Potential of PRODAN derivatives as chemosensors of the microacidity of cyclodextrin host-guest complexes

Hannah R. Naughton
College of William and Mary

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Potential of PRODAN derivatives as chemosensors of the microacidity of cyclodextrin host-guest complexes

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science with Honors in Chemistry from the College of William & Mary in Virginia,

by

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Accepted for (Honors)

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Williamsburg, Virginia
May 2012
Potential of PRODAN derivatives as chemosensors of the microacidity of cyclodextrin host-guest complexes

Hannah Rose Naughton

May 10, 2012
Abstract

Fluorescent chemosensors facilitate the characterization of materials and biological systems. Cyclodextrin (CD), a conical sugar oligomer with a hydrophobic interior and exterior hydroxyl groups, is water-soluble and presents a binding site for fluorescent probes such as PRODAN (6-propionyl-2-dimethylaminonaphthalene). The quenching of PRODAN-based probes occurs as their environment is better able to donate hydrogen bonds, an effect which is enhanced by a twisted conformation of the carbonyl group of the probe. After titrating six structurally distinct probes with β-CD, emission spectra were analyzed for binding constants, maximum increase of fluorescence quantum yield, and effective solvent acidity of the β-CD environment. Probes with twisted conformations give an approximately twenty-fold increase in maximum quantum yield and may bind more strongly to cyclodextrin. While the ideal sensor for microacidity should have increased response to changing environment, the increase should not come at the expense of the range of detectable solvent acidities.
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Acknowledgements

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Chapter 1

Introduction

1.1 Chemosensors

Chemical sensors (chemosensors) are molecules with observable properties that change in a quantifiable manner in response to a change in the environment [1]. The environment encompasses the solvent, other molecules, and physical conditions such as temperature or pH. Fluorescent chemosensors especially draw attention for their capacity to characterize the properties of materials and biological systems [2].

The chemosensors studied in this project are derivatives of the fluorescent molecule Prodan, and were previously synthesized by the Abelt lab. These sensors are manipulated to selectively detect changes in micropolarity, microbasicity and microacidity, also known as hydrogen-bonding capability, in their immediate environment [3, 4]. They will be interchangeably referred to as probes. It is noteworthy, however, that any chemosensor may respond to four solvent parameters: microacidity (SA), microbasicity (SB), polarizability (SP), and dipolarity (SdP) [5]. Comparison of a pair of probes with one uncharacterized solvent effect parameter allows for the calibration of that parameter. [3].

These chemosensors’ information lies in their fluorescence spectra. Electromagnetic radiation at a specific wavelength, usually within the blue region (with differences depending
on the solution composition), hits the molecule, excites an electron, and is either given off at a different wavelength after reorientation of the molecule in its excited state or lost to the environment through nonradiative pathways [6]. This phenomenon of changing emission wavelength with changing solvent characteristics, polarity in particular, is known as solvatochromism. A schematic of the transitions described is shown in Fig. 1.1. The left-hand green arrow shows resonance fluorescence, for which the energy of photons absorbed is equal to that of photons emitted. The right-hand green arrow shows Stokes-shifted fluorescence. Radiative transfers (ie. emission and absorption) are depicted as straight lines and non-radiative transfers are depicted as squiggly lines.

**Figure 1.1:** Theoretical Jablonski diagram indicating energy transfer possibilities.

### 1.2 Prodan

Prodan, (6-Propionyl-2-(dimethylamino)naphthalene,) was initially synthesized in 1979 as an environmentally sensitive fluorescent probe. [7].

#### 1.2.1 History of Prodan and its analytical potential

The structure of Prodan satisfies the requirements of an environmentally sensitive fluorescent probe: mainly, to have an electron donor and an electron acceptor separated with greatest
distance possible from one another by an aromatic ring system [7]. Prodan has an alkylamino group as a donor ideally separated over the naphthalene structure from the carbonyl group, one of the best charge acceptors (see Figure 1.2). Prodan exhibits a red shift—a decrease in energy of its maximum emission peak—when studied in solvent systems of increasing polarity. During the charge transfer between the donor and acceptor groups in Prodan, the dipole moment of the excited state molecule is enhanced relative to that of the ground state due to the partial charge that develops across the molecule. A partial positive charge develops between the amino nitrogen and naphthalene core and a partial negative charge is delocalized over the carbonyl group in the excited state. Polar solvents stabilize the excited state dipole, which gives way to a Stokes shift for which the energy emitted is less than that absorbed. It is this phenomenon that makes Prodan a suitable probe of solvent polarity.

![Prodan PICT excited state](image)

**Figure 1.2:** Diagram of theoretical planar intramolecular charge transfer state for Prodan.

In addition to the wavelength; the intensity of the emission peak of Prodan also changes as the probe experiences different environments. Specifically, Prodan’s fluorescence is increasingly quenched to a limit as the solvent system increases in polarity for aprotic solvents and as it increases in hydrogen bonding capability for protic solvents such as alcohols [8]. The ability of the solvent to donate H-bonds is calculated using Catalán’s solvent acidity parameter [5]. This could be measured as quantum yield, or the ratio of molecules un-
dergoing photoreactions to the number of photons absorbed [6]. For the purposes of this experiment, however; quantum yield is not as informative as a comparison of normalized fluorescence counts between probes in solutions of different solvent acidity and dipolarity (see equation B.0.0.5 and preceding derivations).

