Melanoma is one of the deadliest types of cancer, having a high recurrence rate but no effective chemotherapy (1, 2). It is the eighth most common U.S. malignancy, and recent years have seen significant increases in the incidence rate of melanoma and especially of early stage melanomas (3–6). Disulfiram (DSF) is an FDA-approved drug used in alcohol-aversion therapy for over 50 years (Figure 1); it is a potent inhibitor of aldehyde dehydrogenase and people treated with this drug have a violent and noxious reaction upon imbibing alcoholic spirits (7). Recent reports from several labs have shown DSF to have high and selective toxicity towards melanoma cancer in vitro (8, 9). Cell viability studies show that this toxicity is dependent on the presence of metal ions and suggests that the active agent is likely a Cu complex, bis(diethyldithiocarbamate)Cu(II) or Cu(deDTC)₂, which is formed in situ (10).

Dithiocarbamates (DTCs) have been used extensively over the years as chelating agents, pesticides, and vulcanizing catalysts (11, 12). A previous article in this Journal discussed the metal-catalyzed oxidation of related tetraalkyl trithiolanes (13). In the last ten years, a number of groups have demonstrated that DTC chelators can induce metal-uptake and apoptosis in a variety of cell types (14–22). The relation of this laboratory with cell biology and cancer research enhances the interest of many students and points to the centrality of chemistry in all the biological sciences. As further background, a synopsis of the original research in this area was given to the students as part of the course material (23).

The laboratory procedures are divided into parts A through D, which span two laboratory sessions. In part A, simple metathesis reactions are used to synthesize DTC complexes of Cu(II), Ni(II), Zn(II), and Fe(III), and all complexes are isolated by precipitation in good yields (24). In part B (Scheme I), a more complicated redox reaction is carried out by mixing disulfiram (DSF) with excess CuCl₂. When H₂O is added to the mixture, a disproportionation reaction promoted by the CuCl₂ is proposed to occur. The decomposition of one DSF molecule to sulfate and other byproducts releases 30 electron equivalents, which are then available to reduce 15 DSF molecules and in the presence of CuCl₂ form 15 Cu(deDTC)₂ complexes (10). This stoichiometry gives a theoretical yield of 93% Cu(deDTC)₂ in a 1:1 stoichiometric reaction of DSF and CuCl₂ and also predicts a ca. 25% molar yield of sulfate (10). Qualitative detection of SO₄²⁻ is done by adding metal salts (MgCl₂, NaCl, FeCl₃, and BaCl₂) to the filtrate of the DSF, CuCl₂ reaction. A successful test would only show precipitate for BaCl₂ since it is the only reagent that reacts with sulfates to form an insoluble salt.
In part C (Scheme II), Cu(deDTC)$_2$ is obtained from the decomposition of bitt-4 triiodide, a possible intermediate in the decomposition of DSF. In the first step, bitt-4 triiodide is generated by treatment of DSF with I$_2$ in CHCl$_3$ (25). A crystalline solid of bitt-4 triiodide is formed overnight and isolated by filtration. This isolated product is then reacted with excess CuCl in a CH$_3$CN/H$_2$O mixture, which leads to the formation of Cu(deDTC)$_2$ in ca. 60% isolated yields. In part D, the metal complexes made in parts A–C were analyzed by UV–vis spectroscopy. In addition, the purity of the Cu(deDTC)$_2$ complexes was determined by analyzing the spectrum and also by calculating the molar absorption coefficient at $\lambda_{\text{max}}$ of the 435 nm.

**Experimental Procedures**

**Part A**

A sample of CuCl$_2$⋅2H$_2$O (0.10 g, 0.74 mmol) is added to 15 mL of methanol in an Erlenmeyer flask. A sample of Na(deDTC)⋅3H$_2$O (2 eqiv) is dissolved in ~20 mL of methanol and is added to a second Erlenmeyer flask. The CuCl solution is then added dropwise with stirring to the Na(deDTC) solution, resulting in the formation of a precipitate. The reaction is stirred for an additional 10 min, and the solution is then filtered and the precipitate dried, yielding a dark solid. The metathesis reaction is repeated with NiCl$_2$⋅6H$_2$O, ZnCl$_2$, and FeCl$_3$⋅6H$_2$O [3 eqiv of Na(deDTC)⋅3H$_2$O needed for Fe] and all products are isolated by filtration.

**Part B**

A sample of DSF (0.10 g, 0.38 mmol) is added to 20 mL of methanol in an Erlenmeyer flask. A sample of CuCl$_2$⋅2H$_2$O (1.2 equiv) is dissolved in 15 mL deionized H$_2$O and then added dropwise to the DSF solution with stirring. A black precipitate forms, and the reaction is stirred for an additional 20 min (if no precipitate is formed, let stand for 10 min on ice). Keeping the reaction mixture on an ice bath increases or induces precipitation; a clear filtrate is needed for the qualitative analysis. Filtration and air-drying yields a dark solid. The filtrate is saved and tested for anions by addition of BaCl$_2$, NaCl, MgCl$_2$, and FeCl$_3$ salts; formation of a cloudy precipitate indicates a positive test.

