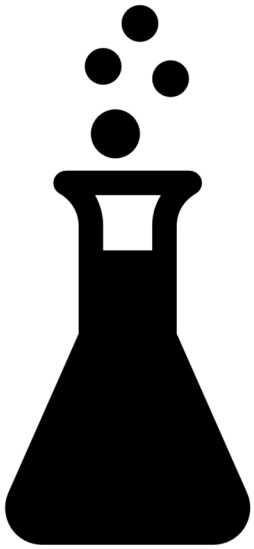
Supramolecular Chemistry



Laboratory sessions

TFYA30-HT2017

Instructions for the Reports

All writing connected to the lab must be done by each student independently.

LABORATORY REPORT

The outline should be:

- ✓ **Introduction** — Describe the relevance of the exercise in respect to the scientific field of supramolecular chemistry.
- ✓ **Method** — Describe shortly how the methods used in the laboratory exercise works. Explain how and why relevant information can be obtained from these methods.
- ✓ **Results** — Include all essential calculations and plots from the experiments.
- ✓ **Discussion** — Interpret and explain the obtained experimental results. Estimate possible errors and their magnitude.

The report should be written in English.

The report should not exceed 1500 words.

Use appropriate scientific references.

Also, make sure to answer **ALL** questions asked in each laboratory exercise.

DEADLINE

The deadline for handing in the report is **one week** after the laboratory session.

If the first version of the report is not approved, there will only be one chance to correct errors and missing parts.

The report will be graded U (fail) or G (pass). If you fail after one correction, there will be a chance to do an additional task to pass the laboratory session.

All reports should be send by e-mail to Michael Jury (michael.jury@liu.se)

Laboratory session 1

The Hydrophobic Effect

Background

The hydrophobic effect is central in many supramolecular interactions, including the assembly of biological and biomimetic lipid membranes, the folding and oligomerization of proteins, and the absorption and distribution of drug molecules within an organism.

The hydrophobic effect is a consequence of certain molecules inability to form hydrogen bonds with water. We refer to these molecules as hydrophobic. Molecules with aliphatic and aromatic groups tend to be more hydrophobic whereas charged and polar groups tend to be more hydrophilic.

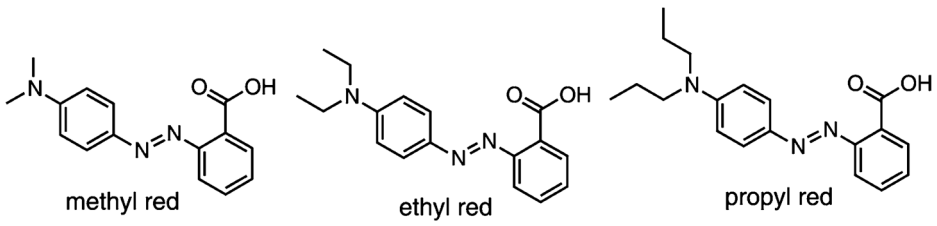
When dissolving a hydrophobic molecule in water, the water molecules surrounding the solute are forced into a more ordered state in order to maintain their hydrogen bonding network. The hydrophobic effect is mainly caused by the entropic gain when hydrophobic molecules aggregate which releases the ordered water molecules.

Make sure you read the chapter about the hydrophobic effect in Steed & Atwood (page 38-39), before the laboratory exercise.

For a quick repetition of entropy, enthalpy and Gibbs free energy check out the Youtube-video "Gibbs free energy" by Bozeman Science (www.youtube.com/watch?v=DPjMPeU5OeM).

The Indicators

In this laboratory exercise we will investigate how three different molecules dissolve in aqueous and a non-polar organic solvent. The three indicators that will be used are methyl red, ethyl red and propyl red that have a distinct yellow color at pH ≥ 7. The strong color enables us to use UV-Vis spectroscopy to determine the concentrations of the indicators in the two solvents.



Indicator	Molar absorptivity at 445 nm (ε_{445})
Methyl Red	21900 L/(mol*cm)
Ethyl Red	19000 L/(mol*cm)
Propyl Red	25100 L/(mol*cm)

There are some small differences in the molecular structure between the molecules. How do you think these differences in structure will affect the solubility of each molecule?

