Fluorescence-Based Thermodynamic Studies of the Inclusion of Anilinonaphthalene Sulfonates by Cyclodextrins

By

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List of Abbreviations

2,6-ANS – 2-anilinonaphthalene-6-sulfonic acid

2,6-TNS – 6-(p-toluidino)-2-naphthalenesulfonic acid

mM - millimolar

nm - nanometer

UV -ultraviolet

Vis - visible

CD- cyclodextrin

2,6-DM-β-CD - Heptakis(2,6-di-O-methyl)- β-cyclodextrin

SD – standard deviation

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Abstract

Fluorescence spectroscopy can be used to investigate the formation of host-guest inclusion complexes. Host-guest inclusion involves the noncovalent interactions between a host and a guest in which a guest molecule is small enough to enter the cavity of a macrocyclic host molecule. A thermodynamic study of the formation of an inclusion complex allows information to be obtained about the inclusion process, such as the enthalpy and entropy of inclusion. Such studies involve measuring the binding constant K at various temperatures. A pure and homogenous sample of a host molecule is required to perform an accurate thermodynamic analysis. This project involves the thermodynamic study of the inclusion of anilinonaphthalene sulfonates as guests with pure samples of cyclodextrin, with the goal of a full determination of the thermodynamics of these complexations, including the enthalpy and entropy of inclusion. A thermodynamic study of the inclusion of 2,6-ANS and 2,6-DM-β-CD was attempted but due to the unavailability of obtaining more of both the host and the guest, only three temperatures trials were performed. The study was then changed to the thermodynamic study of 2,6-TNS with α-cyclodextrin. After performing fluorescent titrations at seven temperatures, a van't Hoff plot was made and observed to be scattered. The higher temperatures seemed to follow a linear trend and was therefore investigated.

Chapter 1 – Introduction

1.1 Host-guest inclusion and binding constant

Host-guest inclusion is a rapidly growing field in supramolecular chemistry. Supramolecular chemistry focuses on the assembly of a large complex being formed by non-covalent intermolecular forces between two or more molecules.[1] Those forces include van der Waals interactions, the hydrophobic effect, hydrogen bonding, charge transfer, and more depending on the host, guest, and solvent involved.[1] A host molecule is often a macrocyclic molecule with a cavity large enough to accept another smaller molecule (often hydrophobic) which is the guest molecule.[1] The penetration of the guest molecule into the host molecule is a dynamic reversible equilibrium process which occurs spontaneously.[1] Such inclusion can cause a modification of the physical and chemical properties of the guest molecule such as increasing its stability and its solubility, as well as its spectroscopic properties.[2] A representation of a host-guest inclusion complexation is shown in Figure 1.1.

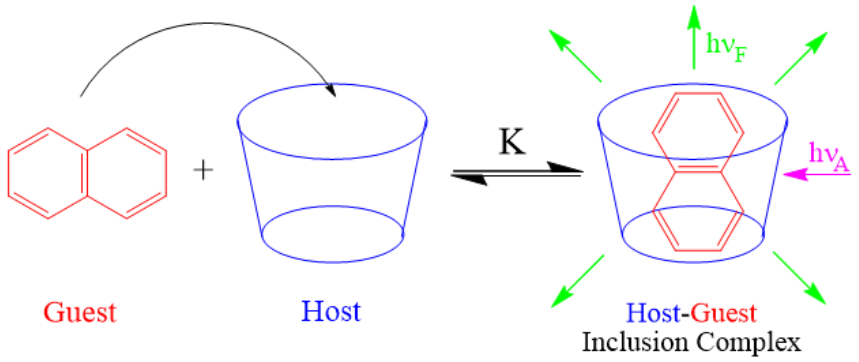


Figure 1.1: A representation of the inclusion of a guest molecule inside a host molecule's cavity.

One major area of host-guest chemistry has been in the pharmaceutical industry, for example for drug delivery.[2] Other applications include acting as agents for chromatography, in the food industry, as catalysts, and in cosmetics as enzymes inhibitors.[1] This work doesn't focus on

such applications but instead on the thermodynamics of a specific host guest inclusion process. In studies of host-guest chemistry, obtaining the binding constant is often the goal of the research. The binding constant, K, is the most important piece of data obtained experimentally as it reveals significant, crucial information about the inclusion process.[2] As is shown in Figure 1.1, the binding constant is an equilibrium constant, often referred to as an association constant. Equation 1.1 shows this equilibrium nature of the binding constant.

$$K=[H:G]/[H][G] \qquad \text{Equation 1.1}$$

The magnitude of the binding constant relates to the strength of the host-guest inclusion; as the binding constant increases, the stronger and more stable is the complex.[2] There exist multiple types of inclusion depending on the number of host and guest required for the host:guest interaction, which is defined as the host:guest ratio. For instance, the most common types of combinations are 1:1, 2:1, 1:2, and 2:2, which can be visualized in Figure 1.2.

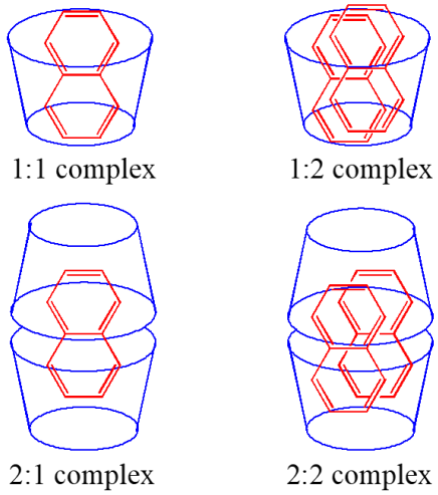


Figure 1.2: A representation of the different combinations of Host:Guest complexes

Each type of complex requires a different equation to solve for the binding constant. In this work, the focus was on 1:1 inclusion complexation, which is the most common combination and therefore Equation 1.2 was used for the calculations performed.[2]

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right)\frac{[host]_0 K}{(1 + [host]_0 K)} \qquad \text{Equation 1.2}$$

1.1.1 Binding constant

As mentioned earlier, the primary measurement that is acquired through the study of an inclusion complex is the equilibrium constant of the interaction, which is also known as the binding constant, K. The binding constant describes the stability of the host-guest complex and how favourable the inclusion is. The larger the value of K, the stronger the binding of the complexation. The binding constant is also related to Gibbs energy ΔG for the inclusion as shown in Equation 1.3. The binding constant can be obtained through different techniques such as NMR or fluorescence spectroscopy, the latter of which is the technique used in this work.[1] The binding constant can be solved using Equation 1.2 which will be described in more details later in this chapter.

$$\Delta G = -RT\, lnK \qquad \text{Equation 1.3}$$

Previously in the Wagner lab, the binding constants were calculated using an in-house software program created mid 1990s which was named Cdeqwin.exe. As this program can be ran only on an older generation computer, an alternative way to obtain the binding constant was investigated. Binding constants in general in the literature, have been determined to only be accurate with a 10 % - 20 % relative uncertainty.[2]

1.2 Cyclodextrins

Cyclodextrins are a family of cyclic oligosaccharides of glucopyranose which were first discovered in 1891 by a French chemist named Antoine Villiers.[3] Cyclodextrin was discovered through a study on the degradation and reduction of carbohydrates in which Antoine Villiers

observed the presence of unexpected crystals, which were later determined to be cyclodextrin.[2] An Austrian scientist named Franz Schardinger described how to synthesize and isolate cyclodextrins from bacterial cultures in 1903.[3] He also described two different cyclodextrins which were first named α-dextrin and β-dextrin. It was not until the 1950s that Freudenberg and Cramer were able to determine the cyclic structure of cyclodextrin.[3] The structure of cyclodextrin is composed of monomers of glucose in the cyclic glucopyranose configuration linked together in a cyclic chain.[4] The glucopyranose rings are linked together through the 1st and 4th position on the ring. Cyclodextrin naturally exists in three sizes depending on the number of monomers present in the molecules, ranging from six to eight glucose monomers. If obtained directly from the degradation of starch, cyclodextrin is referred to as a 'native' cyclodextrin which started to be produced industrially in 1984.[3] Cyclodextrin can be modified synthetically to change its properties or to optimize an inclusion. A six monomer cyclodextrin is referred to as α-cyclodextrin, while β-cyclodextrin corresponds to a structure of seven monomers, and γ-cyclodextrin to eight monomers.[4] This is demonstrated in Figure 1.3.

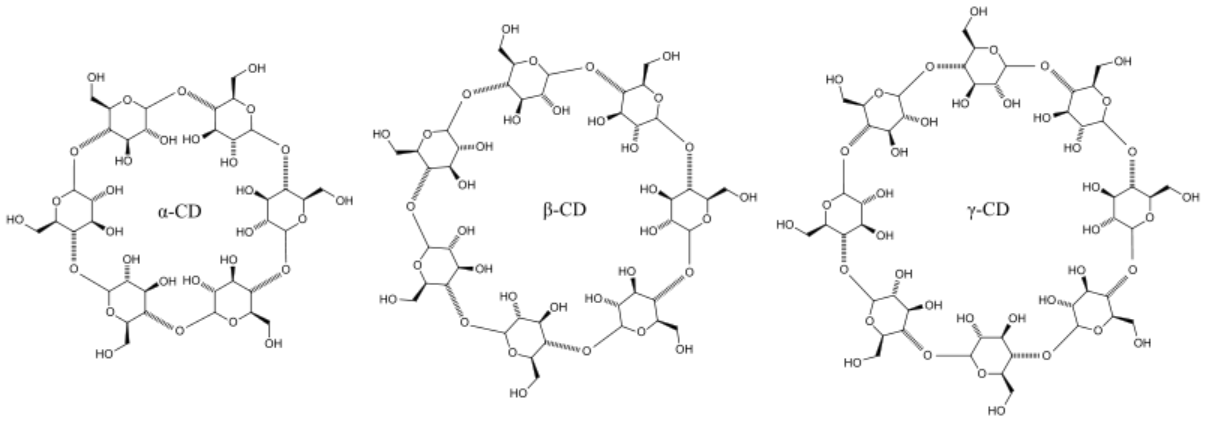


Figure 1.3: Structure of a α-CD, β-CD and γ-CD

The cyclic structure of cyclodextrin forms a truncated cone shape in water often referred to as a "bucket" due to the significant cavity present. The cone shape is due to the presence of two

secondary hydroxyl groups per monomer on one side, forming the larger opening of the cavity also known as the upper rim, while the smaller rim is lined by one primary hydroxyl groups per monomer. The upper rim diameter for the α-CD is 0.57 nm, while a β-CD has an upper rim diameter of 0.78 nm and of 0.95 nm for γ-CD.[5] The difference in the diameters has a significant impact on the formation of stable inclusion complexation with guests, depending on the size of the guest. The number of monomers present in the structure affects the size of that cavity, which has an impact on the inclusion capacity of the CD. Because the strength of a CD complexation is related to the size of the cavity of cyclodextrin, a selective inclusion complexation can be made depending on the type of guest and CD used.[6] Therefore, in a mixture of different cyclodextrins with different guest molecules, the system can be made selective if the difference in the binding constant of each guest with each CD is significantly different.[6] For instance, a larger guest will not fit in a small CD which therefore will only form an inclusion complex with a larger CD while a smaller molecule might not feel the benefits of the internal cavity of the host if the cavity is too large. This allows for a selective complexation.

As mentioned earlier, multiple forces can influence the host-guest inclusion, and cyclodextrin is usually able to form an inclusion complex mainly due to the hydrophobic effect. [7] The inner cavity of the cyclodextrin creates a lipophilic environment which allows a nonpolar hydrophobic guest molecule to penetrate the cavity to avoid the polar solvent, such as water, in which the unfavourable interaction takes place.[7] The hydrophobic nature of the inside cavity of a cyclodextrin is caused by the presence of a ring of glycosidic oxygen bonds. Cyclodextrin is one of the most widely used hosts due to its relatively low cost, easy synthesis, and desirable host cavity characteristics. The rims of the cyclodextrin are hydrophilic which allows cyclodextrin to dissolve in water, which is the most common solvent used for host-guest interactions. As

mentioned, the cavity of cyclodextrin is hydrophobic which allows the guest to penetrate the cavity and become more stable, providing a major driving force which is the hydrophobic effect, for a complexation occurring in an aqueous solution.

1.2.1. Modified cyclodextrins and homogeneity

The primary and secondary hydroxyl groups present on each monomer of a native cyclodextrin molecule can easily be modified as hydroxyl groups are relatively reactive.[2] The purpose of modifying native cyclodextrin is that it can result in some improvement in the characteristics of the nonpolar cavity allowing for a better inclusion of the hydrophobic guests, depending on the substituted group. Modifying a cyclodextrin can also increase the solubility and add specific properties to the host.[8] Modified cyclodextrins have been reported to increase the fluorescent enhancement compared to their corresponding parent cyclodextrins.[8] The main issue encountered with commercially available modified cyclodextrin is their non-homogeneity. The majority of modified cyclodextrin compounds tend to not have all the same molecules of cyclodextrin within a sample. The molecules can have a different number of modified hydroxyl groups and different patterns of substitutions on each monomer. This results in the sample of host molecules not being homogenous which causes problems with reproducibility of experimental data as a pure host is required for accurate studies.

