Determination of Octanol-Water Partition Coefficient (K_{OW}) for Two Contaminants

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1. Objectives

Contaminants in the environment partition between different phases or compartments -- air, water, soil, sediments, biota. Predicting the fate and transport of contaminants in the environment requires knowledge of certain chemical properties. One such property is the octanol-water partition coefficient, commonly denoted K_{OW}. Values of K_{OW} are commonly tabulated and are used frequently by environmental engineers and scientists.

In this laboratory, students will measure K_{OW} for two contaminants, acetophenone and trichloroethene (TCE). The learning objectives of this laboratory are as follows. First, students will learn how contaminants partition between aqueous and organic phases. Second, students will gain an appreciation for the effort that is required to produce the data that are commonly found in tables. Third, students will learn Beer's Law and will learn how UV/visible spectrophotometry can be used to quantify concentrations of contaminants in aqueous samples. Fourth, students will gain experience using laboratory equipment such as separatory funnels.

Experimental measurements can be compared against tabulated values of K_{OW} for the contaminants under study.

2. Background

A. Definition of the Octanol-Water Partition Coefficient

Suppose an organic contaminant is discharged into the environment. For instance, perhaps some pesticides run into a lake during a heavy rainfall, or perhaps a wastewater treatment plant discharges its treated effluent (which still contains low levels of certain contaminants) into a river. Several things could happen to those contaminants. Hopefully, they will degrade (react) to form harmless products; but they might also end up in the sediment of the lake or the river, or perhaps in the bodies of the fish that live in the water. Organic contaminants can be stored in the fatty tissues of fish and other animals (including people). We might want to know how much of a contaminant is likely to end up in the fish (for instance, so we can decide if the fish are safe to eat). One way to do this is to actually gather some fish and to measure how much of the contaminant is in their bodies. Another option would be to measure how much of the contaminant is in the water, and then use that measurement to estimate how much is likely to be

in the fish. That type of estimate is not difficult if we know how the contaminant distributes itself, or "partitions," between the water and the fish that live in the water.
It has been observed that the chemical 1-octanol, which does not mix well with water, is a good surrogate for fatty tissue found in animals. That is, partitioning between water and fatty animal tissue is similar to partitioning between water and octanol. In fact, if we know how a contaminant partitions between water and octanol, that gives us useful information for predicting the partitioning between water and many different organic phases -- not just animal tissue, but also other organic phases such as the organic matter that is found in the lake sediments.

We therefore define a chemical parameter called the octanol-water partition coefficient, commonly denoted K_{OW}. The definition of K_{OW} is the ratio of the concentration of a chemical in octanol relative to the concentration of the chemical in water, when the octanol and water phases are at equilibrium.

$K_{OW} = C^{\text{octanol}} / C^{\text{water}}$ when water and octanol phases are at equilibrium.

A chemical that resides preferentially in the water phase is called *hydrophilic*; a chemical that resides preferentially in the octanol phase is called *hydrophobic* or *lipophilic*, and has a large value of K_{OW} (perhaps 10^3, 10^4, or even greater).

Note that the mathematical relationship above indicates that the concentration in octanol should vary linearly with the concentration in water. If the concentration in the water doubles, then the concentration in the octanol should also double, such that the ratio of the concentrations remains equal to K_{OW}.

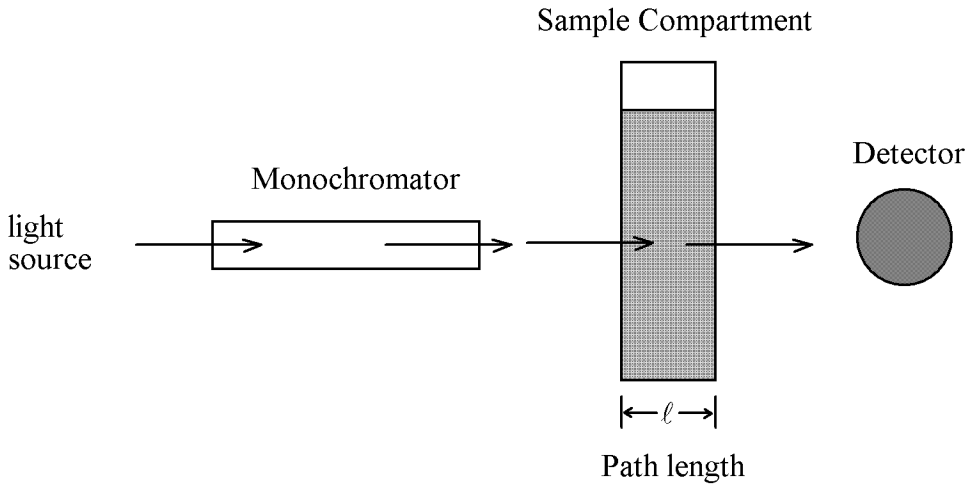
This gives us a way to estimate K_{OW} in the laboratory. Suppose we have several different systems that each contain water, octanol, and a contaminant of concern. Further suppose that, in each system, we can measure or estimate the contaminant concentration in both the water phase and in the octanol phase. Then, if we graph the concentrations in octanol versus the corresponding concentrations in water, then the slope of the line should be equal to K_{OW}.
In this experiment, the target contaminants will be acetophenone and trichloroethene (TCE). Many other contaminants could also be considered using the methods described below. The important characteristics are that the contaminant should have a moderate value of K_{OW} (between, say, 1 and 2×10^3), the chemical should not be excessively hazardous to use in the laboratory, and the concentration of the contaminant should be estimable using UV/visible spectrophotometry.