Because the solvatochromic response of Prodan is primarily incurred by changes in solvent polarity and the quantum yield is primarily affected by quenching via hydrogen bonding protic solvent environments; Prodan and select derivative compounds should make good chemosensors capable of teasing apart the effects of micropolarity and microacidity [3].

Prodan gained popularity for use in fluorimetric assays of various properties of a solution or of a specific protein throughout the late 20th century [9][10][11]. Initially, Prodan was most popularly utilized as an LDL-cholesterol mimic. Due to its nonpolar naphthalene core, lack of charge and comparable size to cholesterol; Prodan binds human serum albumin (HSA) in a similar manner to the natural steroid. Resonance energy transfer between the probe and two fluorescent amino acids in the protein’s binding sites made the probe ideal for a variety of fluorescence assays, most notably drug binding competition studies [12, 13]. We propose the use of structurally modified Prodan probes for analysis of chemical properties of solutions and organized media.

1.2.2 Structural modifications to Prodan

Prodan is an ideal chemosensor for multiple reasons. It is fairly simple to synthesize, as outlined by Weber & Farris, and can respond to its environment with a broad range of emission wavelengths, redshifting from 401 nm in cyclohexane to 531 nm in water. This broad range, along with its neutral charge and decent solubility in aqueous systems, make Prodan and Prodan-derivatives ideal probes of changing environments, physiological fluids in particular [14]. The Abelt lab has been interested in increasing the efficiency of charge transfer by changing the planarity of the Prodan molecule [15]. For structurally twisted compounds, the change in dipole moment of the excited state increases relative to the ground
state, thus maximizing Prodan's photophysical response to increasingly polar environments.

As already mentioned, Prodan responds to micropolarity. This occurs by interaction between the partial dipole of the intramolecular charge transfer (ICT) excited state and the local solvent molecules. However, the probe could take on various excited-state structures to accomplish this ICT state, two of which structures include out-of-plane twisting of either the N-containing or carbonyl containing side groups [16]. Emissions for Prodan and for related probes locked into various planar structures (Prodan, 1 and 2) showed similar solvent-dependent solvatochromism to probes with a forced twisted conformation (3 and 4; see structures of the six probes analyzed in Fig. 1.3) [8]. Thus, the excited state structure is independent of the probe geometry [15]. However, Prodan and 7 fluoresced a magnitude more than carbonyl-twisted 4 and 3. This result opened the door for the use of Prodan-based chemosensors as probes of microacidity.

**Figure 1.3:** Structures of six probes studied. Variations of molecular geometry are indicated.
1.3 Prodan-β-Cyclodextrin Probes

Cyclodextrins are naturally occurring, water-soluble sugars (see figure 1.4. They are conical oligomers of six, seven or eight α-D-glucose rings (α-, β-, and γ-CD, respectively). A well-defined hydrophobic interior facilitates formation of non-covalent inclusion complexes with organic substrates that are not soluble or hardly soluble in aqueous solution.

![Diagram of cyclodextrin structure with dimensions](image)

<table>
<thead>
<tr>
<th></th>
<th>α-CyD</th>
<th>β-CyD</th>
<th>γ-CyD</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>$d_1^0$</td>
<td>4.7</td>
<td>6.0</td>
<td>7.5</td>
</tr>
<tr>
<td>$d_2^0$</td>
<td>5.3</td>
<td>6.5</td>
<td>8.3</td>
</tr>
</tbody>
</table>

**Figure 1.4:** Structure and dimensions of three cyclodextrin forms.

Sugar hydroxyl groups on the larger rim of cyclodextrin are capable of covalently binding an organic fluorophore [17]. Binding of Prodan and its derivatives theoretically creates supramolecular cyclodextrin chemosensors. Fluorescence spectra reveal the placement of the fluorophore as within the CD core (preferable) or outside in solution. Once a given probe-β-CD complex’s behavior is characterized in a specific solvent system and the probe is known to have inserted within the hydrophobic CD cavity, the complex can be used to quantify other solutes by competitive binding experiments.

This project covers the initial step of characterizing the binding of Prodan and Prodan-like probes to β-CD. Effective solvent acidity experienced by the probe tells whether the probe has inserted within the cavity or whether it has remained in solution once binding cyclodextrin. Binding constants and chemosensor complex sensitivity to SA and SdP can be
determined by adding $\beta$-CD aliquots to a solution of probe and then analyzing the emissions spectra. For further information on how absorbance and fluorescence spectra were analyzed to obtain meaningful results, please refer to Appendices A and B.
Chapter 2

Experimental

2.1 Instrumentation

Absorbance readings were taken using an OceanOptics UV/Vis spectrophotometer and detector. Fluorescence readings were taken using an OceanOptics fluorimeter and SLM-Aminco SPF-500 spectrometer as the excitation source. A rectangular quartz cuvette was used to contain samples with one side marked to always face the light source.

2.2 Preparation of standard solutions for study

Standard solutions were created by dissolving approximately 5 mg of solid in methanol in 10 mL volumetric flasks. Final concentrations of Prodan, 1, 2, 3, 4, and 5 were 5.1, 6.2, 5.7, 5.9, 6.0, and 6.6 mg/mL. All solvents used were ≥ 99.5% pure spectrophotometric grade straight from the bottle except for ethylene glycol, which was vacuum distilled in house to remove impurities. Solvents were purchased from Sigma-Aldrich.

