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Laboratory Experiment

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Spectroscopic Determination of Triclosan Concentration in a Series of Antibacterial Soaps: A First-Year Undergraduate Laboratory Experiment

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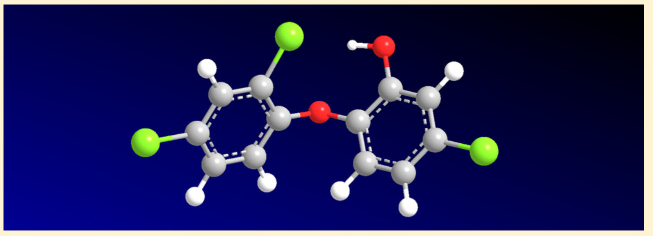
Supporting Information

ABSTRACT: The antibacterial chemical triclosan is a common component in personal care products but recently the subject of many reports concerning environmental toxicity and health impacts. Although triclosan concentration in a soap can be determined simply via high-performance liquid chromatography, this is a technique not routinely available in the general chemistry laboratory. Here we report an experiment, suitable for the first-year undergraduate laboratory, to determine the percentage of triclosan in a series of antibacterial soaps using UV−vis spectroscopy following conversion of the triclosan to an azo-dye through reaction with nitrite and 4-sulfanilic acid. Additional experiments that build on this and look at either triclosan removal from solution or the impact of triclosan on a biological system are also discussed. Although designed for first-year undergraduate students, this laboratory is also adaptable to science outreach programs.



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Much attention has been focused on both the environmental and health impacts of a range of organic chemicals present in pharmaceuticals and personal care products that are now being found in the environment.[1,2] Triclosan (Figure 1) possesses strong antimicrobial properties[3]

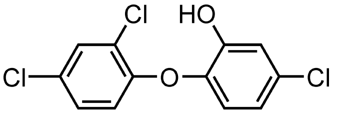

Figure 1. Chemical structure of triclosan (5-chloro-2-[2,4-dichlorophenoxy]-phenol).

and is widely used as an additive to many antibacterial products, particularly hand soaps. The presence of triclosan in the environment[4] is concerning due to not only the possible devastation of aquatic microorganisms,[5] but also the possibility of potentially harmful bacteria developing resistance.[6,7] In addition, while low concentrations of triclosan are not thought to be fatal to humans, this chemical has been detected in the metabolites of users,[8] and studies have linked triclosan to a number of serious health risks including breast cancer,[9] muscle damage,[10] and endocrine disruption.[11] In addition, the photochemical-induced degradation of triclosan to a potentially toxic dioxin[12] raises other concerns about the continued use of this material.

On the basis of these reports, which include a recent front page feature in *Chemical and Engineering News*,[13] triclosan is an extremely relevant material for study in the teaching laboratory since it is something students likely encounter on a regular basis outside the laboratory environment. Although the determination of triclosan concentration in samples such antibacterial soaps and other personal care products is straightforward using a technique such as high-performance liquid chromatography,[14,15] such instrumentation is often not routinely used in the general chemistry laboratory. Direct spectroscopic measurement is not possible despite triclosan possessing a strong absorbance in the UV region (λ_{max} = 282 nm) because of a number of interfering species that absorb in the same region. The use of a colorimetric assay that reacts the target species to produce a new colored compound has been used in analyses of a number of other commonly found materials.[16−19] The reaction of sodium nitrite with 4-sulfanilic acid forms a diazonium complex (Scheme 1), which forms a strong yellow−brown colored complex when reacted with triclosan.[20] By using excess amounts of 4-sulfanilic acid and sodium nitrite, the triclosan is made the limiting reagent in the formation of this colored complex, and the measured absorbance at 475 nm is proportional to the triclosan concentration.

