Ring Structure and Aromatic Substituent Effects on the pKa of the Benzoxaborole Pharmacophore

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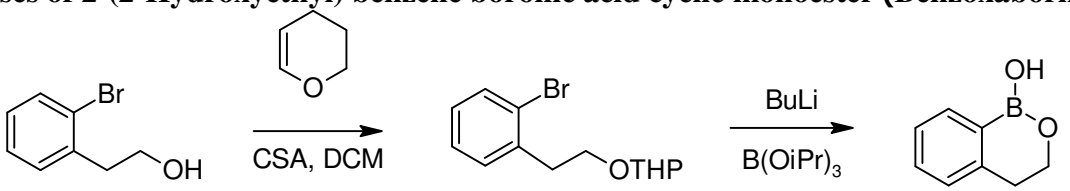
---Supporting Information---

I. Syntheses of 2-(2-Hydroxyethyl) benzene boronic acid cyclic monoester (benzoxaborin, 2)
II. Syntheses of 3,3-Gem-dimethyl-benzoxaborole (3)
III. Spectral data and pKa determination plots
IV. Equations for the 3-component, ARS method of binding constant determination
V. Binding constant determination plots - examples

I. Syntheses of 2-(2-Hydroxyethyl) benzene boronic acid cyclic monoester (Benzoxaborin, 2)



Benzoxaborin (2)

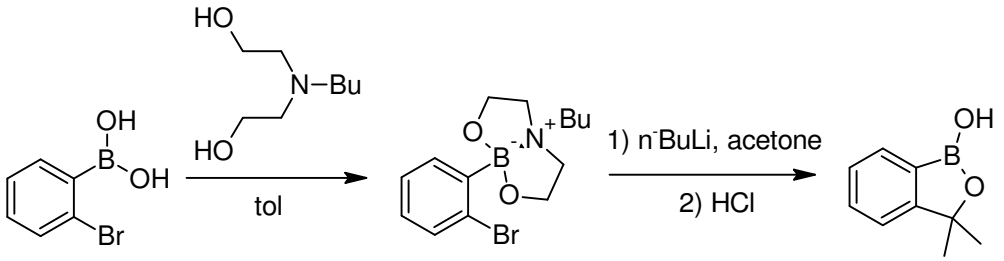
To a solution of 2-(2-Bromo-phenyl)-ethanol (5.0 g, 25.0 mmol, 1.0 eq) in DCM (100 mL) was added 3,4-dihydro-2H-pyran (3.4 mL, 37.5 mmol, 1.5 eq.), followed by camphorsulfonic acid (100 mg). The mixture was stirred at room temperature for 2h. After adding K_2CO_3 (300 mg), the mixture was filtered to remove the precipitate, the filtrate was washed with H_2O (100 mL), brine (100 mL). The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The oily residue was applied to silica chromatography eluting with EtOAc/Heptanes (0:100 to 50:50) to give 2-[2-(2-bromo-phenyl)-ethoxy]-tetrahydro-pyran as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.48 - 7.54 (m, 1H), 7.25 - 7.30 (m, 1H), 7.17 - 7.23 (m, 1H), 7.01 - 7.09 (m, 1H), 4.56 - 4.61 (m, 1H), 3.88 - 3.97 (m, 1H), 3.58 - 3.67 (m, 1H), 3.04 (t, J = 7.2 Hz, 2H), 1.62 - 1.67 (m, 1H), 1.41 - 1.60 (m, 5H), 0.85 - 0.90 (m, 1H)

Amount obtained: 6.9g, 97.1% yield.

To a solution of 2-[2-(2-bromo-phenyl)-ethoxy]-tetrahydro-pyran (1.0 g, 3.5 mmol, 1.0 eq.) in THF (20 mL) at -78 ^{o}C was slowly added BuLi (2.4 mL, 2.5 M solution in THF, 3.8 mmol, 1.1 eq.) under nitrogen atmosphere. Triisopropyl borate (1.2 mL, 5.25 mmol, 1.5 eq.) was then added and the mixture was allowed to warm to room temperature gradually and stirred overnight. After carefully adding HCl (10 mL, 6N), the yellowish solution was stirred at room temperature for another 1h and then poured into a mixture of EtOAc (30 mL) and H_2O (20 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 x 30 mL). Combined organic extracts was washed with H_2O (50 mL), brine (50 mL), dried over MgSO4, filtered and the filtrate was concentrated under reduced pressure. The oily residue was applied to silica chromatography eluting with EtOAc/Heptanes (0:100 to 100: 0) to give 3,4-dihydro-benzo[c][1,2]oxaborinin-1-ol as a light yellow solid. ^{1}H NMR (acetone-d_6) δ: 7.73 (d, J = 7.2 Hz, 1H), 7.33 - 7.39 (m, 1H), 7.15 - 7.26 (m, 3H), 4.10 (t, J = 6.0 Hz, 2H), 2.88 (t, J = 6.0 Hz, 2H).

Amount obtained: 497 mg, 99.3% yield.

II. Syntheses of 3,3-gem-dimethyl-benzoxaborole (GDM, 3)



GDM (3)

To a suspension of 2-bromophenylboronic acid (10.0g, 49.7 mmol) in toluene (70 mL) was added N-butyldiethanolamine (8.5 mL, 52.2 mmol, 1.05 equiv.) via a syringe. The mixture was heated at 50 ^{o}C for two hours. After cooling to room temperature, the toluene was evaporated under reduced pressure and the remaining clear colorless crude oil was treated with heptanes (~ 500 ml). The heptanes mixture was then sonicated ~ 5 min and the resulting suspension was allowed to stand at room temperature overnight. The solid that precipitated was collected by filtration, washed with heptanes, and dried in a vacuum oven overnight to yield a white solid as the titled compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.86 (t, *J*=7.4 Hz, 3 H) 1.14 - 1.25 (m, 2 H) 1.51 - 1.62 (m, 2 H) 2.61 - 2.70 (m, 2 H) 3.01 - 3.11 (m, 2 H) 3.26 - 3.37 (m, 2 H) 4.09 - 4.26 (m, 4 H) 7.10 (td, *J*=7.6, 2.0 Hz, 1 H) 7.24 (td, *J*=7.3, 1.1 Hz, 1 H) 7.51 (d, *J*=7.9 Hz, 1 H) 7.81 (dd, *J*=7.4, 1.9 Hz, 1 H)

Amount obtained, 16.0 g, (98 % yield).

To a solution of 2-(2'-bromophenyl)-6-butyl[1,3,6,2]dioxazaborocan (3.0g, 9.2 mmol) in THF (76 mL) at -78 ^{o}C was added *n*-BuLi (4.4 mL, 2.5M in hexane, 11.0 mmol, 1.2 equiv.) dropwise via a syringe over a period of 10 min while maintaining reaction temperature at -78 ^{o}C. After the addition the reaction solution was stirred 20 min at -78 ^{o}C before acetone (946 μL, 12.8 mmol, 1.4 equiv.) was added dropwise via a syringe over a period of 10 min while maintaining the reaction temperature at -78 ^{o}C. The resulting mixture was allowed to stir for 20 min at -78 ^{o}C then warm to room temperature gradually. Once the reaction vessel reached room temperature, 6M HCl solution (30 mL) was added and the mixture was stirred for 30 min. The mixture was extracted with EtOAc (3X). The EtOAc extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude slightly yellow in color residual oil remaining was then subjected to flash chromatography (Isco Companion, 80g SiO_2 cartridge, solid loaded SiO_2, neat heptane to 20:80 EtOAc gradient at 60 ml/min for 90 min). The product was recovered as clear colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.44 (s, 6 H) 7.31 (d, *J*=1.1 Hz, 1 H) 7.38 - 7.47 (m, 2 H) 7.66 (d, *J*=7.2 Hz, 1 H) 8.99 (s, 1 H).

Amount obtained: 1.76 g (61%).

