Spring 2012

Luminescence studies of Europium (III) - D-glucosamine complexes in water

Triny Trinh Pham
San Jose State University

LUMINESCENCE STUDIES OF EUROPIUM (III) – D-GLUCOSAMINE COMPLEXES IN WATER

A Thesis

Presented to

The Faculty of the Department of Chemistry

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Triny Trinh Pham

May 2012

The Designated Thesis Committee Approves the Thesis Titled

LUMINESCENCE STUDIES OF EUROPIUM (III) – D-GLUCOSAMINE COMPLEXES IN WATER

by

Triny Trinh Pham

APPROVED FOR THE DEPARTMENT OF CHEMISTRY

SAN JOSÉ STATE UNIVERSITY

May 2012

Dr. Herbert Silber	Department of Chemistry
Dr. Roger Terrill	Department of Chemistry
Dr. Karen Singmaster	Department of Chemistry

ABSTRACT

LUMINESCENCE STUDIES OF EUROPIUM(III) – D-GLUCOSAMINE COMPLEXES IN WATER

by Triny Trinh Pham

Lanthanide complexation interactions measured by luminescence have become interesting topic in recent years. Europium is a rare earth metal and the Eu(III) ion is a hard acid; when Eu(III) and a monosaccharide ligand are present, a complex may be formed. The goal is to determine the equilibrium constant K_{EuL} between the europium ion and a D-glucosamine ligand. At 25^{o}C and pH ~ 7.40, a physiologically important pH, adding D-glucosamine enhanced the luminescence of 0.0025 M Eu(III). As the concentration of ligand increased, the emission intensity was also increased. The measurement of a hypersensitive peak at 614 nm indicated a complex reaction at pH ~ 7.40. The equilibrium constant of K_{EuL} was found to be 29.24. Based on the FTIR and laser experiments, the results confirmed that D-glucosamine attached to the europium ion (Eu^{3+}) at pH ~ 7.40; there was no evidence that hydrolyzed Eu(III) was present in the complexation.

ACKNOWLEDGEMENTS

I sincerely give my thanks to my research advisor, Dr. Herbert Silber, for affording me the opportunity to be part of the Silber group. Thank you for guidance, patience, and humor. My special thanks to my former colleague, Victor Maraschin. Thank you for the help with the luminescence instrument. Thanks to Mike Stephens, the instrument technician, for instructing and helping me to use the FTIR spectrometer. Thanks to Dr. Roger Terrill for providing the aqueous Zinc selenide cell for FTIR scanning. Without his help, this thesis would have never been completed. Thanks to Dr. Gilles Muller for allowing me to use the laser instrument and helping interpret the data. Thanks to Dr. Karen Singsmaster for becoming my third committee member. Thanks to Dr. Tran (Ly-Le Tran, MD, JD, Fellow, the American College of Legal Medicine) and James Boyle for editing the thesis. Special thanks to my parents, Friendly Pham and Khai Nguyen, for giving me life, love, and support. Thanks to my brothers, Tuan Pham, David Pham, Andy Pham, Viet Pham, and Kevin Pham for their encouraging me to continue my schooling. To my friends, I wish to express my gratitude for your support and inspiration.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BSA	Bovine serum albumin
$CF_3SO_3^-$	Triflate or trifluoromethanesulfonate
CF_3SO_3H	Triflic acid or trifluoromethanesulfonic acid
Constant	
EDTA	Ethylenediaminetetraacetic acid
Equilibrium	K (unitless)
$Eu(trif)_3$	Europium(III) triflate
Eu_2O_3	Europium (III) oxide
[EuL]	Europium-Ligand concentration
FTIR	Fourier Transform Infrared
GM1	Monosialoganglioside
HCl	Hydrogen chloride (hydrochloric acid)
LASER	Light Amplification by Stimulated Emission of Radiation
M	Molarity (moles/L)
mg	milligram
mL	milliliter
$NaClO_4$	Sodium perchlorate
NaOH	Sodium hydroxide
Nd(III)	Neodymium
nm	nanometer (10^{-9} m)
NMR	Nuclear Magnetic Resonance

NO_3^-	Nitrate
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTf^-	Triflate
SCN^-	Thiocyanate
TMJ	Temporo-mandibular Joint

Chapter 1

Introduction

A. Background

Luminescence, one of the most sensitive spectroscopic techniques, was first discovered by the Irish scientist George Gabriel Stokes (Stokes, 1852). Lanthanide luminescence depends on the contribution of a hypersensitive peak which responds to the environment of lanthanide metal ions. A recent study has shown that europium (III) binds to carbohydrate ligands at a pH of ~7.5 (Leonard, et al., 2007). Lanthanide-ligand complexes may have strong pH-dependent interactions, which can be recorded at various emission wavelengths to determine the hypersensitivity of each complex sample.

Luminescent emission can be detected at times ranging from a few nanoseconds to milliseconds (Hedinger, et al., 1998), and luminescence can be fluorescence or phosphorescence. With regard to phosphorescence, the light absorption causes electronic transitions between different multiplicity levels, and emission persists for 10^{-3} - 10 s (Hamilton, 2003). When the luminescence of a europium-ligand complex is measured, four sharp peaks are detected at J = 1, 2, 3, and 4, the transition peaks are assigned to the $^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$, $^5D_0 \rightarrow {}^7F_3$, and $^5D_0 \rightarrow {}^7F_4$ transitions, respectively (Leonard, et al., 2007 & Skoog, et al., 1998). Leonard et al. (2007) showed that a lanthanide complex exhibits four transitions and the hypersensitive peak has a wavelength of 615 nm. The hypersensitive peak is assigned to a $^5D_0 \rightarrow {}^7F_2$ transition and appears at an emission wavelength of 615 nm.

B. Lanthanide Complexes with Luminescence

The transition energies of lanthanide metal ions are described and illustrated in the form of an energy diagram, and (Skoog, et al., 1998 & Yang, et al., 2004) most lanthanide peaks, except for the hypersensitive peaks, are insensitive to their environment. If a complexation occurs, the hypersensitive peak increases in emission intensity as a function of increased ligand concentrations. Studies have shown that lanthanides, such as neodymium (III), erbium (III), and ytterbium (III), are sensitized when complexed and exhibit a near-infrared luminescence (Hedinger, et al., 1998). Biological experiments like fluoro-immunoassays (Bucella, et al., 2004, Sherry, et al., 1973, Atkinson, et al., 2005, Silber, et al., 2001, & Silber and Nguyen, 1998) and microscopy (Alpturk, et al., 2006 & Diaz and Berger, 2000) have been used for auto-fluorescence (Hedinger, et al., 1998). Other lanthanides that luminesce in the visible region are europium (III) and terbium (III). These lanthanide complexes can also be studied in biological assays.

C. Europium Complexes with Luminescence

Monosaccharide ligands (simple sugars) react with lanthanides and have recently been studied in europium complexes (Alekseev, et al., 1998). Alptürk et al. (2006) reported that a single sugar ligand interacts with Eu^{3+} at pH 7.0 and has an emission wavelength of 615 nm. During the interaction of metal (M) and ligand (L), the metal and ligand complex (ML) is formed:

$$1.1 \qquad M^{+} + L^{-} \rightleftharpoons M-L$$

$$1.2 \qquad K_{eq} = \frac{[M-L]}{[M^{+}] \times [L^{-}]}$$

For the formation of a metal-ligand complex, it is important to consider the pH dependence of the luminescence intensity. Eu^{3+} luminescence spectra exhibit specific emission peaks at 590, 614, 649, and 695 nm, and many lanthanide complexes are formed below a pH of 5. Luminescence spectra indicate that the hypersensitive peak at 614 nm changes in emission intensity as ligand concentrations change. This emission intensity increases as the concentration of ligand increases at pH ~ 7.40 (Leonard, et al., 2007).

The first hydrolysis constant was studied by the Ramírez-Garcia et al. (2003) and Bentouhami et al. (2004) as:

$$1.3 \qquad Eu^{3+} + H_2O \rightleftharpoons Eu(OH)^{2+} + H^{+}$$

$$1.4 \qquad K = \frac{[Eu(OH)^{2+}] \times [H^{+}]}{[Eu^{3+}]}$$

Lopéz-González et al. (2007) performed a recent study of the hydrolysis constant reaction. The authors showed that the hydrolysis equilibrium constant K_{OH} was obtained from the basic hydrolysis reaction (*Equation 1.4*) to form a europium ion complex.

In Figure 1, the Eu (III) hydrolysis constant is plotted as a function of ionic strength (Jimenez-Reyes, et al., 2006) From this graph, pK_{OH} at ionic strength of 0.10 Molar ($NaClO_4$) is 7.41 at 25°C.

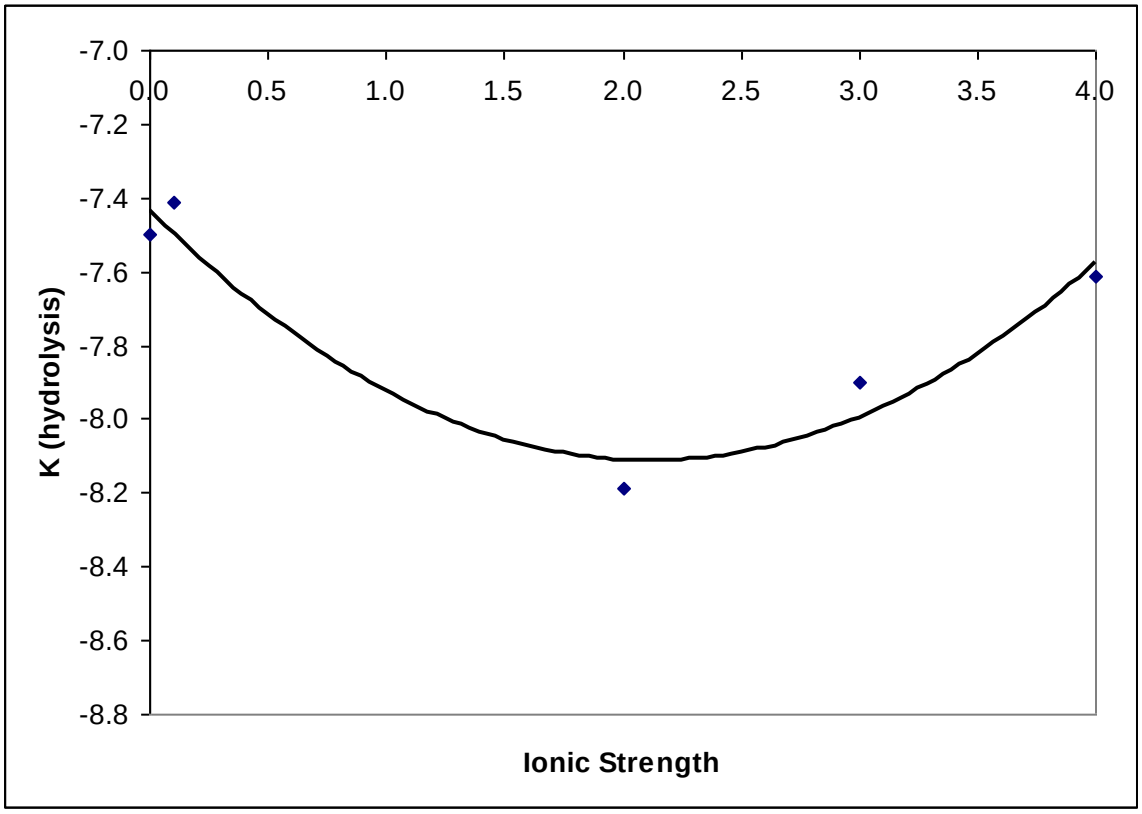


Figure 1. Ionic strength-dependence of the europium hydrolysis constant.

The europium stability constant is substantially influenced by ionic strength. Ionic strength, *I*, is a measurement of the concentrations of all ions in a solution.

$$1.5 \qquad I = \frac{1}{2}\Sigma (Z_i^2 + C_i)$$

where Z_i and C_i are the charges and concentrations of individual species, respectively.

D. pH Dependence of Europium Luminescence

Parker and Yu (2005) studied the europium-citrate complex at pH = 7.40 and emission wavelength of 616 nm. They showed that citrate reacted with europium to form a Eu(III) – citrate complex. Hedinger et al. (1998) confirmed that Eu^{3+} binds to a trinuclear lanthanoid complexes of 1,3,5-triamino-1,3,5-trideoxy-cis-inositol (TACI) between the pH of 7 and 9. In the absence of a complexing ligand, the emission intensity is described as:

$$I_o = k_o + k_{Eu}[Eu] \qquad 1.6$$

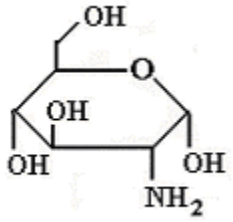
where k_o is the intensity constant due to the instrument and the solvent, and k_{Eu} is the free europium constant at low pH. Then at pH ~ 7.40, europium is hydrolyzed to:

$$I = k_o + k_{Eu}[Eu] + k_{EuOH}[EuOH] \qquad 1.7$$

where $[EuOH^{2+}]$ can be calculated from the hydrolysis reaction (*Equation 1.3*). In *Equation 1.7*, the instrument constants, k_o and k_{Eu} are obtained from the emission intensity of a sample. The constant k_{EuOH} is used to confirm the calculation of K_{OH} from the hydrolysis reaction. However, in the absence of ligand at pH ~ 7.40, substantial $Eu(OH)_3$ may be present.