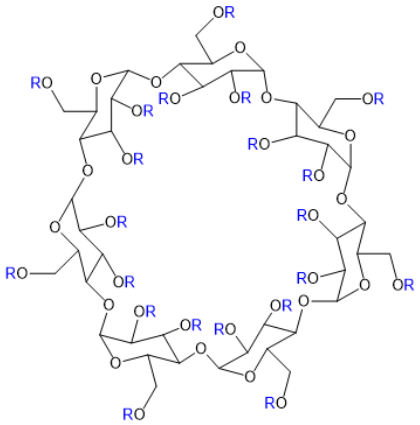


Figure 1.4: General structure of a modified β-Cyclodextrin

1.2.2. Heptakis(2,6-di-O-Methyl)-β-Cyclodextrin

β-Cyclodextrins are the most studied cyclodextrins as their seven-monomer rings offers the most ideal cavity size for most guest molecules which tends to increase the inclusion equilibrium. β-Cyclodextrins are also generally cheaper and more commercially accessible than other cyclodextrins. In this work a chemically modified β-cyclodextrin named heptakis(2,6-di-O-methyl)-β-cyclodextrin was first used but later, the focus changed to α-cyclodextrin due to a lack of the modified β-cyclodextrin. Heptakis(2,6-di-O-methyl)-β-cyclodextrin can be abbreviated to 2,6-DM-β-CD.

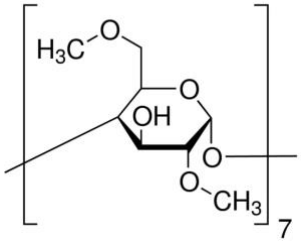


Figure 1.5: Monomer of 2,6-DM-β-CD

Numerous methyl substituted α-, β-, and γ-CD are commercially available; however, they are in general not homogenous. The modified β-cyclodextrin used is a commercially available modified β-cyclodextrin which is substituted with methyl groups identically throughout the cyclic chain. As shown in Figure 1.5, every monomer is disubstituted with a methyl group on the 2,6 positions. This makes the commercially available modified CD ideal for fluorescence based binding studies.

1.3 Anilinonaphthalene sulfonate

The fluorescence study undertaken in this work uses anilinonaphthalene sulfonates as the guest molecule. Anilinonaphthalene sulfonates are commonly studied fluorescent probes which

are composed of a naphthalene group which is responsible for the fluorescent properties of these molecules. One property that makes them good candidates for host-guest inclusion is their polarity-sensitivity, making polar solvents such as water good choices as solvents for the host-guest inclusion studies.[1] Another aspect of the guest molecule which affects the complexation is their hydrophobic properties which results in the hydrophobic effect. The hydrophobic effect is the primary driving force for the inclusions involving CD.[2] It is based on the principle that due to the hydrophobic nature of the guest molecule, there is a greater affinity with the internal cavity of the host molecule which is a non-polar environment.[7]

1-Anilino-8-naphthalene sulfonate or 1,8-ANS is a guest molecule widely used and studied. However, due to its bulky shape, it doesn't undergo the strongest inclusion compared to its isomer 2,6-ANS, 2-anilinonaphthalene-6-sulfonic acid which has a more elongated shape allowing a better entry in the cavity of the host, as shown in Figure 1.6. Another anilinonaphthalene sulfonate less studied than 1,8-ANS and 2,6-ANS is 2,6-TNS, or 6-(p-toluidino)-2-naphthalenesulfonic acid which differs from 2,6-ANS by the addition of a methyl group to the benzene ring as can be seen in Figure 1.6. This work first involved 2,6-ANS as the guest but was later changed to 2,6-TNS due to the subsequent lack of availability of 2,6-ANS.

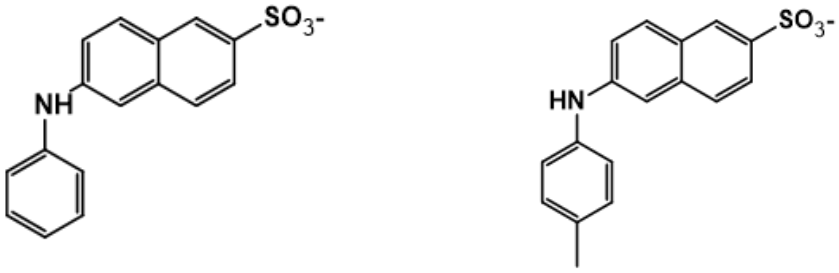


Figure 1.6: Structure of 2,6-ANS (left) and 2,6-TNS (right)

1.4 Fluorescence spectroscopy

Fluorescence can be defined by as the light emitted by electronically excited molecules upon the relaxation of the molecule from a singlet excited electronic state to the ground singlets state. Fluorescence spectroscopy is a technique which can be used to study host-guest chemistry as a change in the emission intensity or wavelength can occur through the fluorescence enhancement of the interaction between the host and guest molecules. Such change can be quantified and analysed to calculate the binding constant. Other techniques can be used to study host-guest inclusions such as NMR, calorimetry, X-ray crystallography but fluorescence spectroscopy is the most sensitive technique and requires less material. [1] Its main drawback is that the guest molecule must be fluorescent.[1] Fluorescence as well as phosphorescence and absorption are known as radiative transitions and are all demonstrated on a Jablonski diagram, a well-known diagram shown in Figure 1.7.

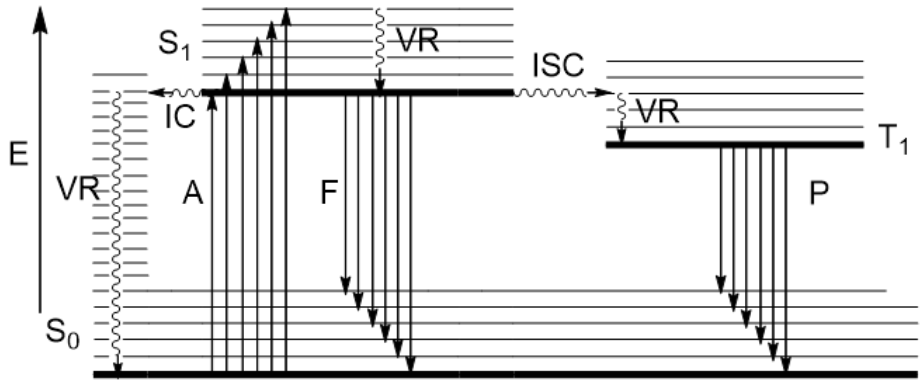


Figure 1.7: Jablonski diagram

For clarification, the labelling of Figure 1.7 is as follows; the A, F, and P, represent the three radiative transitions mentioned above. S_0 represents the singlet ground electronic state while S_1 and T_1 represent the first excited singlet electronic state and the first excited triplet electronic state respectively. This diagram demonstrates the difference between fluorescence and phosphorescence. They differ from which excited state the emission occurs, after the excitation

from the singlet ground state. Fluorescence is the light observed from the relaxation from the first singlet excited electronic state to the singlet ground electronic state while phosphorescence is the relaxation from the first triplet electronic state to the singlet ground electronic state.[9] A triplet state can be reached from the singlet state through a non-radiative transition known as the intersystem crossing.[9] The triplet state differs from the singlet state by the electron spin configuration. This affects the multiplicity which is what defines a singlet or triplet state. A triplet state will have an electron spin flipped compared to the singlet ground state. This process takes more time to relax which explains why phosphorescence can be visible through a long period of time while fluorescence is instantaneous and only visible under a UV light. The polarity of the environment experienced by the guest molecule can be modified upon inclusion with the host caused by the nonpolar inside cavity of the host. This in particular can affect the rate constant for internal conversion which competes with fluorescence.

1.5 Thermodynamics of the inclusion

A thermodynamic study aims to obtain the thermodynamic properties of a complexation process such as obtaining the enthalpy and entropy of inclusion. A thermodynamic study on cyclodextrin as host can lead to interesting information as temperature is known to affect the inclusion dynamics of host-guest complexation. The temperature is linked to the entropy as shown in equation 1.4. In the gas phase, the entropy is negative as the equilibrium goes from two molecules to one complexation. On the other hand, in an aqueous solution, the cavity of the host is not empty like in the gas phase and therefore, upon the entry of the guest molecule into the cavity of the host, more than one solvent molecule in general from the inside of the host cavity is kicked out of the cavity resulting in a positive entropy.[10]

$$\Delta G = \Delta H - T\Delta S \qquad \text{Equation 1.4}$$

Fluorescence spectroscopy is one of the techniques used to perform such studies and it is the method of choice in this work. The acquisition of the binding constant of the host-guest inclusion at various temperatures through a fluorescence titration (see experimental section) enables the thermodynamics of the inclusion process to be calculated. The enthalpy and entropy can be obtained by correlating van't Hoff equation (Equation 1.5) to a plot of ln K vs 1/T with K being the binding constant and T the temperature in Kelvin.[2] As seen in Equation 1.5, the entropy, ΔS, can be calculated from the y-intercept while the enthalpy, ΔH, can be calculated from the slope if the plot obtained is linear.

$$\ln K = -\frac{\Delta H}{RT} - \frac{\Delta S}{R} \qquad \text{Equation 1.5}$$

After obtaining both the entropy and enthalpy, the Gibbs energy can also be calculated as well as directly from the binding constant as shown in equation 1.3. Such information will allow for a better understanding of the interaction between the host and the guest such as the spontaneity and favourability of the host-guest inclusion.

1.6 Previous work

Previous fluorescent studies have been done done, including in the Wagner lab, with all the host and guest molecules mentioned previously but not with the specific combination of the host and the guest used in this work. Every study done with 2,6-TNS and cyclodextrins were performed using β-CD. A paper from another group published in 1989, reports the results of multiple thermodynamic studies between anilinonaphthalene sulfonate and β-cyclodextrin performed using steady state fluorescence.[11] Their results showed that 2,6-ANS had one of the strongest binding constants followed by 2,6-TNS. Their results also showed that for both 2,6-TNS and 2,6-ANS with the β-cyclodextrin, the inclusions were endothermic as can be seen by the

decrease in the binding constants as the temperature increases. That study concluded that 2,6-TNS exhibited both a 1:1 interaction as well as a 2:1 interaction with the native β-cyclodextrin.

Much research has been done in the Wagner lab with anilinonaphthalene sulfonates and cyclodextrins. For instance, a study has been made between 1,8-ANS and 2,6-ANS with the native α-CD, β-CD and γ-CD as well as with modified HP-α, HP-β and HP-γ, with HP referring to hydroxypropyl groups substituted.[8] The results demonstrated that 2,6-ANS had a better fluorescence enhancement and therefore a larger binding constant than 1,8-ANS which can be due to the orientation of the inclusion of the molecule as 2,6-ANS would be included axially compared to 1,8-ANS which would be included equatorially making it more bulky and therefore harder to penetrate the cavity of the CD. It was also demonstrated that the substituted cyclodextrins provided a significantly larger enhancement and that the size of the cyclodextrin also had a significant impact on the inclusion complexation. [8]

1.7 Purpose

The primary goal of this project was to perform an accurate thermodynamic study on the inclusion of 2,6-ANS into 2,6-DM-β-CD using fluorescence spectroscopy. To obtain accurate results, a pure host and guest molecules are required which was not an issue for the guest. However modified cyclodextrins are typically commercially available in a heterogenous compound with the rings not equally substituted with the same number and pattern hydroxyl groups. One of the few commercially available homogenous substituted β-cyclodextrins is heptakis(2,6-di-O-methyl)-β-cyclodextrin. As no study has been done prior to this work on its inclusion with 2,6-ANS, this project was first attempted. Due to an issue with the shipment of the new bottles of the 2,6-ANS and 2,6-DM-β-CD, a new host and guest were subsequently investigated. To navigate around this inconvenience, a guest very similar to 2,6-ANS was chosen as it is only differentiated by the

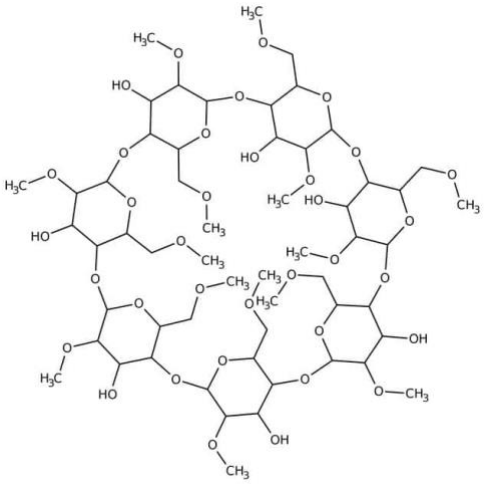
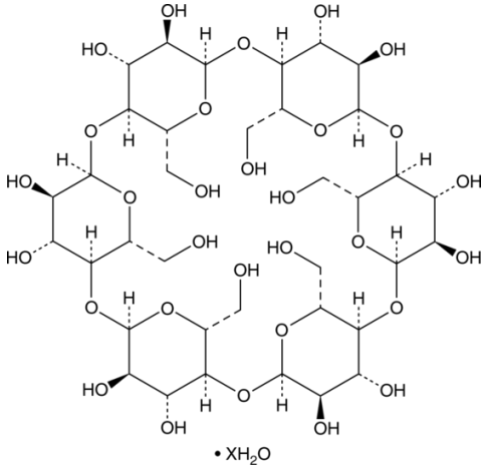
presence of an additional methyl group. This guest molecule is 6-(p-toluidino)-2-naphthalenesulfonic acid and was used throughout the second part of this project. The host chosen to replace 2,6-DM-β-CD was α-cyclodextrin. This host was chosen due to its availability, high aqueous solubility, and purity. It wasn't an ideal choice as α-cyclodextrin is known to have weaker inclusion complexes than β-cyclodextrin due to its small cavity size, but it remained a good host to study after a titration was performed with the 2,6-TNS to assure that an enhancement was occurring. The final purpose of this project was to perform a thermodynamic study on the inclusion of 6-(p-toluidino)-2-naphthalenesulfonic acid with α-cyclodextrin. Such a study has not been previously reported for this specific host and guest inclusion complex. Moreover, an investigation into a potential different method to obtain the binding constant was also investigated to compare with the currently used software in the Wagner lab. The computer on which this software is installed is an old computer and the code language used cannot be read on newer computers resulting in the requirement to find a backup plan.

