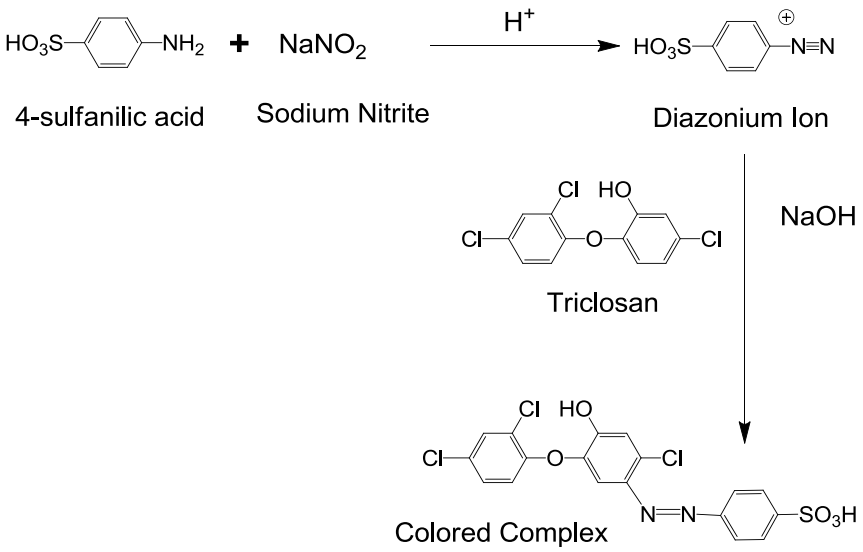
Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

This document contains all supporting information for the 'Spectroscopic Determination of Triclosan Experiment.' Pages 1 - 16 contain instructions for the basic spectroscopic assay laboratory. Pages 16 – 24 describe additional experiments which build upon the basic experiment and use the environmental impact of triclosan as an inspiration, either in looking at impact on a biological organism or investigating methods of removing it from solution. Finally, pages 25 to 30 describe adapting this for a science outreach workshop suitable for teens or interested adults.



Scheme 1. The reaction of sodium nitrite and 4-sulfanilic acid under acidic conditions forms a diazonium ion which reacts with triclosan to form a colored complex.

Spectroscropic Determination of Triclosan in Handsoap - Notes for Instructor:

- Soap samples suitable for analysis can be easily found at most drugstores and supermarkets – the % triclosan is listed on the label (it may be listed as Irgasan) and % values commonly range from 0.17 to 0.45%. These values are ideal for this experiment and work well with the mass of soap to be used listed in the student handout. With the increased media attention on triclosan, it may become harder to find triclosan containing soaps and so stocking up may be recommended. With student groups using ~0.5 g of soap per run, a 6 student group laboratory section uses on average between 2 and 5 g of the soap meaning larger bottles of soap will last a number of years. I originally started development of this laboratory 4 years ago and am still using the same two 250-mL bottles of soap which have shown no signs of degradation or contamination.

- Multiple soap brands have been tested to date and most of our work has used Dial antibacterial soaps – these were chosen due to easy availability and simply by being on special offer at time of this experiment being developed. The brands most commonly tested have been Dial Complete (green) and Dial Gold (orange) antibacterial soaps which contain 0.46 and 0.17% triclosan respectively. Only using 2 brands in the laboratory allows a section with 6 student teams to obtain triplicate results for each. Other soaps subsequently tested in the development of this manuscript have included Walgreen and CVS brand antibacterial soaps (0.17% triclosan) which have also yielded accurate values for % triclosan from this experiment.

- In the reaction scheme, shown on the first page of this Supporting Information and as Scheme 1. in the main manuscript, the colored complex at the end is shown to be fully protonated. At the pH that this species is present (pH 10 – 12), both the sulfonate and the phenol group may be deprotonated. This has not been shown to make the figure simpler for general chemistry students who may not yet have encountered acid-base chemistry and pKa to understand.

- Our laboratory set-up has students work in groups of 3 - 4 students with 5 - 6 such research teams per laboratory section and on average 6 - 8 sections in a semester. Our model for teaching this laboratory has the individual student groups prepare 4 calibration standards (10 – 40 mg/L triclosan) and 1 soap solution (page 12 shows a flowchart for all student solution preparation). From these 5 solutions and distilled water, students prepare 6 solutions for measuring absorbance at 475 nm. Sets of these calibration standards can be prepared for the section if needed to minimize required solution preparation.

- The handout which gives student instructions for the preparation of the shared stock solutions has been developed to best fit the laboratory resources available at Concordia. Whilst volumetric glassware is not necessary for many of the solution, we have chosen to use these to provide students additional experience in use of these flasks and pipettes. Instructors should feel free to redesign the protocols described to better suit their laboratory resources and teaching style.

- Mixing of solutions is absolutely critical. Whilst the method of mixing of solutions is not stated explicitly in the student handouts, this is generally done by either magnetic stirrers or the students placing caps on flasks and shaking/inverting the volumetric flasks, our method of choice for our general chemistry laboratory. Preparation of the initial triclosan master solution and the 4-sulfanilic acid solution can be made easier through use of a sonic bath if one is available or through a stir plate. Instructors should use whatever methods they see fit ensuring solutions are homogenous.

- The % determination of Triclosan student handout (Pages 8 – 13) of the Supporting Information is taken, (following minimal modification to make them stand-alone) from our traditional General Chemistry Laboratory manual. This experiment is taught early in the second semester and as well as fitting in with the theme of pharmaceutical contamination of the drinking water, it also serves to refresh their skills in pipetting and spectroscopy after the end of semester break although such an experiment may also fit earlier in the first semester if desired and is taught at approximately the half-way point in our integrated chemistry and biology laboratory course for freshmen.

- In terms of required accuracy of solution preparation, the most critical measurements should be in the weighing of the triclosan standard (if measured mass is not exactly 0.200 g, the actual mass can be used to calculate the concentration of the master solution and the resulting dilutions may not be exactly 10 -40 mg/L but should be close) Only triclosan containing solutions need be pipetted accurately, the nitrite, 4-sulfanilic acid and glycine could be measured using graduated cylinders if required.

- The majority of our traditional general chemistry second semester requires students be responsible for preparing all solutions they need for their experiments since the second half of the semester is based around a multi-week research project. Hence, the nitrite, 4-sulfanilic acid and 200 mg/L triclosan master solutions along with the glycine buffer are made by the student groups. Rather than make each group make individual solutions of each, these solutions are made in large enough quantities with each student group assigned some task such as preparing the nitrite for the class. As an example, one student group is tasked to make a 4-sulfanilic acid solution which everyone shares while another group makes nitrite solutions for all tables. (I put together small boxes with all required materials for each solution and pages 6-7 provides lists of required materials and student instructions for preparation of all these solutions).

- It is impossible to understate the importance of mixing. The few failures we have had in this laboratory likely arise from students improperly mixing solutions and pipetting or measuring absorbance on non-homogenous solutions. Mixing and complete solvation of the starting solutions, should these be made by students, is also critical. The 4-sulfanilic acid can be slow to dissolve. Placing this solution on a stir plate or use of an ultrasonic bath is recommended. The triclosan is only sparingly soluble in distilled water so dissolving in sodium hydroxide solution and not water is recommended.

- In preparing the soap solution, students should avoid shaking the solution too vigorously. Bubbles formed when this happens can take a long time to dissipate and make accurate dilution in volumetric flasks challenging. Sonication can help with dissipation of bubbles.

- Preparation of 10, 20, 30 and 40 mg/L triclosan calibration standards from the 200 mg/L master solution is done by the student groups. In our labs, students are required to calculate what dilutions must be required and the instructor may check their calculations before allowing them to proceed if desired. A 200 mg/L solution was chosen for the master as it allows students to easily weigh out an accurate amount (0.20 g) and using 1 L provides sufficient quantity for all the laboratory section (and additional sections if desired) – I commonly keep a proven triclosan master (200 mg/L) solution from an early section in the week as a back-up just in case.

- Organization and making sure students understand the order and the solutions to be made is important. From several years of experience running this laboratory, providing a flowchart such as the one on page 12 of the Supporting Information reduces confusion significantly.

- The order of addition of chemicals to make the colored complex is absolutely critical due to a pH dependence. If the procedure is followed, accurate measurement of pH is not required and do not measure this as a routine part of the experiment. It is therefore important that the starting solutions are not made too basic or acidic and follow the protocol described – The first reaction (nitrite and sulfanilic acid) must occur under acidic conditions (pH ~ 1 – 3) and the colored complex forms under basic conditions (the glycine buffer addition results in a final pH lying between 11 and 12). A small cloudiness may be seen when the triclosan is added (which disappears on shaking) but there should be a strong visible yellow/ brown color seen as the glycine is being added or within about 10 seconds. If this does not occur, try adding one drop of a medium strength (6 M) sodium hydroxide solution until final pH >11.

