metals in biology unless they major in chemistry and take upper-division courses where bioinorganic chemistry is introduced. In upper-division inorganic chemistry courses, well-studied metalloproteins or metalloenzymes such as hemoglobin or carbonic anhydrase are introduced to students to show how coordinated metal ions have important functions in living organisms. Yet, bioinorganic chemistry examples that are relevant to simple coordination chemistry might be introduced earlier in the curriculum to pique student interest in this multidisciplinary area of chemistry. Undergraduate laboratory experiments that feature metals in biology may better engage students with these interests.

Here we present an undergraduate laboratory experiment that involves labeling of the common baker's yeast, *Saccharomyces cerevisiae,* with iron complexes with the goal of introducing cellular biology and coordination chemistry of Fe(III). This experiment shows the relative ease of handling yeast in comparison to other types of cells as acknowledged in previous articles in this journal describing beer brewing activities,[1] biochemistry principles,[2] or enzymology of yeast proteins.[3] Coordination chemistry and paramagnetic properties of complexes are also a focus of this laboratory experiment. Magnetic properties of transition metal complexes are central to courses in inorganic chemistry and to the coordination chemistry section in general chemistry courses. Laboratory experiments that illustrate paramagnetic properties in previous issues of this journal focus on the measurement of magnetic susceptibility by Evans method,[4] using an electronic balance and magnets,[5, 6] EPR,[6, 7] or paramagnetic NMR spectroscopy.[8] Here we demonstrate paramagnetic properties by attraction of yeast cells treated with iron(III) complexes to ring magnets, as inspired by a report on magnetic yeast.[9]

Baker's yeast is an ideal organism for studying the effects of metal ion uptake. In addition to being a baking ingredient, this organism has long been utilized as a model system to study metal ion uptake, storage, and mobilization in eukaryotes.[10, 11] For this experiment, the yeast cells are incubated in media which is filled with necessary amino acids and sugars that allow the yeast to grow. Incubation of the yeast in a solution with a paramagnetic metal ion, Fe(III), or a complex of Fe(III), leads to incorporation of the iron into the yeast in sufficient

quantities to produce yeast that demonstrate paramagnetic properties. Magnetic microbes (yeast or bacteria) loaded with iron have potential applications in MRI,[12, 13] new magnetic materials,[12, 14] or biomedical applications such as anti-cancer agents[15, 16] or nutritional supplementation.[11, 17] An analysis of the genes involved in iron uptake into yeast[18] and their effect on magnetization[12] has attracted much interest.

First year chemistry students are introduced to unpaired electrons and paramagnetic properties in O_2 and certain other diatomic molecules as part of an introduction to molecular orbital theory.[19] Magnetic properties are taken up later as a way to distinguish between d-electron configurations produced by strong and weak field ligands in transition metal complexes. Students are taught that each electron has an associated property called spin and, although the electron does not actually spin, it has a magnetic moment that is analogous to a spinning charged particle.[20] Two electrons paired in an orbital have opposed spins that cancel each other, but compounds with unpaired electrons have an associated magnetic moment and can be visualized as bar magnets. Compounds with paired electrons exhibit diamagnetic properties and are weakly repelled from a magnetic field. Compounds with unpaired electrons exhibit paramagnetic properties such as being drawn into, or attracted to, a magnetic field.

