A Spectrophotometric Determination of Barium Complexation with Methylthymol Blue

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A SPECTROPHOTOMETRIC DETERMINATION OF BARIUM COMPLEXATION WITH METHYLTHYMOL BLUE

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

by

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ABSTRACT

Alexander, Wesley. M.S., Department of Chemistry, Wright State University, 2022. A Spectrophotometric Determination of Barium Complexation with Methylthymol Blue

In this work, a spectrophotometric titration of methylthymol blue (MTB) with barium chloride was performed at pH = 7.5, 9.6, and 12.2 to determine the stoichiometry of complexation between the two as well as formation constants and absorptivity coefficients. Complexes of 1:1, 2:1, and 3:1 Ba:MTB were suggested by the experimental data upon fitting with appropriate models using Beer’s Law. The molar absorptivities of free MTB species at 605 nm were determined to be $\varepsilon_{H_2MTB^{4-}} = 5.0 \pm 0.2 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{HMTB^{5-}} = 7.3 \pm 0.3 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{MTB^{6-}} = 1.3 \pm 0.2 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. At pH = 9.6, $\varepsilon_{Ba_2H_2MTB}$ and the formation constant of that complex were determined to be $6.0 \pm 0.2 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, and $7 \pm 4 \times 10^2$, respectively. Similarly at pH 12.2, $\varepsilon_{Ba_2HMTB^{5-}}$, and its formation constant were determined to be $8.2 \pm 0.1 \times 10^3$, and $1.2 \pm 0.4 \times 10^2$, respectively. Formation constants of 3:1 complexes were unable to be determined with certainty due to a lack of constraints at high barium concentration. Binding activity was largely influenced by barium concentration and pH, presumably due to the dominant species of MTB present in solution.
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Introduction

1. Drilling Muds

1.1 Drilling Muds and the Role of Barium

Barium plays a vital role in the gas and oil industry. Barium sulfate, or barite, is commonly used as a densifying agent in drilling muds[1], which are a small but important component to offshore drilling operations. It may be tempting to believe that a drilling mud is the mud created during a drilling operation, but that is not the case. Instead, a drilling mud is a mixture of compounds introduced via a hollowed core in the center of the drill pipe[1]. The primary purpose of a drilling mud is to transport drill cuttings to the surface, but they also cool the drill, provide balance against pressure, and lubricate the drill[1]. Drilling muds are split into three categories: water-based muds (WBMs), oil-based muds (OBMs), and synthetic-based muds (SBMs). WBMs are the most commonly used drilling muds as the environmental regulations are much more lenient than for SBMs and OBM. Barium, in the form of barite, is commonly added to drilling muds due to its high density (greater than 4 g/cm$^3$). The added density from barite assists in combating the pressure experienced by the drill as it digs deeper beneath the surface[1]. Most muds are disposed of by discharge into the ocean[1], and it is through this discharge that barium can be found in wastewater as a contaminant[2,3].
2. Introduction to Methylthymol Blue (MTB)

2.1 MTB Background

Because barium is a relevant environmental contaminant, it is necessary that there be an efficient way to determine aqueous barium concentrations on location without the need for costly instrumentation. Methylthymol blue (MTB) has historically been used as a metal ion indicator via complexometric [4] and spectrophotometric titrations[5], however, the formation of its complex with barium has not been thoroughly studied. MTB is a triphenylmethane dye and a polyprotic acid with pKₐ values at 12.97 associated with the phenolic oxygen; 10.81 associated with the quinoid system; 8.47 associated with a first nitrogen; 6.31 associated with the second nitrogen; and 3.47 and 2.35 associated with acetate substituents[6,7]. In theory all four acetate groups would have acidic hydrogens associated with them, but these pKₐ values have not been reported in the literature as they are not measurable using a spectrophotometric method[7]. The isopropyl and methyl substituents have an inductive effect, contributing to the charge density of two of the aromatic rings. This causes an increase in the pKₐ of MTB, making it more difficult for the protons to dissociate[6]. The fully deprotonated structure of MTB can be seen in Figure 1. MTB experiences notable color changes around several of its higher pKₐ values. Below pH = 6.31, MTB is a deep yellow color, and increasing the pH begins to convert MTB to a pale blue green color. Around pH = 8.47, it becomes a grey blue, and around pH = 10.81, it displays a deep blue color[8]. These changes may also be seen
spectrophotometrically as metal cations are added to solution, making it a popular pH and metal ion indicator.

Previously, EDTA titrations have been completed with barium and MTB. Ueno indicated that the useful range of MTB as an indicator with barium in an EDTA titration is between pH = 11 and 12.5[4]. It is possible that in a more straightforward titration, without the use of EDTA, MTB may be useful over a wider or entirely different pH range. It is for this reason that this work uses a variety of pH values for the titration of barium into MTB both inside and outside the range suggested by Ueno.
Figure 1: Fully deprotonated structure of MTB
3. MTB Binding to Transition Metals

3.1 Trivalent Metal Cations

A variety of studies have been directed toward iron (II) and iron (III) to determine the binding of these metals with MTB in solution, which generally agree with the conclusion that binding occurs as either a tridentate or tetradeutate ligand at the iminodiacetate group. Yoshino et al. discovered that iron (III) monohydroxide is capable of binding 1:1 with MTB in neutral to mildly basic solutions (where $\text{H}_3\text{MTB}^3-$ and $\text{H}_2\text{MTB}^4-$ are the predominant species, respectively)[9]. They further determined that iron (III) can form $\text{FeHMTB}^2-$ and $\text{Fe}_2\text{MTB}$ in alkaline solution although this conclusion was the result of a potentiometric study rather than a spectrophotometric study. The binding of iron (III) was determined to occur at the iminodiacetate as a tetradeutate complex[9]. Two binding constants were spectrophotometrically determined: a single-step addition of Fe (III) to HMTB$^5-$ (forming $\text{FeHMTB}^2-$) and a second single step addition of two ions to MTB$^6-$ (forming $\text{Fe}_2\text{MTB}$). The logK values for each complex was 17.7 ($\text{FeHMTB}^2-$) and 29.8 ($\text{Fe}_2\text{MTB}$), and no uncertainty values were reported. The exact pH of the solutions tested was also not reported. The work of Kantcheva and Nenova generally agreed with these findings, confirming that iron (III) forms a 1:1 complex at pH = 12.0 and expanded on them by discovering that iron (III) can form both a 1:1 and a 1:2 complex with MTB at pH = 5.0 [10]. The reported value for the 1:1 stability constant ($K$) is $20.56 (\pm 0.07)$, whereas the 1:2 complex had a reported stability constant value of $6.6(\pm 0.05)$. Further, this work also reported the molar absorptivities of the 1:1 and 1:2
complexes at 610 nm as $1.73 \pm 0.01 \times 10^4$ L·mol$^{-1}$·cm$^{-1}$, and $3.21 \pm 0.05 \times 10^3$ L·mol$^{-1}$·cm$^{-1}$, respectively. Zhou et al. did similar work with aluminum binding, concluding that Al also forms a 1:1 complex with MTB in mildly acidic conditions[11]. The stability constant was reported as $\log K = 7.38$ without the uncertainty being reported.

3.2 Divalent Metal Cations and the Irving-Williams Series

Several divalent transition metals have also been studied for their binding with MTB. Generally, the trends in stability of the first-row metals follow the Irving-Williams Series, which states that stability of binding generally increases going across the period from manganese to a maximum at copper[8]. Going across the period, this would lead to a trend of binding stability of Mn<Fe<Co<Ni<Cu>Zn. There are three primary reasons that are thought to be the cause of this trend: ionic radii, crystal field stabilization energy (CFSE), and the Jahn-Teller effect[8]. Going across the period, the ionic radius of each metal decreases, which contributes to greater stability. Additionally, CFSE begins at zero with Mn and increases to a maximum at Ni, increasing stability. It may be tempting to believe that nickel, then, might demonstrate the strongest binding ability of the series, but the Jahn-Teller effect provides octahedral copper complexes with additional stability[8]. These divalent transition metals may also form 2:1 stoichiometry[12]. For example, one study determined binding constants for lead, zinc, copper, cobalt, and nickel, demonstrating MTB’s ability to bind both 1:1 and 2:1 in a two-step addition. Copper demonstrated the strongest binding ($\log K_1 = 5.94 \pm 0.04$ and $\log K_2 = 5.17 \pm 0.04$) followed by lead ($\log K_1 = 5.09$
(± 0.02) and log K₂ = 4.60 (± 0.02)), nickel (log K₁ = 5.66 (± 0.08) and log K₂ = 4.01 (± 0.08)), cobalt (log K₁ = 4.50 (± 0.05) and log K₂ = 3.57 (± 0.05)) and finally zinc demonstrated the weakest binding (log K₁ = 3.93 (± 0.05) and log K₂ = 3.66 (± 0.05))[8]. A similar study was conducted more recently using iron (II), zinc (II), copper (II), and cobalt (II), which confirmed the Irving-Williams Series trend with the notable exception of iron[7]. The binding constant values determined in this study were in the order of iron (log K₁ = 6.89 (± 0.02) and log K₂ = 5.30 (± 0.02)), copper (log K₁ = 5.80 (± 0.01) and log K₂ = 3.93 (± 0.02)), cobalt (log K₁ = 4.70 (± 0.03) and log K₂ = 3.32 (± 0.02)), and finally zinc (log K₁ = 4.31 (± 0.01) and log K₂ = 3.03 (± 0.01))[7].

4. MTB Binding to Alkaline Earth Metals

4.1 Literature Trends

Previous work, such as that done by Bremer and Grell [5] and Bremer et al.[13], has supported the theory that divalent metal cations such as alkaline earth metals form two different complexes with MTB in both 1:1 and 2:1, metal:MTB ratios [5,9–16]. The binding is generally thought to occur as either tridentate or tetradentate at either of the iminodiacetate groups and involving the phenoxide group [13,14,16] seen to either side of MTB in Figure 1. The first study done by Bremer and Grell reported on magnesium and calcium binding activity with MTB via spectrophotometric analysis. A wide variety of log K values were presented dependent upon the solution conditions, but the most relevant results to the work presented here would be the binding constants at pH = 7.51 (± 0.1) for magnesium and pH = 7.27 (± 0.1) for calcium. The stability constants for
magnesium were reported to be $\log K_1 = 3.6 \pm 0.1$ and $\log K_2 = 2.7 \pm 0.1$, and those of calcium were $\log K_1 = 3.1 \pm 0.2$ and $\log K_2 = 1.9 \pm 0.1$[5]. This study also reported molar absorptivity values of each species present in solution. The molar absorptivities of the magnesium species at $\text{pH} = 7.51$ were reported in units of $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ as $7.3 \times 10^3$, $9.5 \times 10^3$, and $1.8 \times 10^4$ for the free MTB species, 1:1 complex, and 2:1 complex, respectively. The same species for the calcium complex at $\text{pH} = 7.27$ were reported in units of $\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ as $6.5 \times 10^3$, $7.9 \times 10^3$, and $2.0 \times 10^4$[5]. The follow-up study that Bremer et al. conducted determined different values but confirmed many of the same trends while attempting to confirm the mechanism that magnesium and MTB undergo during complexation[13].