- Once all stock solutions (nitrite, sulfanilic acid, triclosan and glycine) have been prepared, it may be worthwhile for the instructor to quickly demonstrate making a solution which combines all of these in the correct order. This serves both as a demonstration for the class of the formation of the colored complex and a test of the solution viability.

- Our laboratories use small portable spectrometers (Ocean Optics USB 650 Red Tide spectrometers) and are able to quickly collect the whole spectrum, though this experiment is equally possible for single wavelength spectrometers. The spectrometer is baseline corrected using distilled water and all absorbance measurements are made at 475 nm, the wavelength for which the azo-dye absorbs. A pale yellowish color is seen with the blank solution – prepared with distilled water instead of triclosan but no absorbance for this is seen at 475 nm (see Figure 2. in the manuscript).

- It is possible there may be color from the dyes in the soaps adding to absorbance and a correction factor or a blank could be fashioned using the actual soap sample diluted to an equivalent concentration as solution 6 using just distilled water. This has been done for the soaps we use and for the soaps tested, there was no measurable absorbance at 475 nm.

- The issue of baseline correcting the spectrometer with only distilled water is a personal choice for this instructor. First, as seen in the spectral overlay (Figure 2.) a 'blank' solution is made which contains nitrite, sulfanilic acid and glycine but not triclosan. This solution has minimal absorbance at 475 nm. Students may use this as their baseline calibration for the spectrometer instead of distilled water if desired. This solution showing minimal absorbance at 475 nm arises from a small faint color from the nitrite, sulfanilic acid and glycine in the absence of triclosan.

- For reasons discussed in the introduction of the manuscript, care should be taken to avoid disposing of triclosan solution down the drain. While this protocol does generate a large volume of waste (~ 1 L per student group), we have reduced this volume after the laboratory period is done through allowing waste to evaporate to almost dryness in large open basins stored in the fume hoods resulting in small concentrated volumes for proper waste disposal.

- In general, this experiment has proven very successful following implementation in our general chemistry teaching laboratory. The majority of problems have arisen from improper mixing and non-homogenous solutions so the importance of mixing cannot be stressed enough. The order in which solutions are mixed is critical due to formation of the diazonium ion at acidic conditions and the final azo-dye at basic conditions (pH > 11). Following addition of the glycine buffer, there should be the visible formation of a yellowish-brown color in the solution – on one of the rare times this experiment failed to work, the solution became a yellowish color with no hint of the brownish orange. This was later found to be from incomplete solvation of the triclosan to make the master 200 mg/L solution which resulted in no measurable triclosan in the calibration standards. If the orangish-brown color is not seen in the triclosan containing samples, it is recommended perhaps simply remaking the triclosan and starting again. It is also possible to quickly test the other reagents by reacting the 200 mg/L triclosan master solution with the nitrite and sulfanilic acid to check a deep brown color forms following glycine buffer addition. Checking pH with either a pH meter or simple pH paper can also be useful in diagnosing problems.

CAS Registry Numbers

Triclosan (5-chloro-2-[2,4-dichlorophenoxy]-phenol)	3380-34-5
Sodium Nitrite	7632-00-0
4-Sulfanilic Acid	121-57-3
Glycine	56-40-6
Sodium Chloride	7647-14-5
Sodium Hydroxide	1310-73-2
Concentrated Hydrochloric Acid	7647-01-0
Activated Charcoal	7440-44-0

Laboratory Set-up Notes

These notes are based on based on student groups preparing 4 individual triclosan standards, one soap sample and 6 solutions for measurement by UV-Vis spectroscopy.

Required Glassware per Student Group

- 5 x 50-mL volumetric flasks, 6 x 100-mL volumetric flasks
- Volumetric pipettes (2 x 10-mL, 2 x 15-mL, at least 3 x 25-mL) + pipette bulb
- Graduated volumetric pipettes, 5-mL and 10-mL

Recommended Glassware for the Laboratory

- Triclosan containing soap samples (available from local supermarkets, pharmacies)
- Large containers for collection of waste
- Ultra-Sonic Bath to aid in dissolving materials (particularly 4-sulfanilic acid solution below)
- Visible absorbance spectrometer, 1-cm cuvettes or equivalent

In our version of this laboratory, student groups are tasked to produce all solutions from the solids – these are communal solutions with one group making enough sodium nitrite solution for the whole laboratory section. There are separate boxes made up containing the materials to make these solutions, the components of all of these are listed below along with the instructions. Copies of the handout on p.7 of Supporting Information are included in each box. The components of each box are described below.

Triclosan Stock Solution:

- Triclosan (solid) [CAS 3380-34-5]
- 1-L Volumetric Flask

Sodium Nitrite Solution:

- Sodium Nitrite [CAS 7632-00-0]
- 7 x 250-ml volumetric flasks, 15-mL volumetric pipettes, stir plate + stir bar

4-sulfanilic Acid Stock Solution:

- 4-sulfanilic Acid [CAS 121-57-3]
- Concentrated hydrochloric acid [CAS 7647-01-0]
- 10-mL graduated cylinder, 1-L volumetric flask, 1-L beaker, stir plate and stir bar

Glycine Buffer

- Glycine [CAS 56-40-6]
- Sodium Chloride [CAS 7647-14-5]
- 50% Sodium Hydroxide solution [CAS 1310-73-2]
- 2 x 500-mL beakers, 1 x 500-ml and1 x 1-L volumetric flasks, 2 x stir plate and 2 x stir bar

0.01 M NaOH solution

- 50% Sodium Hydroxide solution [CAS 1310-73-2]
- 1-mL volumetric pipette or graduated plastic transfer pipette, 2-L volumetric flask

Student instructions on making class stocks/solutions:

Note: All solutions should be thoroughly mixed and all solid dissolved before use. This can be done using magnetic stir plates, sonication or simple hands-on mixing. Although volumetric flasks are not necessary for all steps, these are used to aid in mixing at the bench top.

0.01 M NaOH solution

- Fill a 2-L volumetric flask approximately ½ full with distilled water.
- Add 1 mL of 50 % NaOH using a graduated pipette, fill to line with distilled water, mix well.
- This will be used first by the group preparing the triclosan standard solution then portions will be used by every group in preparing their soap solutions.

Triclosan Stock Solution (this is made to be 200 mg/L Triclosan):

- The 0.01 M NaOH solution should be obtained from the group assigned to make it.
- Accurately weigh as close to 0.200 g of triclosan (Irgasan) on a piece of weighing paper and then transfer to 1-L flask using water bottle to wash residue off paper into flask. Fill to line with the 0.01 M NaOH then add stir bar and stir till dissolved. Label as 200 mg/L triclosan master.
- This solution can then be used by each group to make their own standards.

Sodium Nitrite Solution (starting solution is 0.5 M, stock is a dilution (0.03 M) from this)

- 8.625 g of sodium nitrite is weighed out and dissolved in 250 mL distilled water in a 250-mL volumetric flask – label this as 0.5 M sodium nitrite master solution.
- Pipette 15 mL of the 0.5 M solution into a 250-mL volumetric flask and dilute to line with water – label as 0.03 M sodium nitrite stock solution.
- Repeat previous step to make enough labeled stock solutions for the whole class (1 per group).

4-sulfanilic acid stock solution

- Prepare a 0.09 M hydrochloric acid solution by adding ~900 mL of distilled water followed by 7.5 mL concentrated hydrochloric acid (measured using a 10-mL graduated cylinder) into a 1-L volumetric flask, fill to the 1-L line with distilled water and mix well.
- Measure 2.50 g 4-sulfanilic acid into a 1-L beaker and add all of the 0.09 M HCl and stir.
- Once the solid has totally dissolved, label beaker and this can be used by all groups.

Glycine buffer (pH ~11.5)

(For a large 5 or 6 group class, you will need to do this twice or simply double the quantities.)

This solution is made in 2 parts which are then combined:

- (1) Make 500 mL of 0.1 M glycine buffer by dissolving 3.65 g of glycine in 500 mL DI water. Add 3.0 g of NaCl to this solution and stir well till all dissolved
- (2) Make 500 mL of 0.1 M NaOH by adding first 400 mL of distilled water then 3 mL of 50% NaOH to a 500-mL volumetric flask, fill flask to the line with distilled H_20 and mix well.
- Mix both NaOH and glycine solutions in 1-L volumetric flask. Mix and label.

