Laboratory 2
Determination of Quinine in Tonic Water by UV-Vis Absorbance Spectroscopy

Introduction to the Lab. The purpose of this lab is to use the UV-Vis spectrophotometer to analyze the amount of quinine and sodium benzoate in tonic water and report how many 8-ounce bottles of tonic water a 140-pound person would have to drink to be supposedly safe from malaria. Although this laboratory is obviously not intended to provide medical advice, the recommended dosage of quinine is typically 8 mg per kg of body weight (when malaria is not severe). Tonic water also has citric acid to give it some acidity, sugars to improve taste, and sodium benzoate, $NaCO_2C_6H_5$, which acts as an antibacterial preservative. Because of its phenyl ring, benzoate also absorbs in the UV-Vis spectral range that we will explore. You will also determine how much sodium benzoate is in your tonic water samples.

This lab will build upon what we learned in last week's experiment. In last week's experiment, you studied the UV-Vis absorbance spectrometer using quinine as a standard, and you already have a copy of its absorbance spectrum, and some idea of its molar absorptivity or extinction coefficient ε. Quinine, whose structure is shown in Fig. 1-1, is a natural product that is isolated from the bark of the cinchoa tree.

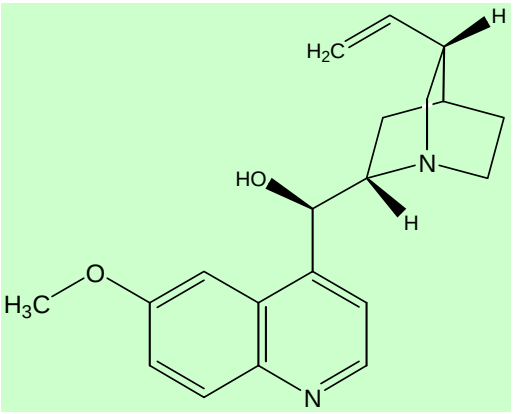

Figure 1-1. The structure of quinine

Background. Quinine has an interesting history in our region because of the infectious disease, malaria. "Malaria is an infectious disease transmitted by the bite of mosquitoes infected with the malaria-causing parasite. After being bitten by an infected mosquito, the parasite infects human liver and red blood cells. Most malaria infections have flu-like symptoms (such as high fever, chills, muscle pain, diarrhea) that come and go in cycles as the disease progresses. One type of malaria may cause more serious problems, including heart, lung, kidney, or brain damage and possibly death. You can become infected with malaria when you are bitten by a certain type of mosquito (*Anopheles* mosquitoes) infected with the malaria parasite. In rare cases, people can get malaria if they come into contact with infected blood or a fetus may get the disease from its mother. You cannot get malaria by being near a person with the disease.

"Malaria occurs most frequently in tropical Africa, parts of Asia, Central America, and South America. It occurs less frequently in Greece, Turkey, and the Middle East. Poor sanitation in some of these countries contributes to the severity of malaria by providing ideal conditions for mosquitoes to breed. In the United States, most malaria cases occur in people who have traveled [to] areas where malaria is present and in immigrants from those areas.

"Malaria can usually be prevented by taking medication before, during, and after travel to an area where malaria is present and by protecting yourself against mosquito bites. Although the use of drugs to prevent malaria is recommended and generally effective, it does not always prevent

infection. This is partly because some parasites that cause the disease are becoming more resistant to some preventive drugs.