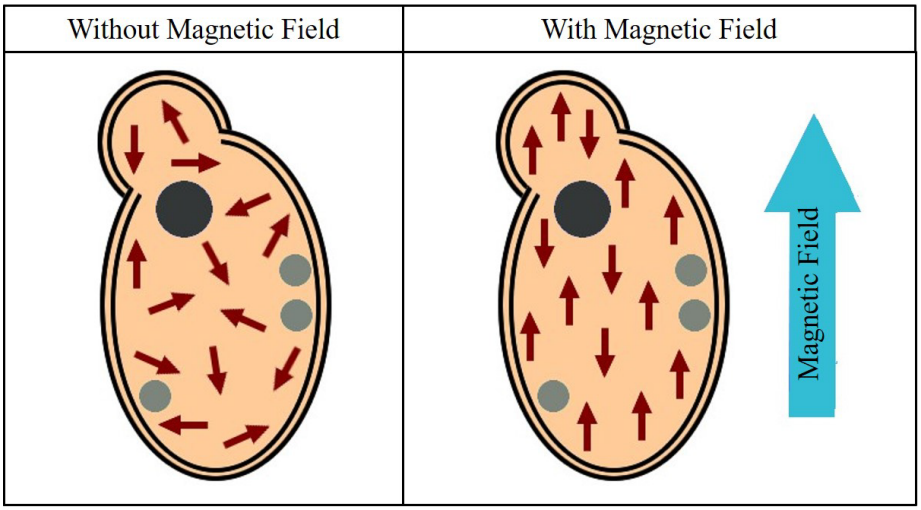

Figure 1. Paramagnetic materials in the absence (left) or presence (right) of a magnetic field. The red arrows represent the magnetic moment of unpaired electrons.

A simple way to visualize this paramagnetic property is shown in Figure 1. The magnetic moments of the electrons (shown as arrows to represent the directionality of the magnetic moment, i.e. N or S on a bar magnet) are not aligned in the absence of a magnetic field but do align in the presence of a magnetic field. A greater proportion of magnetic moments are aligned with the field than against it. This represents the magnetic polarization of the paramagnetic material in the magnetic field. The extent of the magnetic polarization shown in the figure is related to the magnetic susceptibility of the compound as given in more detail in the supplementary *Background Information*. Magnetic susceptibility can be measured by different methods. One of the most common methods uses a Gouy balance that measures the weight of the compound in a magnetic field which is compared to that of the compound in the absence of a field.[5, 21] Importantly, magnetic susceptibility depends on the number of unpaired electrons (n) or total spin quantum number (S). For transition metal complexes, a larger number of unpaired electrons usually translates to a larger paramagnetic effect (magnetic susceptibility). However, in some cases, the orbital angular momentum may contribute to the magnetic susceptibility as well[20] (see Background Information).

In this experiment, students will use the first-row transition metal, iron. Iron has several oxidation states, with Fe(II) and Fe(III) being the most common. Coordination complexes of Fe(II) or Fe(III) are usually six-coordinate and may be in high-spin or low-spin forms, which is referred to as the spin state. Low-spin Fe(II) in an octahedral environment is diamagnetic (containing no unpaired electrons) whereas both the high-spin and low-spin forms of Fe(III) (as well as the high-spin form of Fe(II)) are paramagnetic, but with different numbers of unpaired electrons. The spin state of the iron is influenced by the ligands and coordination environment. Fe(II) and Fe(III) complexes are common in biological systems and are found in many kinds of proteins and enzymes in everything from single-celled microorganisms such as bacteria and yeast to animals and humans.[22] Nearly all living organisms must have a supply of iron in order to survive. One of the iron complexes studied here, $Fe(maltol)_3$ (Figure 2) is in clinical trials as an iron supplement for humans who have iron levels that are too low for them to remain healthy.[23] Yeast require iron to thrive, but is it possible to have too much iron? A simple viability test is introduced here to show differences in yeast survival with $FeCl_3$ versus $Fe(maltol)_3$.