This experiment is ideally suited to the general chemistry laboratory because it requires students to possess volumetric pipetting skills and a familiarity with UV−vis spectroscopy. Results obtained are close to those reported on the packaging of the soaps, and the experiment, while intensive in the number

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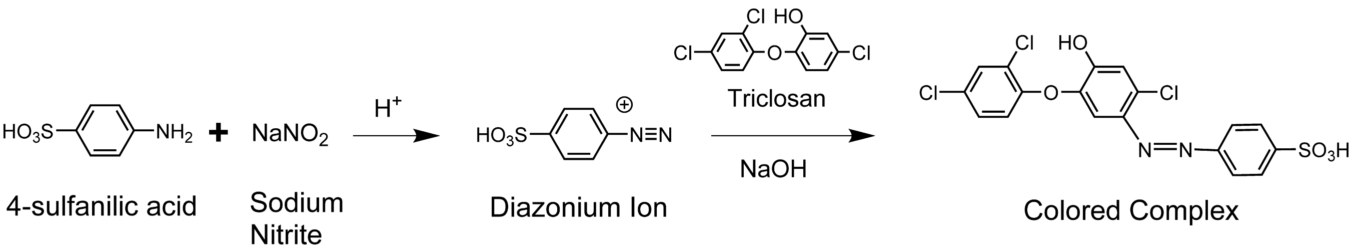
Scheme 1. Reaction of Sodium Nitrite and 4-Sulfanilic Acid under Acidic Conditions Form a Diazonium Ion that Reacts with Triclosan To Form a Colored Complex



of solutions to be prepared (10+), is very simple in terms of data analysis and is ideal for groups of 2–3 general chemistry students. While the bulk of this paper concerns itself with the spectroscopic assay, this method can and has been utilized in a number of subsequent experiments in a general chemistry laboratory curriculum. Further details on these are provided in the Supporting Information.

Our second semester general chemistry laboratory incorporates a multiweek student directed research project that looks at degradation of pharmaceuticals. This incorporates a two week triclosan project in which students investigate the assay of triclosan along with its removal from an aqueous solution using activated charcoal. This two week experiment provides a very relevant real-world example of environmental chemistry in the general chemistry teaching laboratory.[21] In past years, we have also offered an integrated chemistry and biology laboratory experience for freshmen, and the initial assay is linked to a second experiment that is focused on triclosan impacts on a protozoan culture (*Tetrahymena pyriformis*, Kingdom Protista). In both classes, students are required to prepare a formal paper on their work in these two laboratories and actively encouraged to incorporate background research on both environmental and health concerns of triclosan.

■ EXPERIMENTAL PROCEDURE

This experiment has been traditionally taught in smaller (18–24 students) laboratory sections with students that work in groups of 3–4 and can be completed in under 3 h, though it could be easily adapted for larger laboratories or utilized as part of an upper-level analytical laboratory. Full details including student handouts are given in the Supporting Information.

Solutions of sodium nitrite (0.03 M) and 4-sulfanilic acid (0.015 M) along with a glycine buffer can be prepared in advance or assigned to student groups to prepare at the start of the laboratory period. A 0.5 g sample of a triclosan containing soap is diluted to 50.0 mL with 0.01 M NaOH solution. A 200 mg/L triclosan standard is prepared using 0.01 M NaOH as the solvent from which 10, 20, 30, and 40 mg/L triclosan calibration standards are prepared.

Six solutions are prepared by volumetric pipetting of the following, in order, into a 100 mL volumetric flask: 15.0 mL of sodium nitrite solution, 10.0 mL of 4-sulfanilic acid solution, 25.0 mL of one of the solutions under investigation (the four calibration standards, the soap solution, or distilled water (blank)), and 25.0 mL of the glycine buffer. The flask is filled to the line with distilled water and mixed; a yellow–brown color is typically seen to form upon addition of the glycine buffer as the system is made basic, and the absorbance at 475 nm is recorded for each solution using a 1 cm cuvette (Figure 2).