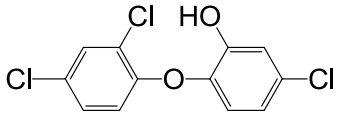
STUDENT HANDOUTS FOR DETERMINATION OF % TRICLOSAN IN ANTI-BACTERIAL SOAP

Keywords: Analytical Chemistry, Spectroscopy, Quantification

Objectives:

- Prepare a series of Triclosan calibration standard solutions
- Determine Triclosan levels in a personal care product

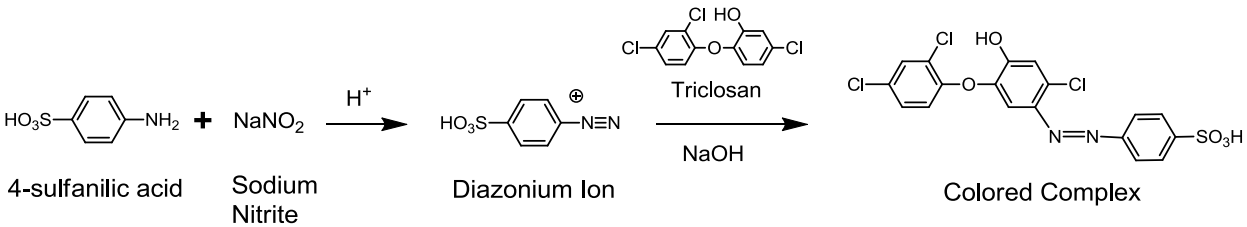
This laboratory period looks at the antibacterial drug triclosan (structure shown below) which has been the subject of a large amount of attention in the scientific literature and the popular media of late over efficacy in terms of environmental impacts and possible negative health effects.[1] In this laboratory, we will have a chance to look at a spectroscopic means of determination of the amount of the drug in a sample of a personal care product (anti-bacterial soap).



This experiment looks at quantification of a single component of a complex mixture. But, due to interfering species such as the other chemicals and dyes in the soap, we cannot measure the concentration of triclosan directly and instead of isolating the material of interest from the reaction mixture, we actually carry out a chemical reaction with it *in situ* to give a much more measurable property (color).

The Spectroscopic Triclosan Assay:

There are many ways to determine the concentration of triclosan in a commercially available product using modern laboratory instrumentation such as High Performance Liquid Chromatography (HPLC) but is there a way to do it just by looking at the color of the solution? Given the triclosan is colorless, one would think not but it does possess a strong absorbance in the UV region (250 – 350 nm) of the spectrum. The problem is so do many other components of soaps and shampoos so isolating what signal (absorbance) arises only from the triclosan is impossible.



However, what if we can react it with something that is colorless as well and make a compound which is not only strongly colored but the color of the resulting solution is also directly proportional to the amount of triclosan in the original sample?

To do this, we react sodium nitrite and 4-sulfanilic acid in the first step of the reaction shown above to make an intermediate species (called a diazonium ion) which then in the second reaction reacts with triclosan and forms a colored complex.[2]

Of course, in any system like shampoo, hand-lotion, or other personal care product, there is more than just triclosan present but only triclosan reacts with the diazonium ion and forms this colored complex, the other components of the soap or shampoo do not react. If we also make sure there is significantly more of the diazonium ion present than triclosan, any and all triclosan molecules will be reacted to form the colored complex. As a result, we can directly relate the intensity of the color of the solution as measured by the spectrometer to the amount of triclosan (triclosan therefore acts as our ***limiting reagent*** in the formation of color in the solution).

A soap solution is prepared by diluting a known mass of soap to a known volume. This is reacted with sodium nitrite and sulfanilic acid to make the colored complex. To make this experiment quantifiable, we will also make a series of standard solutions containing known amounts of triclosan (diluted from a stock solution) and work these up the same way. If we then plot the absorbance at a given wavelength against concentration, we create a calibration plot and can use this to determine the concentration of triclosan in the unknown material. From that, we can calculate the mass of triclosan in the solution made from the soap and finally work out the % triclosan in the soap, a value which can be compared with that listed on the packaging.

UNITS: The units used in this laboratory for the triclosan concentration are mg/L. Stock solutions are prepared in this unit and your unknown personal care product calculated using this.

Safety Information:

Students should wear goggles and follow the appropriate laboratory safety guidelines at all times. All materials can be handled with appropriate care on the bench-top though direct contact with the solid sodium nitrite, 4-sulfanilic acid and triclosan should be avoided. Due to environmental concerns, all waste containing either triclosan, nitrate or 4-sulfanilic acid should be collected at the end of the laboratory and not disposed of down the drain.

Additional References:

1. Kemsley, J. Triclosan Under the Microscope. *Chemical and Engineering News* **2014,** *92(5),* 10-13. (this reference provides a good overview of the chemistry of triclosan along with health and environmental concerns – it is available online at http://cen.acs.org/articles/92/i25/Triclosan-Under-Microscope.html).

2. Huihui Lu, Hongbing Ma, Guanhong Tao, Spectrophotometric determination of triclosan in personal care products, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Volume 73, Issue 5, **1 September 2009**, Pages 854-857.

Experimental Procedure for Determining Triclosan Assay in Personal Care Products

See the schematic on page 12 for an overview of all of the solutions being made – The laboratory section will make 1 of each of the glycine buffer, nitrite, 4-sulfanilic acid and triclosan master stock solutions for the entire section and share these. Each student group will work up one of the soap samples being analyzed to make solution X and also make 4 triclosan standards of 10 – 40 mg/L concentration (A – D). From these standards and solution X, each student group will make 6 solutions for measurement (1 – 6).

Shared Solution Preparation: (The whole laboratory section will have to work on this)

The laboratory requires a number of solutions to be made fresh and this is done by having the groups in the class make these for the entire class. The nitrite, 4-sulfanilic acid, sodium hydroxide solution, triclosan stock and glycine buffer solutions must be made fresh for each section so you will be assigned one and given the relevant box containing all materials and instructions for. Once all of these solutions are made, you and your group may proceed with the following step.

Soap Sample Preparation Procedure: (You will work with your student group only on this)

- You will be provided with a sample of a soap which contains triclosan (% is listed on ingredients – it may instead be listed as irgasan). If you wish, you may bring a sample of a personal care product to laboratory but make sure the ingredients list triclosan or irgasan and how much is present.
- Weigh about 0.4 g (record the exact mass) in the bottom of a 50-mL beaker and add about 20 mL of 0.01 M sodium hydroxide solution. Swirl to mix.
- Place the beaker in the ultra-sonic bath for ~ 5 minutes.
- Transfer the solution to a 50-mL volumetric flask.
- Add ~20 mL 0.01 M sodium hydroxide solution to the beaker, swirl and add to the flask.
- Fill the flask to the line with 0.01 M sodium hydroxide solution and mix. The sample is now ready for analysis (this will be used to prepare solution F below).

Preparing Triclosan Calibration Standards: (You will work with your student group only on this)

A series of stock solutions of triclosan must be prepared from the stock (200 mg/L) master solution being made at laboratory start. This will be diluted by you and your research team to form a series of 4 calibration standards which should span the range 10, 20, 30 and 40 mg/L – all the volumetric glassware you will need to make these will be available in the laboratory.

When doing dilutions whilst working in concentration of mg/L, remember $C_1V_1 = C_2V_2$ where C is concentration in mg/L and V is volume in mL. C_1 will be the volume of the solution you are diluting (our master stock of triclosan which is 200 mg/L), C_2 will be the desired concentration of the dilution, V_1 the amount diluted (pipette size generally) and V_2 the amount of the diluted solution made (flask size). C_1 and C_2 are dictated by what solutions you are making so you will choose either V_1 or V_2 and the expression $C_1V_1 = C_2V_2$ will solve to give you the other.

Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Preparing Samples for analysis: (You will work with your student group only on this)

You will have to prepare 6 samples for measuring the absorbance. These are prepared using the 4 triclosan standards, one blank with distilled water and one sample for the antibacterial soap. See page 12 for a table describing the components of these solutions.

To prepare a sample for analysis, mix the following in a 100-mL volumetric flask. All quantities must be measured volumetrically using volumetric pipettes**. The order in which you mix these is CRITICAL**. Use the order below, do not add any solutions out of order!

- 15 mL Sodium Nitrite Stock Solution ($NaNO_2$)
- 10 mL 4-sulfanilic acid solution
- 25 mL solution to be tested (see table below)
- 25 mL glycine buffer (this alters the pH and stabilizes the product of the reaction)

The sample volume is then made to 100 mL with distilled water and mixed.

You should prepare 6 of the mixtures described above - four use the triclosan calibration standards, one is made with a sample of just distilled water (this is the blank) and one with the sample of hand soap you diluted to a standard volume, The distilled water will act as a blank so can be used to show if there is any background signal we need to worry about. The 25 mL to be tested for each is listed below and on page 12.