As much work as there has been with the binding of MTB and other divalent metal cations, very little work has been done with barium, specifically, to determine complexation stoichiometry and stability constants. It has been reported that barium exhibits weak binding with MTB in near-neutral pH solutions[17], but it is possible that this may be mitigated by working in a higher pH range. In this work we have made use of spectrophotometric titration techniques to determine the complexation of barium and MTB, the stability constants, and molar absorptivity coefficients at three different pH levels, each with a differently protonated species of MTB dominant in solution.
Experimental Methods

Two experiments were performed: a pH titration of MTB in the absence of barium and a barium titration holding pH constant.

1. Chemicals and Materials

Buffer solutions of pH = 10.00 and 7.00 were purchased from Fisher Scientific and used to calibrate a Vernier pH sensor. A buffer solution of pH = 12.46 was purchased from Thermo Scientific to calibrate the probe at pH above 12. Sodium hydroxide pellets (99.99% metals basis, semiconductor grade) were purchased from Sigma-Aldrich to adjust pH as necessary. A tris hydroxymethyl aminomethane base (Tris) was purchased from Fisher Scientific and used to create a pH = 7.5 buffer with addition of dilute HCl. N-cyclohexyl-2-aminoethanesulfonic acid (CHES) (≥ 99.0%, titration grade) was purchased from Sigma and used to create a pH = 9.6 buffer with addition of NaOH. Ethylamine (70 wt. % solution in water) was purchased from Sigma and was used to prepare a buffer solution for pH = 12.2. Tetramethylammonium chloride (reagent grade ≥ 98%) was purchased from Sigma-Aldrich and added to solutions as an ionic strength adjuster (I = 0.1 M). Methylthymol blue tetrasodium salt (indicator grade) was purchased from Sigma-Aldrich and barium chloride dihydrate (certified ACS crystalline) was purchased from Fisher Scientific.
2. pH Titration

A 50 mL solution of 0.125 mM MTB was prepared and adjusted to 12.5 pH units using 0.1748 g of NaOH pellets (2 pellets). The pH of the MTB solution was then lowered using small amounts of 0.5 M HCl. A full UV-Vis spectrum was collected at every half pH unit until the solution reached 7.0 pH value. The absorbance spectra was corrected for the dilutions caused by the addition of HCl, allowing for MTB concentration to be considered constant throughout the additions.

3. Barium Titrations

3.1 Solution Preparation

50 mL of a solution of 0.125 mM MTB was prepared using a concentration of 0.1 M tetramethylammonium chloride to hold the ionic strength of solution essentially constant throughout the titration. A separate solution of 1 M barium chloride was prepared to be used as the titrant. The solutions were adjusted to various pH levels using 0.025 M Tris at pH = 7.5; 0.02 M CHES at pH = 9.6; and 0.75 M ethylamine at pH = 12.2. Both the MTB and barium solutions were given the same concentration of the buffers so as to hold pH constant throughout the additions. The buffered solutions were then allowed to equilibrate at room temperature. The range of temperatures was measured using a thermometer determining a range of 22.4-23.8°C for all experiments performed.

3.2 Titration and Analysis

At each given pH, the 1 M barium chloride solution was titrated into 50 mL of 0.125 mM MTB solution in such a way that the total titrant volume was 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mL after each subsequent
addition. The smallest additions were made via syringe. As the amount of
solution increased, a micropipette was used followed by a series of volumetric
pipettes for the largest additions. Aliquots were taken out of solution and placed
into a glass cuvette and returned to solution after spectrophotometric analysis.
Experiments were run in duplicate to test for reproducibility. Samples were
analyzed by a Varian Cary 50 Bio UV-Visible Spectrophotometer scanning from
800 nm to 200 nm at 5 nm intervals.

Absorbance spectra were corrected for dilution as titrant was added,
allowing for the assumption of constant total MTB concentration throughout the
experiment. Absorbance versus [Ba] data were fit to various barium/MTB
binding models using Matlab code developed to determine molar absorptivities
and formation constants.
Results and Discussion

1. pH Titration Data and Analysis

The spectra of 0.125 mM MTB without barium were collected in half-unit pH increments to demonstrate the effects of pH on the absorbance spectra. The spectra shown in Figure 2 are consistent with trends observed in the literature with the peak around 450 nm flattening and undergoing a hypsochromic shift as the pH was increased[6]. The opposite trend was observed at 605 nm: as the pH increased, the absorbance increased while the peak position did not shift.
Figure 2: Spectra of 0.125 mM MTB without barium from pH 7.0 to 12.5 in half-unit increments.
2. Alpha Fraction Calculation

2.1 Alpha Fraction Plot

A plot of the alpha fractions for the various MTB species was also created (Figure 3) based on the pKₐ values reported in the literature[6,7]. The alpha fractions presented are those that are measurable by spectrophotometric determination. This plot displays the ratio of each species of MTB present at a given pH. Figure 3 contains 5 peaks indicating the point at which each intermediate species of MTB is at its maximum. At each peak there are two other species present at much lower concentration, and the other four species are present at negligible concentration. To reduce the amount of interference caused by minority species, working at or very near the pH of these peaks is ideal. This work examines MTB solutions at three different pH levels: pH = 7.5 in which the dominant species is H₃MTB³⁻; pH = 9.6 in which the dominant species is H₂MTB⁴⁻; and pH = 12.2 in which the dominant species is HMTB⁵⁻. At each pH used in this work, there will be minority MTB species present in addition to the dominant species and these minority species may interact differently with barium. For example, if it is determined that once MTB has been protonated to a point that it will no longer bind, it may be seen that the majority species does not bind, but the lesser protonated minority species may still engage in binding activity.
Figure 3: Plot of the alpha fractions of MTB.
2.2 Using Alpha Fractions to Fit pH Titration Data

Following the $\alpha$ fraction calculations, a fit was created using the $\alpha$ fractions of each species of MTB present within pH = 7.0 - 12.5. Equation 1, a derivative of Beer’s Law, was used to fit the data, multiplying each $\alpha$ fraction by its respective molar absorptivity as a function of pH.

$$A = b \cdot [MTB]_{total} (\alpha_{H4MTB^{2-}} \cdot \varepsilon_{H4MTB^{2-}} + \alpha_{H3MTB^{3-}} \cdot \varepsilon_{H3MTB^{3-}} + \alpha_{H2MTB^{4-}} \cdot \varepsilon_{H2MTB^{4-}} + \alpha_{HMTB^{5-}} \cdot \varepsilon_{HMTB^{5-}} + \alpha_{MTB^{6-}} \cdot \varepsilon_{MTB^{6-}}) \quad (1)$$

Here $b$ is the pathlength of the optical cell (1 cm) and $\varepsilon_i$ and $\alpha_i$ are the molar absorptivities and $\alpha$ fractions of the free MTB species, respectively. Figure 4 displays the absorbance against pH with the calculated fit absorbances at 605 nm.

The fit parameters provided by the function were $\varepsilon_{H4MTB^{2-}} = 3 (\pm 3) \times 10^3$ L·mol$^{-1}$·cm$^{-1}$, $\varepsilon_{H3MTB^{3-}} = 2 (\pm 3) \times 10^2$ L·mol$^{-1}$·cm$^{-1}$, $\varepsilon_{H2MTB^{4-}} = 5.0 (\pm 0.2) \times 10^3$ L·mol$^{-1}$·cm$^{-1}$, $\varepsilon_{HMTB^{5-}} = 7.3 (\pm 0.3) \times 10^3$ L·mol$^{-1}$·cm$^{-1}$, $\varepsilon_{MTB^{6-}} = 1.3 (\pm 0.2) \times 10^4$ L·mol$^{-1}$·cm$^{-1}$. The uncertainties of the $H_4MTB^{2-}$ and $H_3MTB^{3-}$ species is likely the result of low absorbance at 605 nm, making the relative uncertainties large.
Table 1: Parameters for the Matlab fit of the pH titration against alpha fractions using literature pKₐ values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation (L·mol⁻¹·cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\varepsilon_{H_4MTB^{2-}})</td>
<td>3 (± 3) x 10³</td>
</tr>
<tr>
<td>(\varepsilon_{H_3MTB^{3-}})</td>
<td>2 (± 3) x 10²</td>
</tr>
<tr>
<td>(\varepsilon_{H_2MTB^{4-}})</td>
<td>5.0 (± 0.2) x 10³</td>
</tr>
<tr>
<td>(\varepsilon_{HMTB^{5-}})</td>
<td>7.3 (± 0.3) x 10³</td>
</tr>
<tr>
<td>(\varepsilon_{MTB^{6-}})</td>
<td>1.3 (± 0.2) x 10⁴</td>
</tr>
</tbody>
</table>

Figure 4: Fit of absorbance against pH accounting for α fractions in the determination of molar absorptivities at 605 nm.
2.3 An Alternative Approach to Fitting pH Titration Data

A second approach to modeling the alpha fractions was attempted. This model also used Equation 1 at 605 nm, but it did not hold constant the pK\textsubscript{a} values provided by literature. This variance in the pK\textsubscript{a} values gave a greater amount of freedom to the function in how it was able to fit the data, resulting in a more visually pleasing fit. This fit provided values of $\varepsilon_{H_4MTB^2^-} = 0.5 \pm 1.7 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{H_3MTB^3^-} = 8 \pm 3 \times 10^2 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{H_2MTB^4^-} = 3.9 \pm 0.5 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{HMTB^5^-} = 6.2 \pm 0.2 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $\varepsilon_{MTB^6^-} = 9.4 \pm 0.5 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, $K_5 = 2 \pm 1 \times 10^{-10}$, and $K_6 = 9 \pm 5 \times 10^{-13}$. A full list of the parameters from this fit can be found in Table 2. The molar absorptivity of the H\textsubscript{4}MTB\textsuperscript{2-} and H\textsubscript{3}MTB\textsuperscript{3-} appear much more reasonable as a result of the change in fit method.