E. Europium - Glucosamine Reaction

At neutral pH ~ 7.40, the Europium-glucosamine interaction is detected at the hypersensitive emission peak at 614 nm. The complexation is written as:

1.8 Eu^{3+} +  ⟷ Eu – glucosamine

D-glucosamine

Bonding interactions of this europium complex will be studied further using FTIR and laser excitation spectroscopy.

F. Research Goals

The objective of this thesis was to determine the equilibrium constant, K_{EuL}, of europium (III) and D-glucosamine at physiological pH using luminescence at a hypersensitive transition. The experiments were conducted to understand whether D-glucosamine ligand binds to free europium (Eu^{3+}) or to europium hydroxide ($EuOH^{2+}$) at pH ~ 7.40. The data demonstrated that adding ligand to europium increased the luminescence intensity. The experimental data presented describes the peak sensitivity and the lanthanide complex interaction that allows the calculation of the equilibrium constant. The Eu-glucosamine contained the amino group at C_2 and the hydroxyl groups at C_1 and C_3 of D-glucosamine did bind to Eu^{3+}, but did not bind to $EuOH^{2+}$ due to ionic interactions. FTIR and laser excitation measurements provide the evidence that Eu^{3+} binds to D-glucosamine.

CHAPTER 2

Experimental Methods

A. Solutions Preparation

Europium oxide (Eu_2O_3) powder was purchased from Standford Materials Corporation at Irvine, California (99.99% assay), and trifluoromethanesulfonic acid (CF_3SO_3H or triflic acid) was purchased from Alfa Aesar at Ward Hill, Massachusetts (99% assay) and utilized without further purification. A stock solution of 5.049×10^{-2} M $Eu(trif)_3$ was manually prepared from a white solid Eu_2O_3 and triflic acid in deionized water. Prior to the analyses, all necessary glassware was washed and dried in an oven. After drying, 0.880 g of Eu_2O_3 powder and 1.328 ml of triflic acid were slowly dissolved in distilled water under agitation. This process was done under a fume hood because the reactions between Eu_2O_3 and triflic acid give off heat. A 9.701×10^{-3} M ethylenediaminetetraacetic acid (EDTA) solution was also made to standardize the $Eu(trif)_3$ solution. EDTA (3.722 g) solid was dissolved in 100 ml of deionized water. All of the above solutions were manually prepared from solid reagents at room temperature.

1. Eu^{3+} Standardization

After 5.049×10^{-2} M [$Eu(trif)_3$] (alternately known as [Eu^{3+}]) stock solution was prepared, titration was done to standardize [Eu^{3+}] concentration. 0.300 mL of 5.049×10^{-2} M Eu^{3+} was mixed with 3 drops of bromopyrogallol indicator and 1-2 g of sodium acetate and 10 mL deionized water. Titration was done using 9.701×10^{-3} M EDTA. When the indicator was added to the Eu^{3+} solution, it turned purple. At the

endpoint of titration, the purple color had changed to pink. Approximately four to five titration trials were performed and the average net concentration was found to be 0.050 ± 0.001 M.

2. Solutions Dilution

From the above Eu^{3+} stock solution, eight different concentrations were prepared ranging from 5.00 x 10^{-4} M to 4.00 x 10^{-3} M. After eight samples were completely diluted, luminescence data were collected. Emission intensity (Figure 2) was used to prepare a calibration plot as a function of Eu^{3+} concentrations.

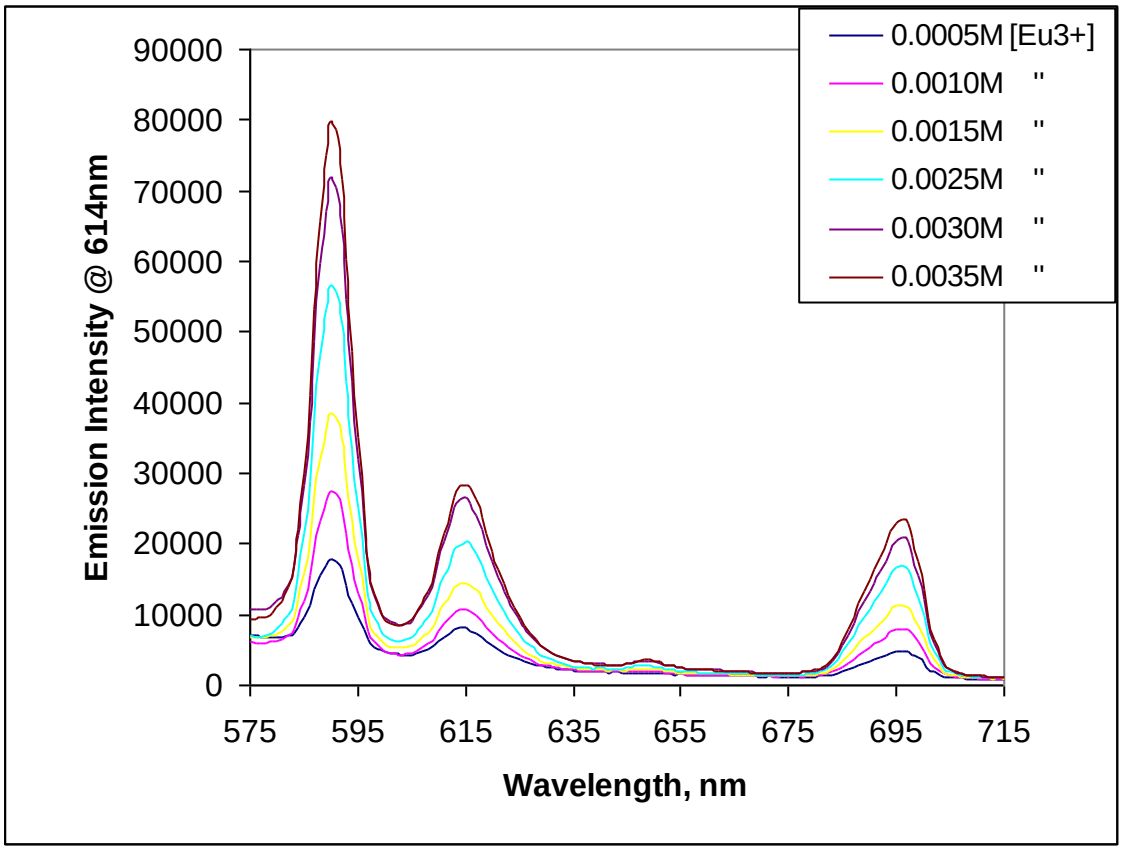


Figure 2. Europium-triflate ($Eu(trif)_3$) solutions measured at pH < 5.0 and excitation at wavelength of 318 nm and 25°C.

a. [Eu^{3+}] and [D-glucosamine] dilutions

A stock [D-glucosamine] solution was made at 0.100 M, and a 2.00 M sodium perchlorate $NaClO_4$ solution was also prepared. A target concentration was selected to be 2.50×10^{-3} M for Eu^{3+} and 0.085 M for $NaClO_4$. D-glucosamine (2.156 g) solid was diluted with deionized water to make a 0.100 M [D-glucosamine] stock solution. Multiple D-glucosamine solutions were prepared ranging from 7.60×10^{-4} M to 2.50×10^{-2} M. A quantity of 24.492 g $NaClO_4$ solid was diluted to make a 2.00 M $NaClO_4$ stock solution. Then 0.425 mL of a 2.00 M $NaClO_4$ solution was added to each of the solution samples to stabilize the ionic strength. Table 1 of the Appendix C shows descriptive details on solution dilution. The 5.00×10^{-2} M sodium hydroxide (NaOH) solution was also made to adjust each sample to pH ~ 7.40.

b. Dilutions of monosaccharide solutions

Eu^{3+} and D-glucosamine samples were measured at excitation wavelengths of 318 nm and an emission wavelength of 614 nm at 25^{o}C. Data on other sugars such as galactose, ribose, and mannose were also collected to measure average intensity (*Equation 1.7*). A 0.100 M concentration of each sugar was diluted. The same dilution method was used to prepare each sugar solution, and 5.00×10^{-2} M NaOH was used to adjust each sample to pH ~ 7.40. These diluted solutions were then ready for measurement of emission intensity. The emission wavelength was arranged between 590 and 715 nm for each luminescence measurement.

3. Ionic Strength

Sodium perchlorate $NaClO_4$ was used in the Eu^{3+} solution to adjust ionic strength. Since the target $[Eu^{3+}]$ was 2.50×10^{-3} M and the total ionic strength in a $[Eu^{3+}]$ solution is 0.100 M, then ionic strength, *I*, was calculated as:

$$\textbf{2.1} \qquad Eu(trif)_3 \rightarrow Eu^{3+} + 3trif^- \qquad \text{where } [trif^-] = 3[Eu^{3+}]$$

$$\textbf{2.2} \qquad I = \tfrac{1}{2}\,\Sigma(Z_i^2 \times C_i) \qquad \text{where } C_{Eu} = 0.0025\ M$$

When all constants were calculated, the ionic strength of $Eu(trif)_3$ was adjusted to 0.015 M with $NaClO_4$. The total ionic strength was therefore 0.100 M. The details of ionic strength are described in the Appendix B.

B. Instrument Operation

After Eu^{3+} and D-glucosamine solutions were prepared, luminescence (Fluorolog®-3) parameters were correctly set and a xenon lamp was chosen for the light source. Luminescence slit widths were set to 5 nm, the excitation wavelength was set to 318 nm and the emission wavelength was set to 614 nm. When all of the parameters were selected and ready to run, numbers of data of initial solutions were collected. Table 2 showed different emission intensities under low and high pH measurements. The emission intensity of lower pH solutions were always lower than that of higher pH solutions.

Using the same scanning method, luminescence data was also collected for other sugars. The recorded emission intensities (cps) at 614 nm of all four sugars were

consistent throughout the scanning. The emission intensity was lower when the lifetime of the xenon lamp decreased; hence causing a discrepancy in data collection. To avoid this discrepancy, a lamp test scan was performed before starting to collect any luminescence data. After collecting data points of this set of initial solutions, more solutions were prepared at higher D-glucosamine concentrations. The findings are in the results and discussion chapter.

C. Equilibrium Constants Calculations

When intensity data for all concentrations were collected, the constants *(k's)* were calculated using the equation:

$$I = k_o + k_{Eu}[Eu] + k_{EuOH}[EuOH] + k_{EuL}[EuL] + k_L[L] + k_{EuOHL}[EuOHL] \quad (2.3)$$

Emission intensity, *I,* is collected from the luminescence instrument. When intensity *I* is known, constant k_{EuL} would be able to obtain a value by calculating from *Equation 2.3*.

As D-glucosamine was a ligand added to the $[Eu^{3+}]$ solution, the equilibrium constant of EuL complex was given by:

$$Eu + L \rightleftharpoons EuL \quad (2.4)$$

$$K_{EuL} = \frac{[EuL]}{[Eu] \times [L]} \quad (2.5)$$

Further calculations are presented in the Appendix A.

D. FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a useful tool to identify functional groups of a chemical compound. When a chemical compound is observed with infrared radiation, vibrational motions can be observed (Skoog, et al., 1998). Under these circumstances, the radiation's electrical field interacts with the molecule in a way that changes the amplitude of the vibrational motions. In this study, spectra were collected between 500-4000 cm^{-1} using a Genesis II FTIR spectrometer, and the spectra were analyzed with WinFirst software.

A white europium trifluoromethanesulfonate ($Eu(trif)_3$, 98% pure) powder and D-glucosamine solid were purchased from Sigma Aldrich at St. Louis, Missouri. A ratio of 1:1 of 0.100 M $Eu(trif)_3$ to 0.100 M D-glucosamine solution was prepared. The prepared stock concentrations for running FTIR were 1.00 M for $Eu(trif)_3$, 1.00 M for D-glucosamine, and 2.00 M for $NaClO_4$. One ml of 1.00 M $Eu(trif)_3$, 1 mL of 1.00 M D-glucosamine, and 0.500 mL of 2.00 M $NaClO_4$ were transferred into a 10-mL volumetric flask, then the solution was diluted to the mark using deionized water and [NaOH] solution to raise pH to 7.40.

Separate solutions of 0.100 M D-glucosamine and 0.100 M $Eu(trif)_3$ were made to run the spectra under 25^{o}C for references. All solutions were measured at pH ~ 7.40. A 1 mL of 1.00 M D-glucosamine was pipetted in a 10-mL volumetric flask, and deionized water was added to the mark. One mL of 1.00 M $Eu(trif)_3$ and 0.500 mL of 2.00 M $NaClO_4$ were transferred into a 10-mL volumetric flask, then deionized water was added to the mark. A zinc selenide attenuated total reflection liquid cell was positioned in the FTIR's sample compartment to run the solutions.