III. Spectral Data and pKa Determinations

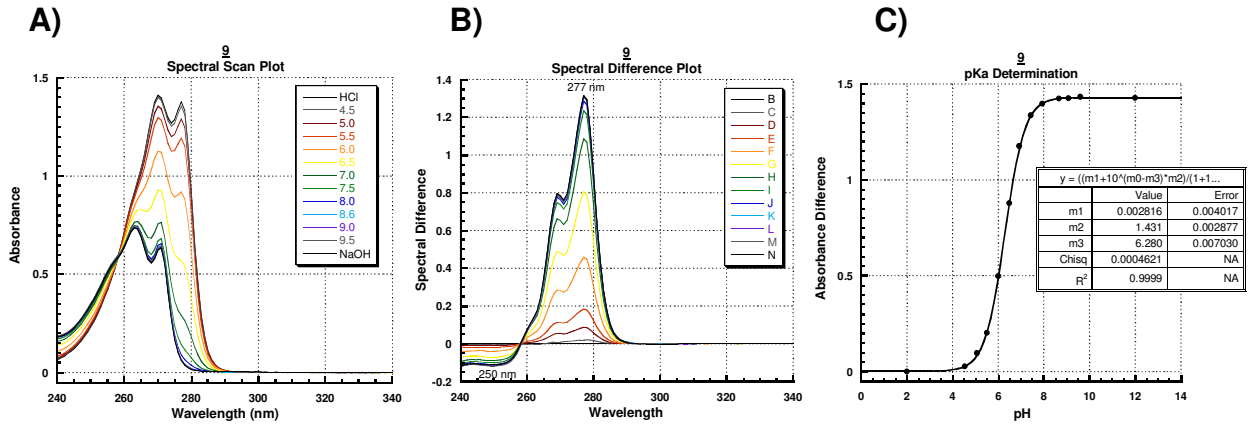
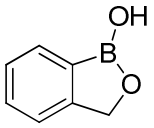
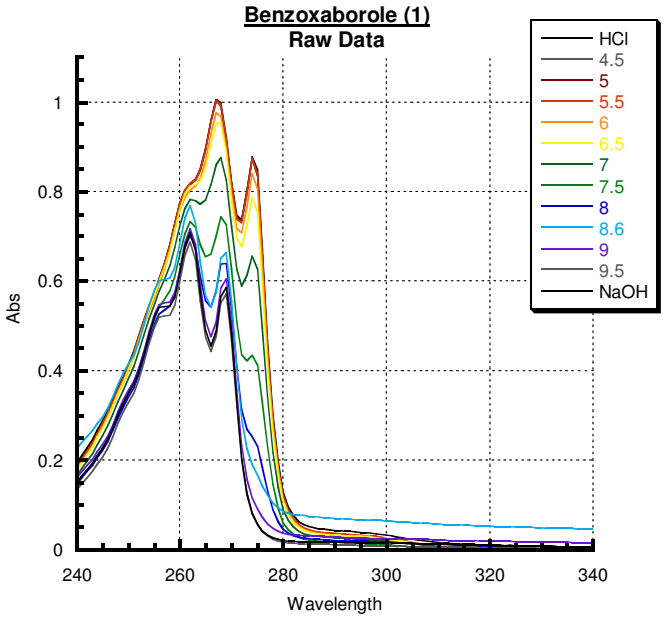
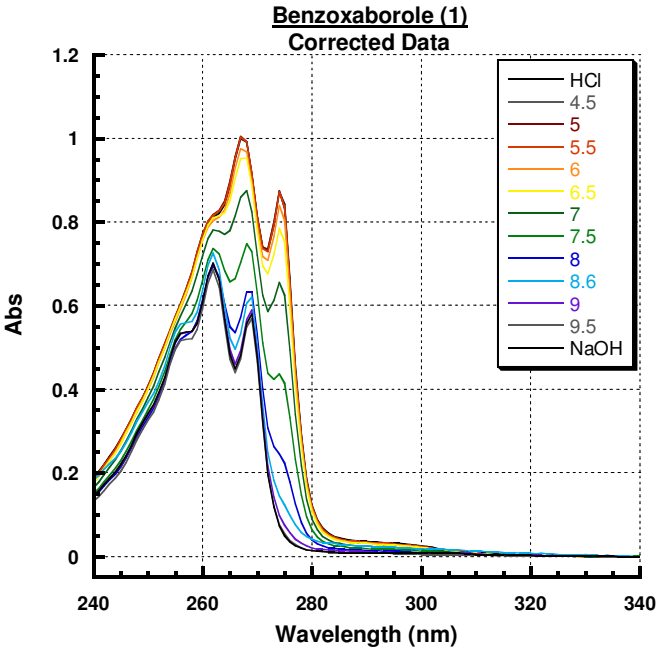


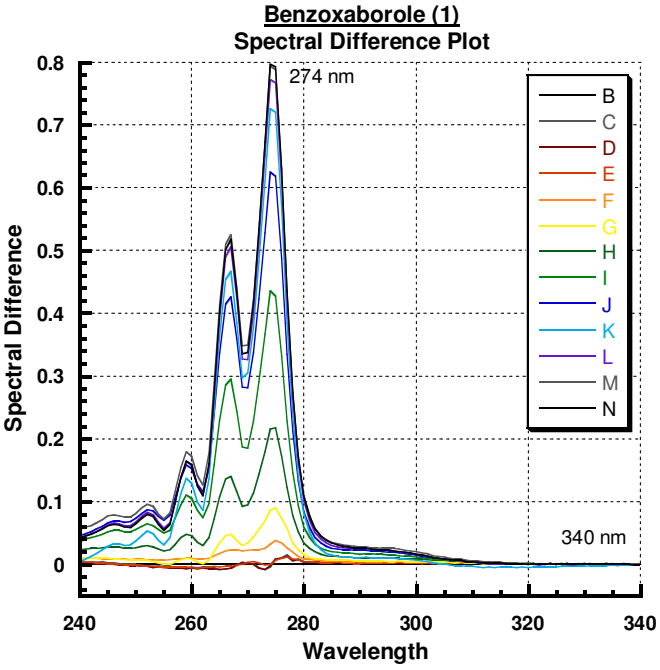
Figure 6 – A) Spectral scans of **9** in aqueous solutions of various pH's.
B) Plot of the spectral difference between different solutions of **9**. The maximum positive deviation occurred at 277 nm while the maximum negative deviation occurred at 250 nm.
C) 9 pKa determination. The total absorbance difference (the sum of the absolute absorbance difference values at 250 and 277 nm at each pH) was plotted against solution pH. The data was then fit to Eq. 1 to obtain the pKa.

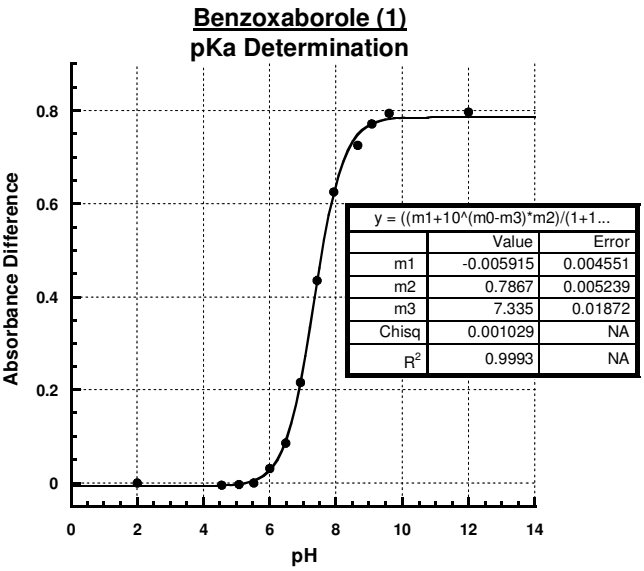


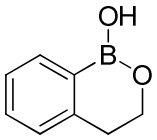
Benzoxaborole (**1**)