Chapter 2 – Experimental

2.1 Materials

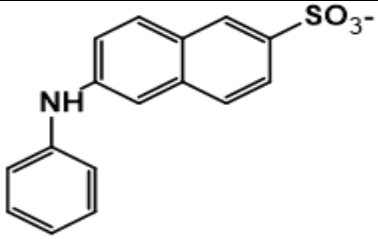
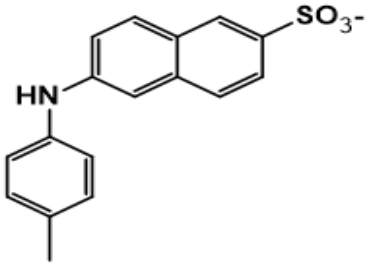
The host first used was heptakis(2,6-di-O-methyl)- β-cyclodextrin as shown in table 1. As the supply of this modified β-cyclodextrin ran low, more was ordered but due to some issue with backorder, 2,6-DM-β-CD would only be available in February 2024, resulting in a change of host necessary. The second host used was α-cyclodextrin shown in table 2.1 as well.

Table 2.1: Host molecules used in the fluorescence titrations performed

Molecule	Structure	Manufacturer
Heptakis(2,6-di-O-methyl)- β -cyclodextrin		Sigma-Aldrich
α-cyclodextrin		Sigma-Aldrich

Similarly, the first guest used was 2,6-ANS (2-anilinonaphthalene-6-sulfonic acid) for which only a small amount was left and was no longer available for purchase, requiring the focus of this work to switch to a similar guest 2,6-TNS(6-(p-toluidino)-2-naphthalenesulfonic acid). Both guests are shown in Table 2.2.

Table 2.2: Guest molecules used in the fluorescence titrations performed

Molecule	Structure	Manufacturer
2,6-ANS		Invitrogen
2,6-TNS		Aldrich

The solvent used was a phosphate buffer made by adding 4.22 g potassium monobasic phosphate and 3.30 g potassium dibasic phosphate into a 500 mL volumetric flask and filling with nanopure water. This creates a buffer keeping the solution at a pH of 6.8.

2.2 Solution preparations

A guest stock solution was made each day of an experiment in a 100 mL volumetric flask with the guest studied diluted in distilled water or the buffer. The 2,6-ANS solutions were made in distilled water while the 2,6-TNS solutions were made in the phosphate buffer. The UV-vis absorbance was taken each time to assure an absorbance of 0.30 - 0.40 at the desired wavelength

of 320 nm for the 2,6-ANS solutions and 330 nm for the 2,6-TNS solutions. By measuring the absorbance each time, a new guest stock solution was made, it was necessary to measure the mass used as the absorbance relates to the concentration through Beer's law shown in equation 2.1 which shows that the absorbance (A) is directly proportional to the concentration (C).

$$A = \varepsilon l C \qquad \text{Equation 2.1}$$

It was determined that the concentration of the guest molecule in 100 mL of nano pure water resulting in an absorbance of 0.30-0.40 is around 5 x 10^{-5} M

Different concentrations of the host were required for the fluorescence titrations; therefore, each concentration was obtained by weighting the host directly in the labelled vials. Once ready to take the fluorimeter measurements, 3.00 mL of the guest stock solution was pipetted into the vials. For the titrations involving 2,6-DM-β-CD, there were eleven different concentrations used which were 0 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 2.5 mM, 5.0 mM, 7.5 mM, and 10 mM. Eight concentrations were used for the α-cyclodextrin, including 0 mM, 2 mM, 4 mM, 6 mM, 8 mM, 10 mM, 15 mM, and 20 mM.

2.3 UV-vis Absorption measurements

As mentioned in the solution preparation, the guest stock solution absorbance was measured using a Cary Bio UV-Vis spectrophotometer to assure a good concentration for the fluorimeter measurements. The solution was placed in a 1 cm^2 quartz cuvette and then placed into the chamber of the spectrophotometer. The absorption spectrum was measured between 500 nm and 250 nm at a medium scan rate.

2.4 Fluorescence Spectra measurements

The fluorescence spectra were measured on a Photon Technology International RF-M2004 Luminescence Spectrometer. The software used to obtain the spectrum was PTI FelixGX. Prior to every titration, the settings were changed to collect an emission spectrum at an excitation wavelength of 320 nm and 330 nm for the modified β-cyclodextrin and α-cyclodextrin respectively, with the emission wavelength starting at a 10 nm increase from the excitation wavelength over a range of 300 nm, in other word between 330 nm to 630 nm for the β-cyclodextrin and 340 nm to 640 nm for the α-cyclodextrin. The emission slits were changed to 1 nm on the software, which is equivalent to a 0.38 mm slit opening on the instrument. The temperature was also adjusted depending on the temperature wanted.

2.5 Thermodynamic study using Fluorescence titration

The thermodynamic study was done by performing a fluorescence titration at different temperatures with a goal of at least five different temperatures. A fluorescence titration is the process by which multiple samples with an increasing concentration of one of the molecules, in this case the host, are measured with a fluorimeter and in which the change in the intensity is measured and used for further study such as calculating the binding constant. The temperatures used for the fluorescence titrations ranged from 8℃ to 45℃. This range was chosen as a temperature lower than 8℃ will cause condensation on the quartz cuvette which will affect the results. Above 45 ℃, evaporation will start to occur and affect the results. For the 2,6-DM-β-CD titrations, only three temperatures of 15 ℃, 25 ℃ and 45 ℃ were obtained before the 2,6-ANS was used up. For the 2,6-TNS and α-cyclodextrin measurements, seven temperatures were

obtained between 8 ℃ and 45 ℃. For each titration, a blank was first measured with either the distilled water or phosphate buffer depending on the guest solution, followed by all the different concentrations of the host-guest solutions. The total integrated areas of the blank and each concentration were obtained from the software and F/F_0 was calculated from those by first removing the area of the blank from every other integrated area. From the equation of fluorescence enhancement (F/F_0), Equation 2.2 which was shown in chapter 1 but is shown again here for convenience, the F can be described as the integrated area of an emission spectrum of a solution containing the host, while F_0 is described as the integrated area of an emission spectrum of a solution which doesn't contain the host, therefore the 0 mM sample.

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right)\frac{[host]_0 K}{(1 + [host]_0 K)} \qquad \text{Equation 2.2}$$

A F/F_0 value can therefore be obtained and calculated for every concentration of a titration which allows for the calculate of the binding constant as will be demonstrated in Chapter 3.

The first goal of this work was to study the thermodynamics of 2,6-ANS with heptakis(2,6-di-O-methyl)-β-cyclodextrin. Only three temperatures were obtained, which were 15 ℃, 25 ℃ and 45 ℃. No other temperatures were acquired as both the 2,6-ANS and the modified β-cyclodextrin were used entirely while performing the 45 ℃ trials. For each temperature, two or three trials were performed to obtain more accurate results. Each fluorescence titration was performed by following the procedure outlined as follows.

The absorbance of a 2,6-ANS stock solution was first measured to determine the wavelength at which the fluorimeter measurements should be recorded at, and it was determined that for 2,6-ANS it was at 330 nm as a peak was observed at 320 nm and that for better measurements, the wavelength chosen is taken slightly at the right of the peak. Similarly for 2,6-

TNS, it was determined that the wavelength for the fluorimeter measurements should start at 340 nm as the peak observed from the absorption spectrum was at 330 nm.

2.6 Binding constant determination

The binding constants were first obtained using Cdeqwin.exe by inputting into the program the values of F/F_0 with their corresponding concentrations. A starting value of AA was approximate based on the F/F_0 vs concentration plot at which values of F/F_0 the graph seemed to level off. The AA constant is a variable used to describe a section of equation 2.2 to simplify the input in the program. AA is defined in equation 2.3.

$$AA = \left(\frac{F_\infty}{F_0} - 1\right) \qquad \text{Equation 2.3}$$

The starting K value for the program was obtained using y-intercept/slope of the double-reciprocal plots. The average binding constants were then calculated and reported as K $\pm$ 1 SD. The binding constants were also obtained using Excel which will be explained in more detail in Chapter 5.

Chapter 3: Thermodynamic study of 2,6-ANS ; result and discussion

At first, a trial was performed using the following concentrations of with heptakis(2,6-di-O-Methyl)-β-Cyclodextrin: 0 mM, 1 mM, 2 mM, 4 mM, 6 mM, 8 mM, and 10 mM. It was then observed that the enhancement values showed a huge gap between the 0mM curve and the 1mM curve of the emission spectrum, so it was decided that smaller concentrations were necessary as this guest-host pair showed strong fluorescent enhancement. The three other trials at 25 ℃ as well as for the other two temperatures were therefore performed using 0 mM, 0.1 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 2.5 mM, 5.0 mM, 7.5 mM, and 10 mM.

3.1 Binding study at 25℃

The emission spectra showing all of the concentrations and the blank is presented in Figure 3.1a. The plots shown for the 25 ℃ trials in this paper are from trial 2 as all three trials were similar in appearance.

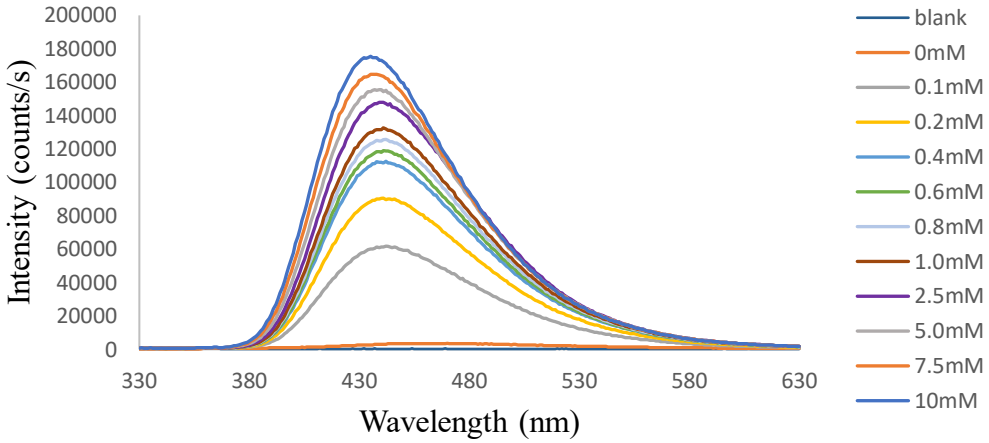


Figure 3.1a: Fluorescence titration of 2,6-ANS with 2,6-DM-β-CD at 25 ℃

After acquiring the total integrated area from Figure 3.1a and calculating F/F_0, a titration plot of F/F_0 versus the concentration of heptakis(2,6-di-O-methyl)-β-cyclodextrin at 25 ℃ was generated as displayed in Figure 3.1b.

