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The Incorporation of a Single-Crystal X-ray Diffraction Experiment into the Undergraduate Physical Chemistry Laboratory

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Background

X-ray crystallography has traditionally been the method of choice in determining molecular structure at atomic resolution. Since the discovery of X-rays in 1896 by Roentgen, more than 17 Nobel prizes have been awarded to scientists who have utilized this method (1). Until recently, the considerable amount of time needed to collect, integrate, and analyze X-ray diffraction data did not lend itself to an undergraduate laboratory experiment unless it was offered as a full-semester class. However, modern X-ray diffractometers are capable of quick and reliable data collections that enable undergraduate researchers to have some experience with the method. A major reason for the enhanced speed of data collection is the development of area detectors. Area detectors collect many diffraction maxima simultaneously and can immediately generate the equivalent of Laue and rotation photographs. These captured-area detector images are an ideal teaching tool to introduce students to the concepts of X-ray diffraction. Here at CMolS, we have incorporated an X-ray crystallography experiment into the undergraduate physical chemistry laboratory.[1] During the first week of class, students mount prepared organic crystals (e.g., benzil, $C_{14}H_{10}O_2$) and collect diffraction data. Before they leave the 3-hour lab, data collection and integration are underway. In the second week of laboratory, students solve and refine the structures.

The results of structure determinations in chemistry are often taken for granted in the general chemistry curriculum and therefore warrant a more in-depth investigation. The purpose of the laboratory is to introduce students to X-ray diffraction methods, not to make them crystallographers in two weeks. However, this laboratory is easily expanded into a semester class on crystallography, where rigorous structure determinations introduce undergraduates to extensive concepts such as packing interactions, thermal motions, and chirality.

The Experiment

Preliminary Laboratory Work

For the last six weeks of CHEM 355 (Physical Chemistry Laboratory), undergraduates decide upon a topic for extensive investigation. This X-ray diffraction experiment is one option. Students who opt to perform it are given

a tour of CMolS's facilities and undergo mandatory safety training. After a brief introduction to the instrument, a question-and-answer session takes place. (The instrument used to collect data at CMolS is a Bruker 3-circle diffractometer equipped with a sealed-tube Mo K_α source and a CCD area detector.) Instructors and students decide which molecules the students will recrystallize and investigate. Students are encouraged to bring samples prepared from their undergraduate research if possible. Some of the molecules investigated by students previously are shown in Figure 1 (2–4). All these structures have been published in refereed journals and were chosen because of their aesthetic beauty, safety, and crystallographic interest.[2] Before beginning the diffraction experiment, students are introduced to the fundamentals of X-ray diffraction with a review of Bragg and von Laue's equations.

The First Week of Laboratory

Students work in teams even though each student will collect and refine data on his or her own sample. Repeated exposure to the instrument and its software is vital for students to comprehend the sometimes difficult concept of X-ray diffraction. Students are required to bring their recrystallized samples to class. They are instructed to pick out the "best" sample: one that is free of cracks and has homogeneous birefringence when viewed between the crossed polarizers of a polarizing microscope. That sample is trimmed with a razor blade to less than 0.5 mm in the longest direction and is mounted with 5-minute epoxy on the end of a glass fiber placed in a goniometer pin. Before the pin is placed on the goniometer head on the diffractometer, sample placement and overall quality are checked by the instructor.

After the sample is prepared, students begin their association with the SMART (5) software that controls the instrument.
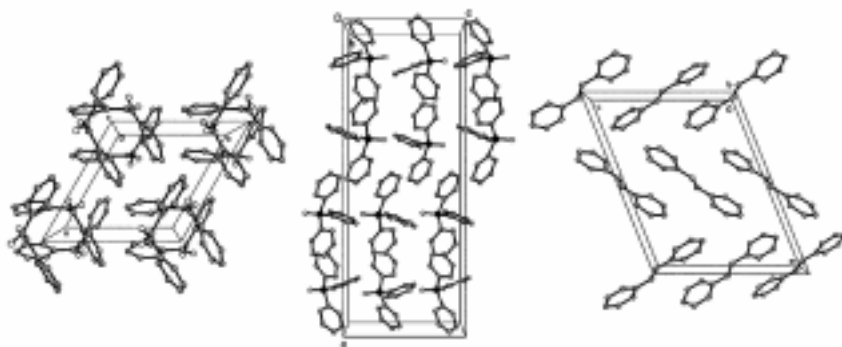

Figure 1. Projections of representative crystal structures of *trans*-stilbene (*2*) (right), benzil (*3*) (left), and one polymorph of triphenylphosphine oxide (*4*) (middle) as generated from the published lattice parameters and coordinates. Hydrogens are omitted in all molecules for clarity.