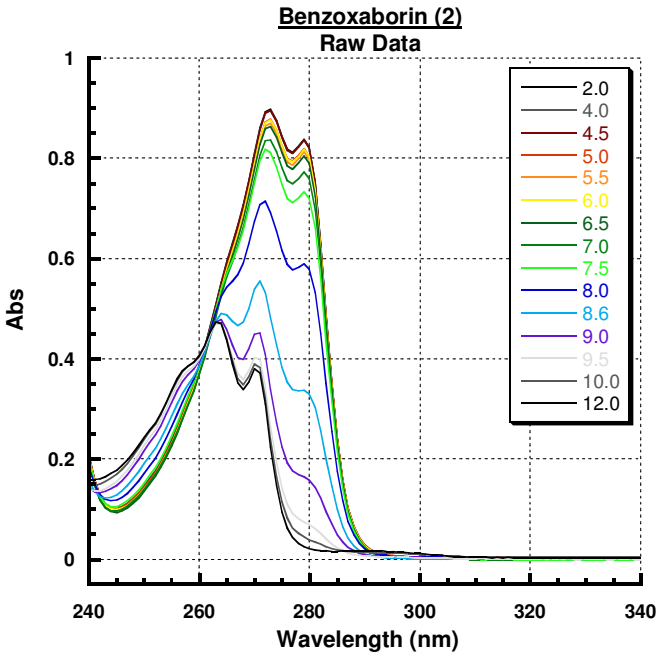


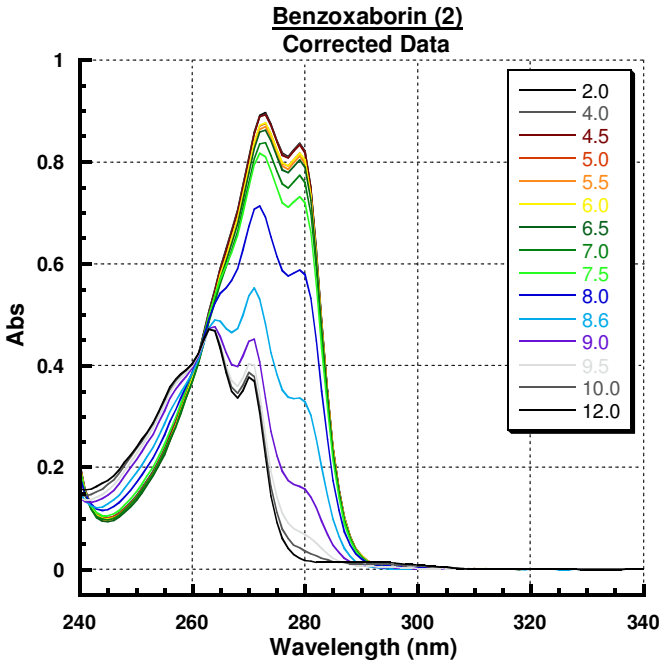


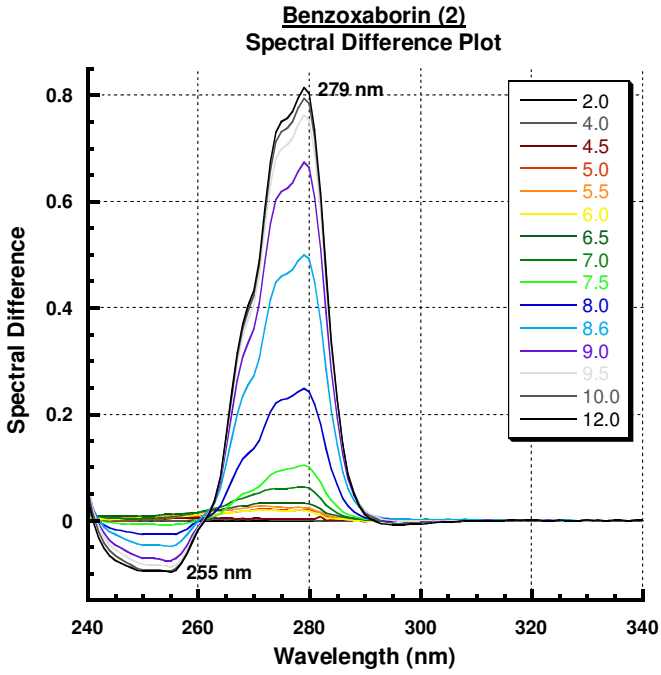


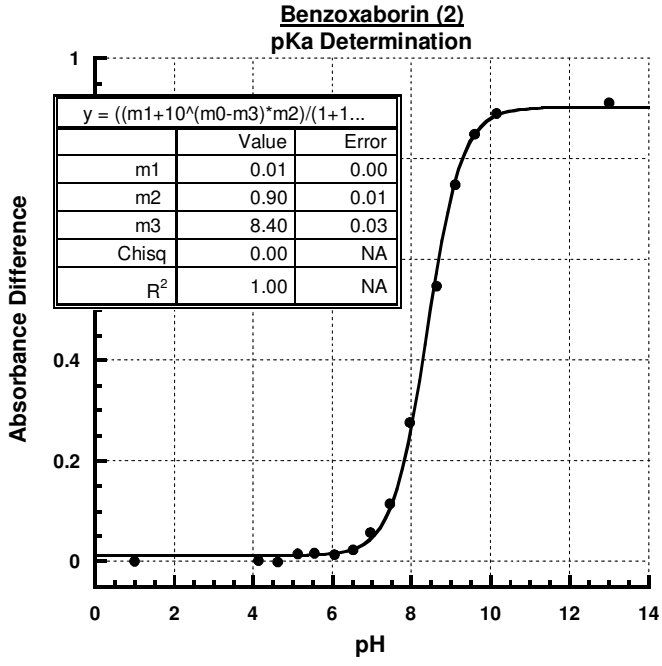


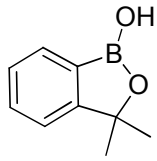
Benzoxaborin (**2**)



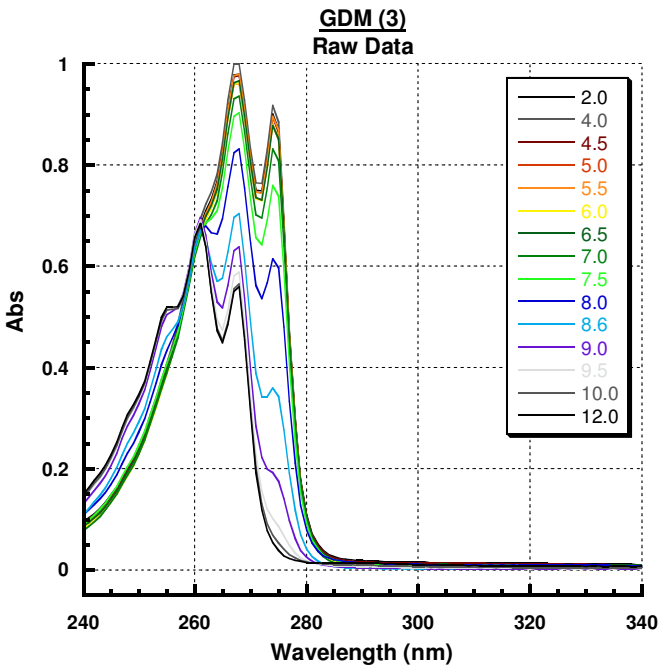


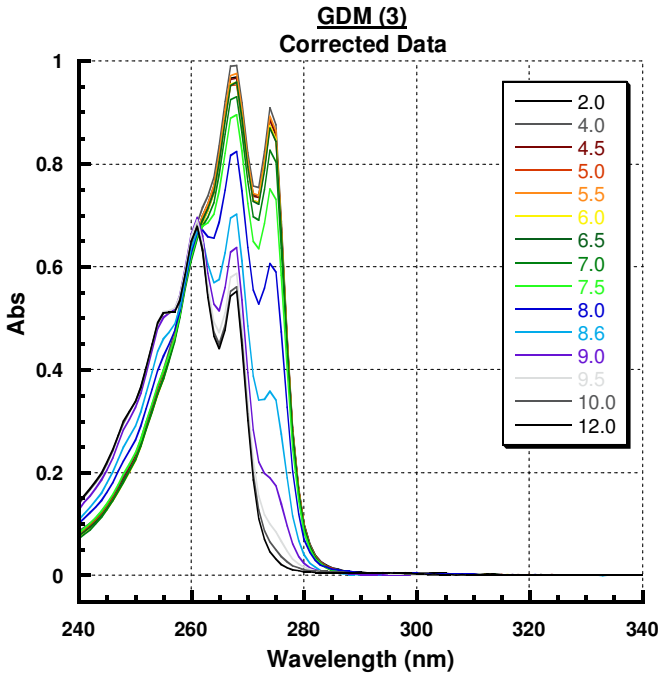


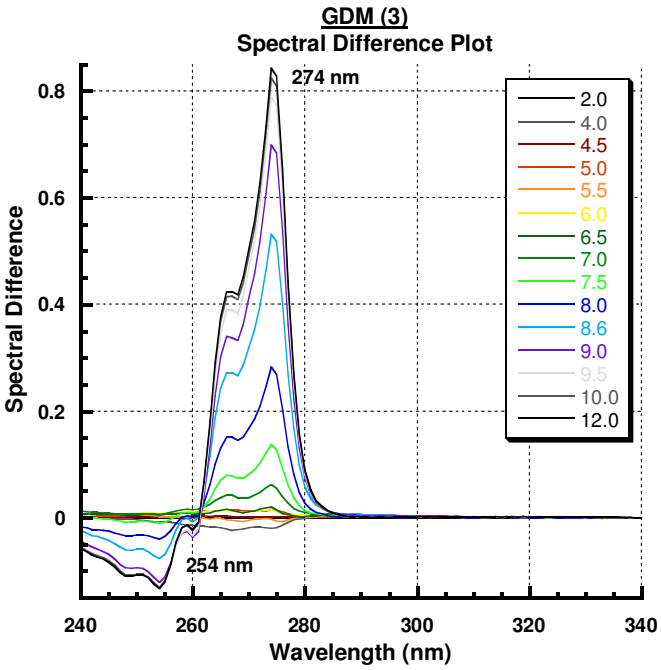


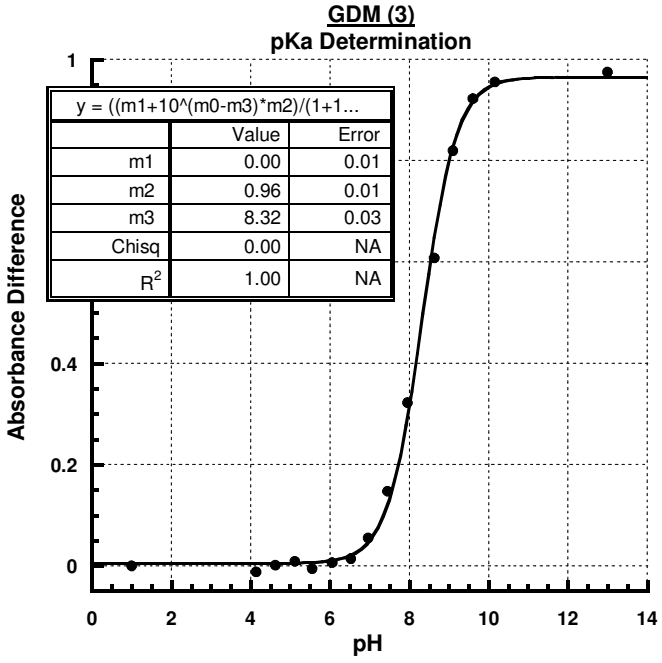


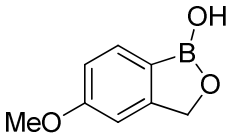
3,3-Gem Dimethyl
Benzoxaorole (**3**)