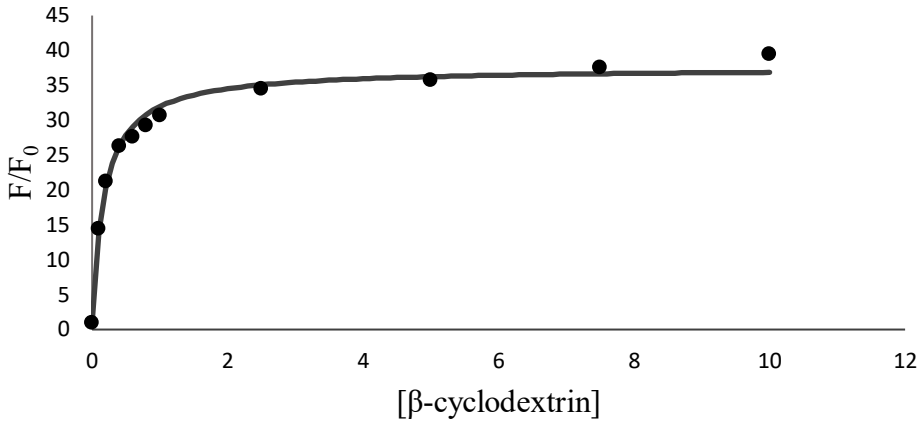


Figure 3.1b: F/F_0 versus the concentration of 2,6-DM-β-CD at 25 ℃

A double-reciprocal plot was then made to confirm the 1:1 nature of the inclusion as a linear double-reciprocal plot indicates a 1:1 inclusion complex.

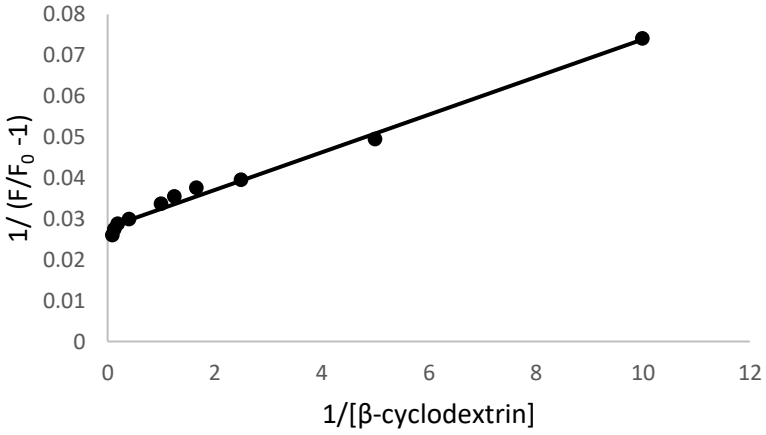


Figure 3.1c: Double-Reciprocal plot of 2,6-ANS with 2,6-DM-β-CD at 25 ℃

As can be seen, a linear slope was observed demonstrating a good correlation as the R^2 values was found to be 0.991 which confirms a 1:1 inclusion complex. The binding constants of the inclusion of 2,6-ANS with heptakis(2,6-di-O-Methyl)-β- cyclodextrin at 25 ℃ were first

obtained using Cdeqwin.exe and are given in Table 3.1 The average binding constant of the three trials was found to be 5470 ± 153 M^{-1}

Table 3.1: Binding constants of the trials of 2,6-ANS and 2,6-DM-β-CD performed at 25 ℃

Trials at 25 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	5290
Trial 2	5560
Trial 3	5550
Avergae	5470 ± 153

The binding constants were found to be very similar and with good agreement of each other.

3.2 Binding study at 15 ℃

Similarly, three trials were performed at 15 ℃. The spectra obtained directly from the fluorimeter is seen in Figure 3.2a. The plots included in this paper are from trial 2 of 15 ℃.

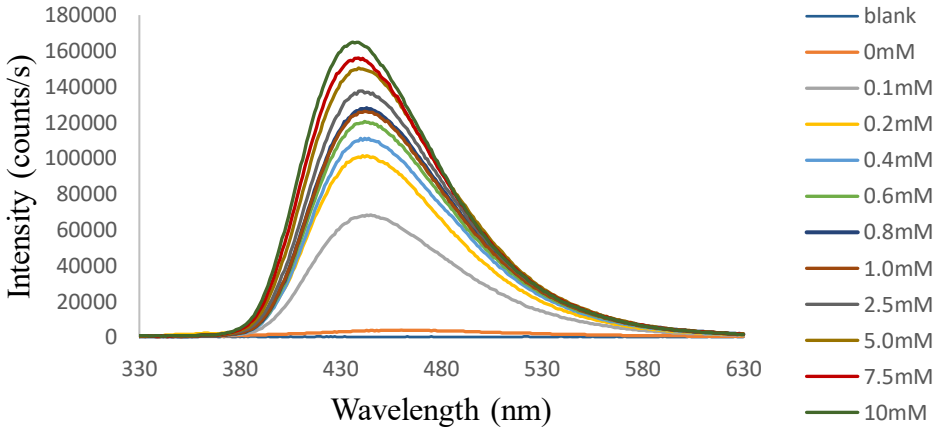


Figure 3.2a: Fluorescence titration of 2,6-ANS with 2,6-DM-β-CD at 15 ℃

After acquiring the total integrated area from Figure 3.21 and calculating F/F_0, a plot of F/F_0 versus the concentration of heptakis(2,6-di-O-methyl)-β-cyclodextrin at 15 ℃ was created in Excel and shown in Figure 3.2b. The solid fit line is not in as good of an agreement with the measured data as it was to for 25 ℃ (Figure 3.1b).

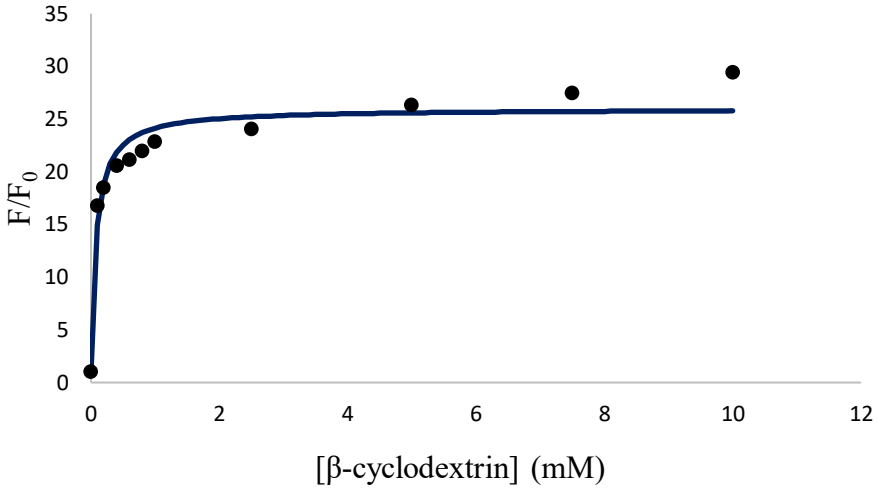


Figure 3.2b: F/F_0 versus the concentration of 2,6-DM-β-CD at 15 ℃

The double-reciprocal plot made using the plot above appears in Figure 3.2c and shows a significant nonlinearity.

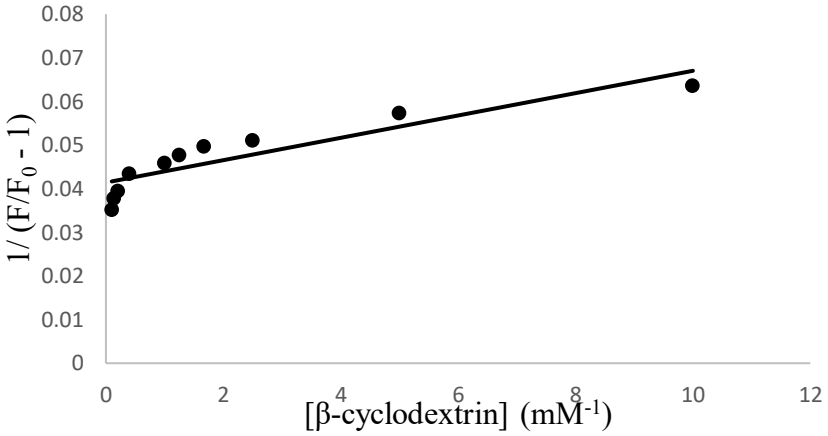


Figure 3.2c: Double-Reciprocal plot of 2,6-ANS with 2,6-DM-β-CD at 15 ℃

Upon acquiring the plot from Figure 3.2c, it was seen that the R^2 wasn't as close to 1 as it should be to confirm a 1:1 interaction. The R^2 value of the Figure 3.2c is 0.8206 which is significantly lower than for 25℃ and 45 ℃. This indicates that higher-order complexation is occurring, beyond a simple 1:1 host-guest inclusion. As all three trials performed at 15 ℃ had a correlation value lower than expected, for the double-reciprocal plot, the binding constant were not acquired as the program used and the focus of this work is 1:1 inclusion complex. Due to the limitation of the guest resources available by the end of the third trial, it was decided that to proceed with a different temperature to determine if the same trend was observed rather than performing more trials at this temperature for further investigation of the inclusion complex at 15℃.

3.3 Binding study at 45 ℃

As mentioned above, reaching this point of the research, the 2,6-ANS available was scarce resulting in only two trials successfully performed at 45 ℃, which was the next temperature obtained as it was the highest temperature goal for the fluorescent titrations. The trials performed at 25 ℃ and 15 ℃ have shown that F/F_0 tends to level out at concentrations higher than 4mM. Therefore, as the amount of 2,6-DM-β-CD started to run low as well, the concentrations for the trials at 45 ℃ were changed to the following: 0 mM, 0.1 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 2.0 mM, 4 mM, 6 mM, and 8 mM.

The emission spectra including all the concentration and the blank is shown in Figure 3.3a. The plots shown for the 45 ℃ trials in this paper are of trial 2, with the graphs of both trials being similar in appearance.

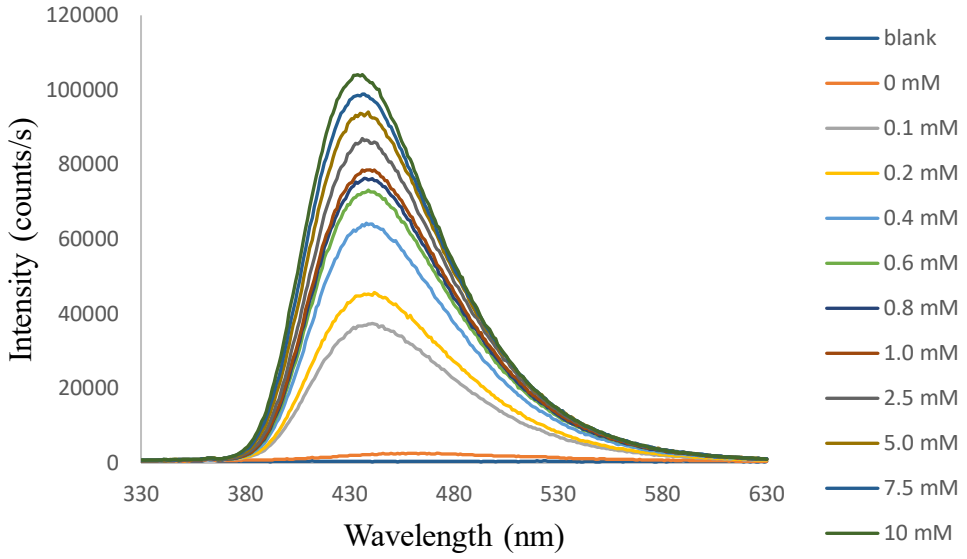


Figure 3.3a: Fluorescence titration of 2,6-ANS with 2,6-DM-β-CD at 45 ℃

A plot of F/F_0 versus the concentration of 2,6-DM-β-CD at 45 ℃ was generated using the integrated area of Figure 3.3a. This graph shows a good fit of the data to the solid 1:1 fit line.

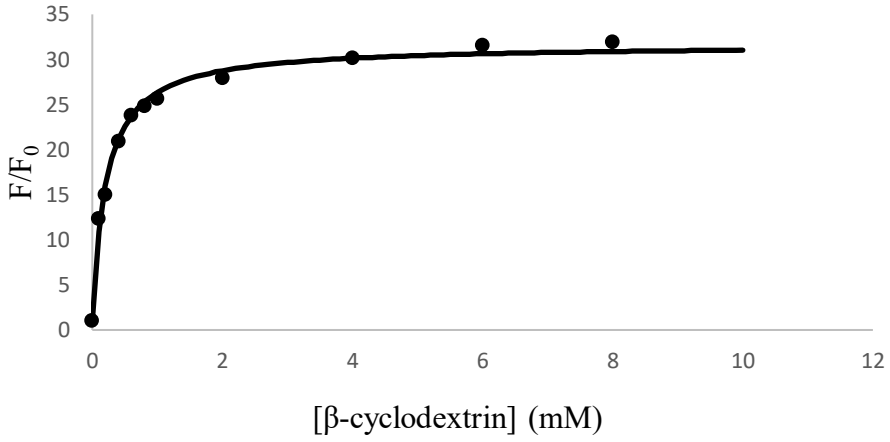


Figure 3.3b: F/F_0 versus the concentration of 2,6-DM-β-CD at 45 ℃

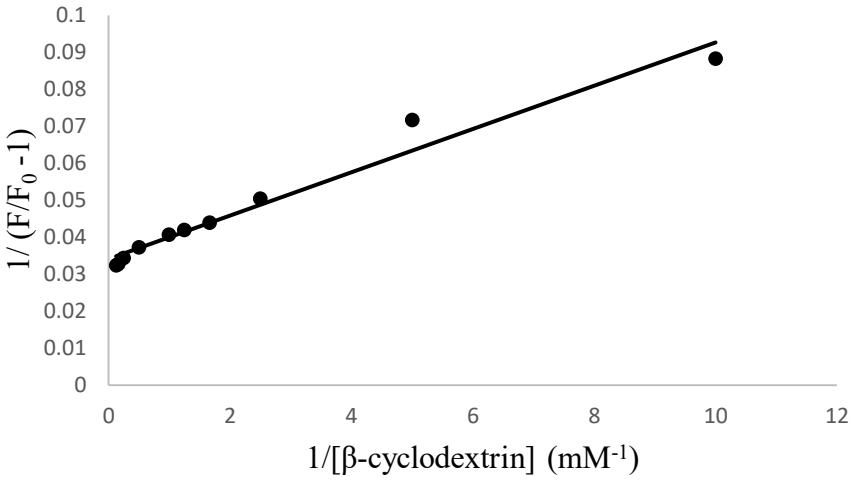


Figure 3.3c: Double-Reciprocal plot of 2,6-ANS with 2,6-DM-β-CD at 45 ℃

The double-reciprocal plot has R^2 value of 0.9664 which isn't a great correlation for this type of plot, but this may be due to the outlier between 4 and 6 mM^{-1}. The average binding constant of the two trials at 45 ℃ was found to be 4830 $\pm$ 80 M^{-1}.