"Malaria often requires treatment with medication[, including quinine] (antimalarial drugs). Antimalarial drugs are usually effective in treating the infection; however, the effectiveness of drugs both to prevent and treat the disease depends on the drug resistance of the malaria parasites you are exposed to" [1]. Quinine has antimalarial activity. Tonic water that you can buy in a grocery store today still has a small amount of quinine added, and hence the term *tonic water*, because one definition of "tonic" is "a medicine that invigorates or strengthens." Today, there are synthetic drugs that are more widely used than quinine for treating malaria. "Malaria is rare in the United States, but it is widespread in other parts of the world. International travelers should be aware of their risk for exposure to malaria" [1]. Malaria still infects hundreds of millions of people today, a large fraction of the human population. It causes 2.5 million deaths annually because severe cases are not always responsive to drugs. Malaria is largely confined to tropical regions, but it used to be more widespread. In colonial times, Philadelphia was larger than New York City, but Philadelphia's population was decimated by a malaria epidemic. Philadelphia never caught up again with New York City in population. In modern times, our country's extensive medical infrastructure has protected us from malaria, the most prevalent infectious disease in the world, although perhaps not widely known as such.

Note for the Future. In a later experiment, you will determine the amount of sodium and compare it with benzoate. Be sure that you are not analyzing diet tonic water because it often has sodium saccharate added, which would preclude the comparison of sodium and benzoate.

PRE-LAB ASSIGNMENT

Turn in typewritten answers to the following questions to your TA at the beginning of your lab period. Keep a second copy to refer to as you discuss it during the pre-lab. Be prepared to discuss the issues.

1. In Lab 1, you were given a spectrum for quinine. Here you are given an absorbance spectrum for 1×10^{-3} M benzoic acid, and absorbance spectra for undiluted and 5-fold diluted Schwepp's tonic water. What two wavelengths will you use to determine quinine λ^{Q}_{max} and benzoate λ^{B}_{max} in tonic water?
2. Given that a 1-cm cell will be used in the analysis, and using your knowledge from Lab 1 about the molar absorptivity ε_Q of quinine, should you analyze the tonic water as it comes from the bottle, or should you dilute it by some factor? Explain your answer in the context of last week's laboratory.
3. You are going to prepare a series of standard solutions for quinine and for sodium benzoate. The spectra of calibration standards will allow you to calculate the molar absorptivities of quinine ε_Q and benzoic acid ε_B at two wavelengths of interest, λ^{Q}_{max} and λ^{B}_{max} , respectively. The molar absorptivities might be different from book values because they are affected by the solution conditions. Briefly explain the principle of using two equations for determining two unknowns (quinine and benzoic acid). How would this problem simplify if one component had a molar absorptivity of zero at the wavelength of the other, and vice versa?

4. In lab 1, you were given an absorbance spectrum of a tonic water sample, as well as a spectrum for quinine plotted in molar absorptivity as a function of wavelength. Attached to the end of this lab in Fig. 2-2 is the absorbance spectrum of 1×10^{-3} M benzoic acid, the principal species of the benzoic acid/benzoate system ($pK_a = 4.20$) at the pH of tonic water (pH ~ 2.9). Also included are absorbance spectra for tonic water undiluted from the bottle (Fig. 2-3) and tonic water after a 5-fold dilution (Fig. 2-4). From these spectra, estimate the concentrations of benzoic acid and quinine in tonic water. (Note that in the lab you will actually use the reagent benzoic acid (not sodium benzoate), which can be made to dissolve in water with a few drops of 6 M NaOH.
5. In this lab, you will use two sets of standards, one of quinine and one of sodium benzoate, for determining the concentrations of these two species in tonic water. Decide on a range of concentrations for each set of 5 standards, and show what absorbance you predict these standards to have on our instrument. Show how you arrived at this. Generate a predicted calibration curve for each before coming to lab. Make sure the axes are carefully labeled with numbers and units.