Determining the Standard Gibbs energy of transfer with a Spectrophotometer

In order to determine the concentrations of the indicators in the two different solvents the absorbance of the solutions will be measured using UV-Vis spectroscopy. The concentration (c) is related to the absorbance (A) by Beer-Lambert's, where ε is the molar absorptivity of the indicator and l is the path length of the cuvette.

$$A = \varepsilon l c$$

The hydrophobicity of a molecule can be estimated experimentally by investigating the partitioning of the molecule in a biphasic mixture of water and an organic solvent. The partitioning of a molecule (X) is governed by the following equilibrium:

$$X_{aq} \Leftrightarrow X_{org}$$

The partition coefficient (P) is an equilibrium constant describing how much of the molecule dissolves in each of the two phases, which in turn tells us how hydrophobic or hydrophilic it is.

$$P = \frac{[X_{org}]}{[X_{aq}]}$$

The standard Gibbs energy of transfer ($\Delta_{trans}G^0$) from the aqueous phase to the organic phase can be calculated by the equation below, where R is the gas constant (R = 8,314472 J/mol*K) and T is the temperature in Kelvin.

$$\Delta_{trans}G° = -RT\ln(P)$$

The Gibbs energy of transfer can also be expressed as: $\Delta_{trans}G° = \Delta H° - T\Delta S°$

$$\Delta_{trans}G° = \Delta H° - T\Delta S°$$

ΔH^0 is the change in enthalpy which is the total energy of the system which is >0 for an endothermic reaction and <0 for an exothermic one. ΔS^0 is the change in entropy which is the magnitude of disorder in the system - the universe spontaneously always strives towards increased disorder.

AIM

In this laboratory exercise you will learn more about the hydrophobic effect and factors that influence the hydrophobicity of molecules. You will also learn how to experimentally determine the thermodynamic parameters involved in hydrophobic interactions, such as the partition coefficient and the standard Gibbs energy of transfer from the aqueous phase to the organic phase.

PREPARATORY TASK

Describe in your own words very shortly what the hydrophobic effect is.

In which phase (aqueous or organic) will you find the indicator in each of the three experiments? Predict the results in this laboratory session and give a short explanation as to why. Are there other factors that can influence the hydrophobicity that you can think of?

Write 300 words before the laboratory sessions starts.
Bring a printout of the text to the lab and hand it in to the tutor.

WHAT TO BRING TO THE LABORATORY SESSION

- ✓ Lab coat (if you do not have one you will be able to borrow one)
- ✓ Computer (one per lab group is sufficient)
- ✓ Printout of the preparatory task

PRECAUTIONS

Always wear lab coat, gloves and glasses. In this lab nitrile gloves will be used due to their increased protection to cyclohexane. Do not inhale fumes from cyclohexane and the indicators, always handle these solutions in a fume hood.

Collect all samples containing cyclohexane and dispose in container marked “Strongly contaminated halogen-free organic solvents” provided by the lab tutor.

Note that the location of this laboratory session is in a real research lab, thus be very careful of other samples present in the lab. In short, please don’t touch anything that is not yours.

EXPERIMENT 1 – NUMBER OF CH_2 GROUPS

How much harder/easier is it for an indicator molecule to go from the MTA-buffer phase to the Cyclohexane phase when the number of CH_2-groups increases? Why is this?

Chemicals

Methyl Red in MTA pH 7
Ethyl Red in MTA pH 7
Propyl Red in Cyclohexane
Cyclohexane
Aqueous MTA-buffer pH 7

GET TO KNOW THE BACKGROUND NOISE AND STARTING CONCENTRATIONS

The first thing you should always start with in an experiment is to make sure you chose a good solution to use for measuring background noise. This is later subtracted from measurements of samples containing one of the indicator molecules to filter out only the signal of interest.

Second it is also important to know as much as possible about your starting solutions. In this lab you are not given the concentration of each indicator, which will be important to know.

1. Add 3 mL of indicator solution to a 13x100 mm glass test tube.
2. Add 3 mL of each solvent to separate tubes and use as references when measuring absorbance.
3. Cover tubes with plugs and seal them with Parafilm.
4. Record the absorbance at 445 nm.
5. Calculate the exact concentration of each indicator. (Beer-Lambert law)

WHICH INDICATOR DISSOLVES IN WHICH PHASE?

Determine in which phase, aqueous or organic or in both, will you find each of the three indicators:

6. Add 3 mL of Cyclohexane or MTA to each tube depending which phase is missing, work in the fume hood.
7. Seal the tubes with plugs and secure them with Parafilm.
8. Vortex for 30 s.
9. Record the absorbance at 445 nm
10. Calculate indicator concentration in both the organic and aqueous phase.

HOW DOES THE $\Delta_{TRANS}G^0$ RELATE TO THE NUMBER OF CH_2-GROUPS?