As would have been expected in the previous fit, the absorptivity of H\textsubscript{4}MTB\textsuperscript{2-} is lower than that of the H\textsubscript{3}MTB\textsuperscript{3-} species at 605 nm. The uncertainty of these values, however, is still quite high, especially for the H\textsubscript{4}MTB\textsuperscript{2-} species. When compared to the previous fit the $\varepsilon_{H_4MTB^2^-}$ experiences a 143% difference; $\varepsilon_{H_3MTB^3^-}$ experiences a 120% difference; $\varepsilon_{H_2MTB^4^-}$ experiences a 24% difference, much less than the previous two; $\varepsilon_{HMTB^5^-}$ experiences just a 16% difference; and $\varepsilon_{MTB^6^-}$ experiences a 32% difference. The values of $K_1 - K_4$ were held constant in an attempt to reduce the codependency of the molar absorptivity and the acid dissociation constants, which is the reason the uncertainty associated with those values in Table 2 have an uncertainty of zero listed. The new values of $K_5$ and $K_6$ result in pK\textsubscript{a}s of 9.70 and 12.05, respectively, though the acid dissociation constants do have a relatively high uncertainty (~50%). These values are lower...
by about one full pH unit from what was reported in the literature, which is a significant difference. This fit was performed as an alternative to the previous alpha fraction fit to determine the reliability of the pK$_a$ values reported in literature. The primary purpose of this work was not to calculate and determine pK$_a$s of MTB, however it may be an interesting topic to explore moving forward since we have seen such significant differences between the literature and the data presented here. Although this fit may appear visually superior, the pKa values from literature will be used for the sake of consistency.
Table 2: Parameters of IgorPro fit of the pH titration against alpha fractions allowing certain pK_a values to vary.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{H_4MTB^{2-}} )</td>
<td>( 0.5 (\pm 1.7) \times 10^3 \text{ (L\cdot mol}^{-1}\cdot \text{cm}^{-1}) )</td>
</tr>
<tr>
<td>( \varepsilon_{H_3MTB^{3-}} )</td>
<td>( 8 (\pm 3) \times 10^2 \text{ (L\cdot mol}^{-1}\cdot \text{cm}^{-1}) )</td>
</tr>
<tr>
<td>( \varepsilon_{H_2MTB^{4-}} )</td>
<td>( 3.9 (\pm 0.5) \times 10^3 \text{ (L\cdot mol}^{-1}\cdot \text{cm}^{-1}) )</td>
</tr>
<tr>
<td>( \varepsilon_{HMTB^{5-}} )</td>
<td>( 6.2 (\pm 0.2) \times 10^3 \text{ (L\cdot mol}^{-1}\cdot \text{cm}^{-1}) )</td>
</tr>
<tr>
<td>( \varepsilon_{MTB^{6-}} )</td>
<td>( 9.4 (\pm 0.5) \times 10^3 \text{ (L\cdot mol}^{-1}\cdot \text{cm}^{-1}) )</td>
</tr>
<tr>
<td>( K_1 )</td>
<td>( 4.47 (\pm 0) \times 10^{-3} )</td>
</tr>
<tr>
<td>( K_2 )</td>
<td>( 3.40 (\pm 0) \times 10^{-4} )</td>
</tr>
<tr>
<td>( K_3 )</td>
<td>( 4.90 (\pm 0) \times 10^{-7} )</td>
</tr>
<tr>
<td>( K_4 )</td>
<td>( 3.37 (\pm 0) \times 10^{-9} )</td>
</tr>
<tr>
<td>( K_5 )</td>
<td>( 2 (\pm 1) \times 10^{-10} )</td>
</tr>
<tr>
<td>( K_6 )</td>
<td>( 9 (\pm 5) \times 10^{-13} )</td>
</tr>
</tbody>
</table>

Figure 5: Fit of absorbance against pH accounting for \( \alpha \) fractions in the determination of molar absorptivities at 605 nm allowing pK_a values to vary.
3. Barium Titrations

3.1 Corrected Absorbance Data

To test for spectroscopic changes in the absorbance spectrum of MTB with added barium, spectrophotometric titrations were conducted on 50 mL of 0.125 mM MTB solutions at pH = 7.5, 9.6 and 12.2, which are very near the peak $\alpha$ values for H$_3$MTB$^3$, H$_2$MTB$^4$, and HMTB$^5$, respectively, seen in Figure 3. After the absorbance spectra were corrected for dilution, the spectra were observed to have several similarities. In Figures 6-8, an example is shown of one full set of spectra at each pH. Replicates of each of the spectra may be found in the Supplemental Information. Absorbance maxima were observed around 605 nm in each of the three data sets. This peak absorbance increased as barium was added to solution, suggesting that barium was binding to MTB in solution. In Figure 6 (pH = 7.5), a peak near 450 nm was observed as was expected from this pH as demonstrated in Figure 2. Furthermore, the peak did not shift nor significantly change in absorbance as barium was added. This may be because there are multiple species of MTB present but only one that is binding. In this case, a doubly protonated minority H$_2$MTB$^4$ species would likely be binding to barium, while the majority triply protonated species of H$_3$MTB$^3$ remains unbound. An H$_3$MTB$^3$ has more difficulty binding than a doubly protonated species due to the presence of extra hydrogen ions at the phenol, quinoid, and iminodiacetate group locations (Figure 1). There is also a minority H$_4$MTB$^2$ species present in solution, which would not engage in significant binding activity with barium.
Each of the data sets at pH = 9.6 and 12.2 (Figures 7 and 8, respectively) had three distinct regions in which spectra were clumped together until a sharp spike in absorbance occurred after a given addition of barium. The presence of these distinct regions may delineate where binding occurs. The dominant MTB species in the pH = 9.6 solution is doubly protonated H$_2$MTB$^4^-$, and that of the pH = 12.2 solution is a singly protonated HMTB$^5^-$. As an effect of pH, the peak once most apparent in pH = 7.5 near 450 nm is flattened and disappears as MTB becomes further deprotonated. The peak at 605 nm becomes the most prominent for these species. It is noteworthy that the absorbance range of the pH = 7.5 solution is well below those of the pH = 9.6 and 12.2 solutions. This is because the absorptivity of MTB at 605 nm increases as it is further deprotonated by increasing pH.
Figure 6: Corrected absorbance spectra of MTB buffered at pH = 7.5 taken after addition of barium chloride. Volume indicates cumulative amount of 1 M BaCl$_2$ added.
**Figure 7**: Corrected absorbance spectra of MTB buffered at pH = 9.6 taken after addition of barium chloride. Volume indicates cumulative amount of 1 M BaCl$_2$ added.
Figure 8: Corrected absorbance spectra of MTB buffered at pH = 12.2 taken after addition of barium chloride. Volume indicates cumulative amount of 1 M BaCl₂ added.
4. pH = 7.5 Barium Titration

4.1 Model I and Equations

Once the absorbance spectra had been collected, three different models were used in determining the stoichiometry of complexation. The first model (Model I) assumes a 1:1 binding stoichiometry between barium and MTB. Model I was initially used to describe the barium titration data at pH = 7.5. A 1:1 Model was used primarily because it is a popular model in literature and seems a logical place to start. The chemical equation and equilibrium equation of Model I is labeled Equation 1.

\[
\text{Ba}^{2+} + \text{H}_2\text{MTB}^{4-} \rightleftharpoons \text{BaH}_2\text{MTB}^{2-} \quad K = \frac{[\text{BaH}_2\text{MTB}^{2-}]}{[\text{Ba}^{2+}][\text{H}_2\text{MTB}^{4-}]} \quad (2)
\]

The H$_2$MTB$^{4-}$ minority species is used for the pH = 7.5 data due to the trends observed in Figure 6. Specifically, there is minimal change in absorbance around 450 nm, while there is incremental increase in absorbance at 605 nm as barium is added. Once the MTB species involved in the binding activity are defined, a fit equation using Beer’s Law was derived (Equation 3).

\[
A = b([\text{H}_2\text{MTB}^{4-}]\epsilon_{\text{H}_2\text{MTB}^{4-}} + [\text{BaH}_2\text{MTB}^{2-}]\epsilon_{\text{BaH}_2\text{MTB}^{2-}}) \quad (3)
\]

Here $b$ is the pathlength of the optical cell (1 cm) and $\epsilon_i$ are the molar absorptivities of the various Ba-MTB and free MTB species. Using equilibrium equations derived in the Supplemental Information, the equation for a 1:1 complex (Model I) becomes Equation 4.

\[
A = [\text{MTB}]_{\text{total}} \left(\frac{\epsilon_{\text{H}_2\text{MTB}^{4-}} + K[\text{Ba}^{2+}]\epsilon_{\text{BaH}_2\text{MTB}^{2-}}}{1 + K[\text{Ba}^{2+}]}\right) \quad (4)
\]
While a fit to the data was possible, the $\varepsilon_{BaH_2MTB^{2-}}$ and K parameters are not reliable due to the lack of an inflection point in the data at high barium concentration, leading to poorly constrained fit quantities. The $\varepsilon_{H_2MTB^{4-}}$ parameter was unable to be determined with certainty due to the majority species having a nonzero absorbance at pH = 7.5. The value is also significantly different than that provided by the alpha fraction fit in figure 4 (129% difference). A better estimate of this value is provided at pH = 9.6, where $H_2MTB^{4-}$ is the majority species. It is also possible that $Ba$ may form a 2:1 complex with MTB in a two-step addition, but the data is not well enough constrained at high $[Ba]$ to attempt using a more complex model. The absence of an inflection point indicates that $H_2MTB^{4-}$ is capable of binding to only one barium cation. This may be due to one of the nitrogen atoms of an iminodiacetate group being protonated at pH = 7.5, therefore disfavoring binding the cation at that location. Figure 10 presents a plausible binding complex using Model I at pH = 7.5. It is also possible that the minority species $H_2MTB^{4-}$ is not the only species in this solution that is binding, but a firm conclusion regarding the exact binding cannot be made.
Table 3: Parameters for Model I (1:1) at 605 nm and pH = 7.5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{H_2MTB^{4-}}$</td>
<td>$1.08 (± 0.02) \times 10^3 \text{ (L·mol}^{-1}·\text{cm}^{-1})$</td>
</tr>
<tr>
<td>$\varepsilon_{BaH_2MTB^{2-}}$</td>
<td>$3.9 (± 0.4) \text{ (L·mol}^{-1}·\text{cm}^{-1})$</td>
</tr>
<tr>
<td>$K$</td>
<td>$2.0 (± 0.4)$</td>
</tr>
</tbody>
</table>

**Figure 9**: Corrected absorbance vs. log[Ba] for the average of two experimental replicates using the Model I fit equation at 605 nm and pH=7.5.
Figure 10: Reaction scheme as $H_3MTB^3$ binds to Ba in a 1:1 ratio forming $BaH_3MTB^-$. 
5. pH = 9.6 Barium Titration