β-Cyclodextrin was available in lab from previous experiments and had been purified by two recrystallizations. Stock solution was prepared by dissolving 0.500 g of solid β-CD in 25 mL of water in a volumetric flask. Final solution was 0.017 M.
2.3 Calibration of the six probes

Two methods were used to calibrate probes based on their emissions in solvent systems of varying hydrogen bonding capability. Prodan and probes 1, 2, and 3 were analyzed over a spectrum of polar, protic solvents including 1-methanol, 1-ethanol, 1-propanol, 2-propanol, 1-butanol, 1,2-ethanediol and 1,2-propanediol. The latter two solvents were removed from the study because they gave anomalous results, perhaps due to high viscosity. Mixtures of solvents were used to create a more even representation of solvent acidities. These mixtures included 75% and 25%, 50% and 50%, and 25% and 75% water with methanol, and 50% methanol with 50% ethanol.

Probes 4 and 5 were calibrated using a water-isopropanol gradient to mimic solvents of intermediate microacidities. Measurements were made starting with pure isopropanol and working towards pure water in 10% increments.

Data was converted from TabDelimited format to Microsoft Excel. Output from absorbance measurements gave values for light absorbed over a spectrum of light source wavelengths. Output from emission measurements gave intensities of fluorescence in counts per minute (an arbitrary unit of intensity) for a spectrum of wavelengths.

Raw emissions spectra were first fixed for absorbance and normalized to the maximum intensity reading. Linear plots were then constructed that allowed for extrapolation of various properties of the given probe (see Figure 3.1).

2.3.1 Spectrum of solvents

Two mL of isopropanol were added to cuvette by 2 mL volumetric pipet. 10 μL of Prodan solution was injected by microsyringe and cuvette was capped. Sample was stirred with micro stirbar, no heat, for 2 mins. The cuvette was tapped to remove bubbles. Absorbance and fluorescence emissions were recorded. For absorbance measurements, a dark reading,

\footnote{Experiments and analyses carried out in the spring and summer of 2011 as preliminary work by H.R. Naughton, Z.B. Nealy, C.J. Abelt, and T. Sun.}
reference reading (solvent alone) and sample reading were taken for each solvent used. For fluorescence measurements, a dark and a sample reading were taken. Dark readings were done by inserting folded notecard in the case of the absorbance spectrophotometer, or aluminum-wrapped notecard for the fluorimeter, in front of the detector. Between runs cuvette and cap were cleaned by acetone and house vacuum to remove extra liquid. The same procedure was then used for other solvents in order of decreasing polarity.

Similar procedures were followed for all six probes. Instrument acquisition parameters and injection sizes were optimized for each probe.

### 2.3.2 Bi-solvent gradient

Two mL of isopropanol were added to cuvette by 2 mL volumetric pipet. 10 µL probe 4 solution was injected by microsyringe and cuvette was capped. The sample was stirred with micro stirbar for 2 mins without heat, then tapped to remove bubbles. Measurements were repeated in same manner as for spectrum of solvents. The next sample was prepared by adding 1.80 mL isopropanol and 0.20 mL water to clean cuvette using graduated pipet. Probe was inserted by microsyringe. Sample was stirred until solvents appeared mixed, noted as lack of film or streaks of undissolved alcohol, and threads of probe were not visible. Following samples were prepared in similar manner by adjusting the volume of each solvent by 0.20 mL until pure water was used. Measurements were repeated for probe 5.
Table 2.1: Solvent acidities as previously determined [5] or calculated as an average weighted by volume ratio.

<table>
<thead>
<tr>
<th>Solvent (for solvent spectrum)</th>
<th>Solvent acidity</th>
<th>Solvent (% 2-Propanol/% Water)</th>
<th>Solvent acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Propanol</td>
<td>0.283</td>
<td>2-Propanol</td>
<td>0.283</td>
</tr>
<tr>
<td>Water</td>
<td>1.062</td>
<td>90% 2-Propanol/10% Water</td>
<td>0.361</td>
</tr>
<tr>
<td>75% Water/ 25% Methanol</td>
<td>0.948</td>
<td>80/20</td>
<td>0.439</td>
</tr>
<tr>
<td>50% Water/ 50% Methanol</td>
<td>0.834</td>
<td>70/30</td>
<td>0.517</td>
</tr>
<tr>
<td>25% Water/ 75% Methanol</td>
<td>0.719</td>
<td>60/40</td>
<td>0.595</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.605</td>
<td>50/50</td>
<td>0.672</td>
</tr>
<tr>
<td>50% Methanol/ 50% Ethanol</td>
<td>0.503</td>
<td>40/60</td>
<td>0.750</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.400</td>
<td>30/70</td>
<td>0.828</td>
</tr>
<tr>
<td>Propanol</td>
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<td>20/80</td>
<td>0.906</td>
</tr>
<tr>
<td>Butanol</td>
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<td>10/90</td>
<td>0.984</td>
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<td>1,2-Ethanediol</td>
<td>0.717</td>
<td>Water</td>
<td>1.062</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>0.475</td>
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<td></td>
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2.4 Binding studies of six probes with $\beta$-cyclodextrin

The probe was injected by microsyringe into 2 mL water in a cuvette. The injection size was determined based on concentration needed to produce an absorbance of 0.2, as determined from solvent calibrations. Absorption and emission experiments were carried out as previously described. Incrementally increasing volume injections of $\beta$-CD solution were added to cuvette as detailed in Table 2.2. Each sample was stirred for one minute before being analyzed. See Appendix A for details regarding the Excel workup of spectral data. Appendix A and B describe further calculations performed on Microsoft Excel and on Easy-Fit Express.
Table 2.2: \( \beta \)-CD Addition volumes for binding studies

