

Guest-Host Complexation by Cyclodextrin¹

Purpose: Determine the equilibrium constant for binding of β -cyclodextrin and β -naphthol. This reaction is a good example of a guest-host complex.

Introduction

UV-Visible absorption spectroscopy is a commonly used technique for the determination of equilibrium constants, particularly in biochemical applications. For example, the binding constant of an inhibitor to an enzyme is a routine determination^{2,3}. In this lab we will study the binding of a cyclic-polysaccharide to a small molecular guest.

The polysaccharide is β -cyclodextrin, CD. Cyclodextrins are often used as active site analogs for enzymes⁴. Cyclodextrins are used to aid the absorption of drugs in the body. Other uses for cyclodextrins include the petroleum industry for separating aromatic hydrocarbons and in agriculture to reduce volatility of insecticides. Cyclodextrins are natural products produced by bacteria from starch. CD is made from seven D(+)-glucopyranose units linked through α -(1 \rightarrow 4) glycosidic bonds⁵, Figure 1.

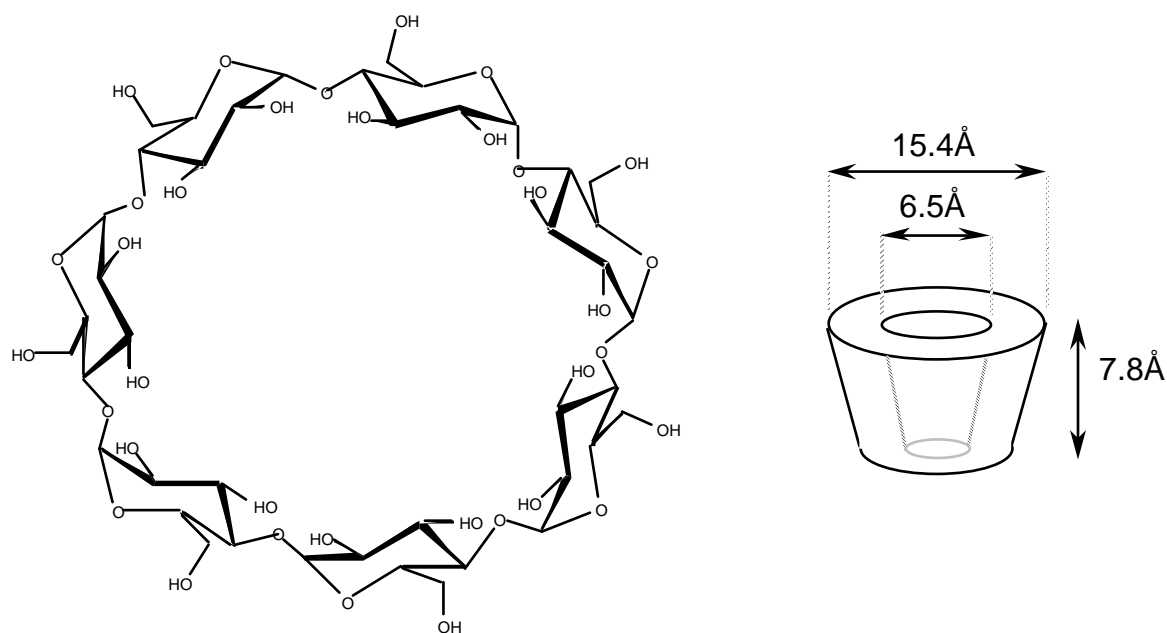
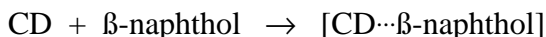


Figure 1. β -cyclodextrin (cycloheptaamylose).

In aqueous solution the CH bonds on the rings point inward producing a hydrophobic cavity inside a cylinder of diameter 15.4 Å. The OH groups extend from the top and bottom of the cylinder, providing sites for strong hydrogen bond formation. On average about 11 water molecules fit inside the cylinder. The cavity volume is 0.14 mL/g. Cyclodextrins bind with a wide variety of substances. Such complexes are examples of guest-host complexes, where cyclodextrin is the host.

β -Naphthol is representative of a wide variety of guests, Figure 2. Many compounds have the same bifunctional nature. β -Naphthol is expected to bind to CD because it has a hydrophobic

group that fits into the cyclodextrin cavity, while the OH group participates in hydrogen bonds with the sugar OH groups. The reaction stoichiometry is 1:1:



Also remember that some water molecules originally in the cavity will be excluded in the complex. This change in the number of water molecules in the cavity has an important effect on the binding enthalpy and entropy.