Solution ID	Made with 25 mL of
1	Triclosan Standard A (40 mg/L)
2	Triclosan Standard B (30 mg/L)
3	Triclosan Standard C (20 mg/L)
4	Triclosan Standard D (10 mg/L)
5	Distilled Water
6	Soap solution X

The solutions will become colored on the addition of the glycine buffer which makes solution basic. You should now have 6 samples for analysis – 4 x standards, 1 x blank, 1 x soap.

NOTE: After the mixing above, a 40 mg/L standard would obviously not be 40 mg/L. We don't use any other dilution in our calculations though as we know our solution we took the 25 mL from was 40 mg/L and this will give a certain reading on the spectrometer. Since we are treating the unknown personal care product the same way, we get the concentration for our soap relative to the calibration standards.

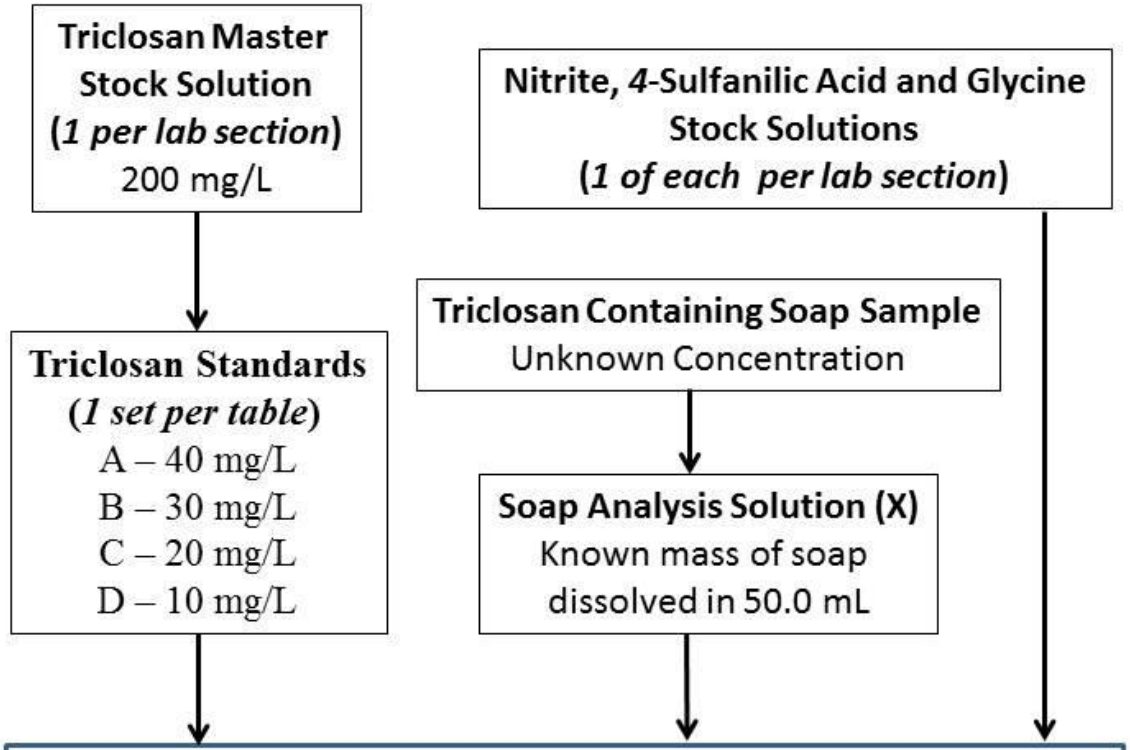
Measuring the absorbance of all samples

The spectrometer should be baseline calibrated using distilled water. Measure the absorbance of all 6 solution at 475 nm using the standard procedure for the spectrometer.

At the end of the laboratory period:

- All solutions should be disposed of using the appropriate waste procedures
- Clean and rinse all glassware with distilled water

Triclosan Solution Preparation Overview



6 x measuring solutions for spectroscopy
(*1 set per table*)
Add the following IN ORDER and make to 100.0 mL with DI water

Solution	Nitrite	sulfanilic acid	Variable	Glycine buffer
1	15 mL	10 mL	25 mL of A	25 mL
2	15 mL	10 mL	25 mL of B	25 mL
3	15 mL	10 mL	25 mL of C	25 mL
4	15 mL	10 mL	25 mL of D	25 mL
5	15 mL	10 mL	25 mL of DI H_2O	25 mL
6	15 mL	10 mL	25 mL of X	25 mL

Data Analysis

The goal is to obtain the % triclosan in the sample of soap being analyzed. To do this, we will calculate the concentration of solution (6) from the absorbance and then the mass of triclosan in the solution. With this, we can calculate the % triclosan in the soap and then compare this value with that listed on the packaging.

- First, construct a Beer-Lambert plot relating the absorbance at 475 nm (y-axis) vs concentration of triclosan for the standards (10 – 40 mg/L) being sure to include the 0,0 point. This should be fit to a linear trend line and the equation of the trend line determined as well as the R^2 value. If any of your points are severe outliers, you may wish to exclude these from the analysis.

- Using the measured absorbance of the unknown soap solution and the Beer-Lambert plot, calculate the concentration of triclosan in solution 6 (in mg/L).

- To convert this value from mg/L to an actual mass, multiply the concentration in mg/L by the number of liters (50 mL = 0.05 L). This gives us the mass of triclosan in mg in solution X.

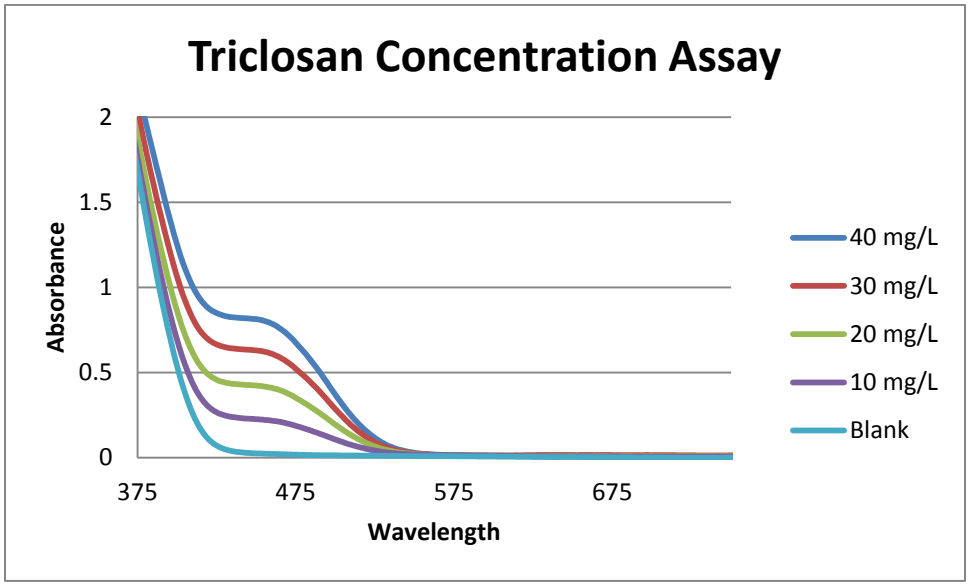
- Convert the mass of triclosan from mg to g and use this to calculate the % triclosan using the following formula:

% Triclosan in soap = (mass of triclosan in solution X / total mass of soap in solution X) x 100

- Compare the value obtained with that listed on the packaging. Is your value close to the value listed? Calculate the % difference between your value and the packaging and comment on possible sources of error. You may also wish to compare data with other groups who analyzed the same soap as you did.

Sample Spectra and Calibration Plot

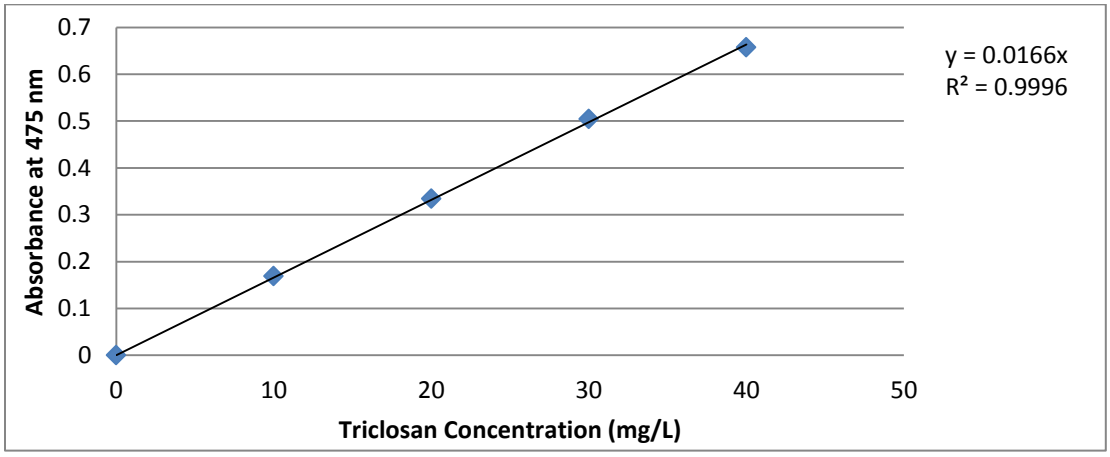
Compiled Spectra of 10 – 40 mg/L triclosan Calibration Standards



Absorbance data at 475 nm

	Absorbance at 475 nm	Corrected by blank subtraction
40	0.674	0.657
30	0.521	0.504
20	0.351	0.334
10	0.186	0.169
0	0.017	0

Beer's Law Plot for Calibration Standards at 475 nm



Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Sample data calculated for % triclosan determination in a sample of anti-bacterial soap

The following data were collected using the procedure as previously described in pages 8 -13.