E. Laser Measurements

A laser was used to study chemical binding between Eu(III) and D-glucosamine groups. Laser excitation spectroscopy was used to induce the excitation of lanthanide ion (Eu^{3+}) from a ground state $^7F_0$ to an excited state $^5D_0$ of the $4f^6$ electron configuration (Albin, Whittle, et al., 1985). The excitation was completed by using a dye laser spectrometer, and the emission was monitored at a corresponding $^5D_0 \rightarrow {}^7F_2$ energy transition at 614nm.

The procedures for 1:1 ratio sample preparation were similar to those used for the FTIR solution mixture at pH ~ 7.40 and pH ~ 6.0. The third $Eu(trif)_3$ sample was prepared with the presence of $NaClO_4$ at pH ~ 7.40, and the fourth $Eu(trif)_3$ sample were made in the absence of $NaClO_4$ at pH ~ 7.40. Four spectra were observed and the intensity recorded as a function of wavelength (nm) at 25^oC.

CHAPTER 3

Results and Discussion

Emission intensity at high pH was formulated in *Equation 2.3*, and each constant, *k*, was determined separately. The constant value k_o was determined from both the instrument and solvent, whereas the constant k_{Eu} is the free europium constant. The constants $k_o + k_{Eu}[\mathrm{Eu}]$ were determined at a low pH in the absence of ligand. The constant $k_L[\mathrm{L}]$ represents the simple sugar (ligand); its value was negligible. Constant k_{EuOH} is determined from the average intensity of the four sugars, galactose, ribose, mannose, and D-glucosamine. The constant $k_{EuL}[\mathrm{EuL}]$ was calculated after a ligand was added to a 0.1M Eu(III) solution. Those constants were a function of instrument wavelength, slit width, and so forth and were not fundamental constants. I will discuss the details of each individual physical term calculating the equilibrium constant from *Equation 2.3*.

A. Luminescence Measurements

1. Determination of Constants k_o & k_{Eu}

A series of [Eu^{3+}] perchlorate and triflate solutions was gathered and recorded at a wavelength of 614 nm at a low pH to eliminate hydrolysis. Figure 2 shows spectra of

different Eu^{3+} concentrations at pH < 5.0. The spectra indicated that there was no peak shift with increasing [Eu^{3+}]; as [Eu^{3+}] increased, the intensity increased at 614 nm. However, the luminescence was emitted at four different wavelengths: 590, 614, 649, and 695 nm. These transition peaks occurred at energy level *J = 1, 2, 3,* and *4,* respectively. The emission intensity is formulated as:

$$\textbf{3.1} \qquad I = k_o + k_{Eu}[Eu]$$

where constant k_o was the instrumental solvent constant, and constant k_{Eu} was the free europium constant.

As spectral results were collected at pH < 5.0, a linear plot of emission intensity as a function of [Eu^{3+}] at wavelength 614 nm was obtained. Figure 3 shows a linear regression with y-intercept (k_o) = 5.27 x 10^3 and the slope (k_{Eu}) = 7.41 x 10^6, where constant k_{Eu} was the free europium constant.

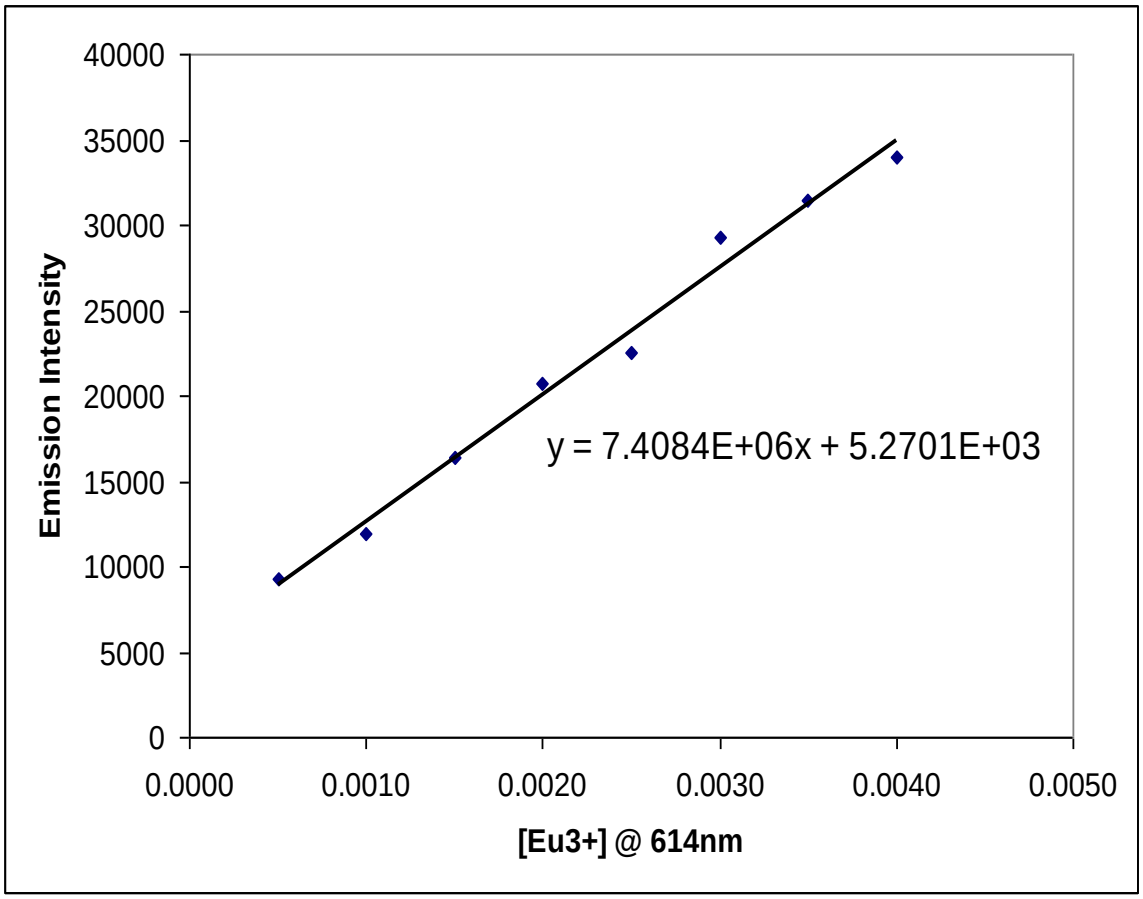


Figure 3. Linear regression line at low pH (pH < 5.0) of Eu(trif)$_3$ at wavelength of 614 nm.

2. Determination of Constant k_L

After free [Eu^{3+}] was characterized, the effects of adding D-glucosamine to [Eu^{3+}] below pH 5 were studied. Although small differences were observed at various concentrations, they were too small to measure constant k_{EuL}. During the first set of initial solutions, [D-glucosamine] was selected to record emission intensity at pH < 5.0. Figure 4 depicts a reaction of Eu (III) – D-glucosamine complex at pH < 5.0.

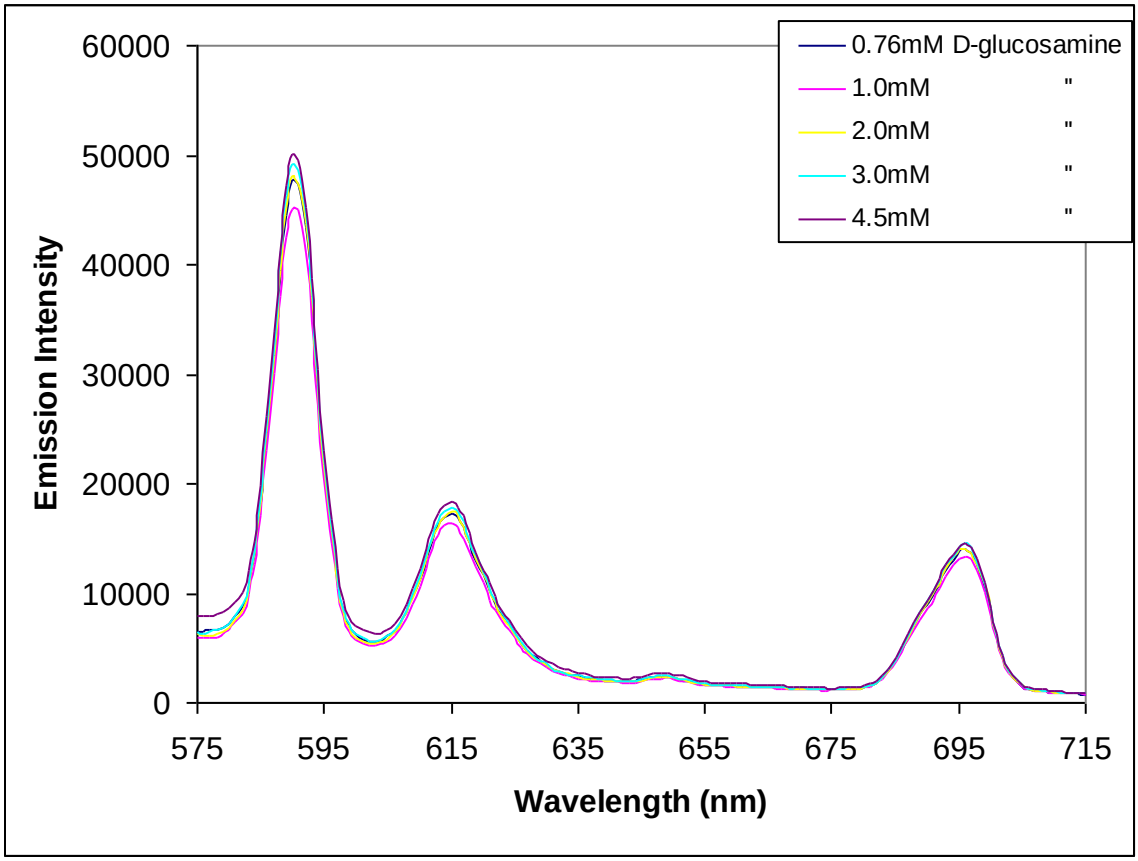


Figure 4. Eu(III) – D-glucosamine reaction at pH < 5.0 and at constant europium concentration.

Wavelength at 614 nm (Figure 4) showed the transition peaks are slightly changed with increasing [D-glucosamine]. These results showed that [D-glucosamine] interacts with [Eu^{3+}] at a low pH indicating a weak complexation. Luminescence spectra illustrated Eu (III) – D-glucosamine reaction at energy level *J = 2* transition, and consequently, this transition obtained a higher intensity when the pH was raised. When [D-glucosamine] increases at a high pH, peak intensity will increase accordingly (Figure 5).

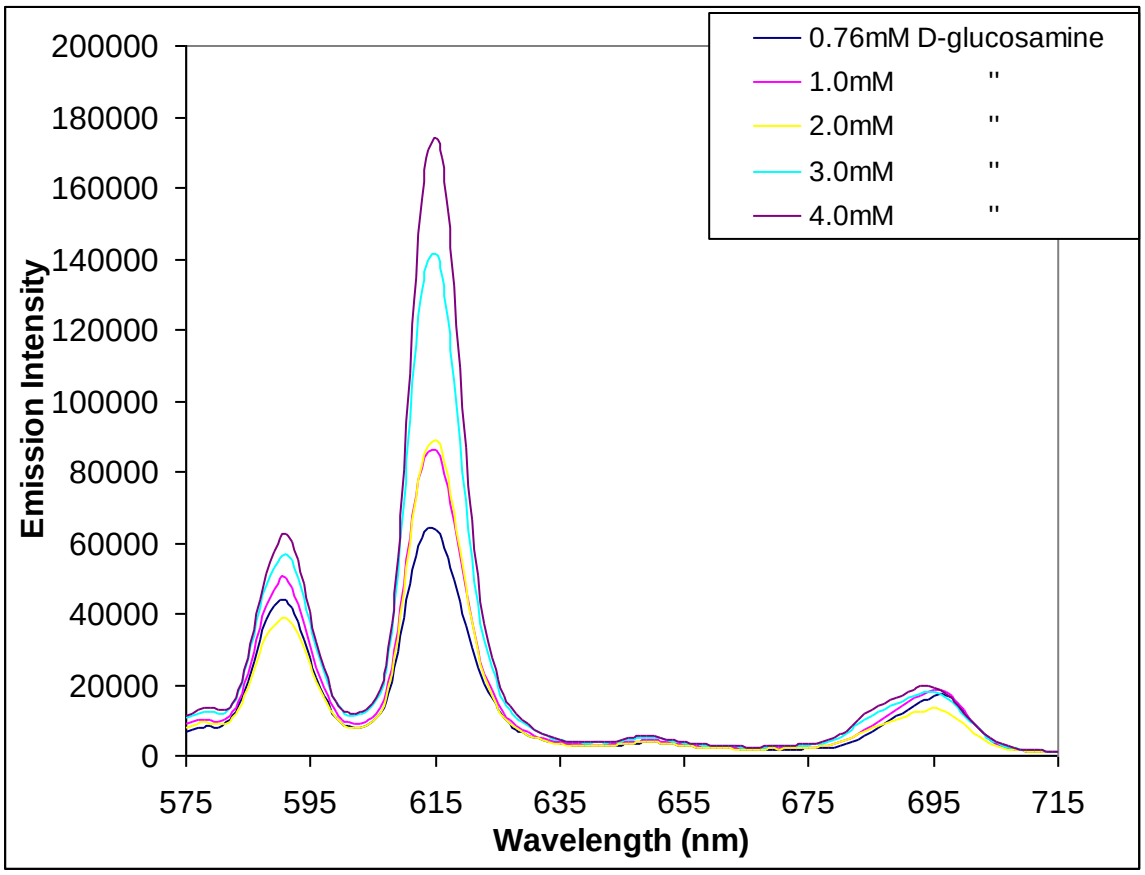


Figure 5. Luminescence spectra of Eu(III)-D-glucosamine complex at emission intensity as a function a wavelength at pH ~ 7.40 and 25°C.