**Part C**

A sample of DSF (0.70 g, 2.4 mmol) is added to a 250 mL round-bottom flask containing 20 mL of dry CHCl$_3$. Three equivalents of elemental iodine are dissolved in 35 mL dry CHCl$_3$ in a 125 mL Erlenmeyer flask. The solution of iodine is stirred until it is completely dissolved and then is added dropwise with stirring to the DSF solution. A dark red color appears. The solution is stirred for an additional 15 min, transferred to a vial, and stored overnight (or longer) in a freezer. The next lab session, this solution is filtered yielding bitt-4 triiodide as a crystalline red solid. A 0.5 g sample of bitt-4 triiodide is placed in a 125 mL Erlenmeyer flask with a minimum quantity of CH$_3$CN (for 0.5 g, use 15 mL) with a magnetic stir bar. A sample of CuCl (1.2 equiv) is dissolved in a minimum quantity of CH$_3$CN (for 0.05 g, use ca. 10 mL CH$_3$CN) and is added with stirring to the bitt-4 triiodide solution. Subsequently 3 mL of deionized H$_2$O is added dropwise with stirring. The reaction is stirred for an additional 15 min and cooled on ice for 20 min. The resulting dark solid is filtered and washed with 3 mL cold CH$_3$CN.

**Part D**

UV–vis spectra and $\lambda_{\text{max}}$ values are obtained for all metal compounds synthesized in part A at concentrations close to $5.0 \times 10^{-5}$ M (Cu and Fe) and $1.0 \times 10^{-5}$ M (Ni and Zn) in CHCl$_3$ (Figure 2). Approximately equal quantities of the dark solids obtained in parts B and C (ca. 1 mg) are dissolved in 50 mL of CHCl$_3$, and the UV–vis spectra are obtained and compared with that of Cu(deDTC)$_2$ from part A.

**Hazards**

Chloroform is a proven animal and probable human carcinogen; it may be fatal if swallowed, inhaled, or absorbed through skin. It may affect the central nervous system, cardiovascular system, liver, and kidneys. Acetonitrile may be fatal if swallowed, inhaled, or absorbed through skin. It affects cardiovascular system, central nervous system, liver, and kidneys. It is a flammable liquid. Iodine causes severe irritation or burns to every area of contact and may be fatal if swallowed or inhaled. Iodine vapors cause severe irritation to skin, eyes, and respiratory tract. The metal salts are harmful if swallowed and can cause irritation to skin, eyes, and respiratory tract. Sodium diethylthiocarbamate, Na(deDTC), may be harmful if swallowed or inhaled and may cause irritation to skin, eyes, and respiratory tract. Tetraethylthiuram disulfide, DSF, is hazardous in case of skin contact, eye contact (irritant), of ingestion, and inhalation.

**Results**

This experiment was used as a two-session lab practical for a class of 146 students; the students found it challenging, but doable in 8 hours. The yields obtained for the isolated DTC complexes in part A were ca. 80%, while the yields for the compounds in parts B and C varied by ability of the students. Many students obtained good yields of Cu(deDTC)$_2$ for the reactions in parts B and C, while others were hampered by incomplete precipitation in the synthesis or qualitative analysis and errors in sample preparation for the UV–vis measurements.

Part C entailed the most demanding procedure: the synthesis of bitt-4 triiodide from the reaction of DSF with I$_2$ required that both reagents be completely dissolved; sonication with applied heat resolved this issue. Another issue was precipitation of Cu(deDTC)$_2$ from the CH$_3$CN/H$_2$O mixture. Cu(deDTC)$_2$
is partly soluble in CH$_3$CN and therefore the students needed to be careful with both the quantities and ratios of solvent used; placing the solution in a dry ice/acetone bath facilitated precipitation.

Analysis of the UV–vis data obtained in part D was difficult for some students since they had not used this characterization method previously. Most students intuitively grasped the utility of this method in product identification. Lab discussions included the assignment of the spectral bands to a ligand–metal charge transfer or $\pi \rightarrow \pi^*$ ligand-based transitions, and a comparison between the complexes and the free ligand (26, 27).

**Conclusions**

This research-based experiment was well-liked by the general chemistry students. Although the mechanistic chemistry involved in the reactions of this lab are complex, the students were able to generate and characterize the products made in each part of the project. The synopsis was presented to the students as an example of graduate student research that crosses into the fields of biological and medical research. In addition, this laboratory tests the students’ abilities in synthesis, qualitative analysis, and reaction stoichiometry, and therefore serves as a good laboratory practical for general chemistry classes.

**Literature Cited**


**Supporting JCE Online Material**


Abstract and keywords

Full text (PDF) with links to cited URL and JCE article

**Supplement**

Student handouts including directions for the experiment, a synopsis of the original research of DSF and DTC complexes with regard to melanoma treatment, and information about crystallization and filtration techniques and qualitative analysis

**Instructor notes**

*JCE Featured Molecules for October 2009* (see p 1248 for details)

Structures of some of the molecules discussed in this article are available in fully manipulable Jmol format in the JCE Digital Library at http://www.JCE.DivCHED.org/JCEWWW/Features/MonthlyMolecules/2009/Oct/.

The structures of a number of the molecules discussed in this article are available in fully manipulable Jmol and Chime format as *JCE Featured Molecules in JCE Online* (see page 1248).

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