Table 3.3: Binding constants of the trials of 2,6-ANS and 2,6-DM-β-CD performed at 45 ℃

Trials at 45 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	4890
Trial 2	4770
Average	4830 $\pm$ 80 M^{-1}

Even though only two trials were obtained instead of three for a more accurate average, the binding constant of the two trials were in good agreement with each other.

3.4 Discussion

Overall, as only three temperatures were obtained, with no binding constant obtained for the trials at 15 ℃ , a plot of ln K vs 1/T could not be made to compare to van't Hoff equation in

order to obtain accurate values of enthalpy and entropy. A plot could have been made with just three data points but as the trials at 15 ℃ were seen to not follow a 1:1 complexation inclusion trend, the binding constants were not obtained as this was beyond the work of this study.

It was first observed in the reciprocal plot obtained from the fluorescent titration performed at 15 ℃ that the first four lower concentration formed a non-linear pattern which was then observed in the reciprocal plot at 25 ℃ and 45 ℃. The double-reciprocal plot at 15 ℃ was determined to be non-linear which invalidated the use of equation 2 to solve for the binding constant. However, based on the R^2 value, the double-reciprocal plots for the other two temperatures, 25 ℃ and 45 ℃, were considered to be more linear although the R^2 value wasn't great either in the case of 45 ℃. A curve was observed in the double-reciprocal plot at the three to four lower concentrations for each temperature but was more pronounced at the 15 ℃ temperature resulting in the non-linearity of the plot. This observation could be the result of the lack of accuracy of the smaller concentrations as the mass of 2,6-DM-β-CD required was extremely low and in the case of 0.1 mM and 0.2 mM, a larger quantity of the 2,6-DM-β-CD had to be weight and diluted with a bigger volume of the 2,6-ANS stock solution compared to the other concentrations. Another explanation which would have to be further looked into and researched if both the host and guest become available would be that at lower concentrations, the complexation of the host and guest might differ and not be a 1:1 inclusion and that upon the increase of the concentration, the interaction becomes a 1:1 host:guest inclusion. Unfortunately, the lack of multiple trials and temperatures performed doesn't allow for a precise conclusion to those results. As mentioned, due to the unavailability of both the 2,6-ANS and 2,6-DM-β-CD, the study on this host-guest pair could not be pursued any further and therefore, the goal of this work switched to the thermodynamic study of 2,6-TNS in α-CD which will be discussed in Chapter 4.

Chapter 4 Thermodynamic study of 2,6-TNS; result and discussion

As the 2,6-ANS was no longer commercially available and the 2,6-DM-β-CD became scarce with the new ordered sample schedule to arrive only in 2024, the purpose of this work switched to a new host and guest. The guest was changed to 2,6-TNS which as mentioned in Chapter 1 is very similar to 2,6-ANS with the presence of an extra methyl group on the benzene ring in 2,6-TNS as the only difference between the two. The host was switched to α-CD as it was one of the pure cyclodextrins available in the lab and for which no thermodynamic studies has been reported with 2,6-TNS, and it has a much higher aqueous solubility than β-CD. A thermodynamic study was therefore performed on the host-guest inclusion of 2,6-TNS and α-CD and the results are displayed in this chapter.

For each temperature, three or four trials were performed to obtain more accurate results. Each fluorescence titration was performed by following the procedure outlined in chapter 2.5.
The following concentrations were used for all the trials of 2,6-TNS with α-cyclodextrin: 0 mM, 2 mM, 4 mM, 6 mM, 8 mM, 10 mM, 15 mM, 20 mM. Due to the smaller difference between the fluorescence titration spectra of 0 mM concentration and the 2 mM concentration, lower concentrations were not necessary. A blank with the buffer was included for every trial. The fluorescence titrations were performed at the following temperatures:
8 ℃, 11 ℃, 15 ℃, 20 ℃, 25 ℃, 35 ℃, 45 ℃. The binding constants of every temperature as well as the plots of one trial per temperature are shown below in ascending order of temperature. However, it is important to note that the data was not collected in that order in the lab. The order of trials performance was the following: 25 ℃, 15 ℃, 35 ℃, 45 ℃, 8 ℃, 11 ℃, 20 ℃. This is mentioned as

the last two temperatures were taken due to the result of the first five as will be explained in Chapter 4.9 where the results of this thermodynamic study will be discussed as well.

4.1 Binding study at 8 ℃

The emission spectra including all the concentrations and the blank are shown in Figure 4.1a. The plots shown for the 8 ℃ trials in this paper are of trial 1, and three trials were obtained.

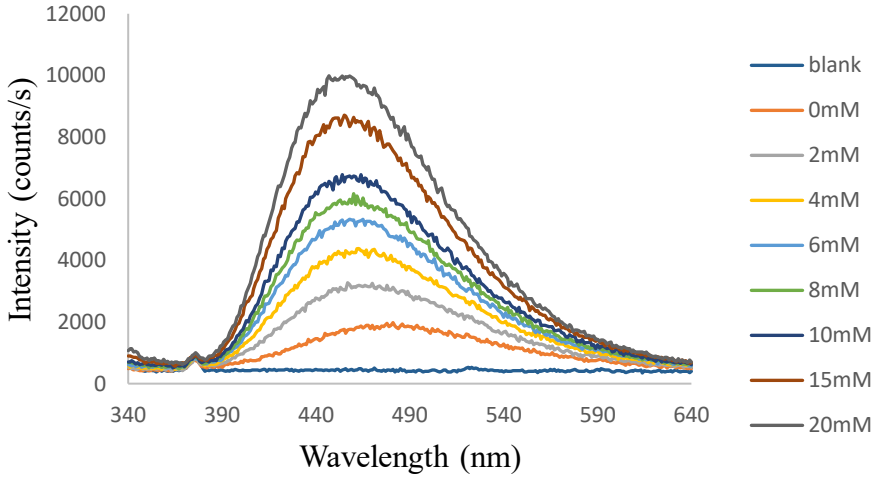


Figure 4.1a: Fluorescence titration of 2,6-TNS with α-CD at 8 ℃

After acquiring the total integrated area from Figure 4.1a and calculating F/F_0, a plot of F/F_0 versus the concentration of α-cyclodextrin at 8 ℃ was generated as shown in Figure 4.1b.

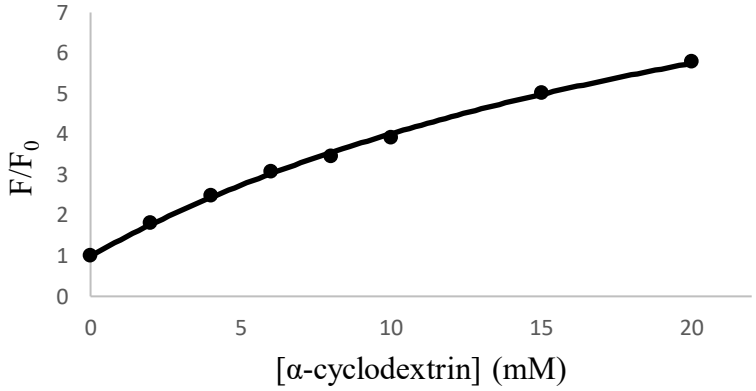


Figure 4.1b: F/F_0 versus the concentration of α-cyclodextrin at 8 ℃

A double-reciprocal plot was made to confirm the 1:1 nature of the inclusion as a linear double-reciprocal plot indicates a 1:1 inclusion complex.

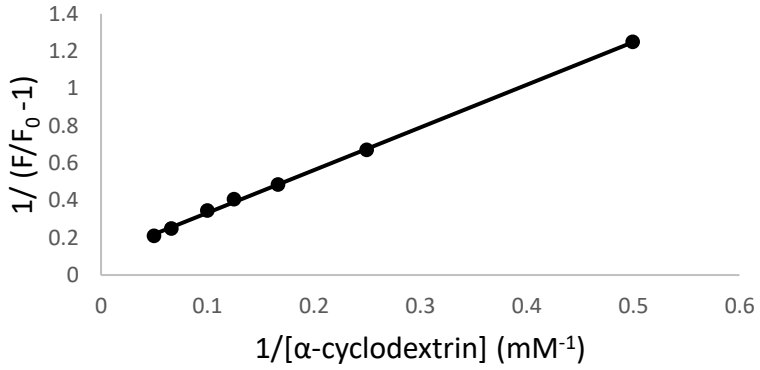


Figure 4.1c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 8 ℃

As can be seen, a linear slope was observed demonstrating a good correlation and a 1:1 inclusion complex. The R^2 value of Figure 4.1c is 0.9993. The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 8 ℃ were obtained using Cdeqwin.exe. The average binding constant of the three trials was found to be 35 ± 2 M^{-1}.

Table 4.1: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 8 ℃

Trials at 8 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	34
Trial 2	37
Trial 3	33

The binding constant were very similar to each other, especially compared to other temperature results. This shows a good agreement between the trials. However as can be seen, the binding constant values are extremely small, especially compared to the binding constant obtained in chapter 3 for 2,6-ANS in 2,6-DM-β-CD, which were around 5000 M^{-1}.

4.2 Binding study at 11 ℃

The emission spectra including all the concentrations and the blank are shown in Figure 4.2a. The plots shown for the 11 ℃ trials in this paper are of trial 2, and three trials were obtained.

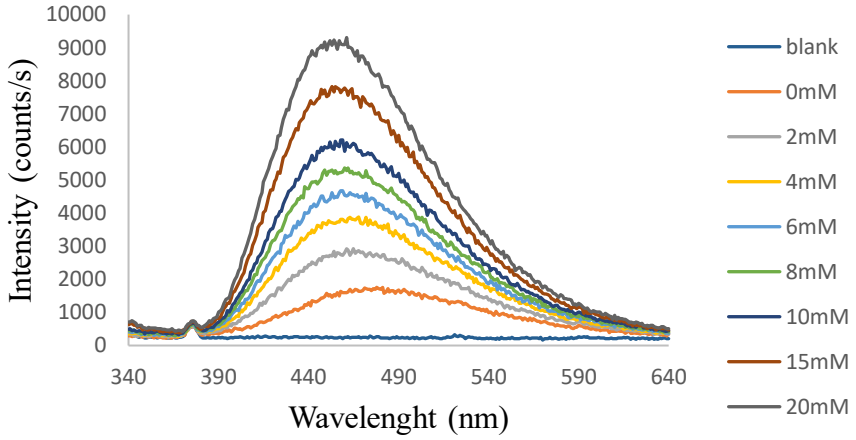


Figure 4.2a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 11 ℃

Using the integrated area of each curve of Figure 4.2a, a plot of F/F_0 vs [α-cyclodextrin] was made to observe the shape of the plot.

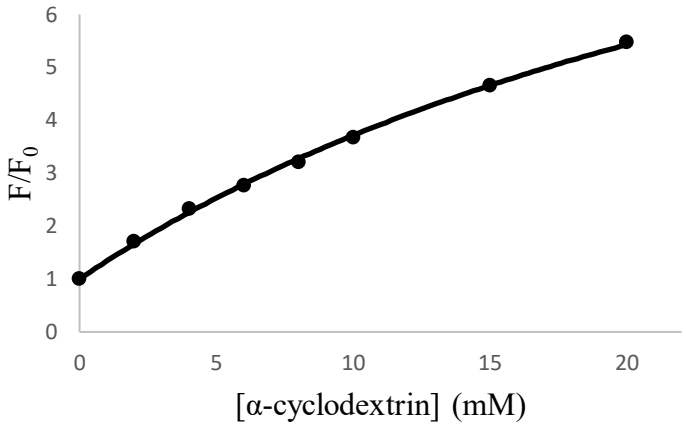


Figure 4.2b: F/F_0 versus the concentration of α-cyclodextrin at 11℃

As can be seen, the shape is similar to the plot at 8 ℃. And from that plot, a double-reciprocal plot was made. It can be observed that the curvature of the F/F_0 vs [α-CD] plot is not as significant as was observed in chapter 3.