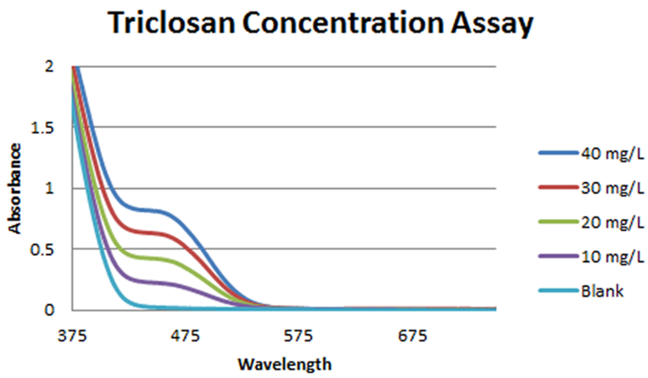


Figure 2. Visible absorbance spectra of triclosan calibration standards and blank (no triclosan).

■ HAZARDS

Students should wear goggles and follow the appropriate laboratory safety guidelines at all times. All materials can be handled with appropriate care on the benchtop, though direct contact with the solid sodium nitrite, 4-sulfanilic acid, and triclosan should be avoided. Because of environmental concerns, all waste containing either triclosan, nitrate, or 4-sulfanilic acid should be collected; this can be concentrated by evaporation to near dryness, which results in smaller volumes of waste for disposal in accordance with local protocols.

■ DISCUSSION

This experiment provides a straightforward means for determination of triclosan concentration in a complex system (antibacterial soap) in the general chemistry laboratory. It has been found that it is critical to add the solutions in the correct order since formation of the diazonium ion only occurs under acidic conditions while the colored complex from the diazonium ion and triclosan requires the system to be basic (pH > 10). The resulting solution is strongly colored with absorbance being measured at 475 nm (Figure 2). The colored complex is stable over the course of the laboratory period, while the absorbance for the solution made using 25 mL of distilled water (blank) is minimal and can be discounted.

The concentration and hence mass of triclosan in soap solution can be determined and lead to determination of the % triclosan in the soap. Student data for the % triclosan in two commercially available soaps are shown in Table 1. These values are comparable to those reported on the soap packaging, which shows that this is a relatively accurate method for determination of % triclosan. For one soap, results have been found to be consistently high, which leads us to postulate that there is some other chemical species present that possesses a small absorbance at 475 nm and gives a slightly elevated value.

Table 1. Comparison of % Triclosan Degradation of Student Groups versus Value Listed on Soap Label

		% Triclosan Determined from	
Soap Color	No. Student Groups	Student Average	Packaging
Green	13	0.51 ± 0.04	0.47
Orange	17	0.17 ± 0.04	0.15

■ ADDITIONAL EXPERIMENTS

Full details on all of these experiments are given in the Supporting Information. This experiment is traditionally taught as a two week laboratory with the first week asking students to determine the accuracy of this protocol in the determination of triclosan concentration. The second week of our traditional general chemistry cycle then looks at the adsorption by activated charcoal.[22,23] Solutions of triclosan are stirred over charcoal for the desired time, the charcoal is removed by filtration, and the remaining triclosan concentration is determined using the nitrite/sulfanilic acid method.

Direct evidence of the impact of chemicals upon biological systems provides an ideal way to demonstrate the potential environmental impacts of this species.[24,25] Therefore, to investigate the effects of triclosan on single-celled organisms, students were tasked to find literature values for the LD50 for the drug on related single-celled species.[26] Solutions of the relevant triclosan concentration were prepared and mixed with a culture of *Tetrahymena pyriformis*, and the results were observed under the microscope.

Finally, this experiment has been simplified to form part of a science outreach workshop. Feedback from participants is favorable as they report enjoying the experience of doing "real chemistry" and also working on a real-world problem.

■ CONCLUSION

An introductory college laboratory experiment to determine the % triclosan, the antibacterial component in a number of personal care products, has been developed. Additional uses of this in related experiments on triclosan removal or ecological impact are also presented. Student response to this experiment has been favorable, and the use of a chemical that has real-world connections proves popular.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Student instructions including instruction sheets to prepare all solutions needed for the laboratory. Notes for instructor and additional material on charcoal adsorption, triclosan effects on a biological system, and instructions for the science outreach workshop. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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