Original Mass of Soap added to create solution X: 0.507 g

Solution	Component	Corrected Absorbance @ 475 nm
1	25 mL of triclosan standard A (40 mg/L)	0.657
2	25 mL of triclosan standard B (30 mg/L)	0.504
3	25 mL of triclosan standard C (20 mg/L)	0.334
4	25 mL of triclosan standard D (10 mg/L)	0.169
5	25 mL of distilled water	0.00
6	25 mL of soap solution X	0.281

Equation of trend line from Beer Lambert plot: $y = 0.0166 x$ (see previous page)

Absorbance of Solution 6 (soap) at 475 nm: 0.281

Concentration of triclosan (mg/L) in soap solution X = (0.281/0.0166) = 16.93 mg/L

Mass of triclosan (mg) in soap solution X = (16.93 mg/L * 0.05 L) = 0.847 mg

Mass of triclosan (g) in soap solution X = 0.000847 g

Mass of soap (g) used to make soap solution X = 0.507 g

% triclosan in soap = (0.000847 g / 0.507 g) * 100

= 0.167 %

% triclosan listed on packaging of soap = 0.17 %

Instructor notes for investigating adsorption of triclosan by activated charcoal

- In this laboratory, students are tasked to investigate the efficacy of activated charcoal in removing triclosan. As our second semester focuses on the themes of pharmaceuticals in the environment and requires students to undertake a 7 week research project on this theme, this experiment serves to provide an introductory research-like experience by removing the structure and expository instructional style of the laboratory manual they are accustomed to and providing simply a problem, some background information and minimal guidance from the instructor.

- This laboratory follows on from the % triclosan determination in soap so students are able to use that technique as a means of determining triclosan concentration in the solutions under investigation. In general, student groups are asked to put forward a hypothesis regarding adsorption of triclosan by charcoal, often whether an additional variable such as temperature or pH will enhance or inhibit this and test this hypothesis through the laboratory period.

- Student groups can be tasked either to investigate the kinetics of the adsorption reaction (the version of the student handout describes this) or set up some combination of triclosan, degassed charcoal with one additional variation present – pH, temperature or salinity for example and see how this variation affects the extent of adsorption. Reactions are allowed to run for the required time period then the solution is filtered to remove the charcoal and stop the reaction. A 25 mL portion of this solution is combined with nitrite, sulfanilic acid and glycine as previously carried out in the preceding week.

- The general format of the laboratory period is that students will have read the information available in the manual (pages 18 to 21 of the Supporting Information) prior to laboratory which begins with a pre-laboratory lecture by the instructor reviewing the concepts and outlining the structure and requirements of the laboratory. Students are then tasked to develop a testable hypothesis and if this is approved by the instructor (based on being achievable within the laboratory period), students carry out the experiment, collect their own data and analyze and share their results.

- The charcoal is used in the form of larger pieces rather than a fine powder to make removal by filter paper easier. In general, we degas the charcoal by slow stirring in a solution of distilled water for 5 minutes prior to use. Stirring the charcoal on a stir plate for the course of the experiment does cause some fragmentation and the solution prior to filtering takes on a greyish tinge but filtering through filter paper removes this dust.

- Based on the observation in the preceding laboratory that a linear relationship is observed between absorbance at 475 nm of the colored complexes and triclosan concentrations of 10 – 40 mg/L without absorbance values exceeding 1.5, it is recommended to the first groups that a working triclosan concentration of the charcoal and triclosan solution is ~ 40 mg/L. Even with significant adsorption, there is still a quantifiable signal observable by spectroscopy.

- Note it is not necessary to calculate the actual triclosan concentration in order to determine the kinetics of the system and plots can be made which simply plot absorbance at 475 nm vs. time. This allows simplification of the data analysis as the reaction order can be determined from the relevant linear plot (absorbance vs. time, ln(absorbance) vs. time or 1/absorbance vs. time). Instructors should feel free to simplify the experiment this way if desired.

- Preparation of all solutions and calibration standards are left to student groups to prepare – instructions on these are described in the preceding pages in the Supporting Information.

Examples of Previous Experiments

- As a starting point, we have found good adsorption is observed when 0.5 g of activated charcoal added to 200 mL of a 40 mg/L triclosan solution and left to stir for 30 minutes. Subsequent assay yielded a final concentration of 26 mg/L (This corresponds to a ~40% decrease in triclosan concentration). The solution was prepared from a 200 mg/L triclosan standard by adding 0.5 g of activated charcoal into 160 mL distilled water and stirred to degas which is followed by addition of 40 mL of the 200 mg/L triclosan standard. We choose 40 mg/L as the starting concentration for the reaction as this corresponds with the upper limit of the calibration standards from the previous week. ~ 30 mL portions are removed at 30, 60 and 90 minutes and filtered through filter paper and then 25.0 mL aliquots analyzed as described in the assay experiment.

- To date, we have investigated a number of parameters as our variant condition experiments – In all cases, at times in the reaction, 25 mL+ portions of the charcoal and triclosan solution are filtered and 25.0 mL volumetrically pipetted into a mixture of nitrite and sulfanilic acid. Addition of glycine then gives the colored complex and absorbance can be measured at 475 nm. These variant experiments have included:
  - **Different Amounts of Charcoal** -Student groups have varied amount of charcoal between 0.1 and 5 g finding at large enough masses, there is no change in rate of adsorbance.
  - **Temperature –** Reactions can be run under a range of temperatures with past examples using either solutions on hotplates or in the fridge or water baths. As expected, the rate of adsorbance is found to increase with increasing temperature. Challenges are faced with obtaining a single stable temperature over time so instructors may wish to plan ahead and prepare water baths if available and also perhaps have distilled water acclimated to the relevant temperatures to aid students – I have 4-L bottles of distilled water stored in the fridge and water baths that students use to make their solutions.
  - **Stirring Speed –** As the reaction rate depends on the triclosan and charcoal coming into contact, stirring at different speeds does affect the reaction with no stirring decreasing the reaction and stirring too fast also resulting in a lower rate.
  - **pH –** Addition of acid or base affects reaction rate though this is rendered a more challenging experiment due to the need to make sure the pH of the measuring solutions containing glycine is basic (pH > 11) at the end. This may require additional NaOH addition and pH of the final solution can be checked by pH paper.
  - **The presence of soap –** Addition of increasing amounts of a non- triclosan containing soap was found to decrease the rate of the reaction.

Student handout for investigating adsorption of triclosan by activated charcoal

Keywords: Physical Chemistry, Kinetics, Spectroscopy, Adsorption

Objectives:

- Investigate the effectiveness of activated charcoal in removing triclosan from solution.
- Investigate the effects of varying conditions on triclosan adsorption.

Activated Charcoal is a form of carbon which possesses a micro-porous structure and a huge capacity for adsorbing a variety of materials from gases to smaller organic molecules. The properties can be modified by further treatment to enhance specific adsorption of the desired materials. In the interaction between charcoal and the triclosan, the drug is adsorbed onto the surface of the charcoal and removed from the solution. ***Obviously the faster the drug is adsorbed and removed from solution, the more efficiently the contaminated water can be cleaned.*** Activated charcoal can also be used in adsorbing harmful biological molecules in the body i.e. as a treatment for drug overdoses and in filters of various types for both gases and liquids. While there is a limit to the capacity of charcoal for adsorbing the various molecules, this is very large and we will not reach this in this laboratory.