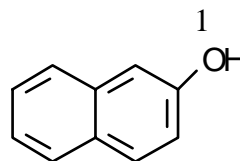


Figure 2 β -Naphthol

Theory

The Beer-Lambert Law describes the absorption of light in solution. The absorbance is defined as $A = -\log I/I_0$, where I_0 is the intensity of light falling on the cuvette, and I is the light intensity leaving the cuvette. Given the path length of the cuvette, b , and the molar concentration of the absorbing species, c :

$$A = a b c \quad 2$$

where a is the molar absorption coefficient. The molar absorption coefficient is unique to each substance and depends on the wavelength of the light used.

The complexation of β -naphthol to CD causes a small red shift in the absorbance maximum and a large increase in molar absorption coefficient, Figure 3. The increase in the molar absorption is presumably caused by the naphthalene ring being complexed in the hydrophobic interior of the cyclodextrin cavity. The change in absorbance with concentration of CD will be used to calculate the equilibrium constant for binding.

The equilibrium constant for the reaction in Eq. 1 is:

$$K = \frac{[\text{CDN}]}{[\text{CD}][\text{N}]} \quad 3$$

where N is uncomplexed β -naphthol and CDN is the β -naphthol-CD complex.

The solutions in this experiment will be made up with a constant concentration of β -naphthol and varying amounts of CD. Let the initial concentration of β -naphthol be C_0 and using Eq. 2 gives the absorbance of the solution with no CD, A^0 , as:

$$A^0 = a_N b C_0$$

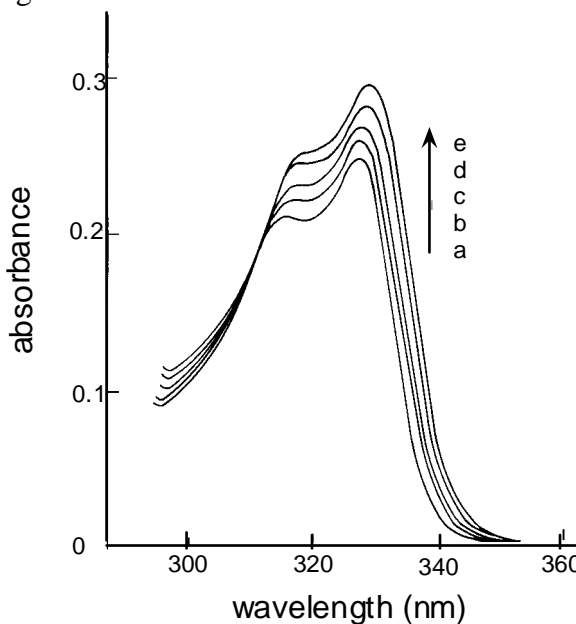


Figure 3. Absorption spectra of β -naphthol ($1.1 \times 10^{-4} \text{M}$) in aqueous CD solutions of concentration: (a) no host, (b) $1.9 \times 10^{-3} \text{M}$, (c) $4.5 \times 10^{-3} \text{M}$, (d) $8.9 \times 10^{-3} \text{M}$, (e) $1.8 \times 10^{-2} \text{M}$.

where a_N is the molar absorption coefficient of uncomplexed β -naphthol . If a large excess of CD is added, all of the β -naphthol will be complexed. The absorbance with a large excess of CD, A^∞ , is:

$$A^\infty = a_{CDN} b C_o \quad 5$$

where a_{CDN} is the molar absorption coefficient of complexed β -naphthol . For intermediate concentrations of CD, β -naphthol will be in uncomplexed and complexed forms. The absorbance of the solution will be the sum of the absorbances of the uncomplexed and complexed forms.

$$A = a_N b [N] + a_{CDN} b [CDN] \quad 6$$

Since the complex is formed with 1:1 stoichiometry:

$$[N] = C_o - [CDN] \quad 7$$

or alternatively solving for [CDN] from Eq. 7 gives

$$[CDN] = C_o - [N] \quad 8$$

We now need to solve for [CDN] and [N] in terms of the measured absorbances. To find [CDN], first substitute Eq. 7 into Eq. 6:

$$A = a_N b C_o - a_N b [CDN] + a_{CDN} b [CDN] \quad 9$$

Next find the difference $A - A^o$ by subtracting Eq. 4 from Eq. 9:

$$A - A^o = - a_N b [CDN] + a_{CDN} b [CDN] = (a_{CDN} b - a_N b) [CDN] \quad 10$$

Finally, solving for [CDN]:

$$[CDN] = \frac{A - A^o}{(a_{CDN} b - a_N b)} \quad 11$$

To find [N], first substitute Eq. 8 into Eq. 6:

$$A = a_N b [N] + a_{CDN} b C_o - a_{CDN} b [N] \quad 12$$

and then subtract Eq. 12 from Eq. 5:

$$A^\infty - A = - a_N b [N] + a_{CDN} b [N] = (a_{CDN} b - a_N b) [N] \quad 13$$