<table>
<thead>
<tr>
<th>( \mu L ) ( \beta )-CD added</th>
<th>Total ( \mu L ) ( \beta )-CD</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>5.0</td>
<td>25.0</td>
</tr>
<tr>
<td>5.0</td>
<td>30.0</td>
</tr>
<tr>
<td>5.0</td>
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<tr>
<td>5.0</td>
<td>40.0</td>
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<td>10.0</td>
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<td>50.0</td>
<td>250.0</td>
</tr>
<tr>
<td>100.0</td>
<td>350.0</td>
</tr>
</tbody>
</table>
Chapter 3

Data and Results

3.1 Preliminary Characterization of Probe Sensitivity to Solvent Acidity

The fluorophores respond to changing solvent conditions differently based on their structure. Response to solvent acidity was studied by measuring the emissions intensity in progressively better H-bond donor solvents. Figure 3.1 shows plots of the log of emissions in a given solvent (normalized by division by the emissions of the sample giving the highest intensity, generally the isopropanol sample) versus the SA of that solvent. This plot gives a slope whose magnitude describes the probe’s sensitivity to changing solvent acidity. Solvent acidity is varied by changing isopropanol-water fractions for 4 and 5 or by changing the protic alcohol used as solvent for Prodan and 1-3. Larger slopes signify larger response to changes in solvent acidity. The three structurally twisted probes have similar slopes on the order of four times larger than the slopes for the structurally planar or uncontrolled probes.
Figure 3.1: Plots of log(fluorescence relative to maximum) against solvent acidity for each probe.

### 3.2 Prodan-β-CD

Prodan absorbance and emission spectra are shown in Figures 3.2 and 3.3. For all absorbance and emissions spectra displayed, series labels refer to measurements of the probe alone in water (series 1), followed by subsequent titrations with β-CD in the following addition sizes: 20, 25, 30, 35, 40, 50, 60, 70, 80, 100, 120, 140, 160, 200, 250, and 350 µL. Series 18 and 19 refer to measurements made of the probe alone in methanol and isopropanol, respectively, in order to use as reference points for subsequent calculations (see Appendix C). Procedures were carried out as described in the Experimental section.
The absorbance spectra are relatively clean for Prodan. They show a redshift once cyclodextrin is added. Upon binding β-CD, the fluorophore experiences an increased micropolarity which stabilizes its excited state. Later additions of cyclodextrin do not change...
the absorbance spectra, signifying that the supramolecular complex is formed immediately and that it involves the majority of the probe molecules.

The fluorescence spectra show the climbing intensity and energy of emissions as β-CD is added to the Prodan solution. This reflects the decreasing polarity and acidity of the environment immediately surrounding the chemosensor complexes. Important to note are the emissions of the probe in methanol (orange line) and in 2-propanol (lilac line). The complex emission is highly quenched in water relative to these two solvents with less hydrogen bonding tendency.

Results from Excel double reciprocal analysis and from Easy-Fit rectangular hyperbolic analysis are shown in Figures 3.4 and 3.5. The double reciprocal analysis yields a binding constant for the probe to the cyclodextrin, a ratio of complex to free probe molar absorptivities, and a ratio of complex to free probe quantum yield. The rectangular hyperbolic analysis similarly yields complex to free probe ratios for both fluorescence and for molar absorptivity and a binding constant. Output values as determined from the two fits, or Figures 3.4 and 3.5 are recorded in Table 3.1. Relevant calculations and derivations appear in Appendices A and B.

![Double reciprocal plot: Prodan- β-CD](image)

**Figure 3.4:** Double reciprocal plot for titration of Prodan with β-CD.
Figure 3.5: Rectangular hyperbolic fit of emission vs. [CD] gives a binding isotherm for titration of Prodan with β-CD.

<table>
<thead>
<tr>
<th>From double reciprocal analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.73</td>
<td>1.83</td>
<td></td>
<td>2140</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.2</td>
<td>0.318</td>
<td></td>
<td>2140</td>
</tr>
<tr>
<td>2 – Propanol</td>
<td>2.3</td>
<td>0.0238</td>
<td></td>
<td>2140</td>
</tr>
<tr>
<td>Average</td>
<td>1.4</td>
<td>n/a</td>
<td></td>
<td>2140</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From rectangular hyperbola binding isotherm analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.76</td>
<td>1.74</td>
<td></td>
<td>3200</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.2</td>
<td>0.302</td>
<td></td>
<td>3200</td>
</tr>
<tr>
<td>2 – Propanol</td>
<td>2.3</td>
<td>0.0226</td>
<td></td>
<td>3180</td>
</tr>
<tr>
<td>Average</td>
<td>1.4</td>
<td>n/a</td>
<td></td>
<td>3190</td>
</tr>
</tbody>
</table>

Table 3.1: Results from double reciprocal and rectangular hyperbolic binding isotherm analyses of Prodan spectral data.

Table 3.1 suggests that Prodan experiences a somewhat high solvent acidity because the average, 1.4, is greater than the SA of water (1.062). It also quantifies the difference in
quantum yield depending on which solvent is used for the baseline measurement. Prodan reports a wide range of solvent acidities depending on which solvent is used as reference. This lack of consensus shows that Prodan is a poor chemosensor of solvent acidity.