B. Using UV/Visible Absorbance to Estimate Concentration

[adapted from an experiment described by W.P. Ball, A.T. Stone, and A.L. Roberts, published in the AEESP Environmental Engineering Process Laboratory Manual, v. 1.0]

Relatively few pollutants absorb light in the visible region; a few that do are dyes and some chemicals used in leather tanning. Many more chemicals, however, absorb light in the ultraviolet (UV) range. We can use this property to estimate the concentration of certain contaminants in aqueous samples. The instrument that we will use is called a UV/visible spectrophotometer.

A spectrophotometer consists of a light source, a monochromator (which separates out a narrow wavelength band), a sample compartment, and a detector, as shown in the figure below.



The spectrophotometer allows us to measure:

- I_o the intensity of light hitting the detector when a "blank" solution (*e.g.*, distilled water) is present in the sample compartment.
- I the intensity of light hitting the detector when the sample is present.

Knowing these quantities, we can calculate:

- T Transmittance = I/I_o
- A Absorbance = $\log_{10}(1/T) = \log_{10}(I_o/I)$

Most spectrophotometers report their measurements in absorbance units. Absorbance values are thus equal to 0 if all of the incident light I_o reaches the detector, and can exceed 1 if more than 90% of the incoming light is absorbed. An absorbance of 2 means that 99% of the incident light is absorbed; an absorbance of 3 means that 99.9% of the incident light is absorbed. Many spectrophotometers will not accurately report absorbance above 3.

The spectrophotometer can be used to estimate concentration because absorbance can be directly related to the concentration of the light-absorbing species (called chromophores) in the sample. The relationship between concentration and absorbance is given by Beer's Law. Beer's Law states that the absorbance of light at wavelength λ owing to the presence of a single solute (X) is given by:

$$A_X = \varepsilon_X \cdot [X] \cdot \ell$$

where

ℓ = path length through the sample compartment (in cm);

ε_X = molar absorptivity of chemical X at wavelength λ (an intrinsic property of X, related to its ability to absorb light of wavelength λ), in $moles^{-1} \cdot L \cdot cm^{-1}$; and

[X] = concentration of species X (in moles/L or M)

Beer's Law can therefore be used to estimate the concentration of the contaminant, [X], if the absorbance is measured on the UV/visible spectrophotometer and if the path length and molar absorptivity are both known. In general, the path length is likely to be known because the UV/visible spectrophotometer will employ cells, cuvettes, or tubes of known path length. However, the molar absorptivity of a chemical X might or might not be known.

If the molar absorptivity for chemical X is not known, we can still use the spectrophotometer and Beer's Law to determine concentration. We must make a calibration curve. The absorbance, A_X, is measured for samples of known concentration, [X]. If Beer's Law holds for chemical X, then it will be observed that A_X varies linearly with [X]. If A_X is graphed versus [X], the result should be a straight line with slope equal to $\varepsilon_X \cdot \ell$. Thus the molar absorptivity is known, and Beer's Law can then be applied to samples of unknown concentration.

C. Use and Care of Separatory Funnels

In this experiment, we will use separatory funnels to allow the contaminant (acetophenone or TCE) to partition between a water phase and an octanol phase. A separate handout has been prepared on "Separatory Funnel Extraction Procedure." Please read that handout carefully *before* coming to the laboratory.

3. Materials and Procedures

A. Safety and Waste Disposal

Acetophenone is not a particularly hazardous chemical. Trichloroethene is believed to be linked to adverse health outcomes, possibly including cancer. Octanol is not particularly hazardous, but it smells strongly, and prolonged exposure can cause headaches or other symptoms of chemical exposure. To the maximum extent possible, handle octanol in a fume hood.