5.1 Model II and Equations

In Figure 7, a sharp increase in corrected absorbance was observed after the 2 mL cumulative addition, indicating a greater binding capacity at pH = 9.6. We can infer that this is caused by the differently protonated states of MTB present in each solution as compared to the pH = 7.5 data. This would appear to indicate that once MTB reaches the majority H$_2$MTB$^4-$ species present at pH = 9.6, it becomes capable of binding to multiple barium cations. This would then require the use of a Model II (Figure 11) to describe the data. The Equations derived for Model II are very similar to those for Model I, however, a second chemical equation, and therefore equilibrium equation, must be created to account for the second step of the reaction.

$$\text{Ba}^{2+} + \text{H}_2\text{MTB}^{4-} \rightarrow \text{BaH}_2\text{MTB}^{2-} \quad (5)$$

$$\text{Ba}^{2+} + \text{BaH}_2\text{MTB}^{2-} \rightarrow \text{Ba}_2\text{H}_2\text{MTB} \quad (6)$$

$$A = [\text{MTB}]_{total} \left( \frac{\varepsilon_{\text{H}_2\text{MTB}^{4-}} + K_1[\varepsilon_{\text{BaH}_2\text{MTB}^{2-}}] \varepsilon_{\text{BaH}_2\text{MTB}^{2-}} + K_1K_2[\varepsilon_{\text{Ba}^{2+}}]^2 \varepsilon_{\text{Ba}_2\text{H}_2\text{MTB}}}{1 + K_1[\varepsilon_{\text{Ba}^{2+}}] + K_1K_2[\varepsilon_{\text{Ba}^{2+}}]^2} \right) \quad (7)$$

The fit parameters provided by Matlab were not reliable for $\varepsilon_{\text{Ba}_2\text{H}_2\text{MTB}}$ or $K_2$ due to lack of constraint at high barium concentration in the data. The parameters for $\varepsilon_{\text{H}_2\text{MTB}^{4-}}$, $\varepsilon_{\text{BaH}_2\text{MTB}^{2-}}$, and $K_1$ were determined to be 5.29 (± 0.03) x 10$^3$ L·mol$^{-1}$·cm$^{-1}$, 6.6 (± 0.4) x 10$^3$ L·mol$^{-1}$·cm$^{-1}$, and 12 (± 7), respectively. This model, however, was not able to provide a sufficient fit to the data as it struggles to account for the rapid rise in absorbance midway through the titration (log[Ba] ~ -1.5). This resulted in the need for a more complex model to describe the binding
activity observed in this set of data. Though it was not able to effectively describe the data, it did provide a reasonable estimate for $\varepsilon_{H_2MTB^4}$ with only a 5.6% difference from the value reported by the alpha fraction fit in Table 1.
Table 4: Parameters for Model II (2:1) at 605 nm and pH = 9.6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{H_2MTB}^{4-}$</td>
<td>$5.29 (± 0.03) \times 10^3$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon_{BaH_2MTB^{2-}}$</td>
<td>$6.6 (± 0.4) \times 10^3$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon_{Ba_2H_2MTB}$</td>
<td>$4.4192 \times 10^5 (± 0.002)$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>12 (± 7)</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$4 (± 2) \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Figure 11: Corrected absorbance vs. log[Ba] for the average of two experimental replicates and Model II fit equation at 605 nm and pH=9.6.
5.2 Model III and Equations

The failure of Model II to sufficiently describe the data observed at pH = 9.6 led to the creation of Model III. This model assumes a 3:1 binding complex in a two-step reaction. The first step is a simultaneous binding of barium at the two identical binding sites on either end of MTB. The second step is the attachment of a third barium ion, presumably attaching at the phenol and/or iminodiacetate. A fit of Model III is shown in Figure 12. Model II clearly fails to describe the midway point in the titration. This is because it does not have complex enough parameters to fit the equation sufficiently, creating a need for a Model III. It is possible that to accommodate the binding of barium to MTB the phenolic proton may be displaced (See Figure 1). The equations for Model III then become Equations 8-10.

\[
2 \text{Ba}^{2+} + \text{H}_2\text{MTB}^{4-} \xrightarrow{K_1} \text{Ba}_2\text{H}_2\text{MTB} \quad K_1 = \frac{[\text{Ba}_2\text{H}_2\text{MTB}]}{[\text{Ba}^{2+}]^2[\text{H}_2\text{MTB}^{4-}]} \tag{8}
\]

\[
\text{Ba}^{2+} + \text{Ba}_2\text{H}_2\text{MTB} \xrightarrow{K_2} \text{Ba}_3\text{HMTB}^{2+} \quad K_2 = \frac{[\text{Ba}_3\text{HMTB}^{2+}]}{[\text{Ba}^{2+}][\text{Ba}_2\text{H}_2\text{MTB}]} \tag{9}
\]

\[
A = ([\text{MTB}])_{total} \left( \frac{\varepsilon_{\text{H}_2\text{MTB}^{4-}} + K_1[\text{Ba}^{2+}]^2\varepsilon_{\text{Ba}_2\text{H}_2\text{MTB}} + K_1K_2[\text{Ba}^{2+}]^3\varepsilon_{\text{Ba}_3\text{HMTB}^{2+}}}{1 + K_1[\text{Ba}^{2+}]^2 + K_1K_2[\text{Ba}^{2+}]^3} \right) \tag{10}
\]

Using Model III, \(\varepsilon_{\text{Ba}_3\text{HMTB}^{2+}}\) and \(K_2\) were again too poorly constrained to be determined with any certainty. It can also be seen in Table 5 that these values are negative. This is a failure of the model due to the codependence of variables upon each other. Though it is not possible for either of these values to be negative, they each have significant uncertainty much greater than their magnitude. However, \(\varepsilon_{\text{H}_2\text{MTB}^{4-}}, \varepsilon_{\text{Ba}_2\text{H}_2\text{MTB}},\) and \(K_1\) were determined to be 5.31 (± 0.02) \(\times 10^3\) L·mol\(^{-1}\)·cm\(^{-1}\) and 6.0 (± 0.2) \(\times 10^3\) L·mol\(^{-1}\)·cm\(^{-1}\), and 9 (± 4) \(\times 10^2\),
respectively. The percent difference (6.0%) of the $\varepsilon_{H_2MTB^4-}$ value is also much more reasonable than the Model I estimate used at pH = 7.5. Figure 13 displays the theoretical scheme of barium binding to H$_2$MTB$^4-$ at pH = 9.6 using Model III, accounting for the possible displacement of the proton previously mentioned.
Table 5: Parameters for Model III (3:1) at 605 nm and pH = 9.6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{H_2MTB^{4-}}$</td>
<td>$5.31 \ (± 0.02) \times 10^3 \ (\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1})$</td>
</tr>
<tr>
<td>$\varepsilon_{Ba_2H_2MTB}$</td>
<td>$6.0 \ (± 0.2) \times 10^3 \ (\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1})$</td>
</tr>
<tr>
<td>$\varepsilon_{Ba_3H_2MTB^{2+}}$</td>
<td>$-6 \ (± 55) \times 10^3 \ (\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1})$</td>
</tr>
<tr>
<td>$K_1$</td>
<td>$9 \ (± 4) \times 10^2$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$-0.2 \ (± 0.7)$</td>
</tr>
</tbody>
</table>

Figure 12: Corrected absorbance vs. log[Ba] for the average of two experimental replicates and Model III fit equation at 605 nm and pH=9.6.
**Figure 13:** Reaction scheme as H$_2$MTB$^+$ binds to Ba in 2:1 and then 3:1 stoichiometries forming Ba$_2$H$_2$MTB and Ba$_3$HMTB$^+$. 
6. pH = 12.2 Barium Titration

6.1 Model II and Equations

A similar trend can be found with the data at pH = 12.2 as was seen in Figures 11 and 12: a rapid rise in corrected absorbance was observed midway through the titration followed by a second rise at higher barium concentrations. This time, the inflection point occurred earlier in the titration, likely because barium binds more readily as MTB becomes further deprotonated. The trend observed indicates the same binding activity as that which was seen at pH = 9.6, nevertheless, an attempt was made using Model II to fit the data in Figure 14. As one may have predicted, Model II does not sufficiently describe the data making Model III necessary once again. The chemical equations used for this model are the same as those at pH = 9.6, simply substituting HMTB\(^5\) as the new majority species. The parameters can be found in Table 6, and two interesting things can be noted about the results. First, the uncertainty of the \(\varepsilon_{Ba_2HMTB^-}\) value is unrealistically small. This is a fault of the software simply being unable to determine a more realistic value on occasion. Finally, the final molar absorptivity and \(K_2\) values will not be reliable due to lack of constraint at high [Ba]. The parameters are not discussed in further detail here as one can see that the model does not effectively describe the data (Figure 14) due to a lack of complexity accounting for the middle of the titration (\(\sim [Ba] = (-1.5) – (-2.5)\)).
Table 6: Parameters for Model II (2:1) at 605 nm and pH = 12.2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{\text{HMTB}^{5-}}$</td>
<td>7.02 (± 0.06) x 10^3 (L·mol^-1·cm^-1)</td>
</tr>
<tr>
<td>$\varepsilon_{\text{BaHMTB}^{3-}}$</td>
<td>8.2 (± 0.1) x 10^3 (L·mol^-1·cm^-1)</td>
</tr>
<tr>
<td>$\varepsilon_{\text{Ba}_2\text{HMTB}^-}$</td>
<td>3.45 x 10^5 (± 2 x 10^-4) (L·mol^-1·cm^-1)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>1.2 (± 0.4) x 10^2</td>
</tr>
<tr>
<td>$K_2$</td>
<td>4 (± 1) x 10^-3</td>
</tr>
</tbody>
</table>

Figure 14: Corrected absorbance vs. log[Ba] for the average of two experimental replicates and Model II fit equation at 605 nm and pH=12.2.
6.2 Model III and Equations

The fit of Model III is presented in Figure 15. It can be seen that Model III provides a much better fit of the data, especially around the inflection point. The derived chemical equilibrium and Beer’s Law fit equations are presented below as Equations 10-12.

\[ \text{Equation 11} \]

\[ 2 \text{Ba}^{2+} + \text{HMTB}^5- \xrightarrow{K_1} \text{Ba}_2\text{HMTB}^- \]

\[ \frac{[\text{Ba}_2\text{HMTB}^-]}{[\text{Ba}^{2+}][\text{HMTB}^5-]} = K_1 \]

\[ \text{Equation 12} \]

\[ \text{Ba}^{2+} + \text{Ba}_2\text{HMTB} \xrightarrow{K_2} \text{Ba}_3\text{HMTB}^+ \]

\[ \frac{[\text{Ba}_3\text{HMTB}^+]}{[\text{Ba}^{2+}][\text{Ba}_2\text{HMTB}^-]} = K_2 \]

\[ \text{Equation 13} \]

\[ A = [\text{MTB}]_{\text{total}} \left( \frac{\varepsilon_{\text{HMTB}^5-} + K_1[Ba^{2+}]^2\varepsilon_{\text{Ba}_2\text{HMTB}^-} + K_1K_2[Ba^{2+}]^3\varepsilon_{\text{Ba}_3\text{HMTB}^+}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \right) \]

Using Model III, \( \varepsilon_{\text{Ba}_3\text{HMTB}^+} \) and \( K_2 \) were again too poorly constrained to be determined with any certainty due to a lack of constraint at high [Ba]. However, \( \varepsilon_{\text{HMTB}^5-} \), \( \varepsilon_{\text{Ba}_2\text{HMTB}^-} \), and \( K_1 \) were determined to be \( 7.11 \pm 0.02 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \), and \( 8.05 (\pm 0.03) \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \), and \( 1.6 (\pm 0.3) \times 10^4 \), respectively. The percent difference (2.6%) of \( \varepsilon_{\text{HMTB}^5-} \) indicates that this fit may provide realistic estimates for the parameters even if we cannot be certain.