### 3.3 Probe 1-β-CD

The absorbance and emission spectra (Figures 3.6 and 3.7) for the addition of β-CD to Probe 1 give a similar pattern to the Prodan plots. The absorbance increases once β-CD is added. The emission intensity increases slightly while the energy significantly increases. The solvent acidities reported all indicate that the fluorophore has entered an environment of lower microacidity. The emission of the complex relative to the probe are similar for all three references used. A binding constant was not possible to calculate from the rectangular hyperbola plot because any starting value used, taken from the double reciprocal analysis, was given back as the most optimal fit. Thus, the complex was not subject to strictly characterizable thermodynamic behavior.

![Probe 1 & β-CD Absorbance](image)

**Figure 3.6:** Absorbance spectra for titration of Probe 1 with β-CD.
Figure 3.7: Emission spectra for titration of Probe 1 with β-CD.

Figure 3.8: Double reciprocal plot for titration of Probe 1 with β-CD.
Figure 3.9: Rectangular hyperbolic fit of emission vs. [CD] gives a binding isotherm for titration of Probe 1 with β-CD.

Table 3.2: Results from double reciprocal and rectangular hyperbolic binding isotherm analyses of Probe 1 spectral data.

<table>
<thead>
<tr>
<th>From double reciprocal analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.87</td>
<td>1.47</td>
<td>2570</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>0.58</td>
<td>1.04</td>
<td>2570</td>
<td></td>
</tr>
<tr>
<td>2-Propanol</td>
<td>0.92</td>
<td>0.278</td>
<td>2570</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.79</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From rectangular hyperbola binding isotherm analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.60</td>
<td>2.49</td>
<td>unresponsive</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>0.45</td>
<td>1.34</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>2-Propanol</td>
<td>0.16</td>
<td>1.29</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.40</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

The fluorescence intensity of 1 does not increase appreciably with addition of β-CD, nor does the probe alone show a good response to changing SA judging from its small slope in
the log(emission) vs. SA workup (see fig. 3.1). This probe should not be a good solvent acidity probe. The behavior seen in the rectangular hyperbolic plot is not understood.

3.4 Probe 2-β-CD

Figures 3.10 and 3.11 show the absorbance and emission spectra for Probe 2. These look similar to those previously shown. The workup from the two graphical analyses gives highly nonuniform solvent acidity values, including negative values. This unpredictability reflects the chemosensor’s unreliable response to the various solvent factors affecting it. A very small slope from the initial calibration of this probe (Figure 3.1) strengthens this explanation for the impossible values obtained in Table 3.3.

![Probe 2 & β-CD Absorbance](image)

**Figure 3.10:** Absorbance spectra for titration of Probe 2 with β-CD.
Figure 3.11: Emission spectra for titration of Probe 2 with β-CD.

Figure 3.12: Double reciprocal plot for titration of Probe 2 with β-CD.
Figure 3.13: Rectangular hyperbolic fit of emission vs. [CD] gives a binding isotherm for titration of Probe 2 with β-CD.

<table>
<thead>
<tr>
<th>From double reciprocal analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>−0.17</td>
<td>5.15</td>
<td>830</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>0.66</td>
<td>0.933</td>
<td>830</td>
<td></td>
</tr>
<tr>
<td>2 – Propanol</td>
<td>1.6</td>
<td>0.173</td>
<td>830</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.70</td>
<td>n/a</td>
<td>830</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From rectangular hyperbola binding isotherm analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>−0.034</td>
<td>12.2</td>
<td>unresponsive</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>−0.011</td>
<td>3.34</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>2 – Propanol</td>
<td>−0.50</td>
<td>2.19</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>−0.18</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3: Results from double reciprocal and rectangular hyperbolic binding isotherm analyses of Probe 2 spectral data.
3.5 Probe 3-β-CD

Probe 3-β-CD gave consistent binding and fluorescence data. The complex absorbed at a slightly higher energy than the free probe. However, complexation did not solvatochromically affect the emissions except for a blueshift upon initial addition of cyclodextrin. This suggests that essentially all of the probe bound with the first addition. A larger binding constant of 5220 supports this interpretation of the spectra.

Figure 3.14: Absorbance spectra for titration of Probe 3 with β-CD.
Figure 3.15: Emission spectra for titration of Probe 3 with β-CD.

Figure 3.16: Double reciprocal plot for titration of Probe 3 with β-CD.
Figure 3.17: Rectangular hyperbolic fit of emission vs. [CD] gives a binding isotherm for titration of Probe 3 with β-CD.

<table>
<thead>
<tr>
<th>From double reciprocal analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>0.54</td>
<td>11.8</td>
<td>5220</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.59</td>
<td>1.46</td>
<td>5220</td>
</tr>
<tr>
<td></td>
<td>2 − Propanol</td>
<td>0.68</td>
<td>0.235</td>
<td>5220</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.60</td>
<td>n/a</td>
<td>5220</td>
</tr>
<tr>
<td>From rectangular hyperbola binding isotherm analysis</td>
<td>Ref. Solvent</td>
<td>Solvent Acidity</td>
<td>Qc/Qref.</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.53</td>
<td>10.5</td>
<td>4890</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.48</td>
<td>1.10</td>
<td>4960</td>
</tr>
<tr>
<td></td>
<td>2 − Propanol</td>
<td>0.32</td>
<td>0.168</td>
<td>5320</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.44</td>
<td>n/a</td>
<td>5060</td>
</tr>
</tbody>
</table>

Table 3.4: Results from double reciprocal and rectangular hyperbolic binding isotherm analyses of Probe 3 spectral data.