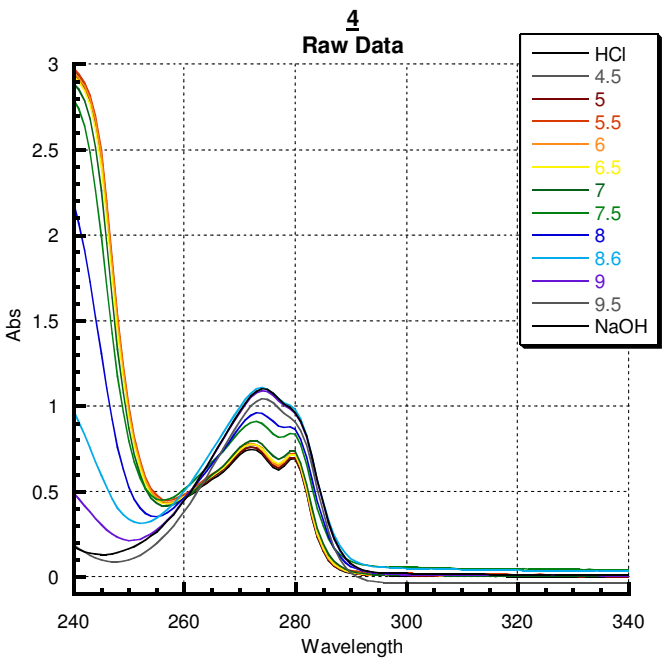







OH
B
O
MeO

4


4
Raw Data
Abs
Wavelength
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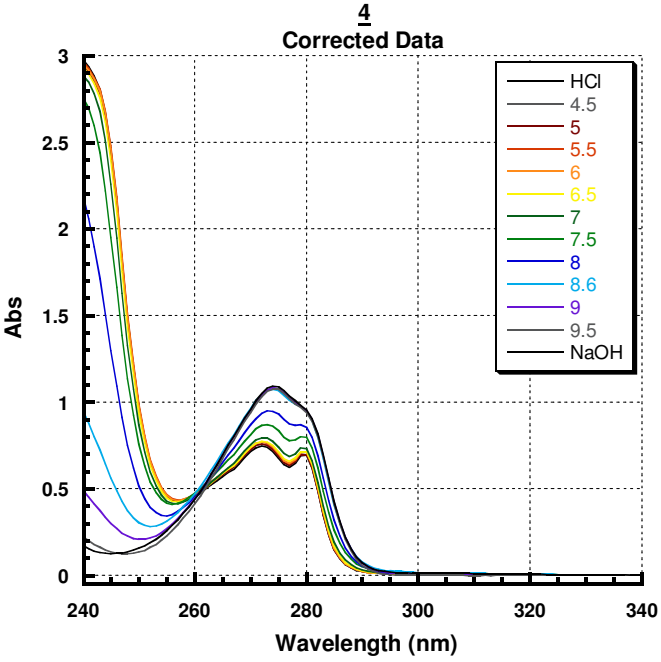
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH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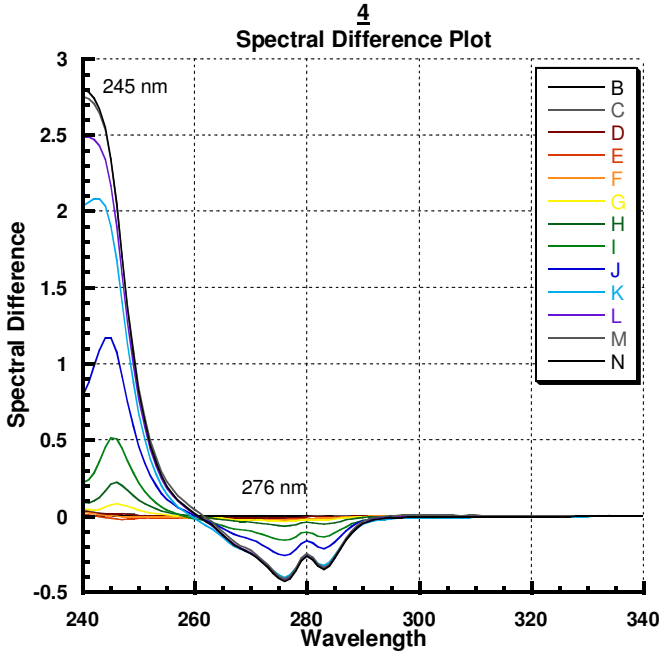
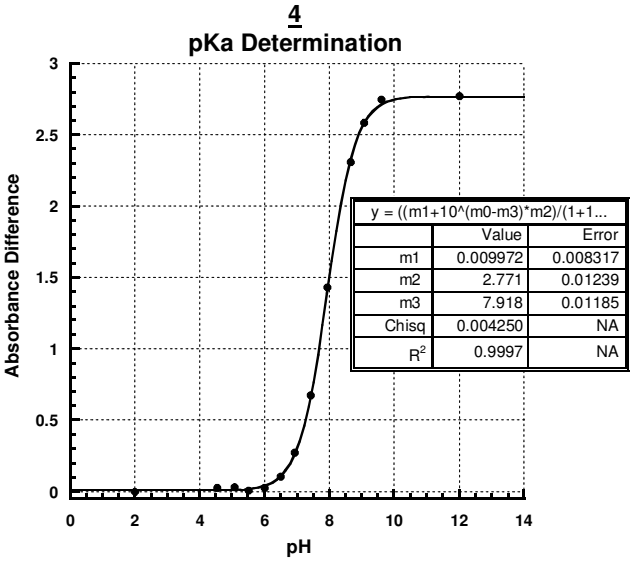
Corrected Data
Abs
Wavelength (nm)
HCl
4.5
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH


4
Spectral Difference Plot
245 nm
276 nm
Spectral Difference
Wavelength
B
C
D
E
F
G
H
I
J
K
L
M
N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pKa Determination
Absorbance Difference
pH
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Chisq
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R²
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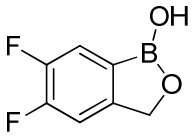
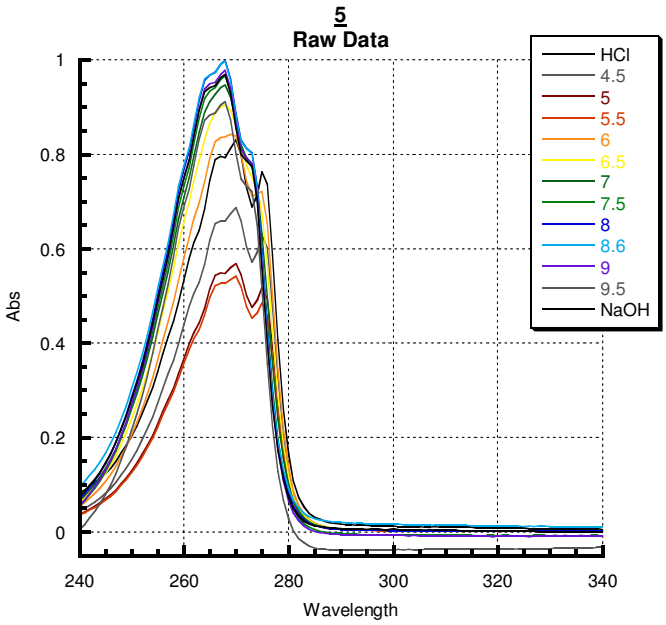