This experiment will focus on measuring the extent of the adsorption of triclosan onto the surface of activated charcoal. Triclosan is mixed with charcoal and allowed to react prior to the removal of the charcoal by filtration. The filtered solution is then reacted with nitrite and sulfanilic acid as described in the preceding laboratory and the absorbance at 475 nm measured. This amount of triclosan containing complex provides a convenient method for monitoring the progress of the reaction. When triclosan is adsorbed onto the charcoal, the resulting concentration decreases, the corresponding color will be less and so the extent of adsorption can be determined from spectroscopic measurements.

Hence, in this laboratory, we will investigate the extent of adsorption of a drug (Triclosan) onto activated charcoal. By filtering out the charcoal from portions of the solution at various times, we can follow the progress of this reaction by determining the concentration of triclosan left unadsorbed in the filtered solution spectroscopically at these times.

Adsorption vs Absorption.

This experiment will use both of these terms throughout but it is worth understanding the difference as they describe quite different processes.

Adsorption is the adhesion of a very thin layer of molecules (such as gases, solutes, or liquids) to the surfaces of a solid bodies or liquids with which they are in contact. In this experiment, the triclosan drug is adsorbed onto the surface of the charcoal.

Absorption is the process by which any material is taken in (or absorbed) by another material. In this case light of specific wavelengths is absorbed by the colored species present. Triclosan will absorb light at specific wavelengths when mixed with the diazonium ion (previous laboratory) while the black activated charcoal absorbs all wavelengths of light

Experiment Overview

A reaction vessel containing a solution of the drug and a suspension of activated charcoal will be set-up and portions of the solution can be withdrawn and analyzed over the course of the experiment. These are filtered to remove the charcoal essentially stopping the reaction in that portion of the solution at that time. The absorbance of the solution can then be measured and used to calculate concentration.

There are several options for how we can best investigate this system. It is possible to look at the kinetics of this reaction and by plotting the concentration of the triclosan remaining vs. time, we can determine the rate law for the system. Ordinarily, the rate law would be:

$$\textbf{Rate of disappearance of triclosan} = -\Delta[T]/\Delta t = k[T][AC]$$

where k is the rate constant, [T] is the triclosan concentration and [AC] is the effective activated charcoal surface area available for the drug to adsorb onto which is related to the amount of charcoal present. But while the concentration of the triclosan [T] will change over the course of the reaction, the capacity of the activated charcoal for absorbing triclosan is unlikely to reach saturation since literature studies have shown mg quantities of charcoal are capable of adsorbing large amounts of materials. Hence the available charcoal surface area remains constant and is unlikely to change during the reaction. Obviously a correlation between the concentration of the charcoal and the rate of the reaction exists since one would expect the more charcoal present, the faster the reaction will proceed. But if we make this amount of charcoal sufficiently large (i.e. an excess is present) then the rate of reaction depends purely on how fast the triclosan will adsorb on to the charcoal. Our rate constant then changes to encompass the charcoal concentration and our rate law simplifies as follows where n is the order of the reaction and $k_{obs} = k[AC]$.

$$\textbf{Rate of disappearance of triclosan} = -\Delta[T]/\Delta t = k_{obs}[T]^n$$

By plotting concentration vs. time, we can determine reaction order and a value for the observed rate constant. Depending on reaction order, a straight line will be observed for one of the following conditions.

n	**x-axis**	**y-axis**	**k_{obs}**
0	Time	[T]	- Slope of the line
1	Time	ln[T]	- Slope of the line
2	Time	1/[T]	Slope of line

We can also vary experimental conditions and see how this affects the reaction order and the value of the observed rate constant (k_{obs}). One option your instructor may suggest is to have different student groups look at the effect of different variables (temperature, pH, salinity for example) and see how these affect k_{obs}. For example, some groups may adjust the pH of their reaction mixture to be acidic and see how this affects their data compared to the control (neutral system). This will be discussed at the start of the laboratory.

Experimental Procedure

It is important all dilutions are carried out precisely and accurately in this section using pipettes and keeping record of all dilutions. The experiment requires accurate determination of concentration at the given time intervals and to determine concentrations, you will withdraw portions of the solution from the reaction vessel over the course of the experiment which are filtered (removing the charcoal and stopping the reaction at the time of filtration).

Measurement of Rate of Adsorption of Triclosan onto Charcoal.

Prior to starting the reaction, the activated charcoal must be degassed. Measure the desired mass of activated charcoal into a 250-mL beaker and pipette in the desired volume in mL of distilled water. Add a stir bar and stir the system on a stir-plate. Observe the bubbling from the charcoal as gases (mainly nitrogen) contained in pores of the charcoal are released. The solution may also become slightly cloudy from dust particles. Once bubbling has stopped, pipette in the required volume in mL of the triclosan stock solution. Once the triclosan is added, record the time - this is the reaction start.

Because we are determining concentration from absorbance, it is important that the solutions are clean and the only absorbance arises from the drug. Unfortunately the charcoal does fragment slightly and dust particles are observed giving a suspension which would interfere with the absorbance. This is unavoidable but can be removed by filtering. In addition, removing the charcoal essentially stops the reaction, if minute particles of charcoal remained in the solution after it was removed, adsorption would continue.

At the first desired time interval, collect the desired volume in mL from the reaction vessel, filter the solution and collect it in a labeled container. You may notice the filtered solution is much clearer than before with none of the black particulate matter you saw in the main reaction vessel. Make sure it is labeled with time collected and keep for analysis later.

Repeat the preceding step at regular intervals collecting each solution in a separate labeled vial until the desired experiment length is reached. Obviously the speed of collection of samples will depend on the length of the filtration process, this will be something you learn as the experiment progresses.

We can then add the triclosan solutions to mixtures of nitrite and sulfanilic acid, prepared as described in the previous laboratory, and add glycine to make the colored complex and measure absorbance at 475 nm

Safety Information:

Students should wear goggles and follow the appropriate laboratory safety guidelines at all times. All materials can be handled with appropriate care on the bench-top though direct contact with the solid sodium nitrite, 4-sulfanilic acid and triclosan should be avoided. Due to environmental concerns, all waste containing either triclosan, nitrite or 4-sulfanilic acid should be collected at the end of the laboratory and not disposed of down the drain.

At the end of the laboratory period:

- All solutions should be disposed of using the appropriate waste procedures
- Clean and rinse all glassware with distilled water

Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Data Analysis.

In this laboratory the concentration of the Triclosan solution is determined via spectroscopic measurements and a Beer Lambert plot. The hypothesis is when triclosan is mixed with activated charcoal, the triclosan can be removed (adsorbed) from the solution by the charcoal.

Your instructor will have discussed whether calibration standards were needed to be prepared fresh this week or you could use your Beer Lambert plot from the preceding week. Either way, you should have a line correlating absorbance with triclosan concentration so use this to convert your absorbance at 475 nm to actual triclosan concentrations.

Once you have these, we can use this data to obtain the observed rate constant (k_{obs}) for the expression

$$\text{Rate of disappearance of the triclosan (T)} = -\Delta[T]/\Delta t) = k_{obs}\,[T]^n$$

where [T] is the concentration of the triclosan, k_{obs} is the observed rate constant and n is the reaction order. Note the absence of the activated charcoal concentration in this expression. As the amount of charcoal remains constant throughout the experiment and we are unlikely to saturate the surface of the charcoal with triclosan, the charcoal concentration is treated as a constant and incorporated into k_{obs}.

Depending on the order of the reaction (n), you should observe a straight line for one of the following:

n	**x-axis**	**y-axis**	**k_{obs}**
0	Time	[T]	- Slope of the line
1	Time	ln[T]	- Slope of the line
2	Time	1/[T]	Slope of line

After plotting the data, fit the relevant graph to a straight line and compare the R^2 values for the quality of the fit. You can then determine k_{obs} from the slope of the relevant straightest line. You can then construct a rate law for the drug absorbance as follows since you have determined values for both k_{obs} and n.

$$\text{Rate of disappearance of Triclosan} = -\Delta[T]/\Delta t) = k_{obs}\,[T]^n$$

You may compare your data results with other groups in your section and see if the changes in experimental parameters affect the kinetics of this system.

Instructor notes for investigating the effects of triclosan on *Tetrahymena pyriformis*

- This experiment has been carried out as part of an integrated chemistry and biology laboratory class which links the first semester laboratories and investigates topics which possess strong connections to each. This might also be worth suggesting to colleagues in biology who are interested in linking a laboratory with chemistry.

- The single celled protozoan chosen for this study is *Tetrahymena pyriformis*, Kingdom Protista. This was chosen solely because it is the protozoa of choice for our biology department and culture samples were readily available for use on request the biology department. This experiment would work equally well with any other single-celled organism and it is worth checking with colleagues in biology for what is available and also recommended for study as they may be able to suggest organisms which are particularly relevant regarding environmental triclosan exposure.