This is the first probe for which the probe-β-CD complex had higher fluorescence yield than the free probe in methanol. The Qc/Qref values second this observation. The effective
solvent acidity values fall within a small range. This probe gives a large and reliable signal.

3.6 Probe 4-\(\beta\)-CD

The high absorbance and fluorescence for the free probe and after the first addition of \(\beta\)-CD suggest the formation of a structurally unique complex at these points. Based on previous work with these probes, it is supposed that they form self-aggregates. Further addition of \(\beta\)-CD appears to break up the aggregates of Probe 4 and respond similarly to the other probes with respect to emission yield intensity and energy. The aggregation makes for a non-responsive plateau region in the double reciprocal plot at low \(\beta\)-CD levels. However, this plot again becomes nonlinear at high concentrations of \(\beta\)-CD. Non-covalent complexes of greater than one-to-one cyclodextrin-to-probe stoichiometries are suspected.

A binding isotherm analysis was not performed on Probe 4 because the range for which quenching occurred linearly with cyclodextrin addition was limited and difficult to determine.

![Probe 4 & \(\beta\)-CD Absorbance](image)

**Figure 3.18:** Absorbance spectra for titration of Probe 4 with \(\beta\)-CD.
Figure 3.19: Emission spectra for titration of Probe 4 with β-CD.

Figure 3.20: Double reciprocal plot for titration of Probe 4 with β-CD.
3.7 Probe 5-β-CD

Probe 5 absorbance and emission spectra show no surprises. Once again, the 5-β-CD complex had a quantum yield on par with that of 5 alone in methanol. However, the enhancement of fluorescence yield in water was less than twice as great as that in methanol. Solvent acidities were very close with methanol and isopropanol reference points. In general, SA’s showed that the probe entered the cavity of the sugar oligomer.

![Absorbance spectra for titration of Probe 5 with β-CD.](image)

**Figure 3.21:** Absorbance spectra for titration of Probe 5 with β-CD.
Figure 3.22: Emission spectra for titration of Probe 5 with β-CD.

Figure 3.23: Double reciprocal plot for titration of Probe 5 with β-CD.
Figure 3.24: Rectangular hyperbolic fit of emission vs. [CD] gives a binding isotherm for titration of Probe 5 with β-CD.

<table>
<thead>
<tr>
<th>From double reciprocal analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>0.78</td>
<td>4.67</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.44</td>
<td>2.77</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>2 – Propanol</td>
<td>0.47</td>
<td>0.314</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.56</td>
<td>n/a</td>
<td>1310</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>From rectangular hyperbola binding isotherm analysis</th>
<th>Ref. Solvent</th>
<th>Solvent Acidity</th>
<th>Qc/Qref.</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>0.76</td>
<td>5.10</td>
<td>1110</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.42</td>
<td>3.25</td>
<td>937</td>
</tr>
<tr>
<td></td>
<td>2 – Propanol</td>
<td>0.45</td>
<td>0.350</td>
<td>994</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.54</td>
<td>n/a</td>
<td>680.</td>
</tr>
</tbody>
</table>

Table 3.5: Results from double reciprocal and rectangular hyperbolic binding isotherm analyses of Probe 5 spectral data.
Chapter 4

Conclusions

4.1 Interpreting the spectra

Similarity of peak intensities of $\beta$-CD chemosensor in water and those of free probe in less acidic (and therefore, less quenching) solvents signifies that the chemosensor complex has an enhanced signal relative to the probe alone water. Enhanced signal was part of the goal in constructing Prodan derivatives with structural torsion. To this end, all three twisted probes (3-5) performed the best, 3 in particular. This is seen both in the emission spectra and in Tables 3.4 and 3.5. Emissions for 3 increased by an order of magnitude when complexed and by half an order of magnitude for 5. Prodan and 1 only showed an increased fluorescence of 1.5 to 2.5 times when complexed with $\beta$-CD. 2 had arguably decent signal when complexed, but the data for this probe were extremely scattered and therefore unreliable. In searching for a chemosensor with high signal in aqueous systems, 3 was most promising.

Qualitative review of the solvatochromic behavior of the probes should reveal how the fluorophore places itself relative to the CD molecule. Maximum emission wavelength for planar probes blue-shifted with additional additions of $\beta$-CD. For twisted probes, wavelength of maximum emission either did not change or else red-shifted upon further addition of $\beta$-CD. The red-shift likely corresponded to binding of additional probe, which would then be
stabilized by placement within the hydrophobic beta-core. Solvent acidities between 0.32 and 0.78 for the twisted structures’ complexes supports this hypothesis. Binding of the planar probes to β-CD, conversely, appears to increase the emission energy, resulting in blue-shifted emissions. According to the average solvent acidity experienced by Prodan-β-CD, the Prodan fluorophore had not inserted into the cavity because the solvent acidities recorded were larger than that of water. However, 1 appeared to have inserted, judging by solvent acidity readings. This result begs the question of whether spectral shifts and effective solvent acidities can be generalized to explain the behavior of one type (planar or twisted) of probe.