OH
B
O
F
F

5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Raw Data
Abs
Wavelength
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5
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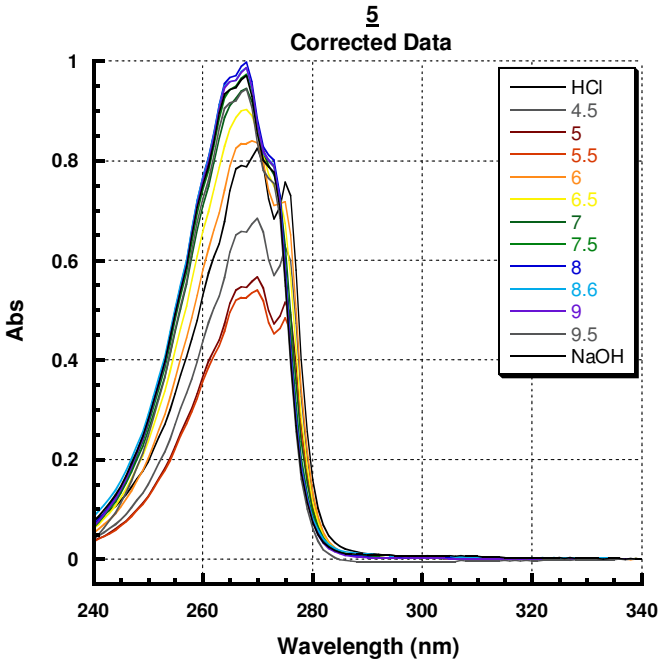
7
7.5
8
8.6
9
9.5
NaOH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Corrected Data
Abs
Wavelength (nm)
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7.5
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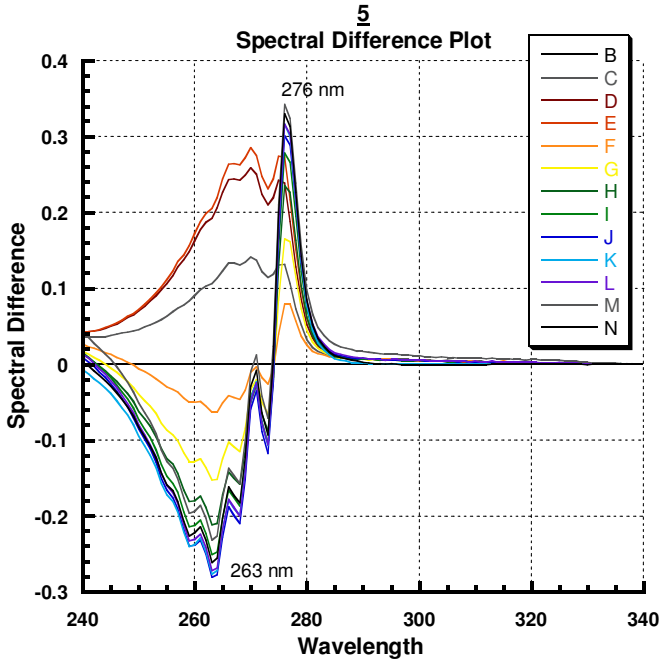
8.6
9
9.5
NaOH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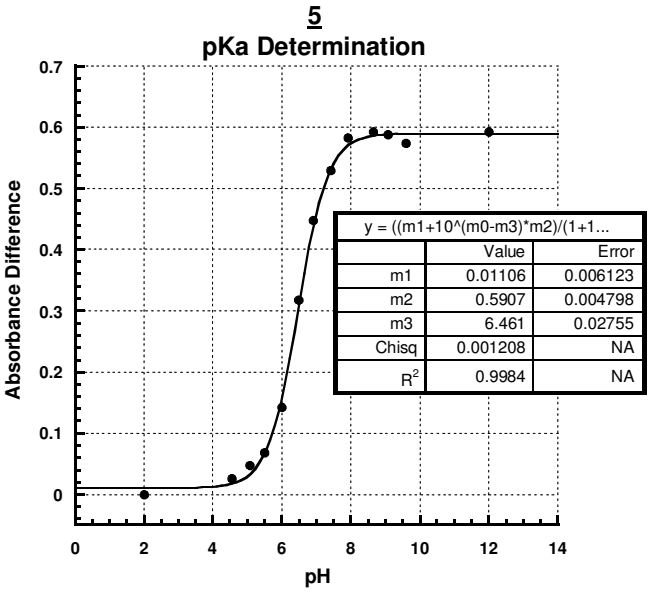
Spectral Difference Plot
276 nm
263 nm
Spectral Difference
Wavelength
B
C
D
E
F
G
H
I
J
K
L
M
N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Absorbance Difference
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Chisq
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R2
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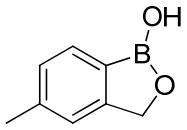
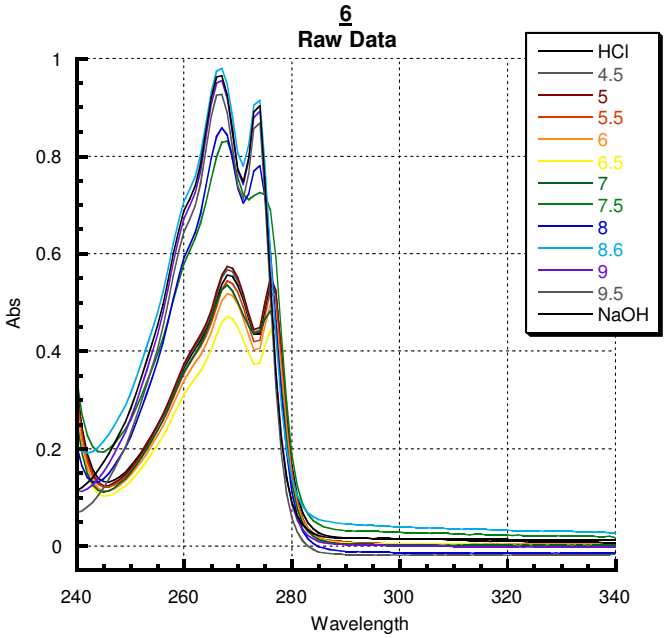