Additionally, when ligand was added to [Eu^{3+}] at pH < 5, the reaction was quantify. Figure 6 illustrates that constant k_{EuL} at pH < 5 was very small compared to all other results.

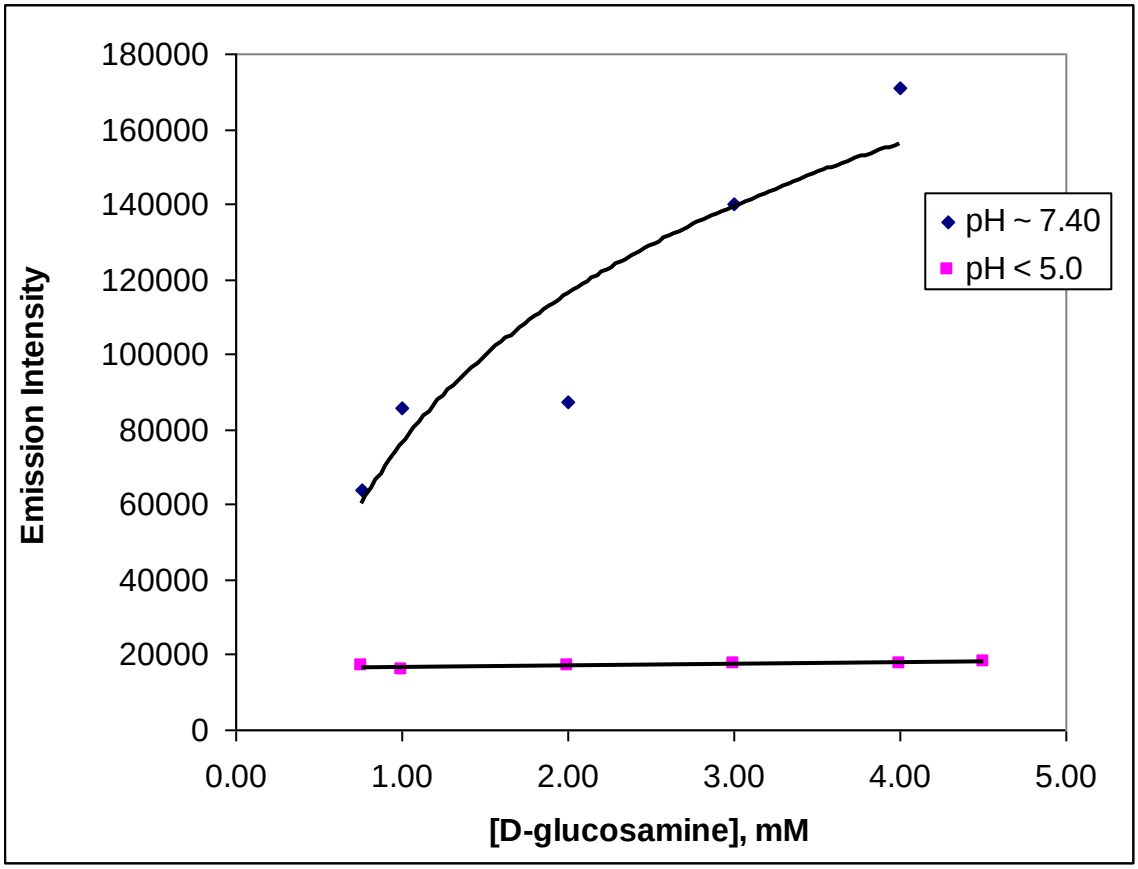


Figure 6. Emission intensity versus [D-glucosamine] at difference pH and at wavelength of 614 nm.

3. Determination of Constant k_{EuOH}

A few data points of three other monosaccharide ligands were studied to measure the intensity. The experiments with the monosaccharide sugars galactose, mannose, and ribose (Table 3) were conducted under the same temperature (25°C) and pH conditions (pH ~ 7.40) as those used for D-glucosamine. Figure 7 shows four different monosaccharide ligand curves. Although the emission intensity of each sugar was different, the y-intercepts are similar.

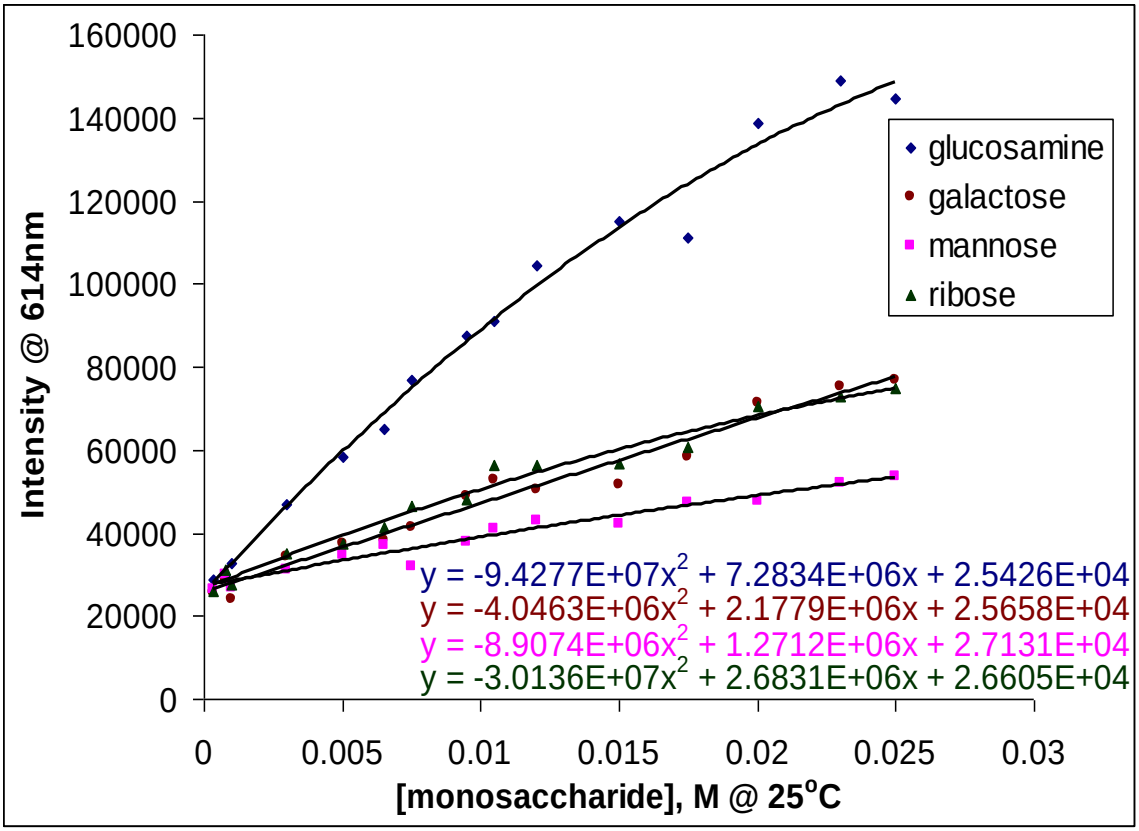


Figure 7. Observation of different monosaccharide ligands at pH ~ 7.40.

Intensities at intercept of the four sugars were similar. The average intensity at zero ligand concentration was calculated. The average intensity, I_o, of these monosaccharide ligands was determined to be 2.62×10^4. From this average intensity, constant k_{EuOH} could be calculated. The intensity I_o value is computed as:

$$3.2 \qquad I_o = k_o + k_{Eu}[Eu] + k_{EuOH}[EuOH]$$

The constants k_o and k_{Eu} have already been determined; therefore, if intensity I_o, $[Eu^{3+}]$ and $[EuOH^{2+}]$ were known, then constant $k_{EuOH} = 1.18 \times 10^7$ could be calculated from Figure 8, where $I_o = 2.62 \times 10^4$, $k_o = 5.27 \times 10^3$ and $k_{Eu} = 7.41 \times 10^6$. When the hydrolysis equation was calculated, the free europium [Eu] and [EuOH] concentrations were obtained, $[Eu] = 1.12 \times 10^{-3}$ M and $[EuOH] = 1.06_6 \times 10^{-3}$ M. All of the calculations were described in the Appendix A. The average intensity calculated from the y-intercept was used to determine the value of the equilibrium constant, K_{EuL}.

The equilibrium constant K_{EuL} of each europium ligand complex was calculated based on the collected data shown in Figure 7. However, steps of calculations for the three ligands were similar to that of D-glucosamine calculations. Table 4 below shows an equilibrium constant, K_{EuL} and intercept intensity for each monosaccharide ligand. The equilibrium constants for the monosaccharide sugars are smaller than the constant for the D-glucosamine ligand.

Table 4: Equilibrium Constant, K_{EuL}, of Each Monosaccharide Ligand

Ligand	K_{EuL} = [EuL] / ([Eu] x [L])	Intensity at y-intercept
Galactose	6.35	2.56×10^4
Mannose	5.35	2.71×10^4
Ribose	13.32	2.66×10^4

4. Determination of the Absence of EuOHL

Before calculating constants k_{EuL} and k_{EuOHL} (*Equation 2.3*), the species that were present at pH ~ 7.40 would be evaluate using FTIR and excitation measurements.

a. FTIR Measurements

FTIR spectra was used to determine the functional groups of the Eu(III) – D-glucosamine complex at pH ~ 7.40. FTIR of the $Eu(trif)_3$ – D-glucosamine appeared to be a perfect sum of the $Eu(trif)_3$ and D-glucosamine spectra. As shown in Figure 8, three stretching bands from the functional groups CO_2, NH, and C=O from $Eu(trif)_3$ were observed.

Triflic (trifluoromethanesulfonic) acid contained carbon, fluorine, sulfur, oxygen, and hydrogen. The small band at 1034 cm^{-1} was assigned to a C-F stretch. The strong sharp band located next to C-F band at 1257 cm^{-1} was assigned to the S=O stretch. A medium bandwidth at 2345 cm^{-1} indicated an S-OH stretch from triflic acid. The three bands provided evidence for the presence of trifluoromethanesulfonic acid. Thus, Eu band located at 570.82 cm^{-1}.

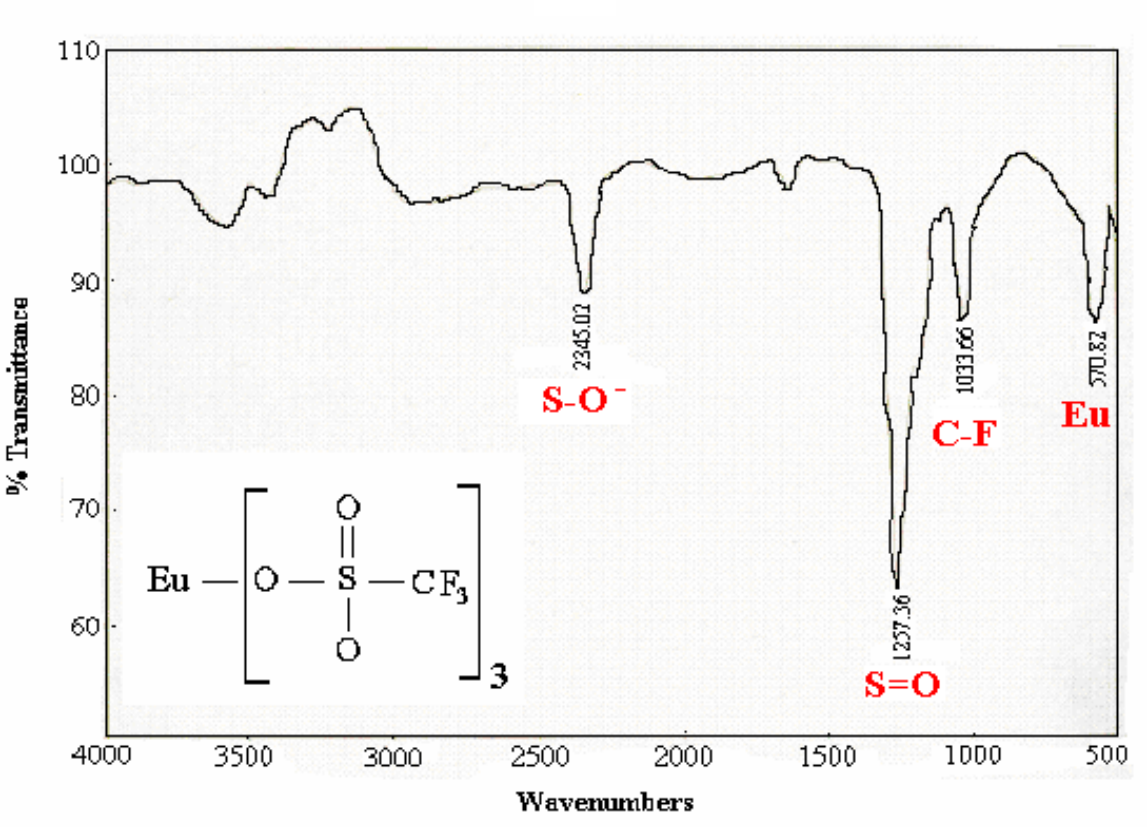


Figure 8. FTIR spectrum shows a 0.100 M Eu(trif)$_3$ at pH ~ 7.40.