The exposure to these chemicals incurred during this laboratory experiment should not be hazardous to your health if the chemicals are handled properly. However, with all laboratory chemicals, it is prudent to keep exposure to a minimum. *Safety glasses, lab gloves, closed-toed shoes, and long pants (or ankle-length skirt or dress) must be worn at all times*. Furthermore, lab coats are highly recommended, but are not required if you wear long sleeves.

Solutions used in this laboratory must be disposed of properly. Do not pour solutions or samples down the sink after use. There should be four separate waste receptacles: aqueous solution with acetophenone, aqueous solution with TCE, octanol with acetophenone, and octanol with TCE. Please be sure to dispose of any waste liquids in the proper waste receptacle.

Do not bring any food or drink into the laboratory.

B. Materials

Common Equipment (shared between all laboratory groups):

- One UV/Visible Spectrophotometer
- Auto-pipettes (100 μL, 500 μL, and 1000 μL) with disposable tips
- Manual pipettes (2.5 mL, 5 mL, 10 mL, and 20 mL) with bulbs
- Squeeze bottles filled with de-ionized water
- Three glass funnels
- Three 10-mL graduated cylinders
- Three 100-mL graduated cylinders

Group Equipment (each lab group should have its own):

- Minimum of 6 disposable cuvettes
- One 125-mL separatory funnel with ground-glass stopper and teflon stopcock
- One ring stand
- One ring for the ring stand to support the separatory funnel
- One clamp for the ring stand to support a buret
- One 25-mL graduated buret
- Two 100-mL volumetric flasks
- Two 15-mL polypropylene conical tubes
- Disposable transfer pipettes

Stock Solutions:

- distilled or de-ionized water
- acetophenone stock solution, concentration C_A (approximately 1000 mg/L in water)
- TCE stock solution, concentration C_B (approximately 800 mg/L in a solution of 95/5 v/v water/methanol)
- 1-octanol

C. Procedures

Step-by-step procedures follow. (In *italics* are *questions to be considered* as the work proceeds.)

Part I: Prepare calibration curves for acetophenone and trichloroethene (TCE).

1. Each group will measure the absorbance of one of the chemicals (either acetophenone or TCE) at two or three different concentrations. Before the lab begins, your instructor should tell you which chemical you will use and what concentrations you will measure. Using the equipment available to you, prepare samples of the specified concentrations. Use as little of the stock solution as you can in order to prepare your samples, so that we don't use it all up. Be sure to record exactly how you prepared your samples: what volume of stock solution did you use, and what is the final volume to which you diluted? For instance, if you are trying to dilute 1000 mg/L down to 10 mg/L, you might take 100 μL of the stock solution and dilute it into 10 mL. However, maybe you didn't get exactly 10 mL -- perhaps it was only 9.8 mL, or perhaps it was 10.5 mL. Be sure to record the volumes that you actually used, so that we can get an accurate calibration curve afterwards.
2. For each of the samples you prepared, transfer approximately 1 mL into a disposable cuvette. (You can use a disposable transfer syringe or a pipette with a 1-mL tip.) Also prepare a cuvette with 1 mL of distilled or de-ionized water to be used as a "blank." Then, using the UV/visible spectrophotometer, measure the absorbance of the samples that you prepared. Please be sure to do the following:
   - Handle the cuvettes carefully, gripping them gently and above the sample line. You want to avoid getting any dirt, smudges, etc., on the surface of the cuvette where the light will pass.
   - Your cuvettes are specially shaped with a long path length and a short path length. When you insert the cuvettes into the spectrophotometer, make sure that the light will pass through the long path length, not the short. (*Why does it matter? Think about Beer's Law.*)
   - Place the blank cuvette into the appropriate cell holder in the spectrophotometer. Put the sample cuvette into one of the other cell holders. Use the blank to "zero" the instrument. This corrects for the absorbance of the water and the cuvette, so that the absorbance you record is the absorbance due to the contaminant only.
   - For acetophenone, measure the absorbance at 246 nm. For TCE, measure the absorbance at 230 nm. Record the absorbance for each of your samples. (*When you measure absorbance, does the instrument reading "drift" over time? If so, does it go up or down? What might be happening?*)
   - After taking your readings, remove your cuvettes. Dispose of the solutions properly. The cuvettes may be discarded assuming that you are using disposable cuvettes.