OH
B
O

6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Raw Data
Abs
Wavelength
HCl
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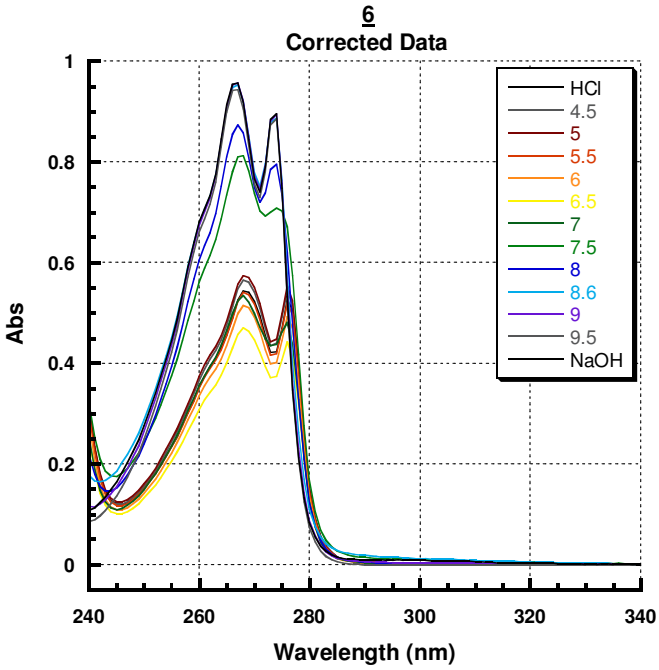
6
6.5
7
7.5
8
8.6
9
9.5
NaOH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Corrected Data
Abs
Wavelength (nm)
HCl
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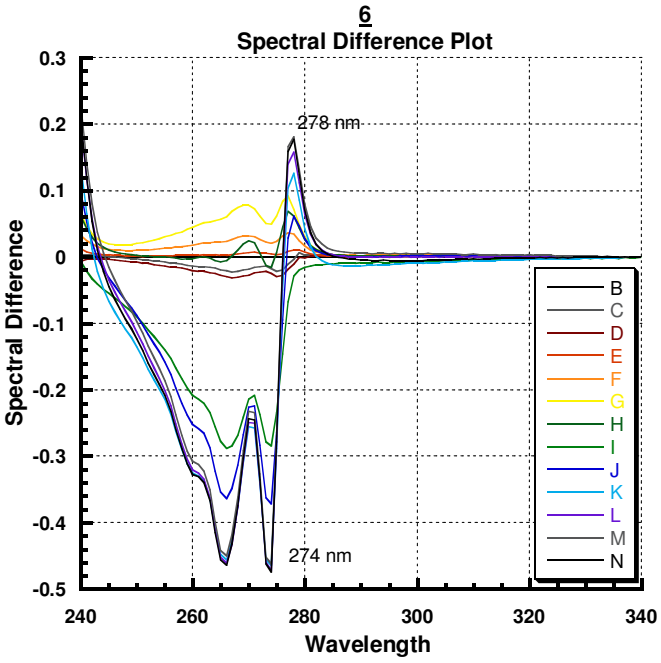
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH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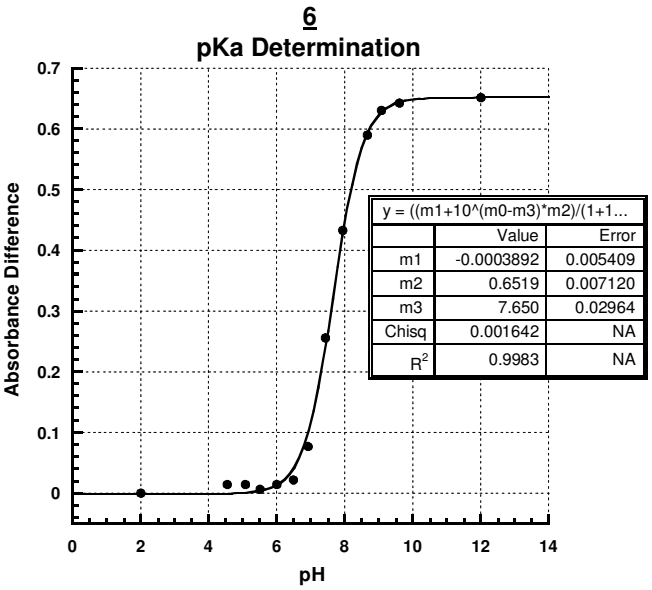
Spectral Difference Plot
Spectral Difference
Wavelength
278 nm
274 nm
B
C
D
E
F
G
H
I
J
K
L
M
N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pKa Determination
Absorbance Difference
pH
y = ((m1+10^(m0-m3)*m2)/(1+1...
Value
Error
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Chisq
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R2
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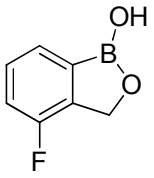
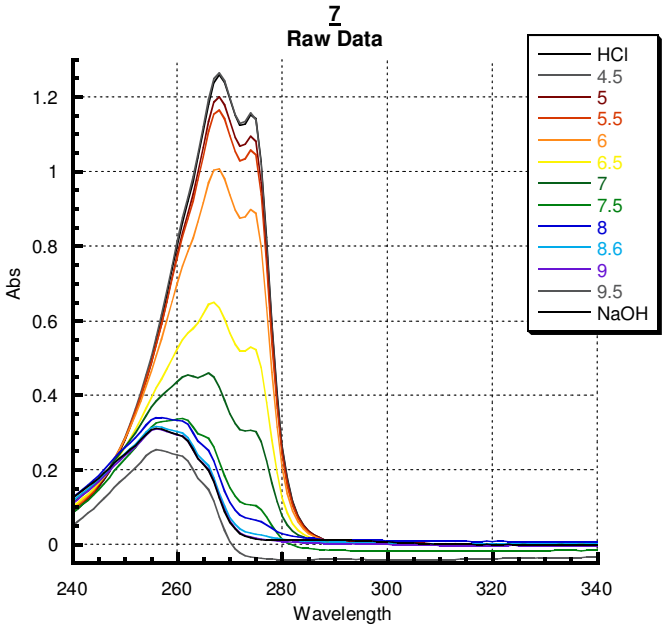
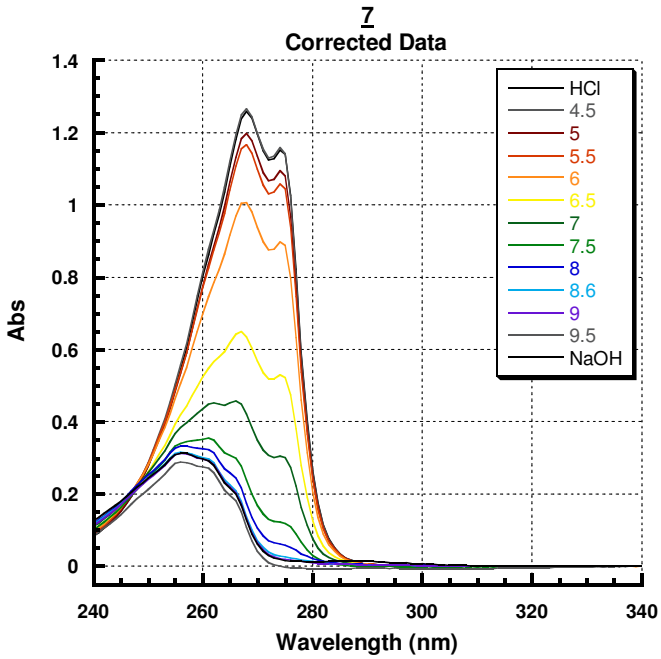
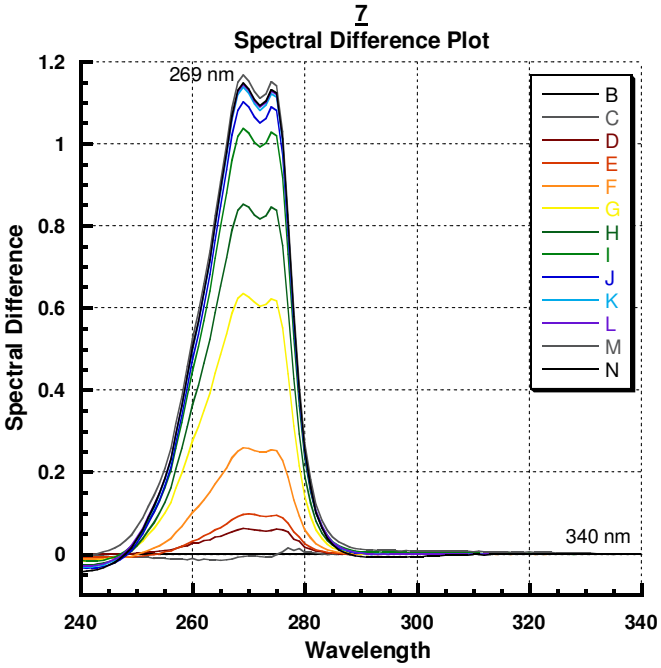
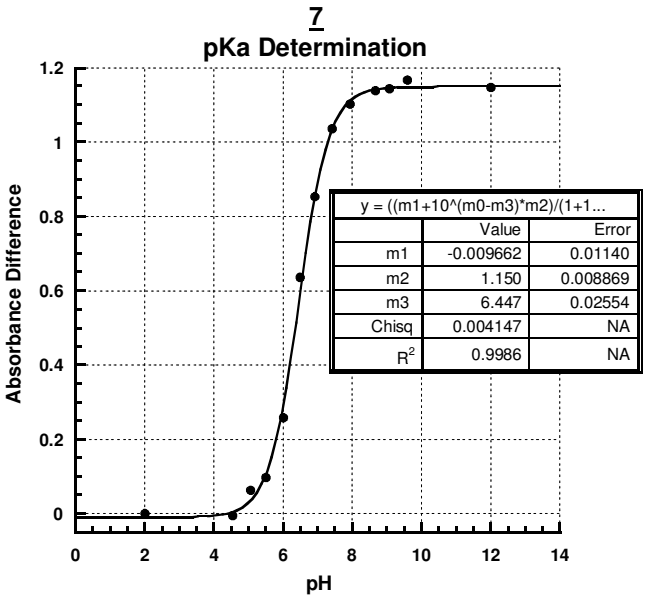