The FTIR spectrum of a free D-glucosamine indicated two main bands (Figure 9). Since D-glucosamine had four hydroxyl (OH) groups attached to the cyclic pyranose ring, a strong OH band appeared at 3420 cm^{-1}. The medium band at 1641cm^{-1} indicated a C-NH stretch.

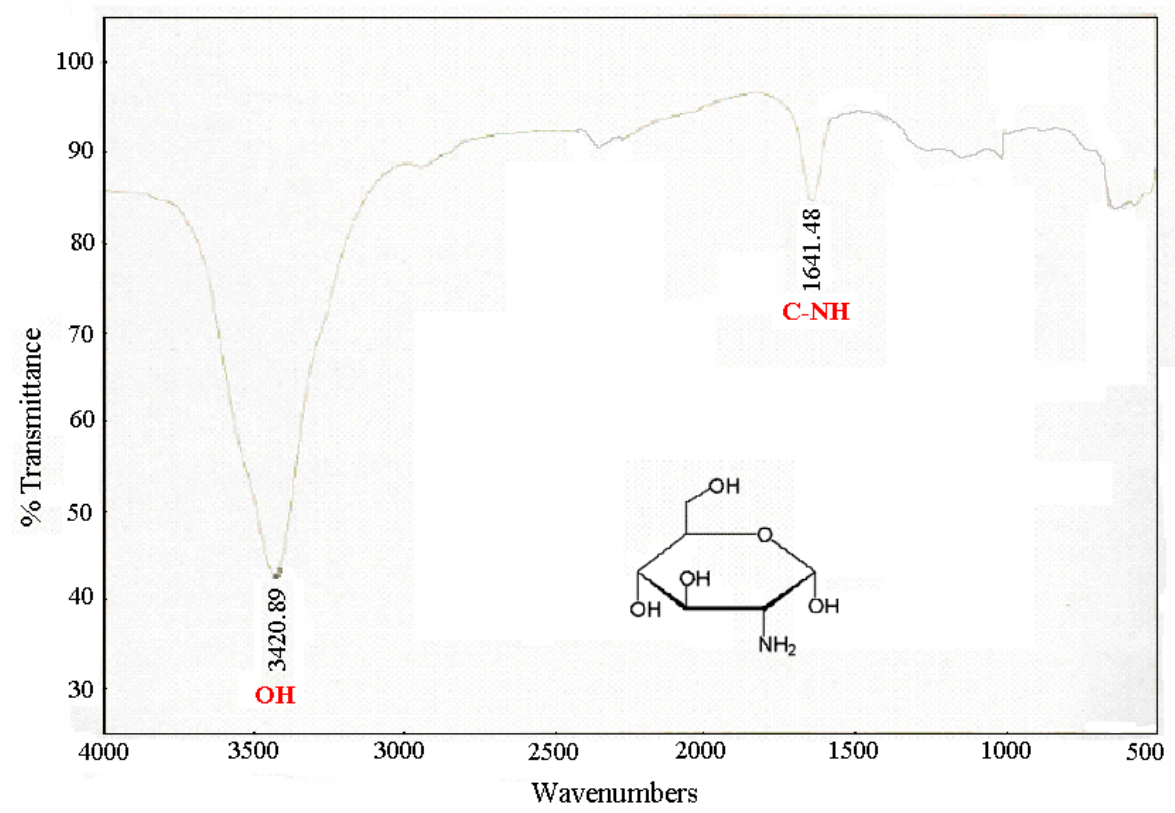


Figure 9. FTIR spectrum shows a 0.100 M D-glucosamine at pH ~ 7.40.

Figure 10 shows the FTIR spectrum of D-glucosamine and $Eu(trif)_3$ at a 1:1 molar ratio. A strong OH band appearing at 3423 cm^{-1} confirmed the presence of free water. Another strong and sharp band observed at 1250 cm^{-1} represented a S=O bond stretching from $Eu(trif)_3$. Two other medium sharp bands at 2345 cm^{-1} and 1054 cm^{-1} confirmed a S-OH and a C-F bond from the $Eu(trif)_3$ solution, respectively. Since europium (Eu) is a heavy metal, its transmittance corresponded to a lower frequency of 633 cm^{-1}. Atoms coordinated to Eu also share lower frequencies of vibration. Shifted bands confirmed there was a complex interaction between D-glucosamine and $Eu(trif)_3$.

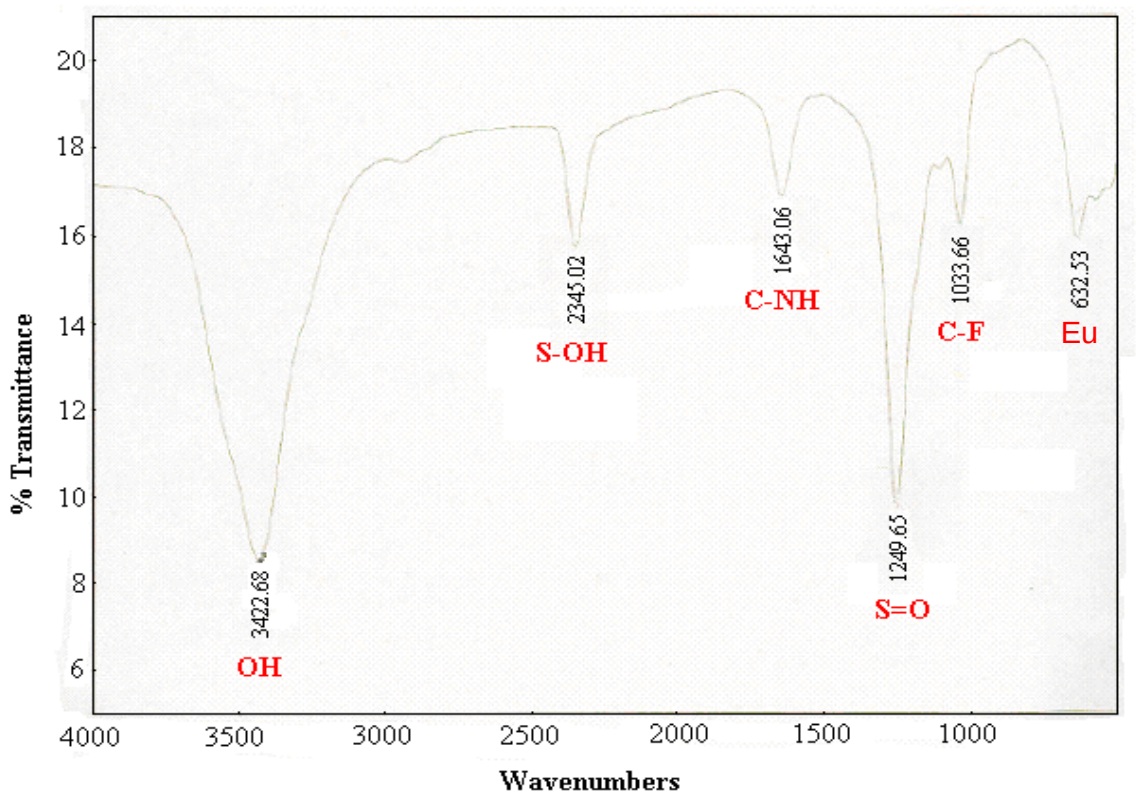


Figure 10. FTIR spectrum of a 0.100 M $Eu(trif)_3$: 0.100 M D-glucosamine at pH ~ 7.40.

The FTIR spectrum shown in Figure 10 indicated two functional groups (Eu and S=O) that shifted when the ligand D-glucosamine was added to the europium-triflic solution. The europium (Eu) band shifted from 571 cm^{-1} (Figure 7) to 633 cm^{-1} (Figure 10). The S = O band indicated a small shift from wavenumber 1257 cm^{-1} (Figure 7) to 1250 cm^{-1} (Figure 10). These band shifts were consistent with complexation but indicated a stronger interaction between triflate and Eu(III) than between D-glucosamine and Eu(III).

b. Excitation Measurements

Excitation spectroscopy was used to determine whether Eu^{3+} or $EuOH^{2+}$ binds to D-glucosamine. When free europium ($Eu(trif)_3$) in $NaClO_4$ solution at pH ~ 7.40 was measured under the dye laser excitation at 617.5 nm, a single peak appeared at wavelength 578.7 nm (Figure 11). Another single peak was observed at 578.5 nm for Eu(III) triflate in the absence of $NaClO_4$ solution (Figure 11). These spectral results indicated that the $NaClO_4$ solution had little or no effect on the intensity. If $EuOH^{2+}$ was present in the sample, a shoulder peak would be expected to appear next to the Eu^{3+} peak. However, no shoulder peak was visible in the spectrum. Therefore only free europium ion (Eu^{3+}) was detected in this solution.

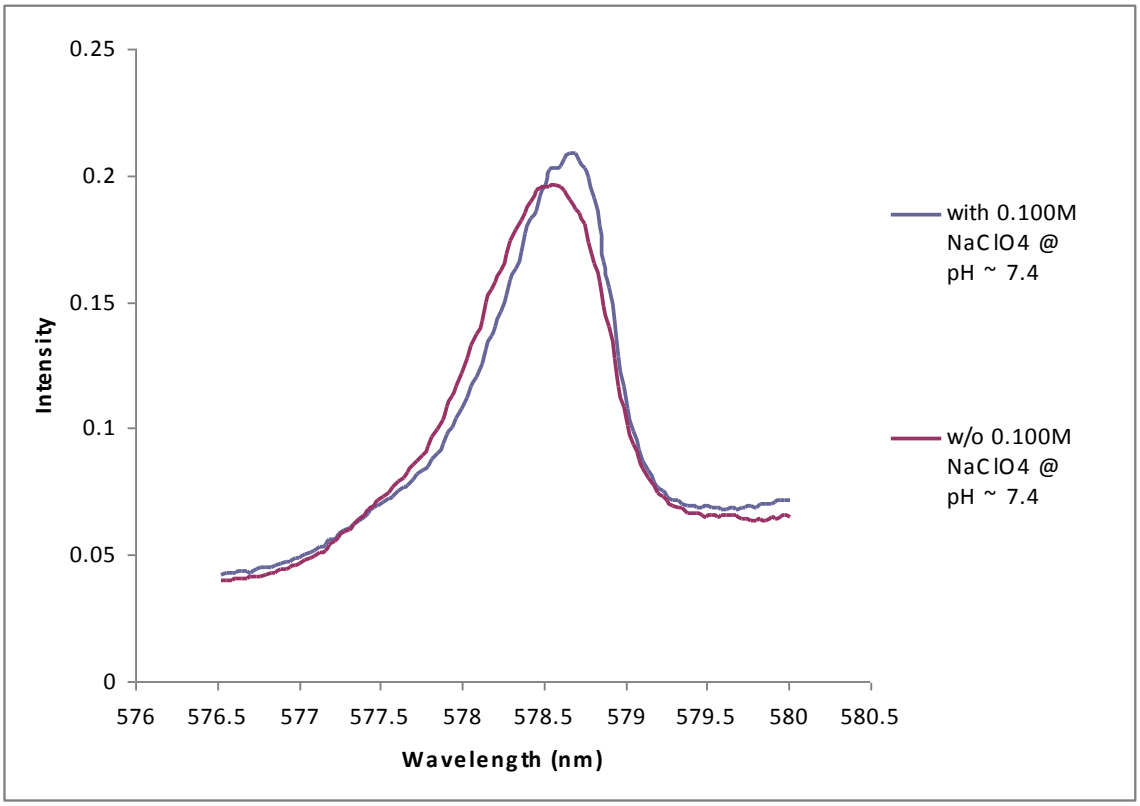


Figure 11. Excitation measurements of 0.100 M Eu(trif)$_3$ with and without 0.100 M $NaClO_4$ at pH ~ 7.40.