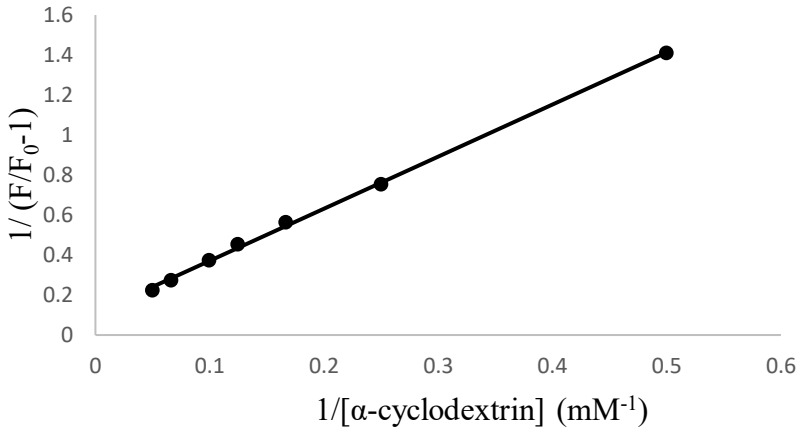


Figure 4.2c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 11 ℃

The plot in of Figure 4.2c can be seen to be linear and the R^2 of 0.9987 demonstrates a very good correlation and a 1:1 inclusion complex. The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 11 ℃ were obtained using Cdeqwin.exe using the slope and y-intercept to estimate the K constant to input in the program. The average binding constant of the three trials was found to be 26 ± 6 M^{-1}.

Table 4.2: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 11 ℃

Trials at 11 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	30
Trial 2	29
Trial 3	20

There was a bigger difference between the binding constant compared to the previous temperature however, the fluctuation isn't dramatically high for a binding constant determination. Therefore, it can be said that there is a good agreement within the three trials.

4.3 Binding study at 15 ℃

The emission spectra of the second trial for the fluorescent titration performed at 15 ℃ is displayed in Figure 4.3a. Four trials were obtained, with the fourth trial being performed after all other trials at every temperature were obtained. The reasoning for this will be discussed in chapter 6.2.

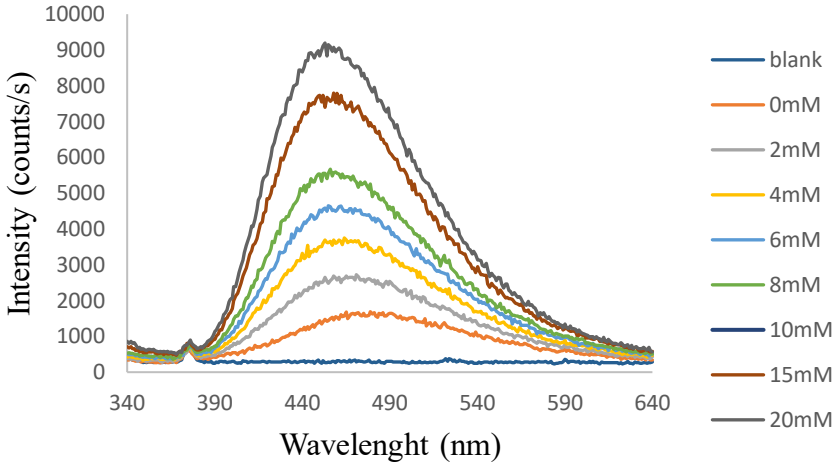


Figure 4.3a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 15 ℃

After acquiring the total integrated area from Figure 4.3a and calculating F/F_0, a plot of F/F_0 versus the concentration of α -cyclodextrin at 15 ℃ was generated as shown in Figure 4.3b.

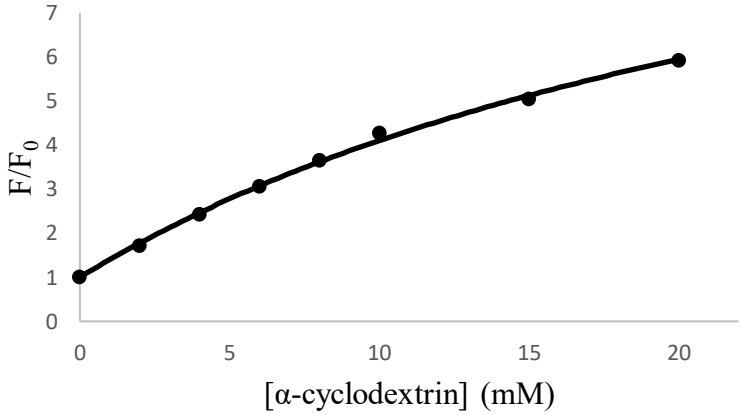


Figure 4.3b: F/F_0 versus the concentration of α-cyclodextrin at 15 ℃

A double-reciprocal plot was made to confirm the 1:1 nature of the inclusion as a linear double-reciprocal plot indicates a 1:1 inclusion complex.

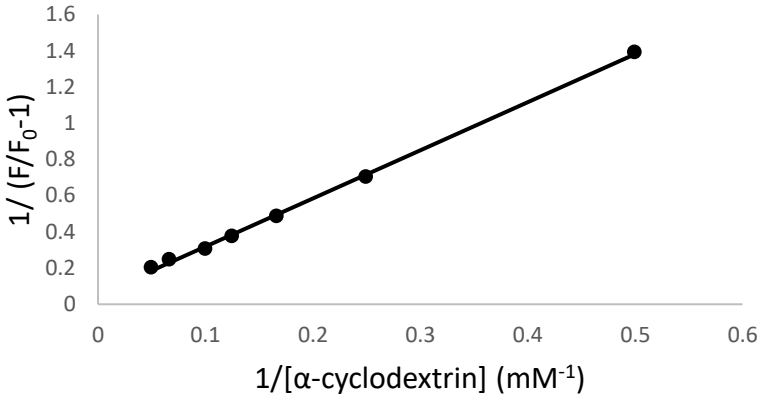


Figure 4.3c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 15 ℃

As can be seen, a linear slope with a R^2 value of 0.9988 was observed demonstrating a good correlation and a 1:1 inclusion complex. The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 15 ℃ were first calculated from equation 1.1 using Cdeqwin.exe. The average binding constant of the four trials was found to be 42 ± 9 M^{-1}.

Table 4.3: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 15 ℃

Trials at 25 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	55
Trial 2	34
Trial 3	40
Trial 4	40

Trial 1 and trial 2 were found to have a big difference of 21 between their binding constants. As all the binding constants are around the same values for the different temperatures, such

difference has a significant impact on the results. Trial 3 and 4 are found to be the same and in between the first two trials.

4.4 Binding study at 20 ℃

The emission spectra including all of the concentrations is shown in Figure 4.4a. Three trials were performed at this temperature. The plots shown for the 20 ℃ trials are of trial 2.

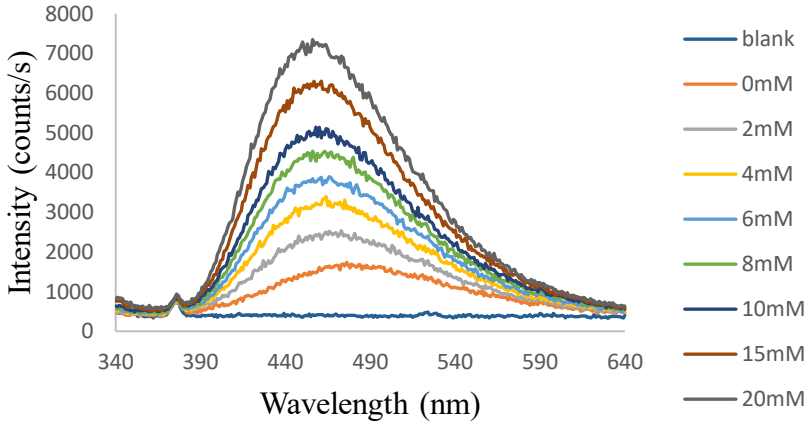


Figure 4.4a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 20℃

Using the integrated area of each curve of Figure 4.2a, a plot of F/F_0 vs [α-cyclodextrin] was made as seen in Figure 4.4b.

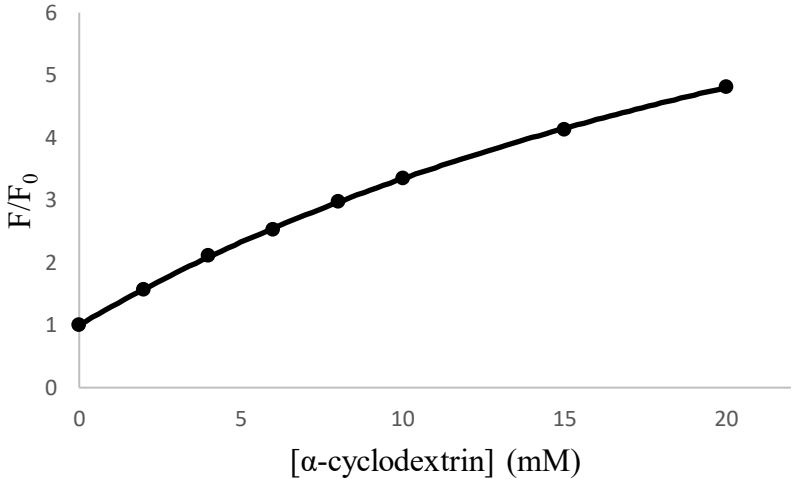


Figure 4.4b: F/F_0 versus the concentration of α-cyclodextrin at 20 ℃

A double-reciprocal plot was made, Figure 4.4c, to observe the nature of the inclusion and confirm that the 1:1 nature of the inclusion is still present at this temperature.

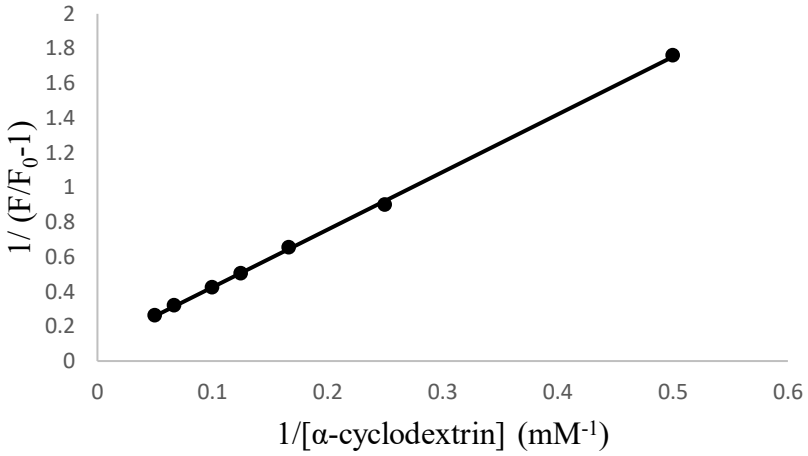


Figure 4.4c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 20 ℃

The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 20℃ were obtained using Cdeqwin.exe after a linear slope with a R^2 value of 0.9995 showed an incredibly strong correlation of each concentration. The average binding constant of the three trials was found to be 31 ± 2 M^{-1}.

Table 4.4: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 20 ℃

Trials at 20 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	33
Trial 2	30
Trial 3	30

The binding constants at this temperature were found to be very similar to each other.

4.5 Binding study at 25 ℃

The emission spectra including all of the emission curves of each concentrations including the blank can be found in Figure 4.5a. The plots shown in this section for the 25 ℃ trials are of trial 1. Four trials were obtained due to the difference between trial 1 and the other two trials as can be seen in table 4.5.

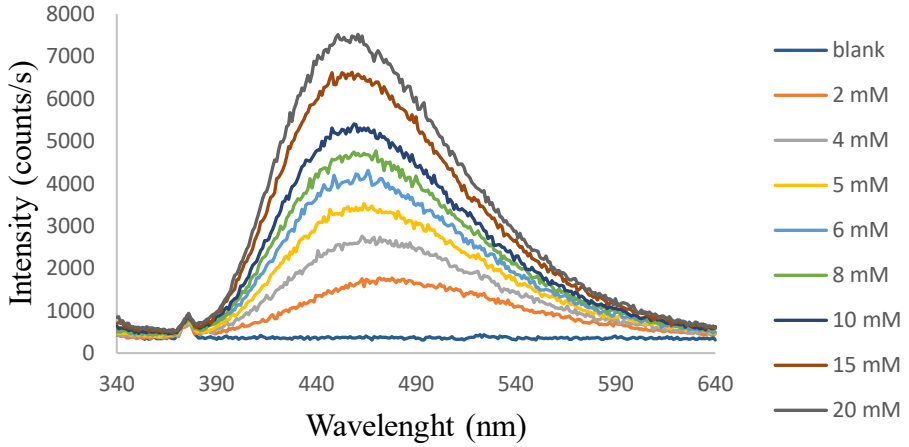


Figure 4.5a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 25 ℃

After acquiring the total integrated area from Figure 4.5a and calculating F/F_0, a plot of F/F_0 versus the concentration of α -cyclodextrin at 25 ℃ was generated as shown in Figure 4.5b.