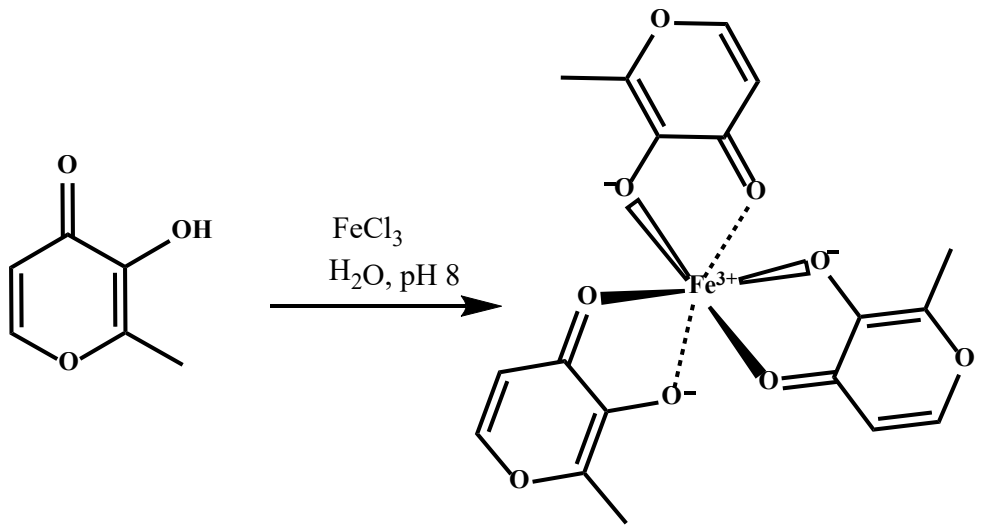

Figure 2. Preparation of ferric maltol by addition of $FeCl_3$ to an aqueous solution containing three equivalents of maltol. Sodium hydroxide is used to adjust the pH to promote complex formation.

Learning Objectives:

- To study uptake of iron complexes in a microorganism (common baker's yeast).
- To gain an understanding of paramagnetic properties of transition metal ions.
- To study the effect of iron compounds on the viability of yeast cells.
- To prepare an iron(III) coordination complex and observe a change in electronic properties by recording the UV-Vis spectrum.
- To determine the extinction coefficient of the iron(III) complex and show how the coefficient can be used to quantitate the concentration of iron(III) complex.

Experiment overview

This experiment was designed for the second semester of general chemistry laboratory. It was piloted in an honors chemistry course at the University at Buffalo, a large public university, as part of an inquiry-based learning approach for the approximately 70 students that sign up each spring. Notably, this course has its own laboratory space and experienced teaching assistants to

address the challenges of implementing more advanced experiments. The teaching assistants were instructed on how to prepare the yeast cultures in advance of the laboratory sections. At Niagara University, a primarily undergraduate institution, several undergraduates at the freshman level carried out the experiment under the supervision of a senior undergraduate student who prepared the yeast cultures with oversight of a faculty member. This experiment requires preparation of the yeast cultures the day before the laboratory section. The media, culture plates, sterile bottles and pipette tips can either be purchased or can be prepared in house if there is an autoclave available for use.

At the University at Buffalo, the experiment was used in three laboratory sections, each meeting once a week with 24 students per section. Two laboratory periods separated by one week were used for the experiment. The students worked in groups of three. Each student answered several prelab questions that included calculations for preparing solutions and for calibrating yeast cell density by using UV-Vis spectroscopic measurements as shown in the supplementary section. The laboratory write-up included questions on several topics; responses to a few topics are tabulated in Table S1 of *Notes for the instructor* as learning outcomes.

In the first lab period, the students prepare a solution of $FeCl_3$ and a solution of $Fe(maltol)_3$. They isolate yeast cells from the yeast culture grown in yeast extract-peptone-dextrose (YPD) media by pelleting the sample and decanting the YPD media. Then the yeast cells are added to YPD alone, to YPD plus $FeCl_3$ solution, and in YPD plus $Fe(maltol)_3$ solution. The samples are left to incubate on a shaker table at room temperature for one week.

The same day, the students prepare their own solution of ferric maltol by the addition of three equivalents of maltol to an aqueous solution of $FeCl_3$ followed by adjustment of the pH with sodium hydroxide to about pH 8. They note the color changes and are asked to explain why base is required for the complex to form. The electronic absorbance peaks are recorded by UV-Vis spectroscopy before and after the addition of $FeCl_3$ to illustrate how the new peaks that appear that are characteristic of an Fe(III) coordination complex (Figure 3). The students carry out a series of dilutions on the $Fe(maltol)_3$ solution they made and use Beer's law to determine the extinction coefficient.