Figure 16 displays the theoretical scheme of barium binding to H\(_2\)MTB\(^4^+ \) at pH = 12.2. This model also accounts for the displacement of a proton on the phenolic oxygen that would be necessary for barium to bind at a 3:1 ratio. It is possible that the quinoid structure may form a resonance structure, removing the need to displace the phenolic proton. The equations for such a model have not been derived as a \( K_2 \) value cannot be successfully determined due to the previously mentioned lack of constraints on the data. The potential scheme for the reaction has, however been shown in Figure 17.
Table 7: Parameters for Model III (3:1) at 605 nm and pH = 12.2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_{HMTB}^{5-}$</td>
<td>$7.11 \pm 0.02 \times 10^3$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon_{Ba_{2}HMTB}^{-}$</td>
<td>$8.05 \pm 0.03 \times 10^3$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$\varepsilon_{Ba_{3}HMTB}^{+}$</td>
<td>$3.85 \times 10^5 \pm 0.1$ (L·mol$^{-1}$·cm$^{-1}$)</td>
</tr>
<tr>
<td>$K_1$</td>
<td>$1.6 \pm 0.3 \times 10^4$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$4.5 \pm 0.3 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

**Figure 15:** Corrected absorbance vs. log[Ba] for both experimental replicates using the Model III fit equation at 605 nm and pH=12.2
Figure 16: Reaction scheme as HMTB$^{5-}$ binds to Ba in 2:1 and then 3:1 stoichiometries forming Ba$_2$HMTB$^{-}$ and Ba$_3$MTB.
Figure 17: Reaction scheme as HMTB\(^{-}\) binds to Ba in 2:1 and then 3:1 stoichiometries forming Ba\(_2\)HMTB\(^{-}\) and Ba\(_3\)HMTB.
Although there are several possibilities when considering exactly how the complexation of barium occurs with MTB, the most plausible explanation begins with 1:1 and 2:1 binding to the iminodiacetate groups as previously expected[5,7,13,14]. To our knowledge, a 3:1 complex has not been proposed previously. This makes any assumption into how it might bind speculative. It is plausible that the 3:1 complex is formed by displacing the proton on the phenolic oxygen, thus having two barium ions attached at a single iminodiacetate group, and the second iminodiacetate with a single barium ion. It is also possible that the quinoid oxygen may create a resonance structure that allows it to receive a second barium ion, which would eliminate the need for the proton to be displaced on the phenolic oxygen. Previous work [16] has suggested that the metal needs to bind to the phenolic or quinoid oxygens to have significant spectrophotometric activity at 605 nm, which would mean a proton would need to be displaced at any pH to achieve the change in absorbance. As the proton(s) is displaced, the oxygen is allowed to bind to the barium giving the metal access to the π-electron system and subsequently causing color change. The stronger the interaction between the metal and the phenolic oxygens, the greater the spectral change will be[16]. Taking into consideration the increase in absorbance observed at higher pH in conjunction with the increase in absorbance with the addition of barium, it may even add credence to the idea that barium binds in a 3:1 ratio or at least displaces protons in order to bind at the phenoxide site. It is unlikely that the sulfonate becomes involved in the binding as it is the weakest base on MTB.
When considering other metal cations binding to MTB in comparison to barium, it is apparent that other alkaline earth metals such as magnesium and calcium and even transition metals experience stronger binding. Work done by Yoshino et al. determined that barium and strontium will have weaker binding than that of magnesium and calcium[14]. Several factors could come into play when considering why this may be the case. The atomic radius of barium is much larger than many of the third and fourth row metals that are commonly bound to MTB, possibly making the charge density of barium a factor. The weak binding of barium to MTB was verified by recent work by Idhe et al. that determined that barium does not significantly bond to MTB near neutral pH[17]. On the other hand, a different rationale comes from Hard-Soft Acid Base (HSAB) Theory. In this theory, barium can be thought of as a moderately soft acid, while MTB is a soft base, which would explain its ability to form a 3:1 complex in solution at higher pH[16]. This theory may not be the best to describe the binding activity, however, as it does appear contrary to the findings of Yoshino et al. in which harder acids such as magnesium and calcium have displayed stronger binding to MTB than barium[14]. It is difficult to compare formation constants in this experiment to those found in literature because the models differ greatly. The comparison of this data to the literature becomes more difficult when one considers that pH also has an effect on binding, and most titrations in literature have been performed near neutral or acidic pH range. The best comparison between this work and that in the literature is the work done by Bremer and Grell [5] determining $\log K_1 = 3.6 \ (\pm 0.1)$ for Mg at pH = 7.51 and $\log K_1 = 3.1 \ (\pm 0.1)$ for
Ca at pH = 7.27 where logK₁ corresponded to a 1:1 stoichiometry. In the present work, K₁ could not be determined due to the weak binding of barium with MTB at pH = 7.5. The logK₂, corresponding to a 2:1 stoichiometry for Mg, at pH = 7.51 was determined to be 2.7 (±0.1) while that of Ca at pH = 7.27 was determined to be 1.9 (±0.1) [5]. The logK₁ for the present experiment corresponds to a 2:1 stoichiometry, whereas logK₂ represents a 3:1 stoichiometry. LogK₂ was unable to be determined with certainty, but logK₁ was determined to be 3.0 (±0.1) at pH = 9.6 and 4.2 (±0.1) at pH = 12.2. This would confirm further the idea that MTB engages in stronger binding activity as it is further deprotonated. For the ease of comparison, a full list of parameters from each data set and model can be seen in Table 8.
<table>
<thead>
<tr>
<th>Model I at pH = 7.5</th>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{H_2MTB}^{4-} )</td>
<td>1.08 (± 0.02) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{BaH_2MTB}^{2-} )</td>
<td>3.9 (± 0.4) (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( K )</td>
<td>2.0 (± 0.4)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model II at pH = 9.6</th>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{H_2MTB}^{4-} )</td>
<td>5.29 (± 0.03) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{BaH_2MTB}^{2-} )</td>
<td>6.6 (± 0.4) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_2H_2MTB} )</td>
<td>4.4192 x 10⁵ (± 0.002) (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( K_1 )</td>
<td>12 (± 7)</td>
<td></td>
</tr>
<tr>
<td>( K_2 )</td>
<td>4 (± 2) x 10⁻³</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model III at pH = 9.6</th>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{H_2MTB}^{4-} )</td>
<td>5.31 (± 0.02) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_2H_2MTB} )</td>
<td>6.0 (± 0.2) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_3H_2MTB}^{2+} )</td>
<td>-6 (± 55) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( K_1 )</td>
<td>9 (± 4) x 10²</td>
<td></td>
</tr>
<tr>
<td>( K_2 )</td>
<td>-0.2 (± 0.7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model II at pH = 12.2</th>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{HMTB}^{5-} )</td>
<td>7.11 (± 0.02) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{BaHMTB}^{3-} )</td>
<td>8.05 (± 0.03) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_2HMTB}^{2-} )</td>
<td>3.85 x 10⁵ (± 0.1) (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( K_1 )</td>
<td>1.6 (± 0.3) x 10⁴</td>
<td></td>
</tr>
<tr>
<td>( K_2 )</td>
<td>4.5 (± 0.3) x 10⁻³</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model III at pH = 12.2</th>
<th>Parameter</th>
<th>Returned Value ± One Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon_{HMTB}^{5-} )</td>
<td>7.02 (± 0.06) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_2HMTB}^{2-} )</td>
<td>8.2 (± 0.1) x 10³ (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{Ba_3HMTB}^{+} )</td>
<td>3.45 x 10⁶ (± 2 x 10⁻⁴) (L·mol⁻¹·cm⁻¹)</td>
<td></td>
</tr>
<tr>
<td>( K_1 )</td>
<td>1.2 (± 0.4) x 10²</td>
<td></td>
</tr>
<tr>
<td>( K_2 )</td>
<td>4 (± 1) x 10⁻³</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

As a solution of barium chloride was titrated into MTB and measured spectrophotometrically at 605 nm, barium was found to bind to MTB in both 1:1 and 3:1 complexes, depending on the pH of the solution. At pH = 7.5, a 1:1 complex was formed as the proton attached to the iminodiacetate at this pH made a second binding site unfavorable. However, at higher pH (9.6 and 12.2) barium is able to form a 2:1 and subsequently a 3:1 complex as evidenced by the model fitting of the spectrophotometric titration data. The Matlab code determined molar absorptivity coefficients and formation constants for the barium-MTB complexes. It can be assumed that the first two barium cations that are complexed by MTB bind at the iminodiacetate groups on either end of the molecule. Our data shows, for the first time, that MTB may form a 3:1 complex with barium at high pH. At pH = 9.6, barium would need to displace the proton from one of the phenolic oxygens and would also likely bind to the nitrogen on the adjacent iminodiacetate group. At pH = 12.2 one of the phenols becomes deprotonated, forming a quinoid structure. We postulate that the third barium would continue to complex at the phenolic oxygen, displacing the proton.
References


[12] Z. Rasouli, Z. Hassanzadeh, R. Ghavami, Application of a new version of GA-RBF neural network for simultaneous spectrophotometric determination of Zn(II), Fe(II), Co(II) and Cu(II) in real samples: An exploratory study of their complexation


Appendices

Appendix A

1:1 Model at pH=7.5

Chemical Equation:

\[ Ba^{2+} + H_2MTB^{4-} \rightleftharpoons K \rightarrow BaH_2MTB^{4-} \]

Equilibrium:

\[ K = \frac{[BaH_2MTB^{2-}]}{[Ba^{2+}][H_2MTB^{4-}]} \]