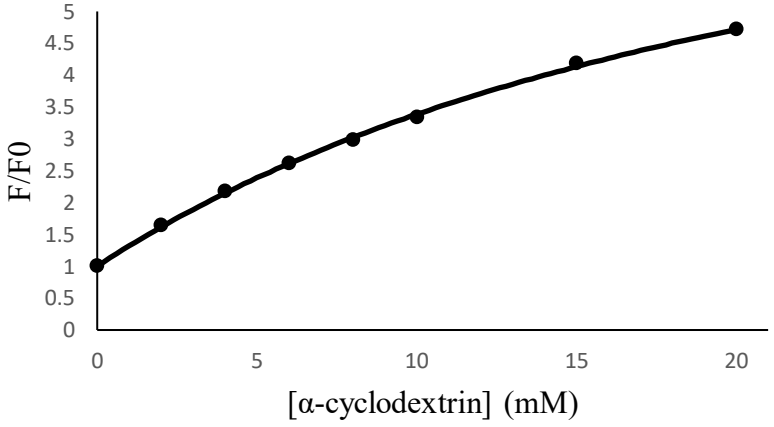


Figure 4.5b: F/F_0 versus the concentration of α-cyclodextrin at 25 ℃

A double-reciprocal plot was made to confirm the 1:1 nature of the inclusion as a linear double-reciprocal plot indicates a 1:1 inclusion complex.

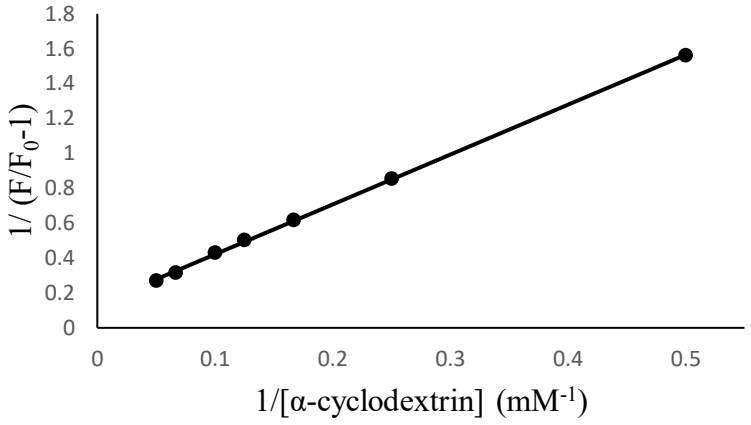


Figure 4.5c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 25 ℃

The slope of Figure 4.5c has a R^2 value of 0.9996, again showing a good correlation between the data of this fluorescent titration. The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 25 ℃ were calculated using Cdeqwin.exe. The average binding constant of the three trials, excluding trial 1, was found to be 40 ± 12 M^{-1}.

Table 4.5: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 25 ℃

Trials at 25 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	180
Trial 2	53
Trial 3	29
Trial 4	39

For this temperature, the binding constant of the first three trials were significantly different and therefore a fourth trial was performed. After obtaining trial 4, a statistical Q-test was conducted

to determine if trial 1 is an outlier and could be removed. It was determined that trial 1 could be considered as an outlier with a 95% confidence level as seen below.

$$Qtest = \frac{(179.97-52.90)}{(179.97-28.86)} = 0.84 > 0.83 \text{ (95\% confidence)}$$

Even without trial 1, the binding constants at this temperature were found to have the biggest variance between the trials.

4.6 Binding study at 35 ℃

The emission spectra including all the concentration and the blank is shown in Figure 4.61. The plots shown for the 35 ℃ trials in this paper are of trial 2, and three trials were obtained.

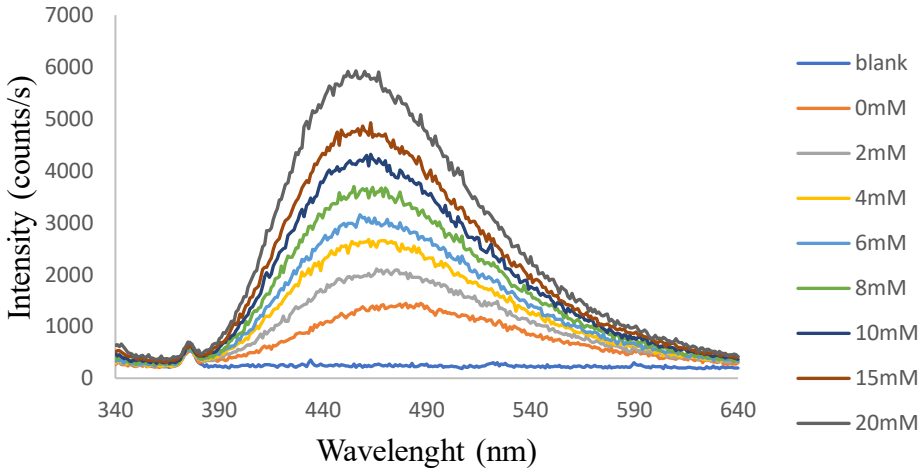


Figure 4.6a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 35 ℃

After acquiring the total integrated area from Figure 4.6a and calculating F/F_0, a plot of F/F_0 versus the concentration of α -cyclodextrin at 35 ℃ was generated as shown in Figure 4.6b.

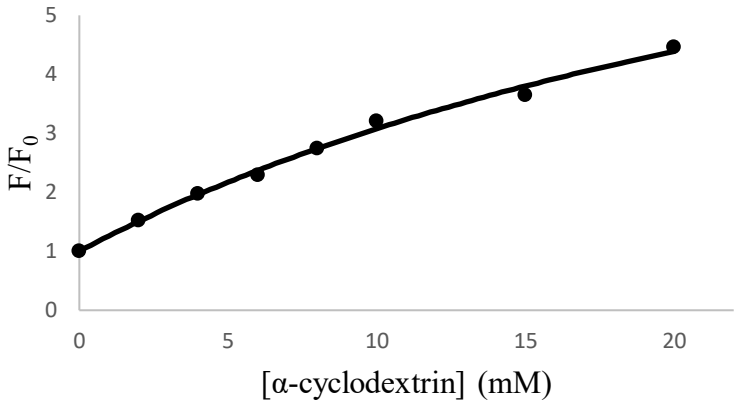


Figure 4.6b: F/F_0 versus the concentration of α-cyclodextrin at 35 ℃

A double-reciprocal plot was made to confirm the 1:1 nature of the inclusion as a linear double-reciprocal plot indicates a 1:1 inclusion complex.

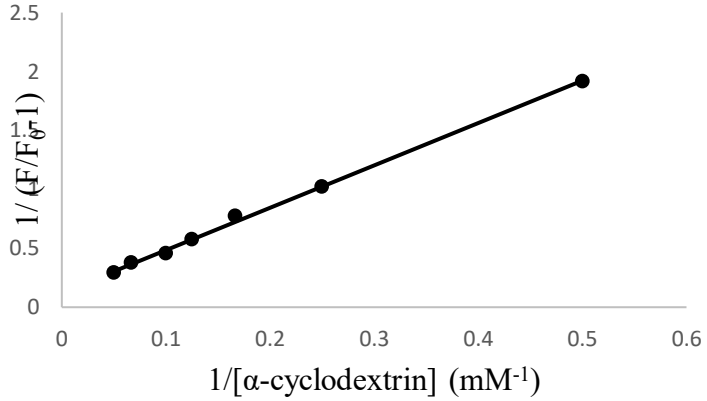


Figure 4.6c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 35 ℃

As can be seen, a linear slope was observed demonstrating a good correlation and a 1:1 inclusion complex, as a R^2 of 0.9981 was obtained. The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 35 ℃ were obtained using Cdeqwin.exe. The average binding constant of the three trials was found to be $31 \pm 8\ M^{-1}$.

Table 4.6: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 35 ℃

Trials at 35 ℃	**Binding Constant (K) (M^{-1})**
Trial 1	40
Trial 2	29
Trial 3	24

There is again a significant difference between the trials, for instance a difference of 16 M^{-1} can be observed between trial 1 and trial 3.

4.7 Binding study at 45 ℃

The emission spectra including all the concentrations is shown in Figure 4.7a. Three trials were performed at this temperature. The plots shown for the 20℃ trials in Figure 4.7a are of trial 2.

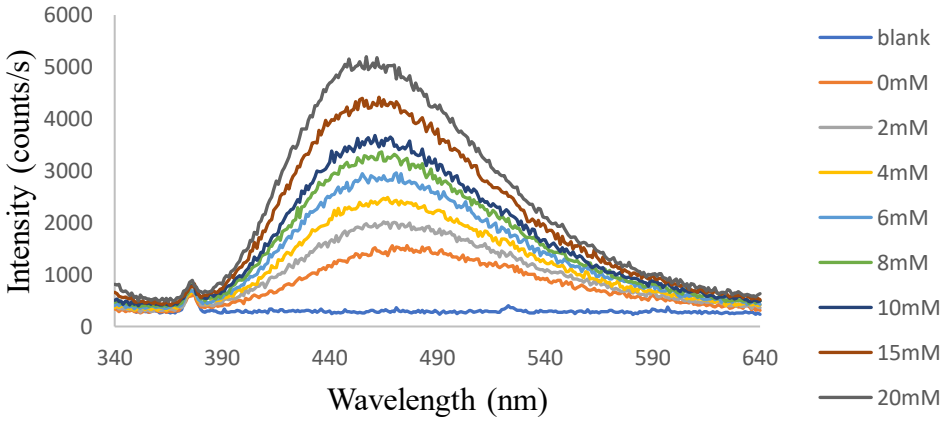


Figure 4.7a: Fluorescence titration of 2,6-TNS with α-cyclodextrin at 45 ℃

After acquiring the total integrated area of all the spectra from Figure 4.7a, a plot of F/F_0 versus the concentration of α-cyclodextrin at 45 ℃ was generated in Excel and is shown in Figure 4.7b.

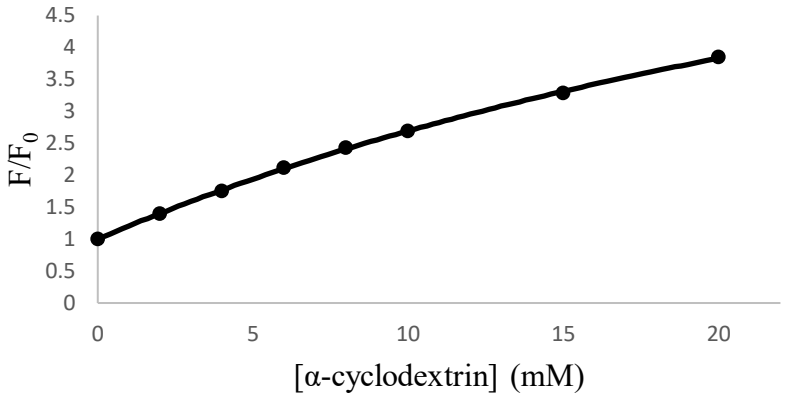


Figure 4.7b: F/F_0 versus the concentration of α-cyclodextrin at 45 ℃

A double-reciprocal plot was made, Figure 4.7c, to observe the nature of the inclusion and confirm that the 1:1 nature of the inclusion is still present at this temperature

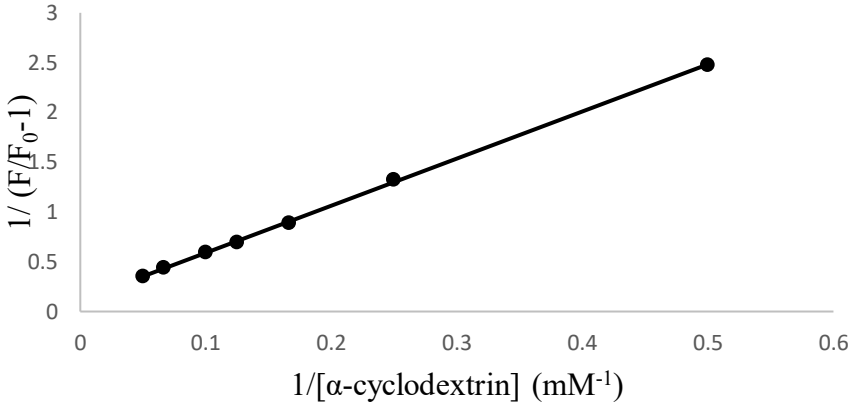


Figure 4.7c: Double-Reciprocal plot of 2,6-TNS with α-cyclodextrin at 45 ℃

The binding constants of the inclusion of 2,6-TNS with α-cyclodextrin at 45 ℃ were obtained using Cdeqwin.exe after a linear plot with a R^2 value of 0.9997 showed an incredibly strong correlation of each concentration. The average binding constant of the three trials was found to be $26 \pm 2\ M^{-1}$.