EXPERIMENTAL

1. Measure the absorbance spectrum of the proper concentration (undiluted or diluted by some factor) of tonic water. What will you use as the blank spectrum that must always be recorded first? Also record the single-wavelength absorbances at the appropriate wavelengths λ^{Q}_{max} and λ^{B}_{max} .. You will actually use the reagent benzoic acid (not sodium benzoate), which can be made to dissolve in water with a few drops of 6 M NaOH. After dissolving the reagent, fill to volumes using the 0.05 M H_2SO_4.
2. Measure the absorbance of each standard at the appropriate wavelengths λ^{Q}_{max} and λ^{B}_{max} . Do they approximately (i.e. within 5 percent or so) match your predictions from the Pre-Lab? Also, do you think you should re-run the blank once in a while?
3. Before leaving lab, input your data into an Excel file and generate a plot to make sure absorbance is linear in concentration for the two sets of standards. Do the absorbances of your standards bracket the absorbance of your quinine and benzoate unknowns in the tonic water?

WRITTEN REPORT

1. Prepare a calibration curve from the experimental absorbance measurements of your standards. Apply least-squares fitting to determine the slope $\pm e_{slope}$ and intercept $\pm e_{intercept}$. Use your textbook to get the errors on the slope and intercept, or get them from the output of the Excel linear regression output. Derive the equation required to calculate the concentration of your quinine and benzoate unknowns, with the correct error.
2. Report the molar absorptivity ε_Q and ε_B and their 95% confidence interval at your selected wavelength for each of the two compounds.
3. Calculate the concentrations of quinine and sodium benzoate in the tonic water.
4. Calculate the 95% confidence intervals for the concentrations of quinine and sodium benzoate in tonic water. Use your textbook as needed.
5. Calculate how many 8-ounce bottles of tonic water a 140-pound person must drink, based on the recommended dosages given above.

6. How many grams of benzoate ion would this 140-pound person have consumed after this many bottles of tonic water?

This laboratory was created by Professor Mary J. Wirth, September, 2002. It was revised by Professor Thomas P. Beebe, Jr., September, 2003.

[1.] Excerpts about malaria were taken from WedMD.com, at the following URL: http://my.webmd.com/content/healthwise/101/25074.htm?lastselectedguid={5FE84E90-BC77-4056-A91C-9531713CA348}; additional information can be found in Taylor TE, Strickland GT (2000), *Malaria*. In GT Strickland, ed., Hunter's Tropical Medicine and Emerging Infectious Diseases, 8th ed., pp. 614–643. Philadelphia: W.B. Saunders.