Mass Balance:

\[ [Cl^-] = 2[Ba]_{total} \quad [Na^+] = 4[MTB]_{total} \]

\[ [MTB]_{total} = [H_2MTB^{4-}] + [BaH_2MTB^{4-}] \]

\[ [Ba]_{total} = [Ba^{2+}] + [BaH_2MTB^{2-}] \]

Derivation of [MTB^{3-}] and [BaMTB^{1-}]:

\[ [BaH_2MTB^{2-}] = K[Ba^{2+}][H_2MTB^{4-}] \]

Add [H_2MTB^{4-}] to both sides, simplifying to [MTB]_{total}

\[ [MTB]_{total} = [H_2MTB^{4-}](1 + K[Ba^{2+}]) \]

Therefore,

\[ [H_2MTB^{4-}] = \frac{[MTB]_{total}}{1 + K[Ba^{2+}]} \]
\[ [\text{BaH}_2\text{MTB}^{2-}] = \frac{K[\text{Ba}^{2+}][\text{MTB}]_{\text{total}}}{1 + K[\text{Ba}^{2+}]} \]

**Charge Balance:**

\[ [\text{Cl}^-] + [\text{OH}^-] + 4[\text{H}_2\text{MTB}^{4-}] + 2[\text{H}_2\text{BaMTB}^{2-}] = 2[\text{Ba}^{2+}] + [H^+] + [\text{Na}^+] \]

Remove \([\text{Cl}^-], [\text{Na}^+], [\text{H}_3\text{MTB}^3],\) and \([\text{BaH}_3\text{MTB}].\)

\[ 2[\text{Ba}]_{\text{total}} + [\text{OH}^-] + [\text{MTB}]_{\text{total}} \left( \frac{4 + 2K[\text{Ba}^{2+}]}{1 + K[\text{Ba}^{2+}]} \right) = 2[\text{Ba}^{2+}] + [H^+] + 4[\text{MTB}]_{\text{total}} \]

Multiply the entire equation by \((1+K[\text{Ba}^{2+}]).\)

\[ 2[\text{Ba}]_{\text{total}} + [\text{OH}^-] + 4[\text{MTB}]_{\text{total}} + (2[\text{Ba}]_{\text{total}} + [\text{OH}^-] + 2[\text{MTB}]_{\text{total}})K[\text{Ba}^{2+}] \]

\[ = [H^+] + 4[\text{MTB}]_{\text{total}} + ([H^+] + 4[\text{MTB}]_{\text{total}} + \frac{2}{K})K[\text{Ba}^{2+}] \]

\[ + K[\text{Ba}^{2+}]^2 \]

Combine like terms setting the equation equal to zero and in terms of \([\text{Ba}^{2+}].\)

\[ -K[\text{Ba}^{2+}]^2 + (2[\text{Ba}]_{\text{total}} + [\text{OH}^-] - [H^+] - 2[\text{MTB}]_{\text{total}} - \frac{2}{K})K[\text{Ba}^{2+}] + 2[\text{Ba}^{2+}] \]

\[ + (2[\text{Ba}]_{\text{total}} + [\text{OH}^-] - [H^+]) = 0 \]

**Beer’s Law fit equation:**

\[ A = ([\text{H}_2\text{MTB}^{4-}]\varepsilon_{\text{H}_2\text{MTB}^{4-}} + [\text{BaH}_2\text{MTB}^{2-}]\varepsilon_{\text{BaH}_2\text{MTB}^{2-}}) \]

Using the equations derived above a usable fit equation can be derived:

\[ A = [\text{MTB}]_{\text{total}} \left( \frac{\varepsilon_{\text{H}_2\text{MTB}^{4-}} + K[\text{Ba}^{2+}]\varepsilon_{\text{BaH}_2\text{MTB}^{2-}}}{1 + K[\text{Ba}^{2+}]} \right) \]
MATLAB Code:

% wavelength (nm)
wl = 605;

% Total MTB
MTBtot = 0.000125;

% Converting pH to H and OH concentrations
pH = 7.5;
H = 10^(-pH);
OH = 10^(-(14-pH));

% Import data from CSV
a = importdata('pH 7.5.csv');
x = a.data;

% Avg Absorbance
Absorbance = x(:,3);

% Barium
Ba = x(:,4); %[Ba]
logBa = x(:,5); % log[Ba]
%% Error
err = x(:,6);

%%% Beers Law model function
modelfun = @(b,Ba)MTBtot.*((b(1)+b(2)*b(3).*Ba)./(1+b(3).*Ba));
% b1=EMTB b2=EBaMTB b3=K;

%%% Initial values of molar absorptivity coefficients
%%% and formation constants
beta0 = [920 3500 2];

%%% Non-linear model fit
mdl = fitnlm(Ba,x(:,3), modelfun, beta0);
bfit = mdl.Coefficients.Estimate';
disp(bfit);
beta0 = bfit;
errorbar(logBa,Absorbance,err)
hold on
plot(logBa,MTBtot.*((bfit(1)+bfit(2)*bfit(3).*Ba)./(1+bfit(3).*Ba)))
Appendix B

2:1 Model at pH=9.6

Chemical Equations:

\[Ba^{2+} + H_{2}MTB^{4-} \rightleftharpoons K_{1} BaH_{2}MTB^{2-}\]

\[Ba^{2+} + BaH_{2}MTB^{2-} \rightleftharpoons K_{2} Ba_{2}H_{2}MTB\]

Equilibrium:

\[K_{1} = \frac{[BaH_{2}MTB^{2-}]}{[Ba^{2+}][H_{2}MTB^{4-}]}\]

\[K_{2} = \frac{[Ba_{2}H_{2}MTB]}{[Ba^{2+}][BaH_{2}MTB^{2-}]}\]

Mass Balance:

\[[Cl^{-}] = 2[Ba]_{total} \quad [Na^{+}] = 4[MTB]_{total}\]

\[[MTB]_{total} = [H_{2}MTB^{4-}] + [BaH_{2}MTB^{2-}] + [Ba_{2}H_{2}MTB]\]

\[[Ba]_{total} = [Ba^{2+}] + [BaH_{2}MTB^{2-}] + 2[Ba_{2}H_{2}MTB]\]

Derivation of \([MTB^{+}], [Ba_{2}H_{2}MTB], \) and \([BaH_{2}MTB^{2-}]\):

Starting with the Equilibrium Equations:

\[K_{1} = \frac{[BaH_{2}MTB^{2-}]}{[Ba^{2+}][H_{2}MTB^{4-}]} \quad K_{2} = \frac{[Ba_{2}H_{2}MTB]}{[Ba^{2+}][BaH_{2}MTB^{2-}]}\]

Solve the first equation for \([BaH_{2}MTB^{2-}]\) and \([Ba_{2}H_{2}MTB]\).

\[[BaH_{2}MTB^{2-}] = K_{1}[Ba^{2+}][H_{2}MTB^{4-}]\]

\[[Ba_{2}H_{2}MTB] = K_{2}[Ba^{2+}]^{2}[BaH_{2}MTB^{2-}]\]

To the \([BaH_{2}MTB^{2-}]\) equation, add \([Ba_{2}H_{2}MTB]\) and \([H_{2}MTB^{4-}]\) to both sides, simplifying the left side down to \([MTB]_{total}\).

\[[MTB]_{total} = K_{1}[Ba^{2+}][H_{2}MTB^{4-}] + [Ba_{2}H_{2}MTB] + [H_{2}MTB^{4-}]\]
By substituting $[\text{Ba}_2\text{H}_2\text{MTB}^+]$ out of the equation, the result is the following.

$$[\text{H}_2\text{MTB}^4^-](1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2) = [\text{MTB}]_{total}$$

Thus, we can deduce $[\text{H}_2\text{MTB}^4^-]$.

$$[\text{H}_2\text{MTB}^4^-] = \frac{[\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2}$$

From here, $[\text{H}_2\text{MTB}^4^-]$ can be used to easily determine $[\text{Ba}_2\text{H}_2\text{MTB}]$ and $[\text{BaH}_2\text{MTB}^2]$ as the following.

$$[\text{BaH}_2\text{MTB}] = \frac{K_1[\text{Ba}^{2+}][\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2}$$

$$[\text{Ba}_2\text{H}_2\text{MTB}^2+] = \frac{K_1K_2[\text{Ba}^{2+}]^2[\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2}$$

**Charge Balance:**

$$[\text{Cl}^-] + [\text{OH}^-] + 4[\text{H}_2\text{MTB}^4^-] + 2[\text{BaH}_2\text{MTB}^2+] = 2[\text{Ba}^{2+}] + [H^+] + [\text{Na}^+]$$

*Note: $[\text{Ba}_2\text{H}_2\text{MTB}]$ is absent because it does not have a net charge.*

Remove $[\text{Cl}^-]$, $[\text{Na}^+]$, $[\text{H}_2\text{MTB}^4^-]$, and $[\text{BaH}_2\text{MTB}^2+]$.

$$2[\text{Ba}]_{total} + [\text{OH}^-] + \left(\frac{4[\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2}\right) + \left(\frac{2[\text{MTB}]_{total}K_1[\text{Ba}^{2+}]}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2}\right) = 2[\text{Ba}^{2+}] + [H^+] + 4[\text{MTB}]_{total}$$

Multiply the entire equation by $(1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2)$.

$$(2[\text{Ba}]_{total} + [\text{OH}^-] + 4[\text{MTB}]_{total}) + (2[\text{Ba}]_{total} + [\text{OH}^-]) + 2[\text{MTB}]_{total}K_1[\text{Ba}^{2+}] + (2[\text{Ba}]_{total} + [\text{OH}^-])K_1K_2[\text{Ba}^{2+}]^2$$

$$= ([H^+] + 4[\text{MTB}]_{total}) + ([H^+] + 4[\text{MTB}]_{total} + \frac{2}{K_1})K_1[\text{Ba}^{2+}]$$

$$+ ([H^+] + 4[\text{MTB}]_{total} + \frac{2}{K_2})K_1K_2[\text{Ba}^{2+}]^2 + 2K_1K_2[\text{Ba}^{2+}]^3$$
Combine like terms setting the equation equal to zero and in terms of $[\text{Ba}^{2+}]$.

$$
-2K_1K_2[Ba^{2+}]^3 + ([\text{OH}^-] + 2[Ba]_{total} - [H^+] - 4[MTB]_{total} - \frac{2}{K_2})K_1K_2[Ba^{2+}]^2
\hspace{1cm}
+ (2[Ba]_{total} + [\text{OH}^-] - [H^+] - 2[MTB]_{total} - \frac{2}{K_1})K_1[Ba^{2+}]
\hspace{1cm}
+ +(2[Ba]_{total} + [\text{OH}^-] - [H^+]) = 0
$$