- This laboratory was taught inquiry style so instructions in the student handout were deliberately minimal, the overall concept is students search literature to find LD_{50} values for triclosan on single celled organisms, prepare solutions of this triclosan concentration and others as desired and add to the culture and observe the effects. Students commonly make a stock solution of triclosan for the class or one can be provided (or reused from previous week) then dilutions of this can be made. Testing of the stock itself which tends to be 10 – 200 mg/L will easily result in *Tetrahymena* decimation, the goal is not to achieve decimation but find concentrations where the population of the organisms is affected but not destroyed. I personally challenge students to find concentration ranges which span perhaps 1 order of magnitude and over which *Tetrahymena* populations go from unaffected to total death.

- Students were previously taught how to set-up and use the optical microscopes in a previous laboratory and have used them in several labs afterwards so no information of how to do this is provided in this handout.

- Mixing of samples can be potentially challenging. The *Tetrahymena* are obtained in aqueous medium which means students must take into account this medium volume when creating the desired triclosan concentration. For example, a 1:1 mixing of a *Tetrahymena* suspension with a 1 µg/L triclosan solution actually yields a net concentration of 0.5 µg/L triclosan. Students commonly do not take into account this final dilution and so stressing the importance of this up front is recommended.

- The physical mixing of samples can also be a challenge. Mixing two aliquots then preparing a slide often results in students missing the initial effects as triclosan acts rapidly especially at higher concentrations. Placing a drop of a *Tetrahymena* suspension and a drop of the triclosan solution on a microscope slide and mixing using a needle or similar tool while observing yields the best results.

- We have found in the desired concentration ranges that the effects on *Tetrahymena* are quite noticeable. Alteration of behavior (swimming in tight circles) is a common precedent to death of the organism and it is possible to find concentrations where only some are affected.

- Students can be encouraged to investigate whether the soap with anti-bacterial properties (contains triclosan) is better than a traditional non triclosan soap in this laboratory should you so desire. Solutions are made from identical masses of triclosan and non-triclosan containing soap and diluted identically. Effects are compared and in general, at the concentrations students work, the soap itself seems to be as destructive as the triclosan. Often membrane disruption of the *Tetrahymena* is observed, they may either be seen to pop or if students wait too long, they appear to have totally vanished.

- As with the preceding laboratory, take care to avoid disposing of triclosan solutions down the drain. In general, these solutions are collected and the waste concentrated through evaporation prior to disposal of this smaller volume according to state regulations.

Student handout for investigating the effects of triclosan on *Tetrahymena pyriformis*

The previous experiment looked at determining the concentration of triclosan in an anti-bacterial hand soap through spectroscopic means but we should also be concerned with the effects of this chemical, not just knowing the concentration in a soap. Triclosan has been shown to possess strong anti-microbial properties but should we simply take this for granted or can this be demonstrated in the undergraduate teaching laboratory? These antibacterial properties are both good and bad as we would like a product which kills off harmful bacteria but does it also kill off less harmful ones too? Not all of the triclosan in the waste water is removed by conventional water treatment plants and so following use of these products, there is some small concentration of triclosan which ends up in the environment. Which leads to the question what concentrations of triclosan are required to actually affect single-celled organisms? The goal of this experiment is to observe through microscopy the effects of varying concentration of an antibacterial chemical on a bacterial population – this should be a direct relationship and as concentration of the antibacterial increases, so should the effects until total die-off is observed.

Connections to Chemistry and Biology:

This laboratory actually provides a great linking of chemistry and biology – the first laboratory period looked at determination of concentration of a chemical in a complex system and then the second laboratory period looks at how it affects a simple bacteria *Tetrahymena pyriformis.* Using microscopy, we can then observe directly the effects of introducing triclosan into the environment of these single celled organisms. One particular goal is to look at effects of varying concentration here and determine the concentration range where *Tetrahymena pyriformis* is affected but not annihilated. This leads into the concept of LD_{50} and we will also try and correlate the observed changes in *Tetrahymena pyriformis* behavior with the reported action of this chemical. One particular challenge will lie in finding the concentrations which affect but do not annihilate the *Tetrahymena pyriformis* population.

Triclosan effects on *Tetrahymena pyriformis*

The goal of this second laboratory period is to observe the toxicity of triclosan on *Tetrahymena pyriformis* under the microscope. While it is easy to add massive quantities of triclosan to the system, the challenge will be to find the range under which the population of these creatures is impacted but not decimated. Some literature research to find effective triclosan concentrations or LD_{50} values for single celled organisms (you will not find the value for *Tetrahymena pyriformis* but there are several other species for which this data has been reported). Triclosan solutions should be prepared, mixed with *Tetrahymena pyriformis* cultures and the effects observed under the microscope and recorded. You should be then able to correlate triclosan concentration vs toxicity to the organisms and in effect determine a LD_{50} value (concentration at which ~50% of population tested dies).

Once you have found the concentration at which 50% of the *Tetrahymena pyriformis* are affected, you should investigate whether the presence of the other materials makes a difference. Dilute a solution of the soap sample to the same concentration and compare the results with just the triclosan solution – is there a difference? Is the soap more effective at the same value meaning the other ingredients in the soap also contribute or is it just the same. We could also look at using the same amount of a non-anti-bacterial soap and seeing if there is a difference in soaps between those with and those without triclosan.

At the end of laboratory period, all waste containing triclosan, should be collected at the end of the laboratory and not disposed of down the drain.

Set-up, notes and modifications for staging experiment as part of science outreach workshop

The general chemistry laboratory version of the experiment to determine triclosan concentration in soap requires extensive use of volumetric pipettes and flasks but the experiment can be simplified somewhat to be run as a high-school workshop or outreach workshop in a venue such as local public library. In general, it is suitable for teens and high school students and provides a valuable interfacing of laboratory science and a real world problem. The workshop is intended to run for approximately 1 hour if moving fast or 90 minutes at a more leisurely and less rushed pace.

The experiment version presented here scales the materials and volumes to use the minimal quantities of volumetric glassware. 250-mL volumetric flasks were selected as our department owned an excessive number of these and so breakages, if they had happened would not impact us significantly. Instead of accurately pipetting all solutions, graduated cylinders are used for the most part and to avoid pipetting soap solutions, the whole of the soap sample prepared is ultimately mixed in the solution with the nitrite and sulfanilic acid. Only one set of calibration standard solutions are prepared, mixed with nitrite and sulfanilic acid and this is done by the workshop leader or an assistant. The use of a small portable spectrometer such as an Ocean Optics USB650 Red Tide Spectrometer also allows this to be easily transported to a range of venues.

The following sheets describe how to prepare for this workshop are described below. A list of general materials is given on this page and modified instructions on how to make the various solutions are given on the next page. Due to the nature of the triclosan as the limiting reagent, the concentrations of nitrite and sulfanilic acid are used in excess and so these solutions need not be extremely accurate in their preparation. Tap water can be used for these preparations so solutions can be prepared at the venue if you want to travel light. We use large 4-L amber glass bottles for collection of waste which is then transported back to the laboratory. It is also important to stress the safety aspects of this experiment – participants should wear eye protection at all time and the host should be prepared to deal with spills.

A sample workshop flier is given on pages 29 and 30 of the Supporting Information – page 29 shows the reaction and provides minimal details on what to do, page 30 allows participants to fill in their numbers and work through the calculations.

General materials needed for workshop:

- 250-mL volumetric flasks – 1 per group and 5 extra for calibration standards
- Medicine droppers, Pasteur pipettes or plastic transfer pipettes
- 25-mL graduated volumetric pipette and pipette bulb
- Funnels to aid solution transfer
- Graduated cylinders – recommended at least 2 x 10-mL, 2 x 25-mL, 2 x 50-mL, 2 x 100-mL.
- Triclosan containing soap samples (available from local supermarkets, pharmacies)
- Balance (should be accurate to 0.01 g or better)
- 50-mL plastic screw cap centrifuge tube with screw cap or 50-mL beaker to measure soap into
- Visible absorbance spectrometer, 1-cm cuvettes or equivalent
- Large containers for collection of waste
- Goggles or other form of eye protection

Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Instructions on making shared stock solutions:

The instructions below describe how to make the solutions for running this experiment as a workshop either in the local community or high schools. To make things easier to transport, it is simpler to pre-measure all chemicals in the laboratory and transport them in vials making the solutions up prior to the workshop start. This makes things simpler for travelling and solutions are fresh. 4 master solutions are required for the workshop, instructions for each of these are given below. For 1-L bottles, we use plastic bottles which are known to hold ~1 L. These need not be accurate as the solutions prepared in these are not limiting reagents.

(1) Sodium Nitrite Solution (0.03 M, 1 L sufficient for up to 20 student groups approx.)