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Since the processes are complex and new to undergraduate researchers, there is a need for constant mentoring during the rest of the lab. The sample is optically centered and a preliminary rotation photograph is taken. A sample rotation photograph for benzil is shown in Figure 2. By inspection of the collected image, students determine whether their crystal is suitable for further analysis; minor crystallites, cracks, or crystal disorder can cause anomalies in rotation photographs.

Before a full data collection is attempted, students determine the unit cell of the sample and the sample's orientation to the detector. they obtain this information by running a routine in SMART that collects runs of diffraction data in three regions of reciprocal space. After the data are collected, the software automatically selects reflections, chooses a preliminary Bravais lattice, and determines the orientation of the sample to the detector. This information allows the diffractometer software to simultaneously collect and integrate data. Students can refine the process further to obtain accurate unit cell parameters for their crystals.

Before leaving the first week of laboratory, students spend the majority of the period exploring the reciprocal space lattice of their sample. Since the lattice parameters and orientation are known, students can input these into the program, ASTRO (6). This program allows students to simulate diffractometer data collection runs in order to map the minimum reciprocal space coverage needed for a complete data set. Formulated data runs from ASTRO are placed into a SMART routine that automatically collects the requested data. After the automated data collection is running as intended, students utilize another software package, SAINT (7), which allows for data integration simultaneously with data collection.

The SMART software package that controls the diffractometer and directs data collection is located on a 120-MHz Pentium IBM[3] networked to a Silicon Graphics Indigo2 (SGI) workstation. Collected frames guided by software on the IBM are written to a scratch drive on the SGI. During data collection, the SAINT software on the SGI uses the orientation matrix to integrate frames as they are collected, periodically refining the orientation matrix during integration of data. After all runs are integrated, final cell parameters and deviations are determined using integrated reflections. By networking SAINT simultaneously with SMART, students are able to refine data almost immediately after data collection. Before leaving the first week of lab, they are collecting and integrating data that will be available for refinement the next week. (Data collections on unique data for most small molecules take on average only 10 hours. This makes the instrument available for use the next morning.)

The Second Week of Laboratory

XPREP in the SHELXTL (8) software package reads integrated data files and suggests other potential Bravais lattices, and displays reflection statistics for cell centering conditions. After assigning a Bravais lattice, students assign a space group based on reflection statistics generated from their cell choices. For situations where the space group is difficult to determine through the inspection of reflection statistics, tolerances for excepted violations of systematic absences can be altered in XPREP, although this is almost never needed. After the space group is determined, the molecular formula and density (if known) are input. Further statistical analysis of collected data gives students the opportunity to apply a high-resolution cutoff, merge data, or remove outliers. Finally, this program prepares the reflection files and atom list files needed for direct methods (XS) and least-squares refinement (XL) programs.

The direct methods program (XS) in the SHELXTL software package generates an initial phase model. Although a complete workup of direct methods structure solution is outside the immediate framework of the lab, students will be introduced to the phase problem via directed questions during refinement. The concepts of localized electron densities and chemically reasonable bond lengths will lead to a small discussion of direct method structure solution. Computationally, the initial phase model determination takes the least amount of time. However, students cannot escape the theoretical ramifications of the loss of phase information and must understand *why* initial phases are important if not exactly *how* they are generated.

The solution from direct methods is inspected in the graphics program XP. Suggested regions of core electron density from the best direct methods solution are assigned to individual atoms. The atoms are written to a file that is inserted into the least squares refinement program XL. Before refinement, the atoms file will have instructions to weight the data, calculate difference Fourier maps, and generate bonding tables during refinement. Four cycles of least-squares refinement occur every time XL is performed. The average solution refinement outline is shown below.

1. Atoms are refined isotropically.

2. Difference Fourier maps are examined for missing atoms; if any are found, isotropic refinement is repeated.

3. Hydrogens are added in idealized positions and refined isotropically.

4. All nonhydrogen atoms are allowed to refine anisotropically.

5. After students evaluate their structural models by inspecting the refinement statistics, tables of bond lengths, bond angles, atomic parameters, and calculated structure factors are generated.
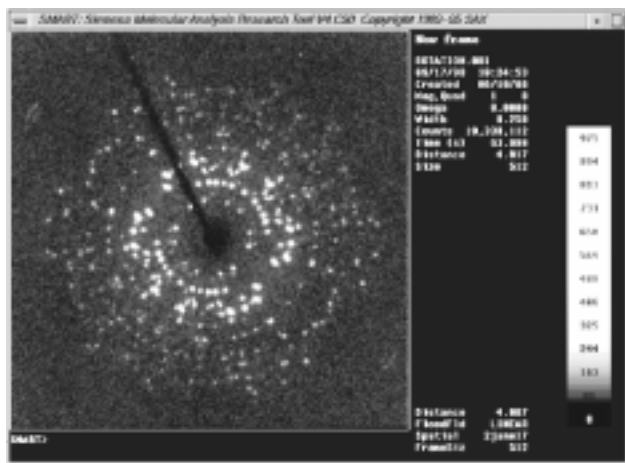

Figure 2. Preliminary rotation photograph of benzil, $C_{14}H_{12}O_2$, as captured on the CCD area detector.