**Beer’s Law fit equation:**

$$
A = ([H_2MTB^{4-}]\epsilon_{H_2MTB^{4-}} + [Ba_2H_2MTB]\epsilon_{Ba_2H_2MTB} + [BaH_2MTB^{2-}]\epsilon_{BaH_2MTB^{2-}})
$$

Using the equations derived above a usable fit equation can be derived.

$$
A = [MTB]_{total} \left( \frac{\epsilon_{H_2MTB^{4-}} + K_1[Ba^{2+}]\epsilon_{BaH_2MTB^{2-}}}{1 + K_1[Ba^{2+}] + K_1K_2[Ba^{2+}]^2} \right)
$$
MATLAB Code:

% wavelength (nm)
wl = 605;

%Total MTB
MTBtot = 0.000125;

% Converting pH to H and OH concentrations
pH = 9.6;
H = 10^(-pH);
OH = 10^-((14-pH));

% Import data from CSV
a = importdata('pH 9.6.csv');
x = a.data;

% Avg Absorbance
Absorbance = x(:,3);

% Barium
Ba = x(:,4); %[Ba]
logBa = x(:,5); % log[Ba]
```matlab
% Beers Law model function
modelfun = @(b,Ba)MTBtot.*((b(1)+b(2)*b(4).*Ba+b(4)*b(5)*b(3).*Ba.^2)./(1+b(4).*Ba+b(4)*b(5).*Ba.^2));
% b1=EMTB b2=EBa2MTB b3=EBa3MTB b4=K1 b5=K2;

% Initial values of molar absorptivity coefficients
% and formation constants
beta0 = [4800 5500 22300 50 .1];

% Non-linear model fit
mdl = fitnlm(Ba,x(:,3), modelfun, beta0);
bfit = mdl.Coefficients.Estimate';
disp(bfit);
beta0 = bfit;
errorbar(logBa,Absorbance,err)
hold on
plot(logBa,MTBtot.*((bfit(1)+bfit(2)*bfit(4).*Ba+bfit(4)*bfit(5)*bfit(3).*Ba.^2)./(1+bfit(4).*Ba+bfit(4)*bfit(5).*Ba.^2)))
```
Appendix C

3:1 Model at pH=9.6

Chemical Equations:

\[ 2Ba^{2+} + H_2MTB^{4-} \rightarrow Ba_2H_2MTB \]

\[ Ba^{2+} + Ba_2H_2MTB \rightarrow Ba_3H_2MTB^{2+} \]

Equilibrium:

\[ K_1 = \frac{[Ba_2H_2MTB]}{[Ba^{2+}]^2[H_2MTB^{4-}]} \]

\[ K_2 = \frac{[Ba_3H_2MTB^{2+}]}{[Ba^{2+}][Ba_2H_2MTB]} \]

Mass Balance:

\[ [Cl^-] = 2[Ba]_{total} \quad [Na^+] = 4[MTB]_{total} \]

\[ [MTB]_{total} = [H_2MTB^{4-}] + [Ba_2H_2MTB] + [Ba_3H_2MTB^{2+}] \]

\[ [Ba]_{total} = [Ba^{2+}] + 2[Ba_2H_2MTB] + 3[Ba_3H_2MTB^{2+}] \]

Derivation of \([MTB^4-]\), \([Ba_2MTB]\), and \([Ba_3MTB^{2+}]\):

Starting with the Equilibrium Equations:

\[ K_1 = \frac{[Ba_2H_2MTB]}{[Ba^{2+}]^2[H_2MTB^{4-}]} \quad K_2 = \frac{[Ba_3H_2MTB^{2+}]}{[Ba^{2+}][Ba_2H_2MTB]} \]

Solve the first equation for \([Ba_2MTB]\).

\[ [Ba_2H_2MTB] = K_1[Ba^{2+}]^2[H_2MTB^{4-}] \quad [Ba_3H_2MTB^{2+}] = K_2[Ba^{2+}][Ba_2H_2MTB] \]

To the \([Ba_2H_2MTB]\) equation, add \([Ba_3H_2MTB^{2+}]\) and \([H_2MTB^{4-}]\) to both sides, simplifying the left side down to \([MTB]_{total}\).

\[ [MTB]_{total} = K_1[Ba^{2+}][H_2MTB^{4-}] + [Ba_2H_2MTB] + [H_2MTB^{4-}] \]

By substituting \([Ba_2H_2MTB]\) out of the equation, the result is the following.
\[ [H_2MTB^{4-}](1 + K_1[Ba^{2+}] + K_1K_2[Ba^{2+}]^2) = [MTB]_{\text{total}} \]

Thus, we can deduce \([H_2MTB^{4-}]\).

\[ [H_2MTB^{4-}] = \frac{[MTB]_{\text{total}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \]

From here, \([H_2MTB^{4-}]\) can be used to easily determine \([Ba_2H_2MTB]\) and \([Ba_3H_2MTB^2+]\) as the following.

\[ [Ba_2H_2MTB] = \frac{K_1[Ba^{2+}][MTB]_{\text{total}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \]

\[ [Ba_3H_2MTB^2+] = \frac{K_1K_2[Ba^{2+}]^2[MTB]_{\text{total}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \]

**Charge Balance:**

\[ [Cl^-] + [OH^-] + 4[H_2MTB^{4-}] = 2[Ba^{2+}] + [+H^+][Na^+] + 2[Ba_3H_2MTB^2+] \]

*Note: \([Ba_2H_2MTB]\) is absent because it does not have a net charge.*

Remove \([Cl^-], [Na^+], [H_2MTB^{4-}],\) and \([Ba_3H_2MTB^2+]\).

\[ 2[Ba]_{\text{total}} + [OH^-] + \frac{4[MTB]_{\text{total}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \]

\[ = 2[Ba^{2+}] + [H^+] + 4[MTB]_{\text{total}} + \left( \frac{2[MTB]_{\text{total}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \right) \]

Multiply the entire equation by \((1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3)\).

\[ (2[Ba]_{\text{total}} + [OH^-] + 4[MTB]_{\text{total}} + (2[Ba]_{\text{total}} + [OH^-])K_1[Ba^{2+}]^2 + (2[Ba]_{\text{total}} + [OH^-])K_1K_2[Ba^{2+}]^3 \]

\[ = ([H^+] + 6[MTB]_{\text{total}}) + 2[Ba^{2+}] + ([H^+] + 4[MTB]_{\text{total}})K_1[Ba^{2+}]^2 \]

\[ + ([H^+] + 4[MTB]_{\text{total}} + \frac{2K_1}{K_2}) K_1K_2[Ba^{2+}]^3 + 2K_1K_2[Ba^{2+}]^4 \]

Combine like terms setting the equation equal to zero and in terms of \([Ba^{2+}]\).
\[-2K_1K_2[Ba^{2+}]^4 + ([OH^-] + 2[Ba]_{total} - [H^+] - 4[MTB]_{total} - \frac{2K_1}{K_2})K_1K_2[Ba^{2+}]^3 \]
\[+ (2[Ba]_{total} + [OH^-] - [H^+] - 4[MTB]_{total})K_1[Ba^{2+}]^2 + 2[Ba^{2+}] \]
\[+ (2[Ba]_{total} + [OH^-] - [H^+] - 2[MTB]_{total}) = 0 \]

**Beer’s Law fit equation:**

\[ A = ([H_2MTB^{4-}]\varepsilon_{H_2MTB^{4-}} + [Ba_2H_2MTB]\varepsilon_{Ba_2H_2MTB} + [Ba_3H_2MTB^{2+}]\varepsilon_{Ba_3H_2MTB^{2+}}) \]

Using the equations derived above a usable fit equation can be derived.

\[ A = [MTB]_{total} \left( \frac{\varepsilon_{H_2MTB^{4-}} + K_1[Ba^{2+}]^2\varepsilon_{Ba_2H_2MTB} + K_1K_2[Ba^{2+}]^3\varepsilon_{Ba_3H_2MTB^{2+}}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3} \right) \]
MATLAB Code:

% wavelength (nm)
wl = 605;

% Total MTB
MTBtot = 0.000125;

% Converting pH to H and OH concentrations
pH = 9.6;
H = 10^-pH;
OH = 10^-{(14-pH)};

% Import data from CSV
a = importdata('pH 9.6.csv');
x = a.data;

% Avg Absorbance
Absorbance = x(:,3);

% Barium
Ba = x(:,4); %[Ba]
logBa = x(:,5); % log[Ba]
%% Beers Law model function

modelfun = @(b,Ba)MTBtot.*((b(1)+b(2).*b(4).*Ba.^2+b(4).*b(5).*b(3).*Ba.^3)./(1+b(4).*Ba.^2+
b(4).*b(5).*Ba.^3));

%% b1=EMTB b2=EBa2MTB b3=EBa3MTB b4=K1 b5=K2;

%% Initial values of molar absorptivity coefficients
%% and formation constants

beta0 = [4750 8100 42000 12.7 .044];

%% Non-linear model fit

mdl = fitnlm(Ba,x(:,3), modelfun, beta0);

bfit = mdl.Coefficients.Estimate';
disp(bfit);

beta0 = bfit;

errorbar(logBa,Absorbance,err)
hold on

plot(logBa,MTBtot.*((bfit(1)+bfit(2)*bfit(4).*Ba.^2+bfit(4)*bfit(5)*bfit(3).*Ba.^3)./(1+
bfit(4).*Ba.^2+bfit(4)*bfit(5).*Ba.^3)))
Appendix D

2:1 Model at pH=12.2

Chemical Equations:

\[ \text{Ba}^{2+} + \text{HMTB}^5^- \xrightleftharpoons{K_1} \text{BaHMTB}^3^- \]

\[ \text{Ba}^{2+} + \text{BaHMTB}^3^- \xrightleftharpoons{K_2} \text{Ba}_2\text{HMTB}^- \]

Equilibrium:

\[ K_1 = \frac{[\text{BaHMTB}^3^-]}{[\text{Ba}^{2+}][\text{HMTB}^5^-]} \]

\[ K_2 = \frac{[\text{Ba}_2\text{HMTB}^-]}{[\text{Ba}^{2+}][\text{BaHMTB}^3^-]} \]

Mass Balance:

\[ [\text{Cl}^-] = 2[\text{Ba}]_{total} \quad [\text{Na}^+] = 4[\text{MTB}]_{total} \]

\[ [\text{MTB}]_{total} = [\text{HMTB}^5^-] + [\text{BaHMTB}^3^-] + [\text{Ba}_2\text{HMTB}^-] \]

\[ [\text{Ba}]_{total} = [\text{Ba}^{2+}] + [\text{BaHMTB}^3^-] + 2[\text{Ba}_2\text{HMTB}^-] \]