Finally, solving for [N]:

$$[N] = \frac{A^\infty - A}{(a_{\text{CDN}} b - a_N b)} \quad 14$$

Let the nominal concentration for CD be C_b . The CD in our solutions will be in excess so we can therefore approximate:

$$[CD] = C_b - [CDN] \cong C_b \quad 15$$

We now substitute Eqs. 11, 14, and 15 into the equilibrium constant formula, Eq. 3, to give:

$$K = \frac{(A - A^0)}{(A^\infty - A) C_b} \quad 16$$

In our experiments we vary the concentration of CD and measure the absorbance of the resulting solutions, A . To conveniently calculate K , we need to rearrange Eq. 16 into straight line form, $y=mx+b$. First, cross multiply in Eq. 16 to give:

$$(A^\infty - A) = \frac{(A - A^0)}{K C_b} \quad 17$$

Now rearrange to give:

$$A = A^\infty - \frac{1}{K} \left(\frac{(A - A^0)}{C_b} \right) \quad 18$$

Therefore, a plot of A verses $\frac{(A - A^0)}{C_b}$ should give a straight line with slope $-1/K$.

Procedure

Equipment

Ocean Optics UV/Vis Diode Array UV/Visible Spectrophotometer, Vernier Logger Pro
 UV plastic cuvette
 automatic pipettor
 plastic pipet

Solutions

pH 6.2 buffer (KH_2PO_4 / borax)
 $1.0 \times 10^{-4} \text{M}$ solution of β -naphthol in pH 6.2 buffer
 0.0150 M β -cyclodextrin and $1.0 \times 10^{-4} \text{M}$ β -naphthol in pH 6.2 buffer

Please read the instrument instructions for the Ocean Optics Spectrophotometers with Vernier Data Acquisition Software Instructions Sections I and II at the back of this manual. Pay particular attention to the instructions for overlaying spectra.

A $1.0 \times 10^{-4} \text{ M}$ solution of β -naphthol in pH 6.2 buffer and a 0.0150 M solution of β -cyclodextrin with $1.0 \times 10^{-4} \text{ M}$ solution of β -naphthol in pH 6.2 buffer will be provided. These compounds dissolve slowly so the solutions must be made in advance. Please note that both solutions have the same concentration of β -naphthol. Note the exact concentration of the solutions, and the calibrated pipet volumes and uncertainties.

We will use UV plastic cuvettes for this experiment. UV plastic cuvettes have a wavelength range starting at 260 or 265 nm (UV quartz cuvettes can be used starting at 190 nm, which is unnecessary for this experiment). Fill a UV plastic cuvette with pH 6.2 buffer, and calibrate (in other words acquire a background spectrum). In a second cuvette, using an automatic pipettor, add 2 mL of the $1.0 \times 10^{-4} \text{ M}$ β -naphthol solution to the cuvette. Acquire the spectrum. Measure the absorbance at the wavelength of maximum absorbance, A_{max} , in the region from 300 nm-360 nm. The reason why we focus on this region is that this band changes the most upon complexation. Record this wavelength and use this wavelength for all the remaining measurements. Also measure the absorbance at 360 nm to act as a background, A_{360} , on each spectrum. For the remaining steps, **leave the cuvette in the cell holder** to avoid small changes in absorbance caused by alignment differences in the cuvette.

Using an automatic pipettor, add 0.1 mL of the 0.0150 M β -cyclodextrin / $1.0 \times 10^{-4} \text{ M}$ β -naphthol solution to the cuvette. To assure complete mixing, using a plastic pipette, fill the pipette and expel the solution several times. Acquire the spectrum. Measure A_{max} and A_{360} . To determine if the solution is well mixed, mix again and reacquire the spectrum and measure the absorbances again. Overlay your spectra for convenient future reference.

Now add 0.2 mL more of the CD/naphthol solution, mix, acquire the spectrum, and measure the absorbances, mix again and reacquire the spectrum and measure the absorbances again. Add 0.3 mL, mix, acquire the spectrum, and measure the absorbances, checking for complete mixing, and then finally 0.5 mL, mix, acquire the spectrum, and measure the absorbances, checking for complete mixing.

To finish up, **leaving the cuvette in place**, use the plastic pipette to empty the cuvette. Rinse the cuvette **in place** with the 0.0150 M β -cyclodextrin / $1.0 \times 10^{-4} \text{ M}$ β -naphthol solution, and fill. The quantity is not critical. Acquire the spectrum, and measure the absorbances.

Wash the cuvette and rinse three times with deionized water.

Calculations

Do your calculations in an Excel spreadsheet. To correct for variations in the background caused by impurities in the cyclodextrin, set $A = A_{\text{max}} - A_{360}$. Calculate the concentration of CD, which is C_b , in each of your solutions. Following Eq. 18, calculate $(A - A^0)/C_b$ for each solution; then do the plot. Use least squares curve fitting to find the slope of the line and calculate K. Report K and the uncertainty in K.