Results and Discussion

By inspection and analysis of generated *cif* files at the end of refinement, students can assess the success of their structure determinations.[4] Specifically, we have them comment on the residual electron density, estimated standard deviations of bond lengths and angles, statistical R values, and anisotropic thermal parameters. All samples investigated so far have refined to $R \le .08$. In CHEM 355, students are required to summarize their results in a journal format and to prepare a poster presentation. The presentation at the end of the semester is open to the entire chemistry department. At the presentations, students engage in question-and-answer sessions concerning their experiment and results. The questions may address anything from the theoretical methodology of the experiment to chemical interpretations of results.

One might ask, "Why were some of the samples chosen, particularly those which display site disorders?" We believe these samples, although not treated rigorously in this laboratory, reflect the role of the contemporary crystallographer in science. Scientists not familiar with the method believe that the advances in software and instrumentation technology have made structural determinations by X-ray diffraction an automated routine. However, structures that accurately reflect the experimental data rely on the scientific intuition of the crystallographer who refines models incorporating all the defects and disorders of single crystals. Nowhere is this clearer than in the refinement modeling of *trans*-stilbene. Neglecting the disorder of the molecular packing still leads to an appreciable R value ($R = .075$); yet the solution fails to reflect the true nature of the solid, where 6% of the molecules in the crystals are disordered on their lattice sites. Therefore we approach the refinement results in our experiment with apprehension and warn our students that crystallography yields only structural models limited by the model builder and experimental data. At the same time, we teach students how to evaluate the success of any crystallographic refinement.

Assessment

Content

We have found that repeated exposure to theoretical explanations and experimental procedures is the best way to introduce students to crystallography. Most students have trouble envisioning the events that lead to diffraction and the basis of the phase problem. The necessary mathematical background required for structure solution and refinement also intimidates them. None of these fears can be instantly alleviated, but clear descriptions, vivid analogies, and visual aids can assist students in comprehending the basics behind these concepts used in determining molecular structure. Students working in teams are more apt to ask questions, whereas assigning each student a molecule to investigate gives each participant an individual goal. The five undergraduate students who have participated in this laboratory found it a positive experience. A more rigorous assessment of the offering is underway as more undergraduates choose to participate.

Logistics

Even through the laboratory portion lasts two weeks, students who have performed this experiment have spent an equal amount of time visiting the center, reviewing the physics of diffraction, learning laboratory safety, and preparing their results. We also accommodate students who, owing to extraneous circumstances, arranged 3-hour laboratory periods outside of the regular meeting time.

The simultaneous collection and integration of data made possible by linking the IBM and SGI computers was central in maintaining operational efficiency but was not central to the success of the lab. Without the IBM-SGI link, integration would have to wait until after data collection was finished on the IBM. Future versions of software mentioned in this paper will operate on Windows NT platforms, eliminating the need for two computers because SMART and SAINT could run simultaneously.

As a final note, we would like to point out that the cost of the entire CCD-equipped diffractometer system is comparable to the cost of a 300-MHz solution NMR system and that interested schools need not purchase the instrumentation. We have initiated a cooperative distance learning laboratory with Juana Acrivos at San Jose State University and John Tate at San Bernardino State University. Over the Internet, students at these institutions have access to the diffractometer and can collect data remotely. Having purchased the refinement software, instructors at these institutions need only transfer the integrated data sets before continuing refinements in classrooms locally.

Conclusions

Historically, X-ray crystallography has provided the best detailed atomic descriptions of molecules. Important concepts such as ionic radii, bond lengths, and bond angles are rooted in crystallographic investigations. With rising interest in structure-based pharmaceutical design, materials chemistry, and preorganized organic substrates, detailed knowledge of the structure of organic solids is needed. X-ray crystallographic investigations address both the nature of the molecule and its arrangement into a solid. It is now possible to expose students to the fundamental and essential experimental method of X-ray diffraction within the context of a two-week physical chemistry laboratory.

Acknowledgments

Notes

1. An excellent introductory text for undergraduates interested in X-ray diffraction is *Crystal Structure Analysis for Chemists and Biologists*, by Glusker, Lewis, and Rossi (Wiley-VCH, 1994).

2. These three molecules lend themselves to further investigations of enantiomorph choice (benzil), site disorder (*trans*-stilbene), polymorphism and molecular conformation (triphenylphosphine oxide).

3. Premier MS-DOS IBM with a 120-MHz Pentium processor, 24 kB RAM, 4-gigabyte hard drive.

4. A *cif* file is a crystallographic information file that sums up the methodologies in and the results of a refinement. Many significant details (*R* values, estimated standard deviations of bond lengths and angles, observed reflections, etc.) are listed.

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