When D-glucosamine solution was added to free europium and $NaClO_4$ solution at pH ~ 7.40, the excitation spectrum exhibited two peaks (Figure 12).

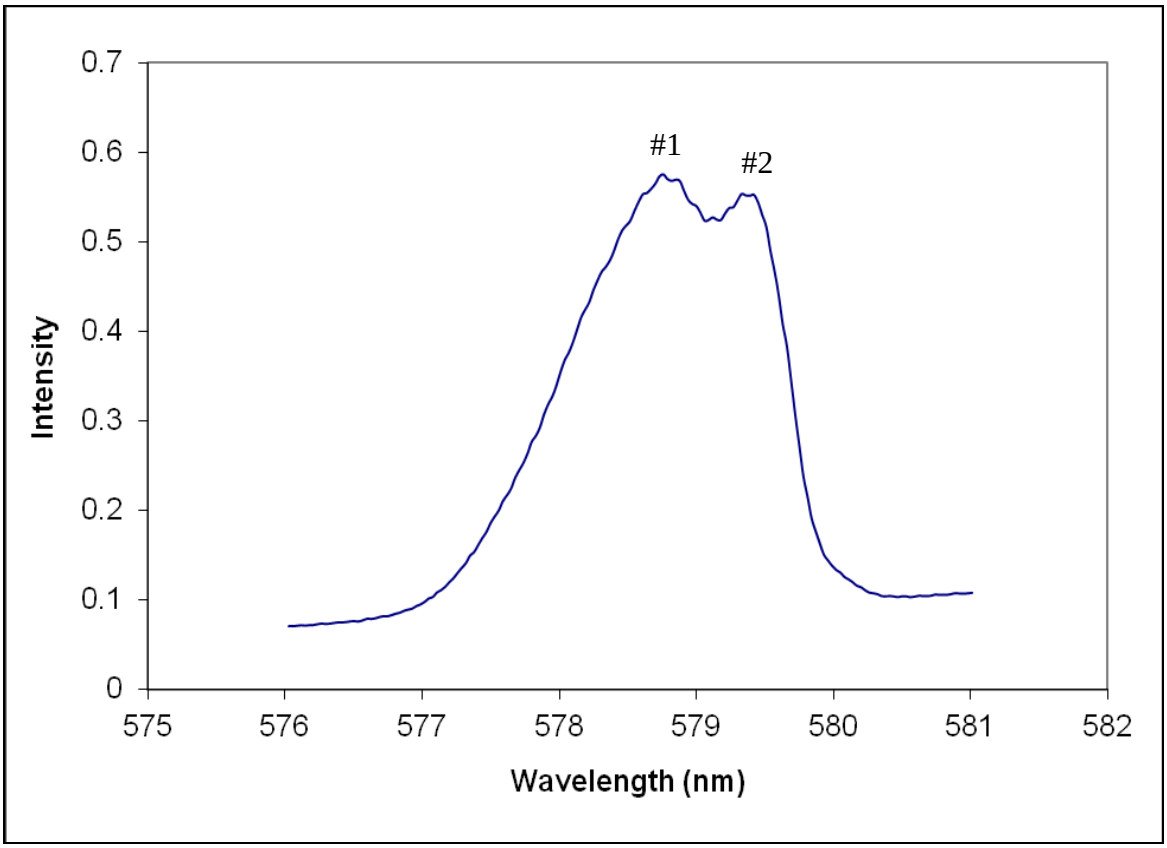


Figure 12. Excitation measurements of 0.100 M Eu(trif)$_3$ + 0.100 M D-glucosamine with 0.100 M NaClO$_4$ at pH ~ 7.40.

No precipitate was formed in the solution while intensity was being measured. However, after several minutes of letting the solutions to settle down, a precipitate was observed. The precipitate indicated that $Eu(OH)_2$ may be forming. Peak #1 appeared at 578.8 nm, and peak # 2 appeared at 579.4 nm (Figure 12) that Eu(III) and D-glucosamine were present at pH ~ 7.40. Since the peak at 578.8 nm appeared in water at pH ~ 7.40, the peak at 579.4 nm was assigned to the D-glucosamine complex. Since no additional peak was observed that might indicate $EuOH^{2+}$ binding, it can be concluded that (Eu^{3+}) bound to the D-glucosamine ligand at pH ~ 7.40.

According to the data presented by Hedinger, et al., (1998), europium complexation (Eu(III) – D-glucosamine) appeared to occur at a pH between 7 and 9, and significant $EuOH^{2+}$ complexation occured at pH above 9. The deprotonation of the hydroxo complex appeared at pH above 9 (Hedinger, et al., 1998).

5. Determination of Equilibrium Constant K_{EuL} & Constant k_{EuL}

A series of spectra with D-glucosamine concentrations were shown at pH ~ 7.40 (Figure 13).

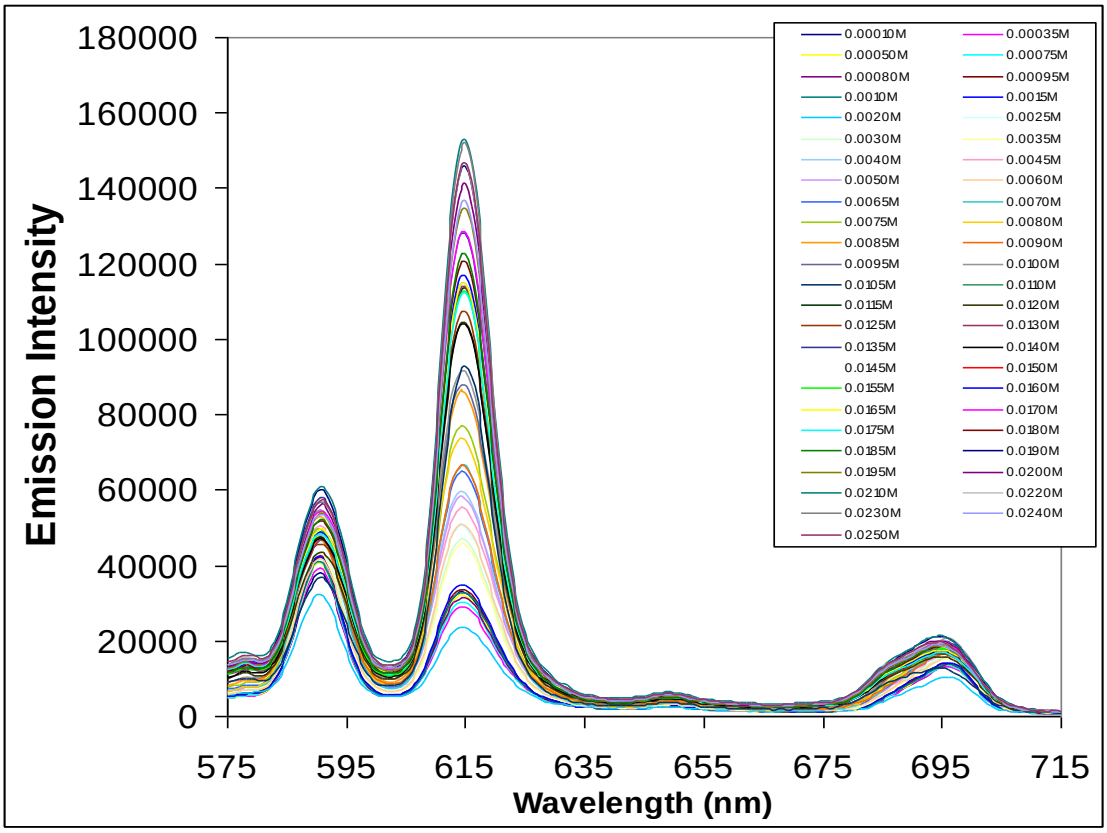


Figure 13. Luminescence spectra of Eu(III) – D-glucosamine excitation at wavelength 318 nm, pH ~ 7.40.

Figure 13 and Figure 14 illustrated a polynomial function that models a reaction between Eu(III) and D-glucosamine.

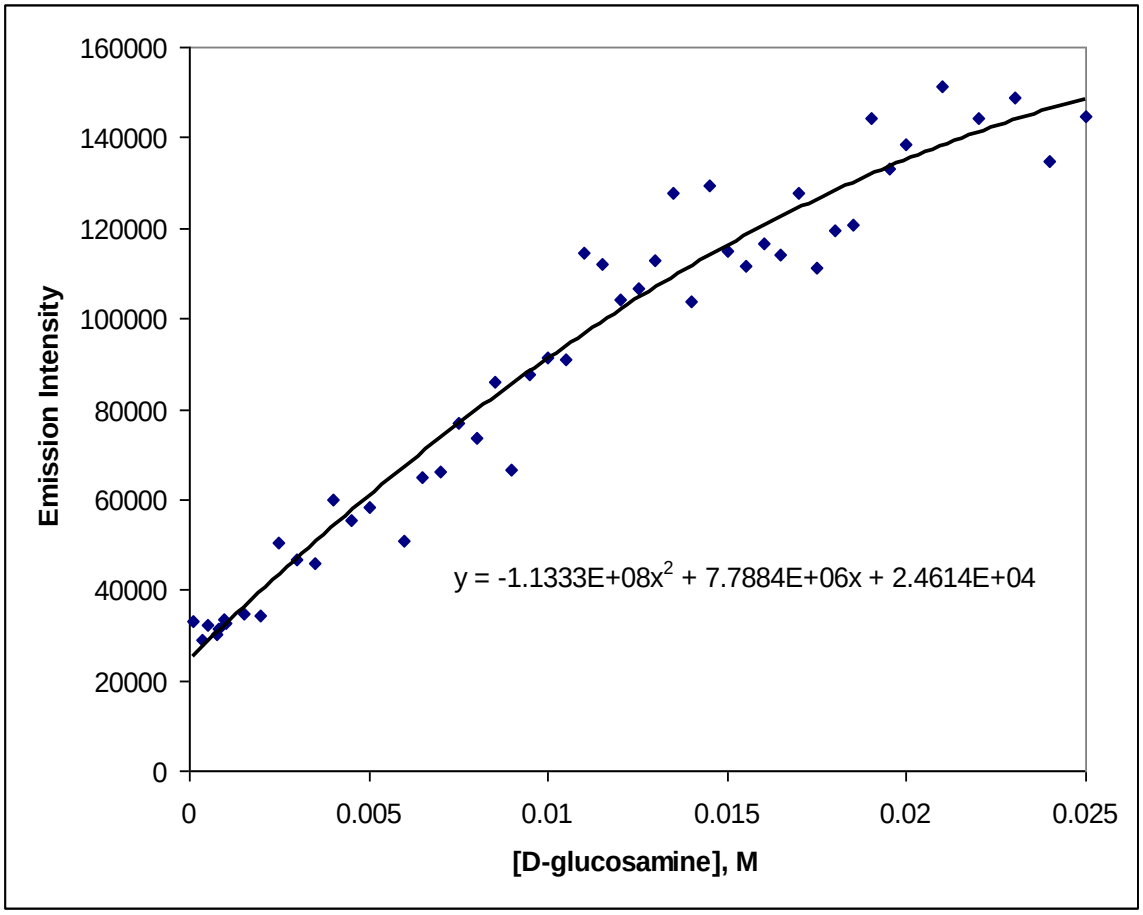


Figure 14. An Eu(III) – D-glucsosamine curve function at pH ~ 7.40 and wavelength of 614 nm at $[Eu]_t = 2.50 \times 10^{-3}$ M.

When constants k_L and k_{EuOHL} were omitted from emission intensity measurements at pH ~ 7.40 (*Equation 2.3*), the response may be expressed as:

$$\textbf{3.3} \qquad I_o = k_o + k_{Eu}[Eu] + k_{EuOH}[EuOH] + k_{EuL}[EuL]$$

As the value of each physical term from *Equation 3.3* has been determined, the initial emission intensity, I_o, value was equal to the sum of each of the above terms. At low pH, the constants k_o and k_{Eu} were found to be $k_o = 5.27 \times 10^3$ and constant $k_{Eu} = 7.41 \times 10^6$, respectively. The free europium concentration value was calculated ([Eu] = 1.12×10^{-3}M), as described in the Appendix A. The europium hydroxide concentration was calculated ([EuOH] = 1.07×10^{-3}M) from the hydrolysis reaction as described in the Appendix A using *Equation 1.4*. The value of constant k_{EuOH} was finally calculated from *Equation 3.2*, yielding a value of 1.18×10^7. The emission intensity, $I_o = 2.46 \times 10^4$ was calculated from the polynomial in Figure 14.

The calculated intensity (*I'*) is computed as:

$$\textbf{3.4} \qquad I' = I - k_o - k_{Eu}[Eu] - k_{EuOH}[EuOH] = k_{EuL}[EuL]$$

where constant k_o and constant k_{Eu} were determined from the measurements of free europium at low pH. The determination of constant k_{EuOH} was calculated based on *Equation 3.2*. The calculated intensity *(I'*) was equal to constant k_{EuL}[EuL]. Therefore, constant k_{EuL} was formulated as:

$$3.5 \qquad k_{EuL} = \frac{I - k_{\circ} - k_{Eu}[Eu] - k_{EuOH}[EuOH]}{[EuL]}$$

The equilibrium constant K_{EuL} was computed by dividing the product by the reactants as shown in *Equation 2.5* and *3.7*. A full description of the calculations for [Eu], [EuL], and [EuOH] is shown in the Appendix A. However, in order to calculate [EuL], an estimated value for the equilibrium constant K_{EuL} was used. When the estimated equilibrium constant K_{EuL} value was obtained, the concentration of Eu and the concentration of EuL were calculated using the equation in Appendix A.