3. After all groups have measured the absorbance of their samples, pool the collected data. You should have measurements of the absorbance of acetophenone for at least six different concentrations, and also the absorbance of TCE for at least six different concentrations. Then, while still in the laboratory, prepare draft plots of the absorbance versus the concentration for each contaminant. This will allow you to check for any major errors, and to help you verify approximate concentrations in "real time." (*Do your calibration curves appear linear over the entire concentration range? Do any of the data points look like they don't fit with the rest? Do you need to re-do any of your samples or any of your measurements?)*
4. If you have time, you can use Excel or another program to fit a straight line through the data; be sure that the line passes through the origin, i.e., the intercept of the line is zero. (*Why must the calibration curves pass through zero? If they didn't, what would that imply about the relationship between absorbance and concentration in the low concentration range?*)
5. If you are exiting the lab after completing Part I, then clean up your work area before you leave.

Part II: Extract acetophenone and trichloroethene into octanol.

6. Each group will work with either acetophenone or TCE. It needn't be the same chemical that you worked with in Part I. Each group will perform either one or two extractions, depending on the availability of separatory funnels and the time available to you. There may be separate glassware for the two different chemicals; please be sure to use the correct glassware for the solution that you are using.
7. When performing the extractions, follow the procedures outlined in the separate handout, "Separatory Funnel Extraction Procedure." Also please note the following:
   - Octanol should be handled in the fume hood as much as possible; it smells strongly.
   - Dispensing the stock solutions out of the bottles may lead to some spillage. **Be sure to wear gloves, eye protection, and long sleeves** (or, preferably, a lab coat). If you spill any of the solution, clean it up thoroughly and immediately with paper towels. At the end of the lab, clean your bench-top with soap and water, and dry with paper towels.
8. For each extraction that you perform, perform the following steps as described in more detail in the handout:
   - Inspect your separatory funnel.
   - Support the separatory funnel in a ring on a ring-stand.
   - Add the contaminant stock solution to the separatory funnel, followed by the octanol. For each extraction, your instructor will tell you what volume of stock solution to use, and what volume of octanol to use. Be sure to record the volumes of solution that you actually use; if you are attempting to measure out 25 mL of octanol, and you get 24 mL or 26 mL, it is fine; but when you perform calculations later, you don't want to base those

calculations on an assumed value of 25 mL. Be sure to record, and then use for computations, the volume that you actually used.

- Shake the separatory funnel -- be sure to follow the steps described in the handout!
- Separate the layers. The separation sometimes takes a while.

9. After the layers separate completely, collect at least a few mL of the aqueous phase. (*Is the aqueous phase on the top or on the bottom? Why? What is the best way to collect the aqueous phase from the separatory funnel?*)
10. Measure the absorbance of the aqueous phase on the UV/visible spectrophotometer. Be sure to measure at the proper wavelength. Also be sure to "zero" your reading against a blank sample. *(Based on your calibration curves, what is the concentration of the aqueous solution after the extraction occurs? Does it agree reasonably well with expectations? If not, locate the source of error and resolve.)*
11. After taking your readings, dispose of all liquid solutions properly. Place glassware by the sink for washing. Clean up your work area.

4. Report Requirements

Write a report about the experiment and submit it to the instructor. You may discuss this experiment with your fellow students, but each student should prepare his/her final report individually. Your report should include the following sections.

A. Purpose

Describe the purpose of the experiment in about 1–2 sentences.

B. Hypotheses

Ordinarily, there might be a "Hypotheses" section between the Purpose and the Experimental Method. However, this particular experiment is based on measuring an unknown quantity rather than testing a scientific hypothesis. Therefore, for your lab report, it is acceptable to omit the Hypotheses section.

C. Experimental Method

Describe the procedure that you carried out to perform the experiment. Describe what you actually did. The goal is to describe your method clearly enough that another scientist, who did not watch you perform the experiment, could re-create your experiment based on your lab report. (The ability to verify another scientist's experimental results is an important aspect of science -- if we can't re-create what you did, then how can we test if you did it right?) It is fine to write this section with bullet points, or with a numbered list, if you like. The format is not so important; the important thing is that the reader can understand each step that you performed.

D. Data Summary

Present a summary or table of the raw data that you collected. This raw data should be consistent with the Experimental Method that you described, above. If, in the method section, you wrote that you used the UV spectrophotometer to measure percent transmittance, then you should include your %T readings here in the Data Summary section. Your data summary should include the data gathered by all the lab groups, not just your own group.

E. Data Analysis

Here, you analyze the data in order to achieve your stated Purpose. How do you take the raw data, and use them to achieve your stated objective?