Calculated binding constants indicated that 3 bound most strongly to β-CD at K ~ 5,000. Prodan and 1 followed at ~ 2,500. Probe 5 came in fourth with a binding constant of ~ 1,100. Rectangular hyperbolic binding isotherms did not optimize binding constant for different specified starting values for probes 1 and 2. This suggests that the probe response to changes in solvent acidity was not large enough to reliably quantify, especially for 2.

Conveniently, the most strongly bound supramolecular sensors were also those with the most enhanced fluorescent signal in water. This amplification of signal could be useful for studies detecting small insoluble molecules in aqueous systems such as blood serum. The most successful chemosensors were those containing 3 and 5. Though 4 would theoretically behave similarly due to its out-of-plane carbonyl, other complications prevented it from making a viable probe. Planar probes both gave nonsensical solvent acidity values and bound less strongly. This would restrict the binding studies to smaller concentrations of competing solute, which would lessen the reliability of the results due to low signal to noise from the spectroscopic methods. Thus, structurally twisted Prodan derivatives form the most promising chemosensors when covalently attached to β-CD.
4.2 Considerations for the future

This preliminary research suggests that Prodan derivative complexes with \( \beta \)-CD have good potential to be used as sensors of solvent acidity in aqueous systems. However, further structural modifications to the covalently bound probe could theoretically improve the quantum yield (or signal to noise) to greater than ten-fold that of probe alone in water. In addition, this study could be redone with re-calibrated SA response factors for mixed solvent systems. It turns out that solvent acidities do not add in linear combinations, which we assumed for our calibration of the probes. However, 3,6-diethyl-1,2,4,5-tetrazine was recently synthesized by the Abelt group to be used to empirically determine the solvent acidity for highly acidic organic solvents [18]. Solvent acidities for the mixed solvents used did not significantly differ between the empirically determined values and the values taken as linear combinations of the SA’s of each component.

The next step in this research is to begin studying the sensing abilities of the most promising cyclodextrin chemosensors with various guest molecules. These guests would be small, hydrophobic inorganics such as naphthalene derivatives, cyclic alkanes, and heterocycles. Binding values could be obtained from the chemosensor and compared to the known concentration of guest molecule. These studies would establish the efficacy of the Prodan-derivative-\( \beta \)-CD chemosensor.
Appendix A

Interpreting the linear regression curve from double reciprocal plots

Below is a list of variables (Table A.1). Subscripts of "c" and "f" refer to complex and free probe, respectively. Similarly, subscript of 0 or "ref" refers to the reference reading (before addition of cyclodextrin).

Table A.1: List of variables used in analysis of spectral data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Absorbance</td>
</tr>
<tr>
<td>F</td>
<td>Fluorescence (a.u.)</td>
</tr>
<tr>
<td>ϵ</td>
<td>Molar absorptivity</td>
</tr>
<tr>
<td>ρ</td>
<td>Density</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
</tr>
<tr>
<td>c</td>
<td>Density- and volume-corrected F</td>
</tr>
<tr>
<td>Q</td>
<td>Quantum yield</td>
</tr>
<tr>
<td>K</td>
<td>Binding constant</td>
</tr>
<tr>
<td>b</td>
<td>y-Intercept</td>
</tr>
<tr>
<td>m</td>
<td>Slope</td>
</tr>
</tbody>
</table>

Equations representing the fluorescence normalized by absorbance are derived below.

\[ A = A_{\text{free}} + A_{\text{complex}} = \frac{\epsilon_f \rho_f}{V} + \frac{\epsilon_c \rho_c}{V} \]  \hspace{1cm} (A.0.0.1)
\[
\frac{F}{A} = \frac{\frac{\rho_f}{V}[c_f + \frac{c_c K_{(CD)}}{V}]}{\frac{1}{V}(\epsilon_f \rho_f + \epsilon_c K_{(CD)})}
\] (A.0.0.2)

\[
\frac{F}{A} = \frac{(c_f + \frac{c_c K_{(CD)}}{V})}{(\epsilon_f + \epsilon_c K_{(CD)})}
\] (A.0.0.3)

\[
\frac{F_0}{A_0} = \frac{c_f \rho_0}{\epsilon_f \rho_0} = \frac{c_f}{\epsilon_f}
\] (A.0.0.4)

With this description, a meaningful interpretation of the \(y\)-intercept and slope from a double reciprocal plot can be derived.

\[
\frac{F}{A} - \frac{F_0}{A_0} = \frac{c_f + c_c K_{(CD)}}{\epsilon_f + \epsilon_c K_{(CD)}} - \frac{c_f}{\epsilon_f}
\] (A.0.0.5)

Multiplying the right-hand expression by \(\epsilon_f / \epsilon_f\) gives the following expression, after canceling an \(\epsilon_f c_f\) term:

\[
\frac{F}{A} - \frac{F_0}{A_0} = \frac{K_{(CD)}(\epsilon_f c_c - \epsilon_c c_f)}{\epsilon_f(\epsilon_f + \epsilon_c K_{(CD)})}
\] (A.0.0.6)

Then,

\[
\frac{1}{\frac{F}{A} - \frac{F_0}{A_0}} = \frac{\epsilon_f^2}{(\epsilon_f c_c - \epsilon_c c_f)K_{(CD)}} + \frac{\epsilon_f \epsilon_c}{\epsilon_f c_c - \epsilon_c c_f}
\] (A.0.0.7)