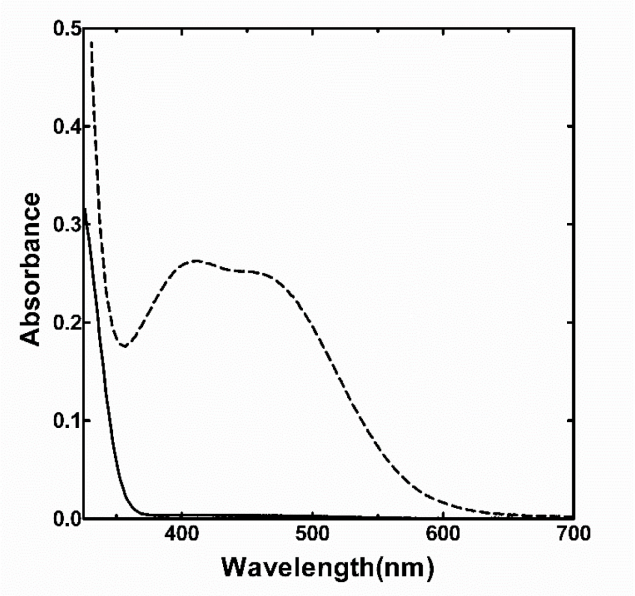

Figure 3. Electronic absorption spectra of solutions containing 0.30 mM maltol, pH 7.5 (solid line) or 0.10 mM Fe(maltol)$_3$, pH 7.83 (dotted line).

On the second day of lab, the students analyze their incubated yeast samples. Viability tests are set up to assess toxicity of the iron salt and iron complex on the yeast using agar plates (Figure 4). The students do a series of dilutions of each sample and then the yeast cells are left to grow on the plate for a couple of days. This activity shows that living cells multiply and that their rate of multiplication is influenced by conditions and ingredients added to the media.

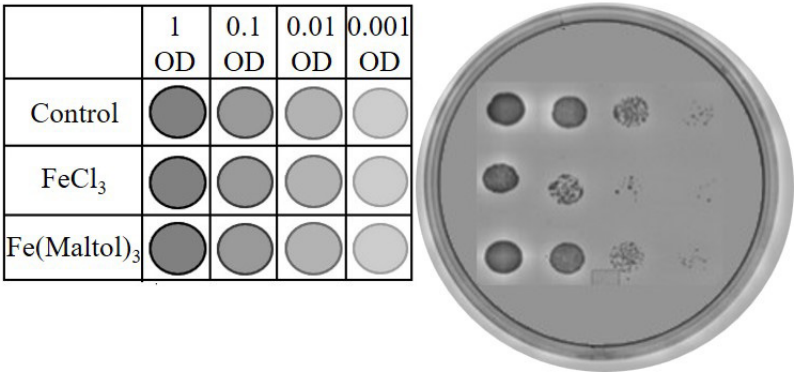

Figure 4. Yeast viability test. The circles on the agar plate represent yeast colonies. Each vertical column is for yeast incubated with no iron (control) or one of the iron treatments. Each horizontal row represents serial dilutions of the yeast cells (differing by a factor of ten).

The remainder of the sample is used for studying the magnetic properties of the incubated yeast. The yeast sample is added to a mixture of water and glycerol in an empty plate and placed on top of magnets. Migration of the magnetized yeast occurs over 24 hours. In Figure 5 is shown the rings that form from the attraction of the yeast to the ring magnets placed under the plate. In this figure, the magnets are removed and the rings formed by yeast migration are captured.

Figure 5. Yeast migration towards two ring shaped magnets with control plate, $FeCl_3$ treated yeast and $Fe(maltol)_3$ treated yeast.

Materials and methods

Chemicals that are required are maltol (3-Hydroxy-2-methyl-4-pyrone) and iron(III) chloride hexahydrate. Supplies include baker's yeast, yeast media and plates, disposable falcon tubes, and microcentrifuge tubes. An autoclave, shaker table, UV-Vis spectrometers, vortexers, and micro- pipettors are also used. A full list of equipment is given in the supplementary section.

Hazards

A list of chemicals and associated hazards are given in Table S2 in instructor notes.

Discussion

The preparation of the ferric maltol complex illustrates many of the principles that are taught about coordination chemistry in first-year chemistry courses. The neutral six-coordinate complex has three anionic ligands, and thus the iron complex is in a trivalent oxidation state. Binding of maltol to the Fe(III) changes the color of the solution to a deep red, with new electronic absorbance peaks that can be used to monitor formation of the complex and quantitation of concentration. The kinetics of the reaction is relatively rapid as shown by the formation of complex within minutes of mixing ligand and iron salt. Fe(III) forms a thermodynamically stable complex with the tris-complex being the major species present in solution at pH 7, with 100-400 μM concentrations of Fe(III) and 3-fold excess of maltol.[24] All of these properties can be discussed further by the instructor, depending on the level of detail desired.