11. Calculate the partition coefficient and Gibbs energy of transfer from the aqueous phase into the cyclohexane phase ($\Delta_{trans}G^0$).
12. Plot $\Delta_{trans}G^0$ versus the total number of CH_2 groups in each indicator.

EXPERIMENT 2 – TEMPERATURE

How much harder/easier will it be for an indicator molecule to go from the MTA-buffer phase to the Cyclohexane phase when the temperature increases?

What can the calculated values of enthalpy (ΔH^0) and entropy (ΔS^0) tell you about the system?

Chemicals

Use the Ethyl Red tube prepared in Experiment 1.

HOW WILL ETHYL RED BEHAVE WHEN TEMPERATURE CHANGES?

How is the hydrophobicity of the indicator molecule affected by temperature?

1. Immerse the tube in 10 °C water for 10 min (not below 6 °C since this is the freezing point of Cyclohexane).
2. Write down the exact temperature.
3. Make sure the plug and Parafilm is still secure.
4. Vortex for 30 s.
5. Place the tube back in the water bath until the two phases are clearly separated (takes about 30-60 s).
6. Record the absorbance at 445 nm.
7. Immerse the tube in 30 °C water for 10 min.
8. Repeat step 2-6.
9. Immerse the tube in 50 °C water for 10 min.
10. Repeat step 2-6.
11. Calculate indicator concentration in both the organic and aqueous phase.

HOW DOES THE $\Delta_{TRANS}G^0$ RELATE TO THE CHANGE IN TEMPERATURE?

12. Calculate the partition coefficient and Gibbs energy of transfer from the aqueous phase into the cyclohexane phase ($\Delta_{trans}G^0$).
13. Plot $\Delta_{trans}G^0$ versus the absolute temperature (Kelvin). Estimate ΔH^0 and ΔS^0 from the plot.

$$\Delta_{trans}G° = \Delta H° - T\Delta S°$$

EXPERIMENT 3 – PH

How much harder/easier will it be for an indicator molecule to go from the MTA-buffer phase to the Cyclohexane phase when the pH increases?

Compare the trends of the two plots "concentration versus pH" and "$\Delta_{trans}G^0$ versus pH", how can it help explain the behavior of the molecule?

Chemicals

Propyl Red in Cyclohexane
Aqueous MTA-buffer pH 7
Different pH-solutions

HOW WILL PROPYL RED BEHAVE WHEN PH CHANGES?

How will the hydrophobicity of the indicator molecule change with changing pH? What can explain this behavior? Look at the structure of the molecule and see if you can figure it out!

1. Add 3 mL of each "pH-solution" to two tubes. One for adding the indicator to and one for reference.
2. Add 3 mL Propyl Red to one tube of each pH.
3. Seal the tubes with plugs and secure them with Parafilm.
4. Vortex for 30 s.
5. Record the absorbance at 445 nm.
6. Calculate indicator concentration in both the organic and aqueous phase.
7. Plot the Concentration in the aqueous phase versus the pH.

HOW DOES THE $\Delta_{TRANS}G^0$ RELATE TO THE CHANGE IN PH?

8. Calculate the partition coefficient and Gibbs energy of transfer from the aqueous phase into the cyclohexane phase ($\Delta_{trans}G^0$).
9. Plot $\Delta_{trans}G^0$ versus the pH.

SUMMARY OF QUESTIONS TO ANSWER IN THE REPORT

PART 1

- ✓ In which phase, aqueous or organic or in both, will you find each of the three indicators and how can you explain the differences?
- ✓ How much harder/easier is it for an indicator molecule to go from the MTA-buffer phase to the Cyclohexane phase when the number of CH_2-groups increases? Why is this?

PART 2

- ✓ How is the hydrophobicity of the indicator molecule affected by temperature?
- ✓ How much harder/easier will it be for an indicator molecule to go from the aqueous MTA-buffer phase to the Cyclohexane phase when the temperature increases?
- ✓ What can the calculated values of enthalpy (ΔH^0) and entropy (ΔS^0) tell you about the system?

PART 3

- ✓ How will the hydrophobicity of the indicator molecule change with changing pH? What can explain this behavior? Look at the structure of the molecule and see if you can figure it out!
- ✓ Compare the trends of the two plots "concentration versus pH" and "$\Delta_{trans}G^0$ versus pH", how can it help explain the behavior of the molecule?

Laboratory session 2

Cyclodextrin Host-Guest Chemistry

Introduction

Cyclodextrins (CD) is a class of cyclic oligosacchariders that have a molecule-sized cavity (Del Valle 2004). CDs consist of six to eight D-glucopyranoside units joined by a 1,4-glycosidic link (Figure 1). CDs are referred to as α-, β- and γ-cyclodextrin consisting of six, seven, and eight D-glucopyranose units, respectively. Smaller and larger ring structures are also known and used.