Table 4.7: Binding constants of the trials of 2,6-TNS and α-cyclodextrin performed at 45 ℃

Trials at 45 ℃	Binding Constant (K) (M^{-1})
Trial 1	24
Trial 2	24
Trial 3	28

The binding constant were very similar to each other, especially compared to the results of 25 ℃ and 35 ℃. The standard deviation shows the good agreement present between the trials.

4.8 Plot of ln K vs 1/T.

After all the binding constants at the seven temperatures were obtained, a plot of ln K vs 1/Temperature could be made to relate to van't Hoff equation (Equation 1.2) to solve for the thermodynamic constants of the inclusion of 2,6-TNS and α-cyclodextrin. That plot is seen in Figure 4.8.

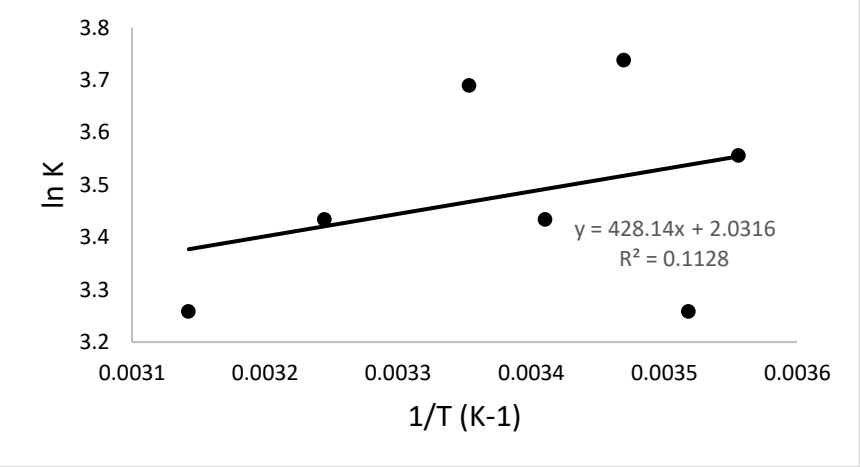


Figure 4.8: Plot of ln K vs 1/T.

The plot obtained was unexpectantly not linear as is required to solve for van't Hoff equation 3. In fact, it is highly scattered, especially at the four lower temperatures. This result

was unexpected and not predicted by any thermodynamic model. As a result of this non-linear plot, as shown in Figure 4.8, van't Hoff equation could not be used, and the enthalpy and entropy could not be obtained.

However, even though no accurate data could be acquired from Figure 4.8, the fit for all the data was taken and the trend is shown in Figure 4.8. The enthalpy for this trend was calculated to be -3.56 kJ mol^{-1} while the entropy was found to be 16.89 J K^{-1} mol^{-1}. Those results although they do not accurately represent the inclusion between 2,6-ANS and α-cyclodextrin as the plot shown in Figure 4.8 is not linear, they indicate some potential information on the inclusion which will be discussed in Chapter 4.9

4.9 Discussion

From the seven fluorescence titration spectra, a hypsochromic shift was observed between the 0 mM and the other concentrations, going from approximately 480nm to 460nm. This blue shift occurs as the energy gap between a polar and nonpolar environment increases in the nonpolar environment. For instance, in the absence of the host at 0 mM, the guest is in a polar environment and has a specific energy gap. Upon the addition of the host molecule, the guest molecule can enter the cavity which is a nonpolar environment. This will increase the energy gap and therefore there is less internal conversion occurring resulting in more guest molecules fluorescing. This energy increase results in a wavelength shift towards smaller wavelengths.

After obtaining the average binding constant of the first five temperatures desired, 25℃, 15 ℃, 35 ℃, 45 ℃, 8 ℃, using fluorescence spectroscopy, a plot of ln K vs 1/T was made, and it was observed that the plot followed an increasing linear trend from 45 ℃ to 25 ℃ followed by 15 ℃ which was higher than 25 ℃ but did not followed the linear trend and curved the plot slightly. It was then observed that the binding constant at 8 ℃ was lower than both the 15 ℃ and

25 ℃. To understand better what was happening on the right side of the plot with the lower temperatures, fluorescence titrations at 20 ℃ and 11 ℃ were performed. The plot obtained with those two new temperatures showed that both data points did not follow any trend and made the plot scattered as can be seen in Figure 4.8. The main explanation to those result is the possibility that due to the binding constants being so low for every trial, less than 50, the required accuracy could not be achieved.

The variation between the trials of some temperature is significant enough that the binding constant of one trial at a specific temperature can be found between two trials of another temperature. For instance, trial 3 at 25 ℃ resulted in a lower binding constant than all the trials at 8 ℃ while trial 4 at 25 ℃ resulted in a higher binding constant compared to all the trials at 8 ℃. Therefore, it shows that the binding constants are too similar between the different temperatures and that this closeness in binding constant as well as their small magnitude doesn't allow for an accurate thermodynamic study of the complexation of 2,6-TNS and α-cyclodextrin.

Even though the overall thermodynamic data for this complexation couldn't be obtained due to the non-linear plot obtained in Figure 4.8, the linear fit of the scattered plot was acquired to obtain an enthalpy and entropy just for the purpose of gaining some information about the inclusion although those values are not accurate. An enthalpy of -3.56 kJ mol^{-1} and an entropy of 16.9 J K^{-1} mol^{-1} were obtained using van't hoff equation. What those values can indicate about the inclusion of this pair is that the inclusion is more likely to be exothermic from the negative enthalpy obtained and that there is a decrease in order due to the negative entropy. Those values agreed with what was predicted for this host-guest inclusion. A negative enthalpy is typically observed for inclusion in solution, as a result of energetically favourable interactions between the host and guest, while a positive entropy is to be expected for inclusion in a solution as one guest molecule entering the

cavity of the host molecule removes more than one solvent molecules, increasing the disorder. Using both Equation 1.3 and Equation 1.4, the free energy value can be calculated for the seven temperatures, and ΔG of the inclusion of 2,6-TNS with α-CD was found to range between -7 and -9 kJ mol^{-1}. A negative ΔG for the inclusion confirms that it is a spontaneous dynamic inclusion process.

Chapter 5 Binding constant programs

As mentioned, in addition to the thermodynamic study of the host:guest inclusion of 2,6-TNS and α-cyclodextrin, obtaining an alternative approach to the Cdeqwin.exe program was investigated. Multiple different programs were looked into such as Python and Mymathlab but due to the lack of knowledge in programing and coding language, the binding constant was not successfully acquired with those methods. A method which was looked deeper into was using Excel which seemed to be an easy and accessible method. Excel can be used to perform a non-linear fit enabling to solve for an equation containing multiple variables by using Solver, which is an extension available on Excel. The binding constants obtained from this method were all found to be very similar to the ones obtained from the Wagner lab computer program however, all binding constants were found to be smaller.

5.1 How to use solver extension in Excel

Using solver with Excel is easy and straightforward. First, the concentrations and F/F_0 are inputted in two columns and equation 5 is substituted in a third column to solve for F/F_0.

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right)\frac{[host]_0 K}{(1+ [host]_0 K)} \qquad \text{Equation 5}$$

That equation contains two unknowns which are the binding constant K and $\left(\frac{F_\infty}{F_0} - 1\right)$ which can be replaced by a variable, AA. Two cells in the spreadsheet can be dedicated for each of those variables and a random number can be inputted. In a fourth column, the difference between F/F_0 obtained from the graph and the F/F_0 obtained from the equation can be calculated for every concentration. Finally, in a fifth column, the square of that difference can be obtained, and the sum of the squared difference should be calculated in a cell. All the calculations can be performed

directly in Excel. Once that is obtained, solver can be opened and the sum of the square difference should be picked as the set objective, the parameters should be set to minimize, and the changing variables cells should be the two cells containing the number of the AA variable and K. Solver will then solve for the closest solutions to the variables to fit the data.

5.2 Binding Study using Excel

The binding constants for every trial of both the inclusion of 2,6-ANS with 2,6-DM-β-CD and 2,6-TNS with α-cyclodextrin were obtained using Excel and solver as described above. The average of each temperature was then calculated. The results were then compared to the results obtained with Cdeqwin.exe. The difference between the average binding constants and the percent difference between those two methods were also calculated for each temperature. Table 5.1 shows the results for the inclusion of 2,6-ANS with 2,6-DM-β-CD.

Table 5.1: Difference of K between Excel and Cdeqwin.exe of 2,6-ANS with 2,6-DM-β-CD

Temperature	Average K using Cdeqwin.exe (M^{-1})	Average K using Excel (M^{-1})	% Difference
25 ℃	5470	5100	6.8
45℃	4830	4560	5.6

As can be observed, the binding constant obtained with Excel are smaller compared to the ones obtained with Cdeqwin.exe. Due to the high magnitude of the binding constant of the inclusion of 2,6-ANS with 2,6-DM-β-CD, ΔK might appear to be significant but after obtaining the percent difference, the difference can be said to be small enough that it doesn't cause inaccuracy of the results as binding constants can only be accurately measured within a 10% range. Similarly, Table 5.2 shows the results of the inclusion of 2,6-TNS with α-cyclodextrin.

Table 5.2: Difference of K between Excel and Cdeqwin.exe of 2,6-TNS with α-cyclodextrin

Temperature	Average K using Cdeqwin.exe (M^{-1})	Average K using Excel (M^{-1})	% Difference
8°C	35	33	5.7
11°C	26	25	3.8
15°C	42	40	4.8
20°C	31	31	0.0
25°C	40	38	5.0
35°C	31	30	3.2
45°C	26	25	3.8

After acquiring the binding constant for the inclusion of 2,6-TNS with α-cyclodextrin using Excel and the program Cdeqwin.exe used in the Wagner lab, for all the trials at every temperature, the same observation as for the inclusion of 2,6-TNS and 2,6-DM-β-CD can be made regarding the magnitude of the binding constant as it was observed that each time, the binding constants obtained with Excel were a little bit lower than the ones obtained from Cdeqwin.exe. Even though ΔK is significantly smaller in Table 5.2 compared to Table 5.1, the percent difference is in the same range and is below 10 % for each temperature.

As the binding constants are very similar, the results demonstrate that the binding constant can be solved using this method with Excel with enough accuracy compared to Cdeqwin.exe. Using Excel has the advantages that it can be easily obtained on any computer at any time, compared to Cdeqwin.exe which can only be used in the Wagner lab, on a very old computer that may fail at any time. This makes Excel a good alternative in case Cdeqwin.exe was not available, for instance if the computer on which this program is stopped working.

Chapter 6 Conclusion and future work

Regarding the first goal of this project including 2,6-DM-β-CD and 2,6-ANS, as the binding constant data was only acquired at two temperatures due to the unavailability of the host and guest commercially, the thermodynamic constants, specifically the enthalpy and entropy were not successfully obtained. However, good results were obtained at 25 ℃, showing a very high binding constant of 5460 M^{-1}. This shows that between 2,6-ANS and 2,6-DM-β-CD, there is a very strong inclusion complexation being formed. A non-linear trend on the double-reciprocal plot was observed at lower temperature but could also occur at lower concentrations as the double-reciprocal plots indicated.

After performing fluorescence titrations on the host:guest inclusion of 2,6-TNS and α-CD, it was determined that due to the small magnitude of the binding constants, which ranged from 26 to 42 M^{-1} as well as the similarities in the binding constant values between the different temperatures, the study could not be concluded with accurate results. This was observed through the obtention of a non-linear, scattered van't Hoff plot. Out of curiosity, the enthalpy and entropy for the scattered plot was calculated to be -3.56 kJ mol^{-1} and 16.9 J K^{-1} mol^{-1} respectively. Those values are to be considered inaccurate and were obtained as an overall indication of the inclusion. It was concluded that inclusion was more likely to be exothermic due to the negative enthalpy obtained from the plot linear fit equation, and that the order decreased due to the positive entropy. The Gibbs free energy was then calculated for the seven temperatures. The ΔG was found to be negative meaning that the inclusion is spontaneous.

Using Excel to obtain the binding constants was concluded to be a good alternative to the use of the program named Cdeqwin.exe in the Wagner lab as the percent difference between the

two program was calculated to be less than 10% which is a range under which the results are deemed accurate.

6.1 Future work

Future work would include performing a full thermodynamic study on 2,6-ANS and heptakis(2,6-di-O-methyl)-β-cyclodextrin as well as with 2,6-TNS and 2,6-DM-β-CD. The binding constant for the inclusion of 2,6-ANS and 2,6-DM-β-CD were significantly large which would therefore be a good inclusion pair to study as such a strong interaction is present. Such a study could also result in the clarification of the presence of a different complexation than a 1:1 inclusion complex as more trials and temperatures would be obtained. Performing a fluorescent titration of this host:guest inclusion at a different temperature than 15 ℃ but lower than 25 ℃ would allow for the determination if a lower temperature affects the number of hosts and guests involved in the inclusion complexation.

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