The growth of yeast cells in media and the effect of iron added as a salt or as a complex is central to this experiment. Yeast cells in this experiment are cultured in YPD (yeast extract, peptone, dextrose) media. Students who have made bread know that dextrose (D-glucose) is an important component for initiating growth of the yeast cells. The viability studies presented here demonstrate that the cellular toxicity of a compound depends on its concentration. Although iron is essential for life and is the most abundant of the transition metal ions in the human body,[22] no compound is completely non-toxic. In fact, $Fe(maltol)_3$ is shown to be less toxic than $FeCl_3$ to the yeast under the conditions of the experiments, as can be seen with the viability test. There are several differences between Fe(III) bound to maltol compared to the free salt that might explain this observation. For example, the YPD media has components that bind the Fe(III) in solutions of $FeCl_3$ whereas the $Fe(maltol)_3$ already has a strongly chelating ligand. Moreover, the iron from $FeCl_3$ incubation is more likely to binding to cell wall components[25] and to form iron oxides in yeast cells.[9]

Outstanding questions concern the localization and form of the iron that associates with the yeast cells. Excess iron normally gets sequestered in yeast vacuoles as yeast cells lack the mammalian storage protein, ferritin.[18, 26] Some Fe(III) complexes distribute between vacuoles and the negatively charged cell wall. Importantly, even though the yeast are washed thoroughly, it is likely that some Fe(III) is bound tightly to the anionic groups of protein or chitin in the cell wall as characterized previously.[25] In fact, studies of the surface analysis of the yeast treated with

Fe(III) show a puckered surface, consistent with binding to the cell wall. Scanning electron microscopy (SEM) images of the iron treated yeast are shown in Figure S1 of the *Background section*. The more pronounced yeast migration to the magnet when incubated with $FeCl_3$ in comparison to $Fe(maltol)_3$ suggests that there is either more iron associated with the yeast cell or that the form of the iron in $FeCl_3$ treated cells has larger magnetic susceptibility, perhaps through formation of superparamagnetic iron oxides.[9]

Transmission electron microscopy, TEM, studies showed that yeast treated with Fe(III) citrate complexes produced electron-dense deposits in round particles associated with membranes, possibly vacuoles.[9] Magnetic susceptibility studies of the iron-loaded yeast cells by using a SQUID (superconducting quantum interference device) showed magnetic properties that were mostly consistent with paramagnetic or superparamagnetic iron species.[9] The latter result suggests that dense material contained iron oxides. Most likely, the $FeCl_3$ solutions studied here give similar electron-dense deposits as do the Fe(III) complexes of citrate. On the other hand, the form and location of the Fe(III) maltol complex within the yeast cells is not known. The light pink color of the yeast that were treated with $Fe(maltol)_3$ suggests that maltol ligands remain bound to the Fe(III) center. Moreover, it has been noted that lipophilic α-hydroxy ketones such as maltol restore growth to yeast streaked onto low iron agar plates containing 10 μM $FeCl_3$ consistent with uptake of the Fe(III) maltol complex into the cell.[27] These experiments may form the basis for a discussion about metals in biology and their transport into cells.

Notably, these studies are not limited to iron salts and compounds. Additional paramagnetic metal ion salts and complexes may be studied including those of transition metal ions and lanthanides. Moreover, there are many possibilities for undergraduate experiments of metal ion complex uptake by yeast that may be used in more advanced labs as example applications in medicine, metal ion scavenging, or magnetic materials.[12, 16, 28] Suggested metal salts that the students may choose for additional experiments are given in the *Notes for the Instructor*.

ASSOCIATED CONTENT

Supporting Information

Supporting Information is available at xxxx

Student handout, instructor's notes (homework and pre-lab questions, notes on synthesis and procedures with yeast culture), and additional background information.

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