OH
B
O
F

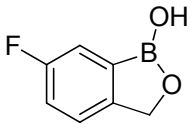
7


7
Raw Data
Abs
Wavelength
HCl
4.5
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH

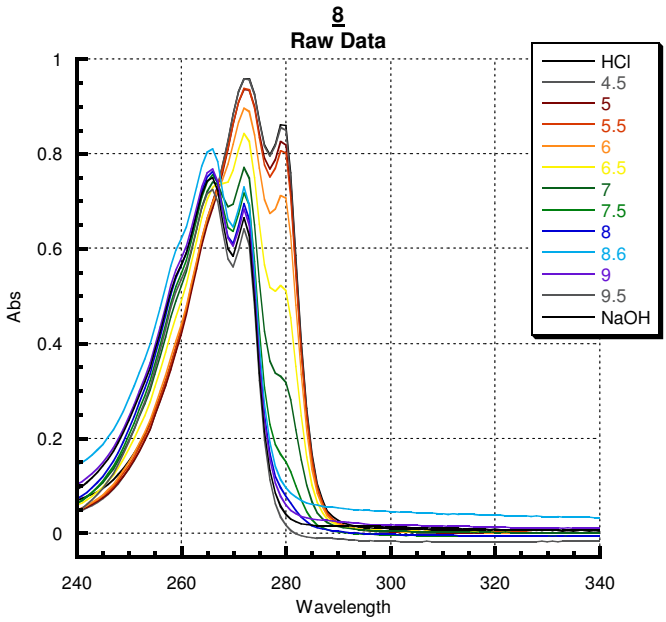

7
Corrected Data
Abs
Wavelength (nm)
HCl
4.5
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH

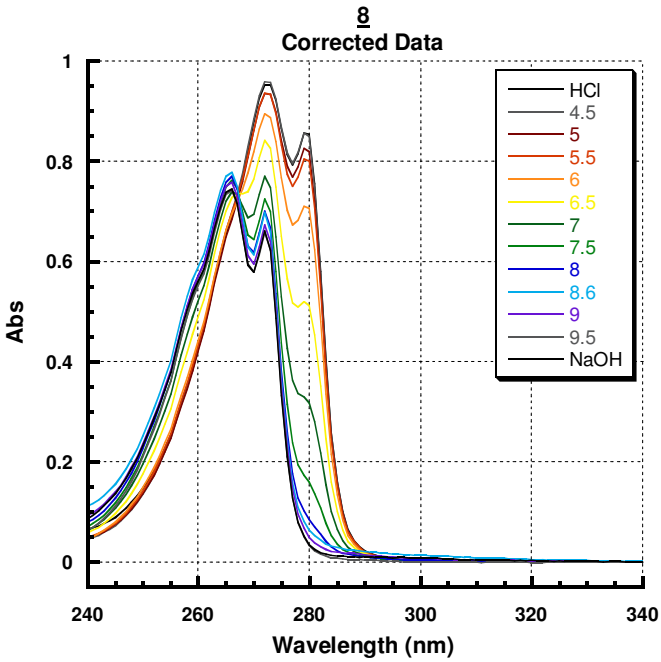

7
Spectral Difference Plot
269 nm
340 nm
Spectral Difference
Wavelength
B
C
D
E
F
G
H
I
J
K
L
M
N

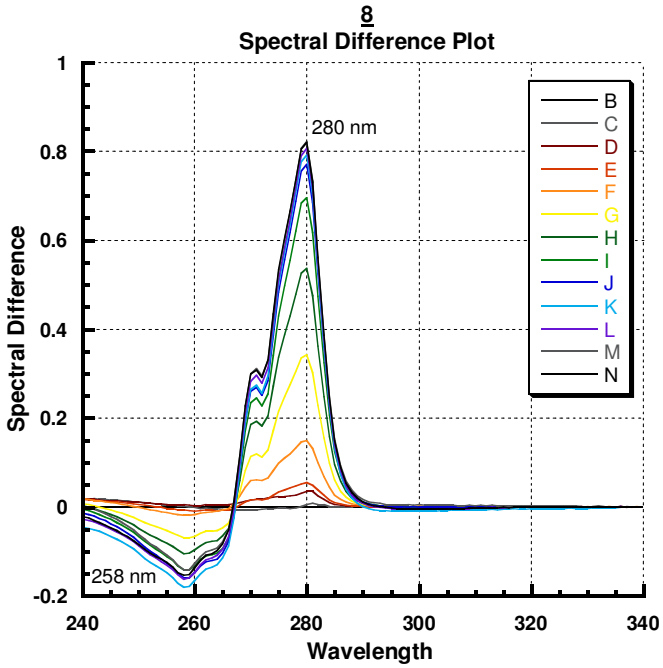

7
pKa Determination
Absorbance Difference
pH
y = ((m1+10^(m0-m3)*m2)/(1+1...
Value
Error
m1
-0.009662
0.01140
m2
1.150
0.008869
m3
6.447
0.02554
Chisq
0.004147
NA
R2
0.9986
NA

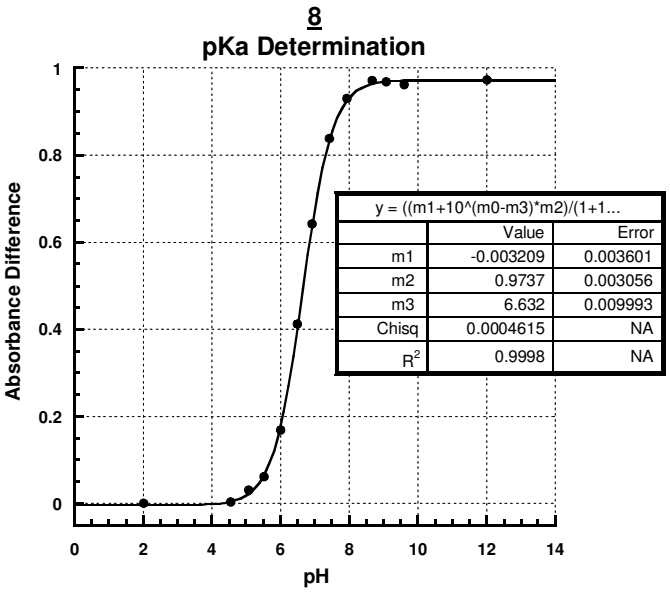

OH
F
B
O

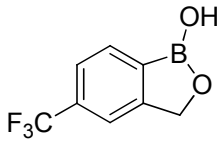
8


8
Raw Data
Abs
Wavelength
HCl
4.5
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH

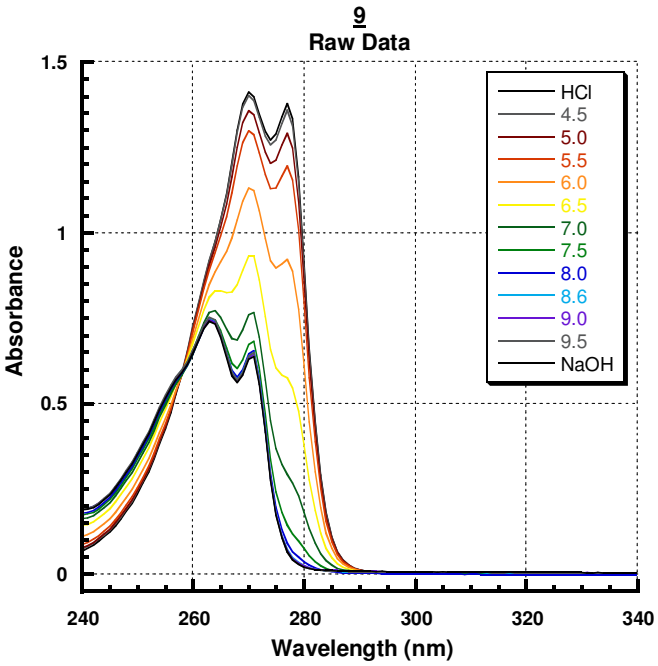

8
Corrected Data
Abs
Wavelength (nm)
HCl
4.5
5
5.5
6
6.5
7
7.5
8
8.6
9
9.5
NaOH

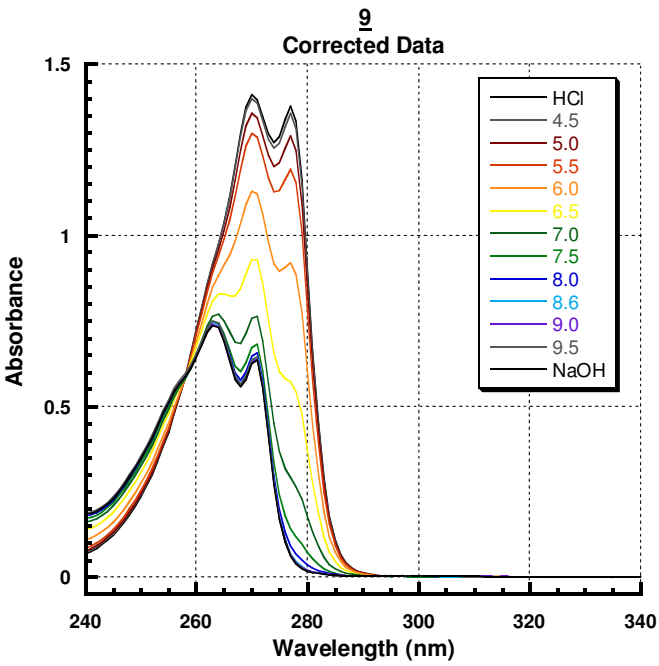

8
Spectral Difference Plot
Spectral Difference
Wavelength
280 nm
258 nm
B
C
D
E
F
G
H
I
J
K
L
M
N

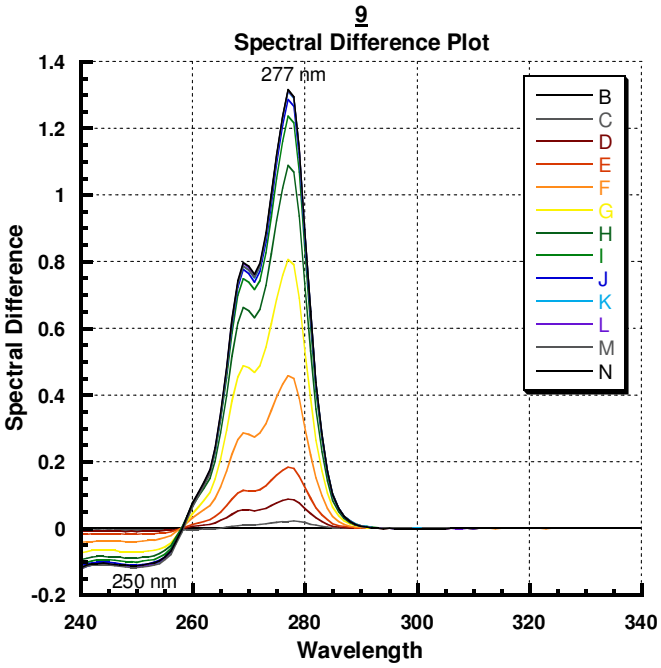

8
pKa Determination
Absorbance Difference
pH
y = ((m1+10^(m0-m3)*m2)/(1+1...
Value
Error
m1
-0.003209
0.003601
m2
0.9737
0.003056
m3
6.632
0.009993
Chisq
0.0004615
NA
R2
0.9998
NA

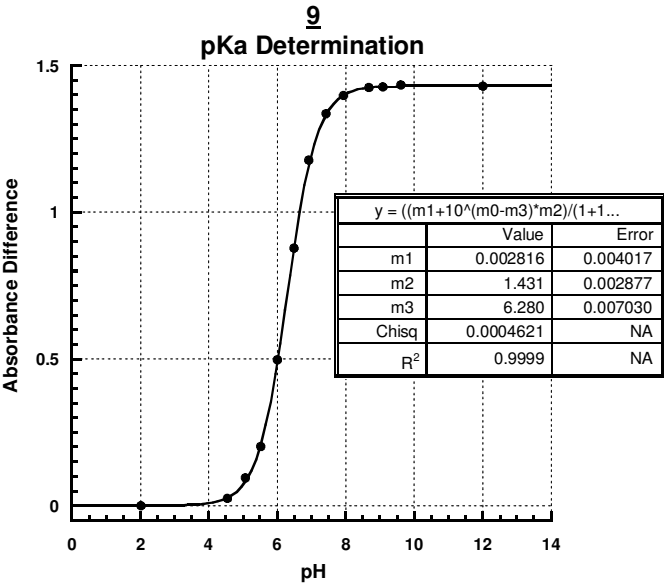

OH
B
O
F3C

9


9
Raw Data
Absorbance
Wavelength (nm)
HCl
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.6
9.0
9.5
NaOH


9
Corrected Data
Absorbance
Wavelength (nm)
HCl
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.6
9.0
9.5
NaOH


9
Spectral Difference Plot
277 nm
250 nm
Spectral Difference
Wavelength
B
C
D
E
F
G
H
I
J
K
L
M
N


9
pKa Determination
Absorbance Difference
pH
y = ((m1+10^(m0-m3)*m2)/(1+1...
Value
Error
m1 0.002816 0.004017
m2 1.431 0.002877
m3 6.280 0.007030
Chisq 0.0004621 NA
R² 0.9999 NA

IV. Equations for the 3-component, ARS method of binding constant determination

In the complex mixture of boronic acid receptor (R), ARS indicator (I), and sugar substrate (S), there exist the following equilibria:

$$R + I \overset{K_{ars}}{\rightleftharpoons} RI$$

$$RI + S \rightleftharpoons RS$$

where RI is the boronic acid-ARS complex and RS is the substrate complex.