- From the data gathered in Part I of the experiment, generate calibration curves for both acetophenone and TCE, showing how absorbance depends upon concentration. Report the best-fit line for the calibration curves. Make sure the best-fit line passes through the origin. You can perform the linear regression by hand, or you can use Excel or another software program to perform the linear regression.
- Next, demonstrate how you use these calibration curves to determine the concentration of acetophenone or TCE in the aqueous samples from Part II of the experiment. We'll denote these aqueous concentrations with the notation C^{water}.
- Next, demonstrate how you can estimate or calculate the contaminant concentration in the octanol, C^{octanol}, at the end of the extraction. Your expression for C^{octanol} should be based upon the concentration in the stock solution (let's denote it C^{initial}), the concentration in the aqueous phase (C^{water}), the volume of the stock solution you placed in the separatory funnel (V^{water}), and the volume of the octanol that you placed in the separatory funnel (V^{octanol}).
- For each chemical, graph C^{octanol} versus C^{water}. Determine the best-fit line for the data, making sure it passes through the origin. You can perform the linear regression by hand, or use a computer program to do it. How can you estimate K_{OW} from these graphs?
- For each chemical, graph $(C^{\text{initial}}/C^{\text{water}} - 1)$ versus $(V^{\text{octanol}}/V^{\text{water}})$. Determine the best-fit line for the data, making sure it passes through the origin. You can perform the linear regression by hand, or use a computer program to do it. How can you estimate K_{OW} from these graphs?

F. Results

What are your experimental values of K_{OW} for the two compounds? Present the results both as K_{OW} and as $\log_{10}(K_{OW})$. Estimate the uncertainty associated with your results.

G. Discussion

How close are your measured values to the literature values? Do you consider this to be good agreement, or poor agreement? If the agreement is poor, what reasons can you think of that might have led to the discrepancy? You can list as many reasons as you can think of, but try to indicate which you think might be the most likely.

5. Additional Questions

Below is a list of questions that your instructor might ask you to include in your lab report. Your instructor will indicate to you which of the following questions should be included. However, to gain the most from this laboratory exercise, think about how you might respond to all of these questions, even if not asked to include them specifically in your report.

A. When you performed your data analysis, you probably ignored the head space of the separatory funnel. That is fine as long as the contaminant mass in the head space is negligible. Demonstrate that the mass of acetophenone and TCE in the head space is negligible compared to the mass in each of the two liquid phases.

B. Which of the two compounds has a higher value of K_{OW}? What does that tell you about the relative hydrophobicity of the two compounds? How is this related to the molecular structure of the two compounds?

C. Estimate the molar absorptivity, ε, for each of the two chemicals at the relevant wavelength. If possible, compare your estimates to values found in the literature. How close are your estimates to the literature values? What might cause any differences between your estimates and the literature values?

D. Probably, you based your calculations on the assumption that $C^{initial}$ = 1000 mg/L for acetophenone, and $C^{initial}$ = 800 mg/L for TCE, because these are the approximate concentrations for the stock solutions. Suppose you now find out that the stock solutions were prepared incorrectly, and each one was only half the strength that it should have been. That is, $C^{initial}$ = 500 mg/L for acetophenone, and $C^{initial}$ = 400 mg/L for TCE. How does this affect your estimates of K_{OW} for the two compounds? Demonstrate or explain your answer.

E. [**adapted from an experiment described by W.P. Ball, A.T. Stone, and A.L. Roberts, published in the AEESP Environmental Engineering Process Laboratory Manual, v. 1.0**] Think some more about your calibration curves.

- Compare the slopes of your calibration curves with values expected based on the expected molar absorptivity of these chemicals at the relevant wavelengths. Do they differ substantially? If so, offer some possible explanations.
- For each point on your "best fit" calibration curve, calculate the amount of error between the line and the data point – *i.e.*, calculate the "residual" as the difference between "actual" versus "model-fit" absorbance for each concentration.
- For each chemical, prepare a plot showing "residual" (difference between measured and modeled absorbance) on the y-axis versus concentration on the x-axis. Note that residuals can (and should!) be both negative and positive. Are the residuals scattered randomly and uniformly about zero, or do they show a trend with concentration? If the magnitude of the residuals increases systematically with concentration, it is possible that

"relative error" is more uniform among the calibration points than is the absolute error– see the next part, below.

- For each chemical, prepare a plot showing absorbance-normalized residuals instead of absolute residuals on the y-axis – *i.e.*, divide each residual by the (calculated) absorbance value before plotting. Are these "relative" residuals scattered randomly and uniformly about zero, or do they show a trend? Compare with the "absolute" residuals from above.
- Use least-squares regression techniques to find the "best fit" line through your data, but use the *relative* residuals instead of the *absolute* residuals. How much does this change your best-fit calibration curves? Does it affect your estimates of K_{OW}?
- Construct confidence intervals about the regressions for the best-fit lines of your calibration curves.

6. References and Related Reading

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