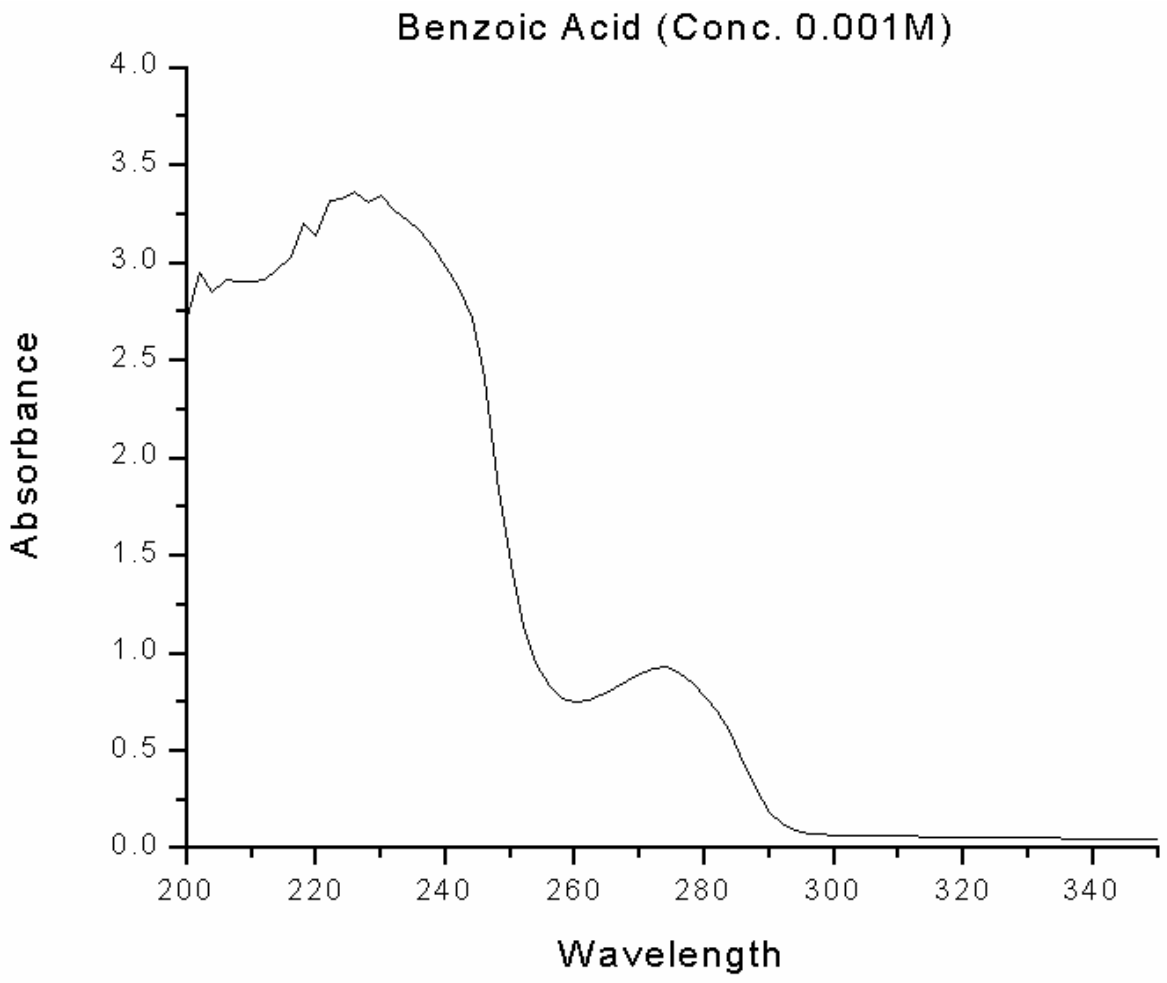

Figure 2-2. Absorbance spectrum of 0.001 M sodium benzoate at pH ~ 2.9

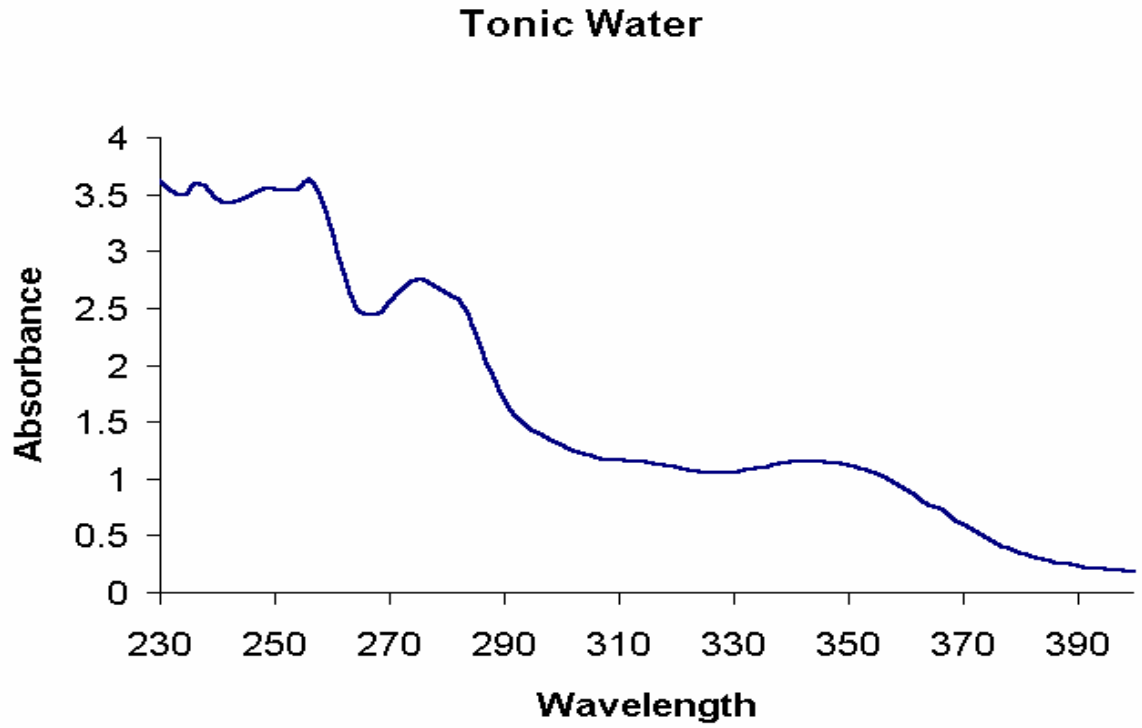