The shape of the CD is often represented as a funnel with an upper wider rim and lower narrower rim. The upper rim consists of the secondary hydroxyl groups and the lower with primary hydroxyl groups. The larger number of hydrophilic hydroxyl groups at the rims and the hydrophobic cavity make CD a useful host for hydrophobic guest molecules under water.

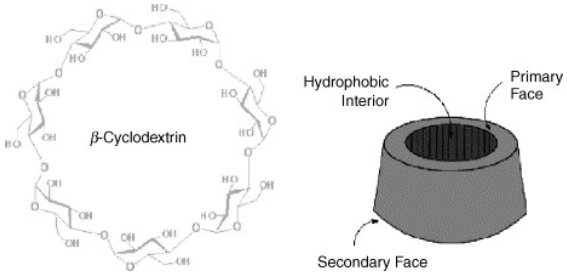


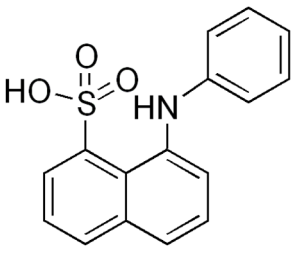
Figure 1. Structure of β-cyclodextrin. From (Del Valle 2004).

Aim

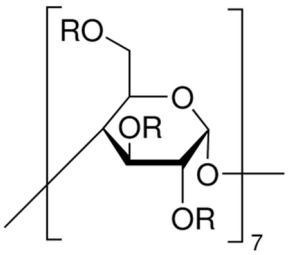
In this laboratory exercise we will study host-guest interactions between a small molecule (**8-anilino-1-naphthalenesulfonate** (ANS, Figure 2a) and three types of β-CDs; **β-CD**, **Methyl-β-CD** (ME β-CD) and **(2-Hydroxypropyl)-β-CD** (HP β-CD). The latter two are modified to give a more hydrophobic cavity (Figure 2b).

ANS displays increased fluorescence intensity in a hydrophobic (non-polar) environment compared to in a polar solvent such as water. Therefore, when ANS associate to β-CD an enhancement in fluorescence can be seen, which will be examined in this lab session. Furthermore, binding constants for one of the complexes will also be estimated in this lab session.

a)



b)



β-CD: R = –H

ME β-CD: R = –H or $–CH_3$

HP β-CD: R = –H or $–CH_2CH(OH)CH_3$

Figure 2. a) 8-Anilinonaphthalene-1-sulfonic acid (ANS). b) Structure of the three types of β-cyclodextrins to be used in the laboratory exercise.

Preparatory task

Read about cyclodextrins (page 327-336 in the book).

Read about binding constant and different methods to measure it (page 9-17 in the book).

Fill in the table associated with Part 2.

What to Bring to the Laboratory Session

- ✓ Lab coat (if you do not have one you will be able to borrow one)
- ✓ Computer (one per lab group is sufficient)

Precautions

Always wear lab coat, gloves and glasses.

Dispose all samples in Non-halogenic waste container provided by the lab tutor.

Note that the location of this laboratory session is in a real research lab, thus be very careful of other samples present in the lab. In short, please don't touch anything that is not yours.

EXPERIMENT 1 - FLUORESCENCE ENHANCEMENT OF ANS

Part a – Using different solvents

How will different solvents affect ANS fluorescence?

Chemicals

400 µM ANS-solution
Methanol
Ethanol
2-Butanol
Phosphate buffered saline (PBS)

1. Add 100 µl of ANS to 1.9 ml of the different solvents in 2 ml micro tube.
2. Incubate for ~ 5 min in an ultrasonicator.
3. Measure the fluorescence (excitation: 340 nm, slit nm = 1 / emission: 400-600 nm, slit nm = 1).
4. Write down peak position and maximum fluorescence intensity.
5. Divide each value of fluorescence intensity by the maximum intensity value of the PBS-sample ($\frac{F_{solvent}}{F_{MQ}}$).

Part b – With different β-cyclodextrins

In part b you will use the knowledge gained from part a to identify which of the three β-CD (see Figure 2b) *that are in solution A, B and C.*

Chemicals

40 µM ANS in PBS
A-solution
B-solution
C-solution

1. Dilute 1 ml of 400 uM ANS with PBS to make a 40 uM ANS solution.
2. Add 1 ml of ANS to 1 ml of each β-CD solution in a cuvette.
3. Incubate for ~ 5 min.
4. Measure fluorescence (excitation: 340 nm, slit nm = 1 / emission: 400-600 nm, slit nm = 1).
5. Write down peak position and maximum fluorescence intensity.
6. Divide each value of fluorescence intensity by the value of maximum intensity of the PBS-sample from part a.