Discussion

In the Theory section of your report:

1. Derive Eq. 16 from Eqs. 11, 14, and 15.
2. Show that Eq. 18 is in straight line form with slope $-1/K$.

In the Discussion section of your report answer the following questions:

1. Why do both of the solutions you used contain β -naphthol?
2. Measure the width of the naphthalene ring and the β -cyclodextrin cavity and compare. To obtain this estimate, you may use MOE, Chime (see the 3D-Molecule Structures section of the "Resources for Chemistry" of the Chemistry Department Web Pages), or other molecular mechanics program. Don't forget to include the Van der Waals radii of the H atoms.

Report

Include an Introduction, Theory, Procedure, Results, and Discussion. In the Introduction, describe the experiment and the expected result in a few sentences. For the Theory section, just reference the write-up, but also do the requested derivations. In other words, the Theory section is just a reference (e.g.: please see "**Guest-Host Complexation by Cyclodextrin**" in the CH341 Lab Manual for the theory and procedure) and the two short derivations.

For the Procedure section, describe enough of your procedure so that another student could easily repeat your experiments. Tell exactly what you did using explicit volumes, weights, and temperatures. Give the manufacturer and model of any major instrumentation (UV-Vis spectrophotometer in this experiment). Use past tense to describe your procedure. Don't copy the procedure from the write-up; state exactly what *you* did.

For the Results section, provide the data in a tabular format, including **all information necessary to repeat your calculations**. Please format your tables in a fashion similar to the literature (i.e. don't attach an Excel spreadsheet). Attach your graph. Graphs should be at least half-page in size with axes labeled with units. Slopes and intercepts from curve fitting should always be given with uncertainties. Include the uncertainty for the equilibrium constant propagated from the curve fit values (see the Error Analysis handout for instructions for representing uncertainties). (You do not need to propagate the uncertainties of the pipettors through to the final results. Just start with the uncertainties in the fit coefficients).

In the Discussion section, comment on the uncertainty of the final results: what is the predominate random experimental error? Note that correctible student mistakes are not random experimental errors. For example, spills or not following the instructions produce systematic errors, so you should not report them as random errors. Compare your final results to literature values. Is the difference between your equilibrium constant and the literature value larger than the technique is capable of? In other words, is there some unaccountable source of error? To help you answer this question, please note the following helpful hint on error propagation for this experiment.

In the Discussion section, also discuss the chemical significance of the results. In other words, state why these results are useful and important. State how this experiment and technique fit into the larger world of chemistry. Discuss why someone might need to do a study of this type. Are the results for this system unusual or do they fall within the normal range for other systems? Answer the questions in the Discussion section of the lab write-up.

Error analysis hint for your Discussion of random experimental errors:

The expected uncertainty in most spectrophotometric absorbance measurements is about 2%. The procedure in this lab where the cuvette remains in place and background subtraction is used gives a better uncertainty of around 1%. What uncertainty in K would you expect given a 1% error in the absorbances? An easy way to answer this question is to rearrange the equation used for curve fitting to solve for K directly. The equation used for the curve fitting is:

$$A = A_{\infty} - \frac{1}{K} \left(\frac{A - A_0}{C_b} \right)$$

Solving for K gives

$$K = - \frac{(A - A_0)}{(A - A_{\infty})} C_b \quad 19$$

Then use a single typical data point and propagation of errors rules to find the uncertainty in this single equilibrium constant value. The uncertainty in your calculations will be better since you used curve fitting. Curve fitting is the optimal way of minimizing the effect of the overall random experimental error. But how can you estimate the error in your final results from the single-value error you got from Eq. 19?

Assume you have done a curve fit using N data points. Very approximately it can be assumed that the final uncertainty will be improved by \sqrt{N} over the uncertainty of a single value, just like an average.

Report the uncertainty that is expected in your final equilibrium constant value based on a 1% uncertainty in the absorbance in the Discussion section of your report.

Literature Cited

- (1) Yorozu, T.; Hoshino, M; Imamura, M.; *Journal of Physical Chemistry*, **1982**, 86(22), 4422-26.
- (2) Dryer, R. L.; Lata, C. F.; *Experimental Biochemistry*, Oxford University Press, New York, NY, 1989. pp101-108, 356-359.
- (3) Chingnell, C. F. *J. Biol. Chem.* **1975**, 250, 5622.
- (4) Furuki, T.; Hosokawa, F.; Sakurai, M.; Inoue, Y.; and Chûjô, R. *J. Am. Chem. Soc.* **1993**, 115, 2903-2911.
- (5) Diaz, D.; Vargasa-Baca, I.; and Garcia-Mora, J., *J. Chem. Ed.* , **1994**, 71, 708.