Figure 2-3. Absorbance spectrum of Schwepps tonic water, undiluted from the bottle.

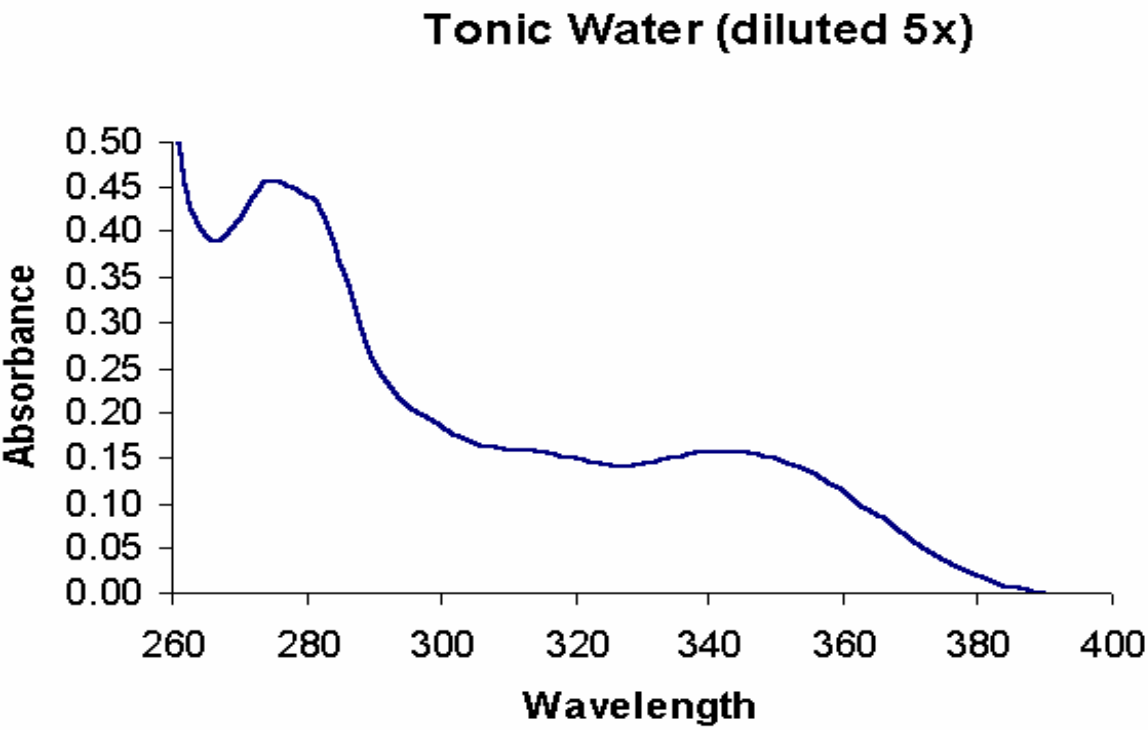

Figure 2-4. Absorbance spectrum of Schwepps tonic water, diluted 5x from the bottle.