Derivation of [\text{MTB}^4^-], [\text{BaHMTB}^3^-], and [\text{Ba}_2\text{HMTB}^-]:

Charge does not affect the derivation, so we can simply modify those equations from the pH 9.6 model.

\[ [\text{HMTB}^5^-] = \frac{[\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2} \]

\[ [\text{BaHMTB}^3^-] = \frac{K_1[\text{Ba}^{2+}][\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2} \]

\[ [\text{Ba}_2\text{HMTB}^-] = \frac{K_1K_2[\text{Ba}^{2+}]^2[\text{MTB}]_{total}}{1 + K_1[\text{Ba}^{2+}] + K_1K_2[\text{Ba}^{2+}]^2} \]
Charge Balance:

\[ [Cl^-] + 5[HMTB^{5-}] + 3[BaHMTB^{3-}] + [Ba_2HMTB^-] + [OH^-] \]
\[ = 2[Ba^{2+}] + [Na^+] + [H^+] \]

Remove \([ Cl^-], [Na^+], [HMTB^{5-}], [BaHMTB^-], \) and \([BaHMTB^{3-}]\).

\[ 2[Ba]_{total} + [OH^-] \]
\[ + \left( \frac{5[MTB]_{total} + 3K_1[Ba^{2+}][MTB]_{total} + K_1K_2[Ba^{2+}]^2[MTB]_{total}}{1 + K_1[Ba^{2+}] + K_1K_2[Ba^{2+}]^2} \right) \]
\[ = 2[Ba^{2+}] + [H^+] + 4[MTB]_{total} \]

Multiply the entire equation by \((1 + K_1[Ba^{2+}] + K_1K_2[Ba^{2+}]^2)\).

\[ (2[Ba]_{total} + [OH^-] + 5[MTB]_{total}) + (2[Ba]_{total} + [OH^-]) \]
\[ + 3[MTB]_{total}K_1[Ba^{2+}] + (2[Ba]_{total} + [OH^-]) \]
\[ + [MTB]_{total}K_1K_2[Ba^{2+}]^2 \]
\[ = ([H^+] + 4[MTB]_{total}) + ([H^+] + 4[MTB]_{total} + \frac{2}{K_1})K_1[Ba^{2+}] \]
\[ + ([H^+] + 4[MTB]_{total} + \frac{2}{K_2})K_1K_2[Ba^{2+}]^2 + 2K_1K_2[Ba^{2+}]^3 \]

Combine like terms setting the equation equal to zero and in terms of \([Ba^{2+}]\).

\[ -2K_1K_2[Ba^{2+}]^3 + ([OH^-] + 2[Ba]_{total} - [H^+] - 3[MTB]_{total} - \frac{2}{K_2})K_1K_2[Ba^{2+}]^2 \]
\[ + (2[Ba]_{total} + [OH^-] - [H^+] - [MTB]_{total})K_1[Ba^{2+}] + (2[Ba]_{total} \]
\[ + [OH^-] - [H^+] + [MTB]_{total}) = 0 \]

Beer’s Law fit equation:

\[ A = ([HMTB^{5-}]e_{HMTB^{5-}} + [BaHMTB^{3-}]e_{BaHMTB^{3-}} + [Ba_2HMTB^-]e_{Ba_2HMTB^-}) \]

Using the equations derived above a usable fit equation can be derived.

\[ A = [MTB]_{total} \left( \frac{e_{HMTB^{5-}} + K_1[Ba^{2+}]e_{BaHMTB^{3-}} + K_1K_2[Ba^{2+}]^2e_{Ba_2HMTB^-}}{1 + K_1[Ba^{2+}] + K_1K_2[Ba^{2+}]^2} \right) \]
MATLAB Code:

% wavelength (nm)
wl = 605;

%Total MTB
MTBtot = 0.000125;

% Converting pH to H and OH concentrations
pH = 12.2;
H = 10^(-pH);
OH = 10^(14-pH);

% Import data from CSV
a = importdata('pH 12.2.csv');
x = a.data;

% Avg Absorbance
Absorbance = x(:,3);

% Barium
Ba = x(:,4);  %[Ba]
logBa = x(:,5);  % log[Ba]
%%Error
err = x(:,6);

%% Beers Law model function
modelfun = @(b,Ba)MTBtot.*((b(1)+b(2)*b(4).*Ba+b(4)*b(5)*b(3).*Ba.^2)./(1+b(4).*Ba+b(4)*b(5).*Ba.^2));

%% b1=EMTB b2=EBa2MTB b3=EBa3MTB b4=K1 b5=K2;

%% Initial values of molar absorptivity coefficients
%% and formation constants
beta0 = [6500 7800 8000 50 .5];

%% Non-linear model fit
mdl = fitnlm(Ba,x(:,3), modelfun, beta0);
bfit = mdl.Coefficients.Estimate';
disp(bfit);

beta0 = bfit;
errorbar(logBa,Absorbance, err)
hold on
plot(logBa,MTBtot.*((bfit(1)+bfit(2)*bfit(4).*Ba+bfit(4)*bfit(5)*bfit(3).*Ba.^2)./(1+bfit (4).*Ba+bfit(4)*bfit(5).*Ba.^2)))
Appendix E

3:1 Model at pH=12.2

Chemical Equations:

\[2Ba^{2+} + HMTB^{5-} \overset{K_1}{\rightarrow} Ba_2HMTB^-\]

\[Ba^{2+} + Ba_2HMTB^- \overset{K_2}{\rightarrow} Ba_3HMTB^+\]

Equilibrium:

\[K_1 = \frac{[Ba_2HMTB^-]}{[Ba^{2+}]^2[HMTB^{5-}]}\]

\[K_2 = \frac{[Ba_3HMTB^+]}{[Ba^{2+}][Ba_2HMTB^-]}\]

Mass Balance:

\[[Cl^-] = 2[Ba]_{total} \quad [Na^+] = 4[MTB]_{total}\]

\[[MTB]_{total} = [HMTB^{5-}] + [Ba_2HMTB^-] + [Ba_3HMTB^+]\]

\[[Ba]_{total} = [Ba^{2+}] + 2[Ba_2HMTB^-] + 3[Ba_3HMTB^+]\]

Derivation of [MTB\(^+\)], [BaMTB\(^3\)], and [Ba\(_2\)MTB\(^-\)]:

Charge does not affect the derivation, so we can simply modify those equations from the pH 9.6 model.

\[[HMTB^{5-}] = \frac{[MTB]_{total}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3}\]

\[[Ba_2HMTB^-] = \frac{K_1[Ba^{2+}]^2[MTB]_{total}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3}\]

\[[Ba_3HMTB^+] = \frac{K_1K_2[Ba^{2+}]^3[MTB]_{total}}{1 + K_1[Ba^{2+}]^2 + K_1K_2[Ba^{2+}]^3}\]
Using the equations derived above a usable fit equation can be derived. 

Beam’s Law fit equation:

\[ A = \left( [H_{MTB}^{5-}] \varepsilon_{H_{MTB}^{5-}} + [Ba_2H_{MTB}^-] \varepsilon_{Ba_2H_{MTB}^-} + [Ba_3H_{MTB}^+] \varepsilon_{Ba_3H_{MTB}^+} \right) \]

Using the equations derived above a usable fit equation can be derived.

\[ A = [MTB]_{total} \left( \frac{[H_{MTB}^{5-}] \varepsilon_{H_{MTB}^{5-}} + K_1[Ba_2^{2+}]^2 \varepsilon_{Ba_2H_{MTB}^-} + K_1K_2[Ba_2^{2+}]^3 \varepsilon_{Ba_3H_{MTB}^+}}{1 + K_1[Ba_2^{2+}]^2 + K_1K_2[Ba_2^{2+}]^3} \right) \]
MATLAB Code:

```matlab
% wavelength (nm)
wavelength = 605;

%Total MTB
MTBtot = 0.000125;

% Converting pH to H and OH concentrations
pH = 12.2;
H = 10^(-pH);
OH = 10^(14-pH);

% Import data from CSV
a = importdata('pH 12.2.csv');
x = a.data;

% Avg Absorbance
Absorbance = x(:,3);

% Barium
Ba = x(:,4); % [Ba]
logBa = x(:,5); % log[Ba]
```
%Error
err = x(:,6);

% Beers Law model function
modelfun = @(b,Ba)MTBtot.*((b(1)+b(2)*b(4).*Ba.^2+b(4)*b(5)*b(3).*Ba.^3)./(1+b(4).*Ba.^2+b(4)*b(5).*Ba.^3));

% b1=EMTB b2=EBa2MTB b3=EBa3MTB b4=K1 b5=K2;

% Initial values of molar absorptivity coefficients
% and formation constants
beta0 = [6000 7500 10000 223000 .05];

% Non-linear model fit
mdl = fitnlm(Ba,x(:,3), modelfun, beta0);
bfit = mdl.Coefficients.Estimate';
disp(bfit);
beta0 = bfit;
errorbar(logBa,Absorbance,err)
hold on
plot(logBa,MTBtot.*((bfit(1)+bfit(2)*bfit(4).*Ba.^2+bfit(4)*bfit(5)*bfit(3).*Ba.^3)./(1+bfit(4).*Ba.^2+bfit(4)*bfit(5).*Ba.^3)))
Figure S1: 2\textsuperscript{nd} replicate of titration at pH=7.5
Figure S2: 2nd replicate of titration at pH=9.6
Figure S3: 2nd replicate of titration at pH=12.2
Appendix G

Figure S4: The structures of every species of MTB as it is protonated. It should be noted that some pKₐ values cannot be determined spectrophotometrically and are therefore not present in literature.
Figure S5: Image of MTB before (left) and after (right) barium addition at pH = 7.5. These solutions were made separately, so no dilution effect has occurred. All concentrations with that exception are the same as they were before and after the titration, respectively.
**Figure S6**: Image of MTB before (left) and after (right) barium addition at pH = 9.6. These solutions were made separately, so no dilution effect has occurred. All concentrations with that exception are the same as they were before and after the titration, respectively.
Figure S7: Image of MTB before (left) and after (right) barium addition at pH = 12.2. These solutions were made separately, so no dilution effect has occurred. All concentrations with that exception are the same as they were before and after the titration, respectively.
Figure S8: Image of MTB before and after barium addition at each of the three tested pH levels. From left to right the solutions are without barium at pH = 12.2, with barium at pH = 12.2, without barium at pH = 9.6, with barium at pH = 9.6, without barium at pH = 7.5, and with barium at pH = 7.5. These solutions were made separately, so no dilution effect has occurred. All concentrations with that exception are the same as they were before and after the titration, respectively.