Equation A.0.0.7, once parced out, is in the form of a linear regression where the inverse increase in fluorescence of sample relative to the reference reading is plotted against the inverse concentration of cyclodextrin. This is convenient because the dividend of \(b/m\) is then the binding constant for the \(\beta\)-CD-probe complex, normalized for change in molar absorptivity.
between the complex and the free probe.

\[
m = \frac{\epsilon_f^2}{(\epsilon_f \epsilon_c - \epsilon_c \epsilon_f)K} \tag{A.0.0.8}
\]

\[
b = \frac{\epsilon_f \epsilon_c}{\epsilon_f \epsilon_c - \epsilon_c \epsilon_f} \tag{A.0.0.9}
\]

\[
\frac{b}{m} = K \cdot \frac{\epsilon_c}{\epsilon_f} \tag{A.0.0.10}
\]
Appendix B

Derivation of equations for binding isotherm analysis

\[
\frac{F_{\text{ref}}}{A_{\text{ref}}} = \frac{c_{\text{ref}}}{\epsilon_{\text{ref}}} \quad (B.0.0.1)
\]

\[
\frac{F}{A} = \frac{c_f + c_c K[CD]}{\epsilon_f + \epsilon_c K[CD]} \quad (B.0.0.2)
\]

\[
\frac{F}{F_{\text{ref}} A_{\text{ref}}} = \frac{c_{\text{ref}} c_f + c_c K[CD]}{c_{\text{ref}} \epsilon_f + \epsilon_c K[CD]} = \frac{c_f + (c_c c_{\text{ref}}) K[CD]}{\epsilon_f + (\epsilon_c c_{\text{ref}}) K[CD]} = \frac{c_1 + c_2 K[CD]}{\epsilon_1 + \epsilon_2 K[CD]} \quad (B.0.0.3)
\]

\[
\frac{F}{F_{\text{ref}} A_{\text{ref}}} - 1 = \frac{c_f + (c_c c_{\text{ref}}) K[CD] - c_f - (\epsilon_c \epsilon_{\text{ref}}) K[CD]}{\epsilon_f + (\epsilon_c \epsilon_{\text{ref}}) K[CD]} \quad (B.0.0.4)
\]

When the concentration of \(\beta\)-CD is large, the following simplification is found:

\[
\frac{F}{F_{\text{ref}} A_{\text{ref}}} \approx \frac{c_c}{\epsilon_c} \frac{c_{\text{ref}}}{\epsilon_{\text{ref}}} = \frac{c_2}{\epsilon_2} \quad (B.0.0.5)
\]
\[
\frac{F}{A} = \frac{1 + \frac{c_2}{c_1} K[CD]}{\frac{c_1}{c_1} + \frac{c_2}{c_1} K[CD]} \quad (B.0.0.6)
\]

Let \( \frac{c_2}{c_1} = f_1 \) and \( \frac{c_2}{c_1} = f_2 \). Then, \( L = \text{limiting} \frac{F}{A_{\text{ref}}} = \frac{c_2}{c_2} \).

\[
e_1 = \frac{c_2}{c_1} \frac{f_2}{f_1} = \frac{f_1}{Lf_2} \quad (B.0.0.7)
\]

\[
e_2 = \frac{c_2}{c_1} \frac{f_2}{f_1} = \frac{f_1}{L} \quad (B.0.0.8)
\]

\[
e_1 = \frac{1 + f_1 K[CD]}{\frac{f_2}{L} + \frac{f_1}{L} K[CD]} = L \left( \frac{1 + f_1 K[CD]}{f_1 \left( \frac{1}{f_2} + K[CD] \right)} \right) \quad (B.0.0.9)
\]

\[
\frac{L f_2}{f_1} = \frac{\frac{c_2}{c_1} \cdot \frac{c_2}{c_1}}{\frac{c_2}{c_1}} = \frac{c_1}{e_1} \quad (B.0.0.10)
\]
Appendix C

Calculating effective solvent acidity experienced by probe in complex with $\beta$-CD

Effective solvent acidity experienced by the probe in complex with cyclodextrin can be found from both the double reciprocal plot and the rectangular hyperbola plot. Fluorescence emission response is used to extrapolate effective SA. Equations C.0.0.1 and C.0.0.2 show how emission response is calculated from the double reciprocal and the rectangular hyperbola plots, respectively.

\[
\frac{c_c}{c_{\text{ref}}} = \frac{1}{b} + \frac{c_{\text{water,free}}}{c_{\text{ref,free}}} \quad \text{(C.0.0.1)}
\]

\[
\frac{c_c}{c_{\text{ref}}} = f_1 + \frac{c_{\text{water,free}}}{c_{\text{ref,free}}} \quad \text{(C.0.0.2)}
\]

Solvent acidity is found by one method once the maximum fluorescence increase is found.

\[
\frac{Q_c}{Q_{\text{ref}}} = \frac{c_c}{c_{\text{ref}}} \cdot \frac{\epsilon_{\text{ref}}}{\epsilon_c} \quad \text{(C.0.0.3)}
\]
Because the slope from the initial calibrations (see figure 3.1) measure change in quantum yield of the probe relative to change in solvent acidity, the following calculation is appropriate to determine effective solvent acidity.

\[
SA_{eff} = SA_{ref,sol} - \frac{\log\left(\frac{Q_c}{Q_{ref}}\right)}{m_{calib}}
\]  
(C.0.0.4)
Bibliography