- Pre-weigh 2.2 g $NaNO_2$ (sodium Nitrite [CAS 7632-00-0]) into a small vial for transport.
- Prior to workshop, transfer vial contents into 1-L bottle, wash vial with water to ensure complete transfer and fill and mix well till all contents dissolved.

(2) 4-sulfanilic acid stock solution (0.015 M, make 1 L for up to 20 student groups)

- Weigh 2.50 g 4-sulfanilic acid [CAS 121-57-3]into a small vial for transport.
- Prior to workshop start, make ~1 L of 0.09 M hydrochloric acid by adding ~7.5 mL of concentrated hydrochloric acid [CAS 7647-01-0] to 1 L of water (Mix well).
- Add vial contents (4-sulfanilic acid) to the 1 L of hydrochloric acid.
- This solution needs to be mixed well, maybe stir if you have a stir plate available.

(3) Glycine buffer – make 1 L per 10 student groups

- Weigh 3.65 g of glycine [CAS 56-40-6] and 3.0 g of sodium chloride [CAS 7647-14-5] into a small vial for transport.
- Prior to workshop start, add 4 mL 50% sodium hydroxide [CAS 1310-73-2] to 1 L of water and mix.
- Mix in contents of solid vial, once totally dissolved, this is now ready to use.

(4) Triclosan Stock Solution (this is 500 mg/L Triclosan):

- Weigh 0.125 g of triclosan [CAS 3380-34-5] into a small vial for transport (weigh as accurate as possible).
- Prior to workshop start, add 1 drop of 50% sodium hydroxide [CAS 1310-73-2] and ~5 -10 mL water to the vial.
- Mix/shake as well as possible to dissolve as much triclosan as possible in this small volume.
- Transfer vial contents to a 250 mL volumetric flask and wash residue across with small amount of water.
- Try to get complete solvation of the solid while keeping the volume small (triclosan is more soluble the more basic it is but we do not want the solution to be too strongly basic so keeping volumes for initial solvation small works well then when all water is added, the base is diluted)
- Once it is completely dissolved, fill flask to the line with water and mix well.

Workshop Leader instructions for preparing the 4 calibration standard solutions (250 ml)

The following allows the workshop leader to prepare calibration solutions containing nitrite, sulfanilic acid and one of four different triclosan concentrations without the need to prepare four separate calibration standards of triclosan (which is normally done as part of the general chemistry laboratory variant of this experiment) but instead uses one triclosan master solution for the direct preparation of them all. The preparation of the solutions (1) – (4) used to make these solutions are described on the preceding page. These solutions should be made just prior to the workshop start.

- Add 40 mL Nitrite Solution (1) + 25 mL 4-sulfanilic acid solution (2) to 250-mL flask and mix.
- The goal of this step here is to add the same total volume of triclosan and water (100 mL) to all flasks but by varying the relative amounts of each, we get different final volumes of triclosan. It has been found to work better if the triclosan is added after the water so first add a volume of water so water + triclosan volume added = 100 mL (i.e. 95, 90, 85 and 80 mL).
- Now, pipette in to flasks (A) to (D) 5, 10, 15 and 20 mL respectively of triclosan standard (500 mg/L) (solution (4) from preceding page) such that the volume of water previously added and the volume of triclosan added = 100 mL.
- This makes solutions which correspond to 10, 20, 30 and 40 mg/L triclosan respectively.
- Add ~70 mL glycine buffer (solution 3) to each, should see color change then (yellow / brown)
- Make flasks to line with DI water and mix well.

Calibrate a visible absorbance spectrometer and record absorbance at 475 nm. Plot absorbance at 475 nm of (A) to (D) against concentrations of 10 – 40 mg/L along with 0,0 to get calibration plot y = mx – this can be done in Excel or on graph paper as desired.

Workshop Leader Note on Safety

Taking an experiment out of the more secure locale of the college chemistry laboratory and into a more public location such as a high school or my case the local public library can be stressful at times but this experiment uses materials which are relatively benign and poses no risks beyond that routinely encountered when handling chemicals. All patrons should be provided safety glasses and asked to wear them at all times though latex gloves are not necessary. The worst of the solution preparation is handled by the workshop leader or an assistant who is likely more experienced in chemical handling and at the workshop, the largest problem will be the inevitable spills or drops on the table. I recommend having copious amounts of towels and water for clean-up and if you have people assisting, regularly wiping the work areas is recommended. In the case of a major spill, handle clean-up yourself – I don latex gloves and use absorbent towels to soak up the liquids and then wash the area with plenty of water to be sure. Do not forget to advise all patrons to wash hands at end of workshop.

Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Instructions for participant soap solution preparations

These instructions can be distributed to participants as a handout or projected on screen as desired. Using a follow-along with the workshop leader style is preferred to ensure things proceed smoothly. The soap solution is initially made in a 50-mL plastic centrifuge tube with lid and then all reactants combined in a 250-mL volumetric flask.

- All volume measurements can be made with graduated cylinders.
- Weigh the empty 50-mL plastic screw cap centrifuge tube, record mass on worksheet.
- Weigh 0.5 g of soap into the 50-mL plastic screw cap centrifuge tube, add 1 drop of NaOH and 25 mL water and mix with lid on. Record the mass used on the worksheet.
- Add 40 mL Nitrite Solution + 25 mL 4-sulfanilic acid solution to 250-mL flask and mix.
- Transfer contents of soap container to measuring solution volumetric flask (250-mL).
- Add ~ 25 mL water to soap container, mix and add to 250-mL flask.
- Repeat twice more for ~ 25 mL additions of water to soap container, mix and add
- Total volume of soap added ~ 100 mL
- Add ~70 mL glycine buffer to the 250 mL volumetric flask, you should see a color change
- Make to line with DI water and mix well

Record absorbance at 475 nm on the worksheet, use data provided from the calibration plot and you should be able to solve for the % triclosan in the soap solution

Workshop Leader Note on Calculations:

The 250 mL volume calibration standards are essentially 10, 20, 30 and 40 mg/L as made. This means we are essentially plotting 10 – 40 mg/L vs measured absorbance and we calculate the concentration of the whole 250 mL soap solution.

We then get mg/L for the soap solution and since we have 250 mL, we multiply this by 0.25 to get actual number of mg.

Then we divide by 1000 to get from milligrams to grams of triclosan present.

Then divide (g of triclosan)/(g of soap) x 100 to get % triclosan in soap.

Compare this value with that listed on the soap packaging and calculate % difference between packaging and experimentally determined value.

Great Chemistry!

(for TEENS)

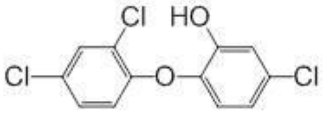
Experiment Overview:

Introduction
Making Solutions
Measurements
Data Analysis

Laboratory Objective:

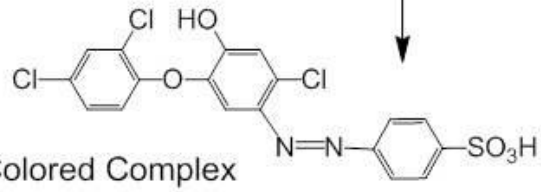
Determine the Concentration of triclosan in an antibacterial soap and see how value compares to that listed on the label

4-sulfanilic acid Sodium Nitrite Diazonium Ion



Triclosan

NaOH



Colored Complex

Solution Instructions:

- **40 mL Nitrite Solution**
- **25 mL 4-sulfanilic acid**
- **50 - 100 mL soap solution**
- **75 mL Glycine Buffer**
- **Make to 250 mL with water**

Supporting Information for Spectroscopic Determination of % Triclosan in Anti-Bacterial Soaps

Worksheet for determining % triclosan in antibacterial soap

Name:_____

Soap used (Color):_____

Mass of Soap Added to Beaker (to make solution): _____ g (1)

Absorbance of Standard A (40 mg/L): _____

Absorbance of Standard B (30 mg/L): _____

Absorbance of Standard C (20 mg/L): _____

Absorbance of Standard D (10 mg/L): _____

Absorbance of Soap Solution: _____ (2)

Slope of Line from Calibration Plot (y = mx): m = _____ (3)

Triclosan Concentration for Solution X: (2) / (3) = /

= _____ mg/L (4)

Mass of Triclosan in 250 mL flask = (concentration x volume) = (4) x 0.25

= _____ mg (5)

Convert to g by dividing by 1000 = _____ g (5*)

We can now calculate % Triclosan in Soap

% Triclosan = mass of triclosan / mass of soap used = (5*) / (1) x 100

= (/) x 100

= _____ % (6)

Value from Soap Label = _____ % (7)

Difference between experimental value and packaging = (7) – (6) = _____ (8)

% Difference between experimental value and packaging = ((8) / (7)) x 100 = _____ %