To discuss in report:

1. How will the different solvents affect ANS? Explain the differences.
2. Which β-CD are in which solution? Discuss why you think so and relate it to the knowledge you gained from part a.

EXPERIMENT 2 – BINDING CONSTANTS FOR ANS: ME β-CD

What are the binding constants for ANS:ME β-CD?

Chemicals

400 µM ANS in PBS
20 mM ME β-CD in PBS
PBS

1. Add 500 µl of ANS to 12 cuvettes.
2. Add X µL of ME β-CD and Y µL of PBS according to the table below to increase the concentration of ME β-CD from 0 mM to 15 mM.
3. Incubate for ~ 5 min.
4. Measure fluorescence (excitation: 340 nm, slit nm = 1 / emission: 400-600 nm, slit nm = 1).
5. Write down the maximum fluorescence intensity in the table (*F*).
6. Plot $\frac{F}{F_0}$ versus concentration ME β-CD (*[CD], mM*).
7. Create a Benesi-Hildebrand Plot by plotting $\frac{F_0}{F}$ versus $\frac{1}{[CD]}$.
8. Estimate binding constant (K_a and K_d).
9.

To discuss in report:

1. Is the complexion ANS: ME β-CD of a 1:1 stoichiometry?
2. Your estimated binding constants. Compare your value with other well-known complexes (e.g. Biotin/Streptavidin); is this a strong or weak interaction?
3. Describe some other methods/plots that could have been used to determine the stoichiometry and/or binding constants.

[ME β-CD]	X µL of ME β-CD (20 mM)	µL of ANS (400 µM)	Y µL of PBS	Fluorescence intensity (F)	F/F_0
0 mM	0	500	1500	$= F_0$	1
1 mM		500			
2 mM		500			
3 mM		500			
4 mM		500			
5 mM		500			
6 mM		500			
7 mM		500			
8 mM		500			
9 mM		500			
10 mM		500			
15 mM		500			

Determine the required volumes of ME β -CD and PBS for each measurement. Total volume should always be 2 ml. The stock solution of ME β-CD has a concentration of 20 mM and the concentration of ANS is 400 µM.

Determine binding constants using fluorescence spectrometry

For a 1:1 ANS:CD complex, the association constant K_a can be defined as:

$$ANS + CD \overset{K_a}{\Leftrightarrow} ANS{:}\,CD$$

$$K_a = \frac{[ANS{:}\,CD]}{[ANS][CD]}$$

The value of K_a can be obtained from the observed fluorescence enhancement $\frac{F}{F_0}$ as a function of added cyclodextrin concentration (*[CD])(Peña, Salanas et al. 1993)* :

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right)\frac{[CD]K_a}{1 + [CD]K_a}$$

where *F* is the measured fluorescence intensity in the presence of CD, F_0 is the measured fluorescence intensity in the absence of CD, and F_∞ is the measured intensity when all ANS molecules have been complexed by CD molecules (that is, no major increase in fluorescence can be seen with increased concentration of CD).

This equation is only true for 1:1 complexes, which need to be proven before it can be used to determine K_a. By creating a Benesi-Hildebrand plot, that is plotting $\frac{F_0}{F}$ versus $\frac{1}{[CD]}$, it is possible to confirm 1:1 stoichiometry. If this stoichiometry is true it should be possible to fit the data points with a linear function. The neat thing with this plot is that you not only can confirm 1:1 stoichiometry, you will also be able to use it to estimate the K_a. To do this, you need to calculate the linear equation (***y = kx + m***) for your data points and estimate at which value of ***x*** that ***y = 0***. From this K_a (and dissociation constant, K_d) can be calculated by:

$$K_a = \frac{1}{K_d} = -\text{“}value\ of\ \boldsymbol{x}\ when\ \boldsymbol{y} = \mathbf{0}\text{”}$$ (note the minus sign)

References

Del Valle, E. M. M. (2004). "Cyclodextrins and their uses: a review." Process Biochemistry **39**(9): 1033-1046.

Peña, A. M., F. Salanas, et al. (1993). Absorptiometric and spectrofluorimetric study of the inclusion complexes of 2-naphthyloxyacetic acid and 1-naphthylacetic acid with β-cyclodextrin in aqueous solution. Journal of inclusion phenomena and molecular recognition in chemistry, Kluwer Academic Publishers. **15:** 131-143.