The complexation reaction was written as the equilibrium:

$$3.6 \qquad \text{Eu(III)} + \text{D-glucosamine} \longleftrightarrow \text{Eu(III)} - \text{D-glucosamine}$$

Thus the equilibrium constant, K_{EuL}, was simplified to:

$$3.7 \qquad K_{EuL} = \frac{[\text{Eu(III)} - \text{D-glucosamine}]}{[\text{Eu(III)}] \times [\text{D-glucosamine}]}$$

The calculated equilibrium constant K_{EuL} was determined using a Microsoft Excel spreadsheet based on the calculated total europium and total D-glucosamine concentrations. *Equation 1.7* was also rearranged to give a determinant equation:

$$\boldsymbol{3.8 \qquad I - k_0 - k_{Eu}[Eu] - k_{EuOH}[EuOH] = k_{EuL}[EuL]}$$

Equation 3.8 was a straight line with a zero intercept. Once the constant k_{EuOH} was determined, an estimated value of equilibrium constant K_{EuL} was obtained. Calculations were performed for free complexed europium and free D-glucosamine. The plotted graph $I - k_0 - k_{Eu}[\text{Eu}] - k_{EuOH}[\text{EuOH}]$ versus $k_{EuL}[\text{EuL}]$ determined the linear regression line. If the estimated value of equilibrium constant K_{EuL} was high, the result was a negative intercept, as shown in Figure 15a.

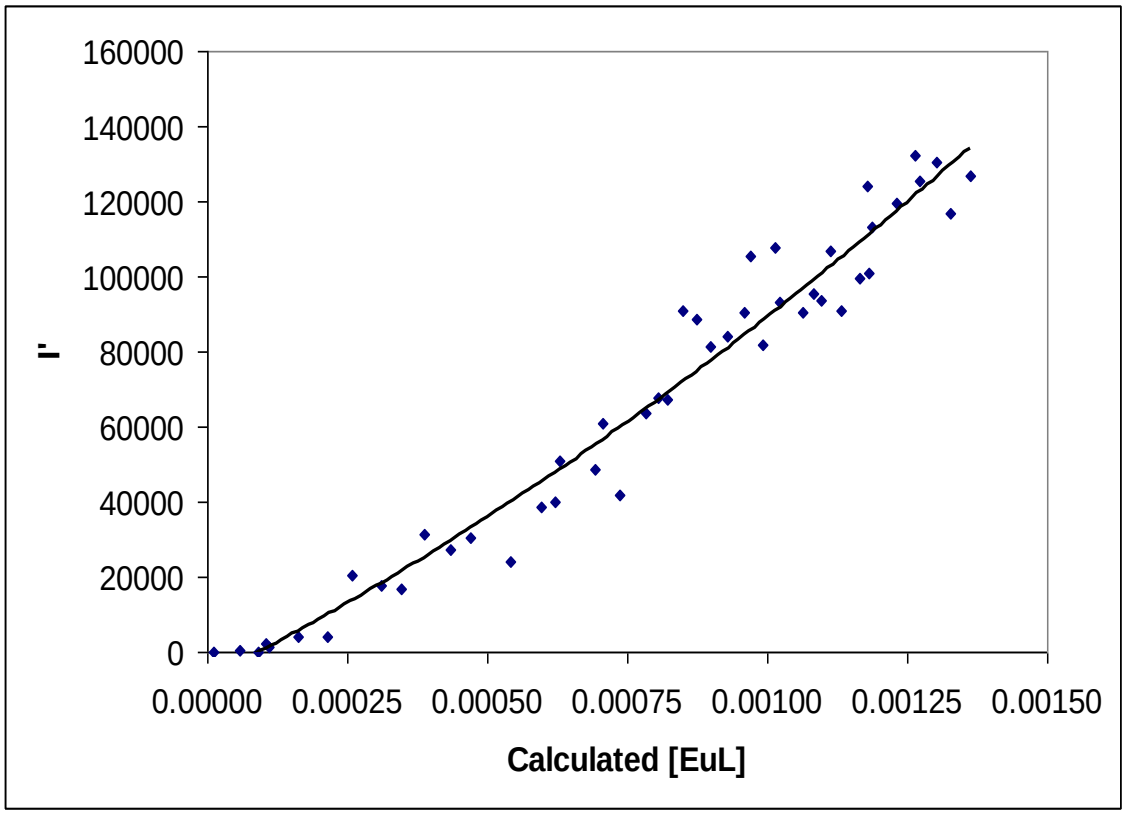


Figure 15a. A calculated intensity (*I'*) as a function of [EuL] (Eu(III) – D-glucosamine) when equilibrium constant K_{EuL} is assumed to be 100.

However, if the equilibrium constant K_{EuL} was low, then the result was a positive intercept, as shown in Figure 15b.

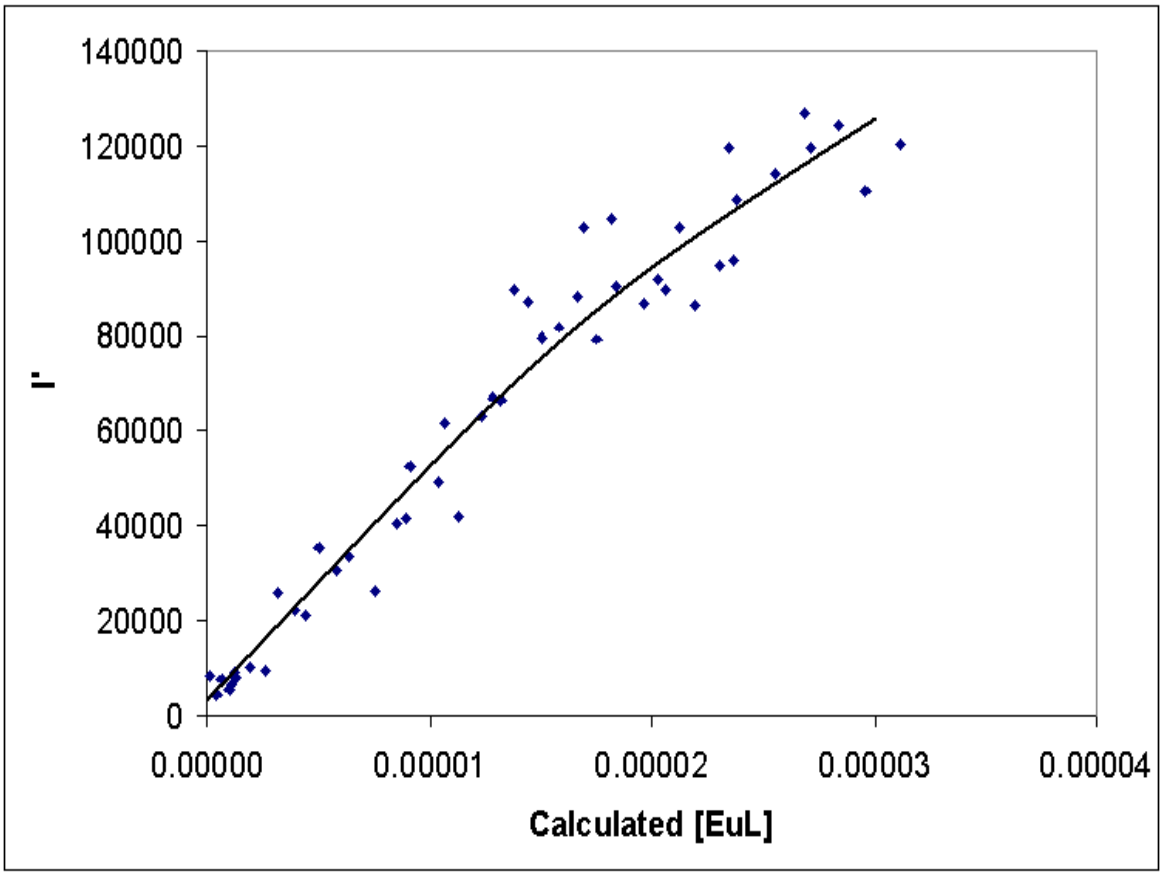


Figure 15b. A calculated intensity (*I'*) as a function of [EuL] (Eu(III) – D-glucosamine) when equilibrium constant K_{EuL} is assumed to be 1.

When the best value of equilibrium constant was estimated, a good correlation between experimental data and the best straight line was an intercept at zero. The graph of the calculated intercept as a function of equilibrium constant K_{EuL} was a curve that intersects at zero. The best equilibrium constant K_{EuL} value was determined (Figure 15c).

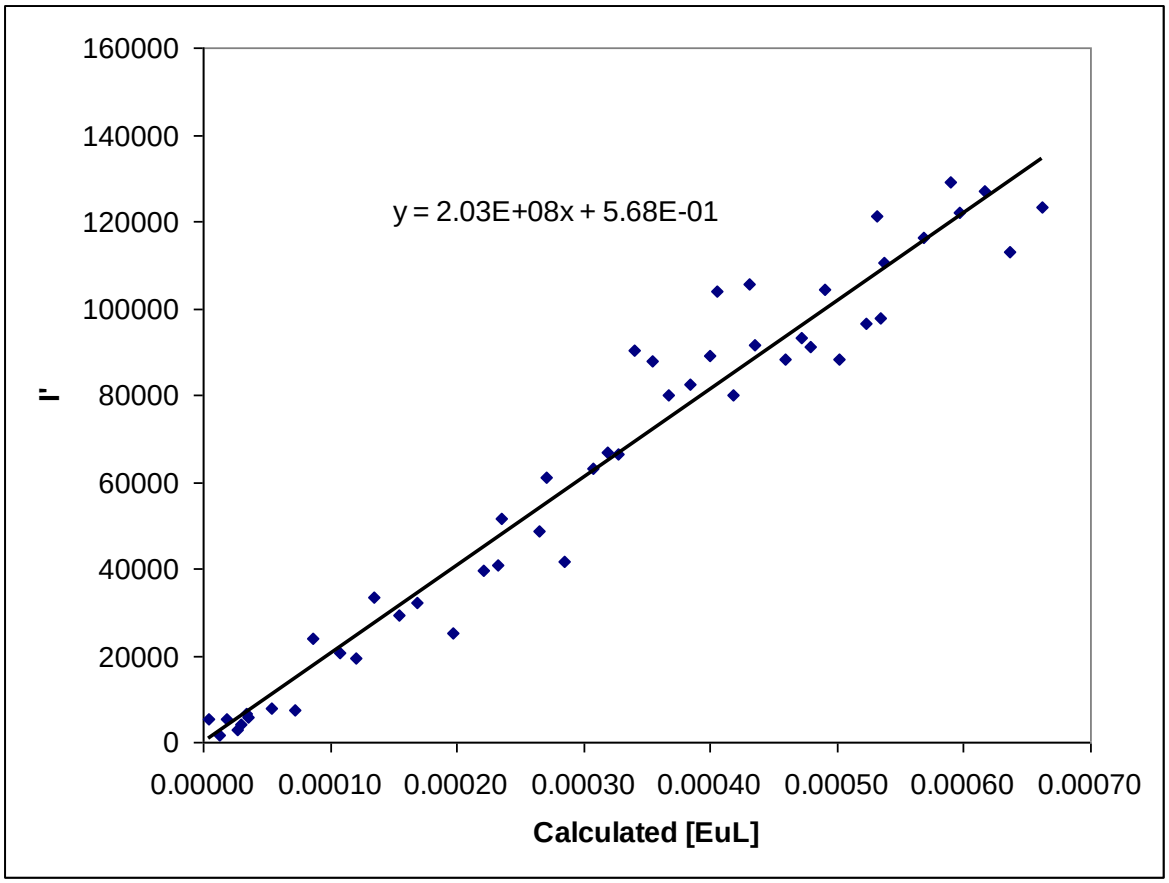


Figure 15c. A calculated intensity (*I'*) as a function of [EuL] (Eu(III) – D-glucosamine) when equilibrium constant K_{EuL} is 29.24, the best fit of the data at 25°C.

Chapter 4

Conclusion

The two main goals of this research were the determination of the equilibrium constant K_{EuL} and identification of complex formation at pH ~ 7.40 and 25 ^{o}C. The hypersensitive emission peak at 614 nm was studied and indicated a complex reaction at pH ~ 7.40. An increase of the D-glucosamine concentration resulted in an increase of the emission intensity at this wavelength. An equilibrium constant K_{EuL} of the Eu(III) – D-glucosamine complex was found to be 29.24.