Equations for association constant determinations:

$1/\Delta A = (\Delta K\ p_o I_o K_{ars})^{-1}\ 1/[S] + (\Delta K\ p_o I_o)^{-1}$ (1)

where ΔK, p_o, I_o are all the parameters of the UV spectrophotometer.

$Q = [I]/[RI] = (A_{RI} - A)/(A - A_I)$ (2)

Where A is measured absorbance, A_{RI} is absorbance of the receptor-indicator complex, and A_I is absorbance of free indicator.

$P = [R] - 1/(QK_{ars}) - [I_0]/(Q+1)$ (3)

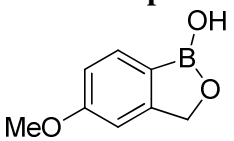
where $[I_0]$ is total indicator concentration (ARS).

$[S]/P = (K_{ars}/K_a)Q + 1$ (4)

where [S] is substrate concentration.

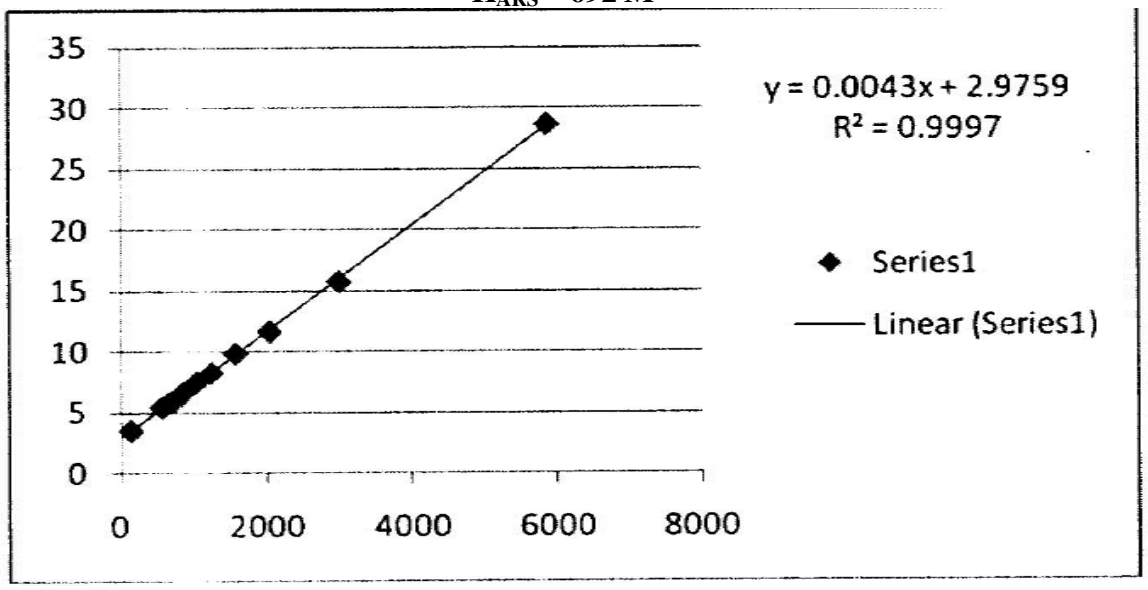
The association constant of the ARS-boronic acid (K_{ars}) is the quotient of the intercept and the slope in a plot of $1/\Delta A$ versus 1/[S] (Equation 1).

V. Binding constant determination plots - examples

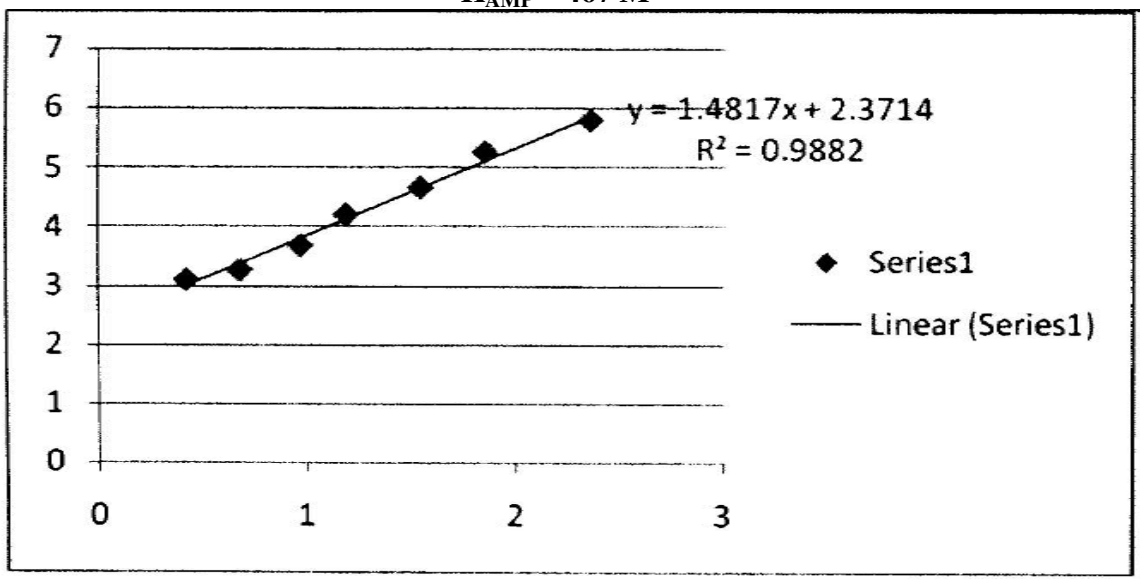


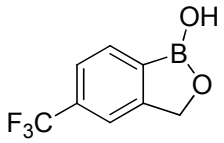
4

K_{ARS} = 692 M^{-1}



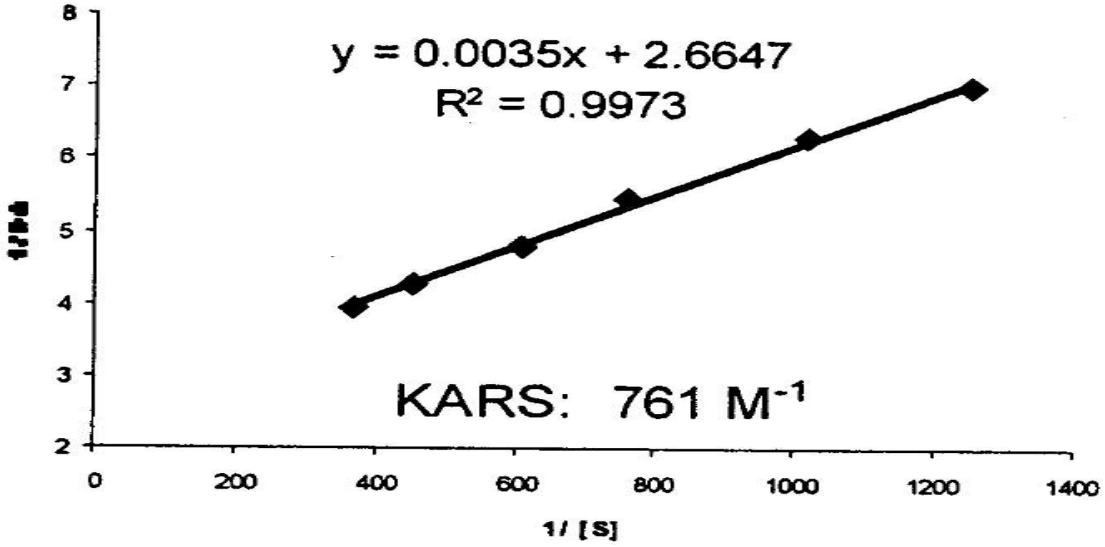
K_{AMP} = 467 M^{-1}





9

K_{ARS} = 761 M^{-1}



K_{AMP} = 716 M^{-1}