Functional groups of D-glucosamine and Eu(III) were successfully observed in a solution-phase of the FTIR experiment. Laser excitation spectroscopy was used to determine if D-glucosamine bound to europium ion (Eu^{3+}), or bound to the europium hydroxide ion ($EuOH^{2+}$). Therefore, evidence concludes that [Eu(III)]:[D-glucosamine] complex indicated D-glucosamine bound to Eu^{3+} at pH ~ 7.40, but there was no evidence that the complexation bound to hydrolyzed Eu (III) .

Future Studies

The results obtained here provide insights into the binding reaction and suggested further studies on temperature dependence of Eu binding with different monosaccharide ligands. Equilibrium constants K_{EuL} can be determined at different temperatures under pH ~ 7.40.

References

Albin, M., Whittle, R. R., & Horrocks, W. Jr. (1985). Laser spectroscopic and x-ray structural investigation of europium (III)-oxydiacetate complexes in solution and in the solid state. *Inorganic Chemistry*, *24*(26), 4591-4594.

Alekseev, Y. E., Garnovskii, A. D. & Zhdanov, Y. A. (1998). Complexes of natural carbohydrates with metal cations. *Russian Chemical Reviews*, *67*(8), 649-669.

Alptürk, O., Rusin, O., Fakayode, S. O., Wang, W., Escobedo, J. O., Warner, I. M., Crowe, W. E., Král, V., Pruet, J. M., & Strongin, R. M. (2006). Lanthanide complexes as fluorescent indicators for neutral sugars and cancer biomarkers *PNAS*, *103*(26), 9756-9760.

Atkinson, P., Bretonniere, Y., Parker, D., & Muller, G. (2005). NMR and luminescence binding studies of ytterbium, thulium, and europium macrocyclic complexes with phosphorus (V) oxy anions. *Helvetica Chimica Acta*, *88*(3), 391-405.

Bentouhami, E., Bouet, G. M., Meullemeestre, J., Vierling, F., & Khan, M. A. (2004). Physicochemical study of the hydrolysis of rare-earth elements (III) and thorium (IV). *Comptes Renduschimie*, *7*(5), 537-545.

Bucella, S., Riello, P., Scremin, B. F., Calvelli, P., Polloni, R., Speghini, A., Bettinelli, M., & Benedetti, A. (2004). Synthesis and luminescence properties of ZrO_2 and ZrO_2/SiO_2 composites incorporating Eu(III)–phenanthroline complex prepared by a catalyst-free sol–gel process. *Optical Materials*, *27*, 249-255.

Diáz, M. D. & Berger, S. (2000). Studies of the complexation of sugars by diffusion-ordered NMR spectroscopy. *Carbohydrate Research*, *329*(1), 1-5.

Hamilton, K. (August, 2003). *Synthesis, characterization, and application of water-soluble chiral calix[4]arene derivatives in spectroscopy and capillary electrokinetic chromatography*. Ph.D. Thesis, Louisiana State University and Agricultural and Mechanical College, Baton Rouge, LA.

Hedinger, R., Ghisletta, M., Hegetschweiler, K., Tóth, E., Merbach, E. A., Sessoli, R., Gatteschi, D., & Gramlich, V. (1998). Trinuclear lanthanoid complexes of 1,3,5-triamino-1,3,5-trideoxy-*cis*-inositol with a unique, sandwich-type cage structure. *Inorganic Chemistry, 37*, 6698-6705.

Jiménez-Reyes, M., Solache-Ríos, M., & Rojas-Hernández, A. (2006). Application of the specific ion interaction theory → the solubility product and first hydrolysis constant of europium. *Journal of Solution Chemistry, 35*(2), 201-214.

Leonard, J. P., Dos Santos, C. M. G., Plush, S. E., McCabe, T., & Gunnlaugsson, T. (2007). pH driven self-assembly of a ternary lanthanide luminescence complex: the sensing of anions using a β-diketonate-Eu (III) displacement assay. *Journal of the Chemical Society, Chemical Communications*, *2*, 129-131.

Lopez-Gonzalez, H., Jiménez-Reyes, M., Solache-Ríos, M., & Rojas-Hernández, A. (2007). Solubility and hydrolysis of lutetium at different $[Lu^{3+}]_{initial}$. *Journal of Radioanalytical and Nuclear Chemistry, 274*(1), 103-108.

Parker, D., & Yu, J. (2005). A pH-insensitive, ratiometric chemosensor for citrate using europium luminescence. *The Royal Society Chemistry, Chemical Communications*, *25*, 3141-3143.

Ramírez-García, J. J., Jiménez-Reyes, M., Solache-Ríos, M., Fernández-Ramírez, E., López-González, H., & Rojas-Hernández, A. (2003). Solubility and first hidrolysis constants of europium at different ionic strength and 303 K. *Journal of Radioanalytical and Nuclear Chemistry*, *257*(2), 299-303.

Sherry, A. D.; Yoshida, C.; Birbaun, E. R., & Darnall, D. W., (1973). Nuclear magnetic resonance study of the interaction of neodymium (III) with amino acids and carboxylic acids. Aqueous shift reagent. *Journal of the American Chemical Society*, *95*(9), 3011-3014.

Silber, H. B., Chang, T., & Mendoza, E. (2001). Europium (III)–asparagine complexation in aqueous methanol. *Journal of Alloys and Compounds, 323-324*, 190-192.

Silber, H. B., & Nguyen, Y. (1998). Lanthanide complexation with amino acids: Eu(III) with alanine in aqueous methanol. *Journal of Alloys and Compounds, 275-277*, 811-814.

Silber, B. H., Marachin, V., Paquette, S., & Smith, S. (2004). Luminescence studies of lanthanide complexation reactions: europium (III) with saccharin in water. *Journal of Alloys and Compounds*, *374*(1-2), 339-343.

Skoog, D. A., Holler, F. J., & Nieman, A. T. (5^{th} ed.). (1998). *Principles of Instrumental Analysis.* Orlando, FL: Harcourt Brace & Company, Chapter 15.

Skoog, D. A., Holler, F. J., & Nieman, A. T. (5^{th} ed.). (1998). *Principles of Instrumental Analysis*. Orlando, FL: Harcourt Brace & Company, Chapter 16.

Stokes, G. G. (1852). On the Change of Refrangibility of Light. *Philosophical Transactions of the Royal Society of London, 142*, 463-562.

Yang, C.; Fu, L. M.; Wang, Y.; Zhang, J. P.; Wong, W. T.; Ai, X. C.; Qiao, Y. F.; Zou, B. S.; Gui, L. L. (2004). A highly luminescent europium complex showing visible-light-sensitized red emission: direct observation of the singlet pathway. *Angewandte Chemie International Edition*, *43*(38), 5010-5013.

Appendices

APPENDIX A

Instrumental Constants

1. At low pH (pH ~ 5.0)

$$Eu^{3+} + H_2O \rightleftharpoons EuOH^{2+} + H^+$$

$$K_{OH} = \frac{[EuOH^{2+}]\,[H^+]}{[Eu^{3+}]} \quad ; \quad K_{OH} = 10^{7.41} = 3.89 \text{ X } 10^{-8}$$

$$[EuOH^{2+}] = \frac{K_{OH}\,[Eu^{3+}]}{[H^+]} \quad ; \quad [H^+] = 4.08 \text{ X } 10^{-8} \text{ M}$$

$[EuOH^{2+}] = 1.07 \text{ x } 10^{-3}$M ; $[Eu^{3+}] = 1.12 \text{ x } 10^{-3}$ M

Find instrument constants, k_o, k_{Eu}:

$I = k_o + k_{Eu}\,[Eu]$ $I = 7.41x10^6 \text{ X} + 5.27x10^3$

$k_{Eu} = 7.41 \text{ x } 10^6$
$k_o = 5.27 \text{ x } 10^3$

2. At high pH (pH ~ 7.4)

$$Eu + L \rightleftharpoons EuL$$ (for clarity, charges were neglected)

$$K_{EuL} = \frac{[EuL]}{[Eu]\,[L]}$$; $[EuL] = 3.15 \text{ x } 10^{-4}$ M

estimated K_{EuL} value

Calculations of instrument constants, k_o, k_{Eu}, k_{EuOH}, k_{EuL}:

$$I_o = k_o + k_{Eu}[Eu] + k_{EuOH}[EuOH]$$

$$k_{EuOH} = \frac{I_o - k_o - k_{Eu}[Eu]}{[EuOH]} = 1.04 \times 10^7$$

$$I' = I - k_o - k_{Eu}[Eu] - k_{EuOH}[EuOH] = k_{EuL}[EuL]$$

$$k_{EuL} = \frac{I - k_o - k_{Eu}[Eu] - k_{EuOH}[EuOH]}{[EuL]}$$

APPENDIX B

Ionic Strength

$$I = \tfrac{1}{2} \Sigma(Z_i^2 \times C_i)$$

$$Eu(OTf)_3 \rightarrow Eu^{3+} + OTf^-$$

$$I = \tfrac{1}{2} [(3^2 \times C_{Eu}) + (1^2 \times C_{OTf})] \quad ; \quad \text{where } C_{OTf} = 3C_{Eu}$$

$$I = 0.015M \quad ; \quad \text{where } C_{Eu} = 0.0025M$$

APPENDIX C

Additional Tables

Table 1: Stock Solution Dilutions.

Solution #	Stock Solution, 0.0505M [Eu^{3+}]		Stock Solution, 0.100M [D-glucosamine]		Stock Solution, 2.00M [$NaClO_4$]	
	[Eu^{3+}], M	Vol, mL	[D-gluco], M	Vol, mL	[$NaClO_4$], M	Vol, mL
1	0.0025	0.495	0.00076	0.076	0.085	0.425
2	0.0025	0.495	0.0010	0.100	0.085	0.425
3	0.0025	0.495	0.0020	0.200	0.085	0.425
4	0.0025	0.495	0.0030	0.300	0.085	0.425
5	0.0025	0.495	0.0040	0.400	0.085	0.425

Table 2: Initial Data Points of 0.0025 M Eu(III) – D-glucosamine.

[D-glucosamine], M	Intensity @ 614 nm at pH < 5.0	Intensity @ 614 nm at pH ~ 7.4
0.00076	16988	64086
0.0010	16139	85551
0.0020	16996	87586
0.0030	17491	139815
0.0040	17571	170695

Table 3: Initial Data Points of 0.0025 M Eu(III) – Monosaccharide at pH ~ 7.4.

Galactose		Mannose		Ribose	
Concentration (M)	**Intensity**	**Concentration (M)**	**Intensity**	**Concentration (M)**	**Intensity**
0.00035	25797	0.00035	26600	0.00035	26111
0.00075	28892	0.00075	29952	0.00075	31159
0.0010	23968	0.0010	26757	0.0010	27764
0.0030	34323	0.0030	30958	0.0030	35126
0.0050	37526	0.0050	34863	0.0050	37449
0.0065	38174	0.0065	37028	0.0065	41555
0.0075	41307	0.0075	32026	0.0075	46631
0.0095	48898	0.0095	37899	0.0095	48085
0.0105	52685	0.0105	41047	0.0105	56245
0.0120	50330	0.0120	42917	0.0120	56353
0.0150	51734	0.0150	42154	0.0150	56665
0.0175	58515	0.0175	47487	0.0175	60514
0.0200	71278	0.0200	47836	0.0200	70362
0.0230	75194	0.0230	52001	0.0230	73089
0.0250	76932	0.0250	53671	0.0250	74866

Table 5: Data Points of 0.0025 M Eu(III)– D-glucosamine at Wavelength of 614 nm.

Number of Scanning	**$[Eu]_t$ (M)**	**$[Ligand]_t$ (M)**	**pH**	**Measured Intensity (cps)**
1	0.0025	0.00010	7.40	32980
2	0.0025	0.00035	7.37	28914
3	0.0025	0.00050	7.35	32281
4	0.0025	0.00075	7.37	30112
5	0.0025	0.00080	7.35	31246
6	0.0025	0.00095	7.38	33670

7	0.0025	0.00100	7.38	32831
8	0.0025	0.00150	7.38	34930
9	0.0025	0.00200	7.37	34190
10	0.0025	0.00250	7.39	50624
11	0.0025	0.00300	7.36	46799
12	0.0025	0.00350	7.39	45750
13	0.0025	0.00400	7.40	59818
14	0.0025	0.00450	7.38	55221
15	0.0025	0.00500	7.39	58279
16	0.0025	0.00600	7.40	51011
17	0.0025	0.00650	7.36	64838
18	0.0025	0.00700	7.38	65997
19	0.0025	0.00750	7.43	76930
20	0.0025	0.00800	7.37	73796
21	0.0025	0.00850	7.40	86096
22	0.0025	0.00900	7.40	66561
23	0.0025	0.00950	7.37	87681
24	0.0025	0.01000	7.38	91403
25	0.0025	0.01050	7.40	90980
26	0.0025	0.01100	7.40	114611
27	0.0025	0.01150	7.40	112000
28	0.0025	0.01200	7.40	104295
29	0.0025	0.01250	7.39	106520
30	0.0025	0.01300	7.38	112797
31	0.0025	0.01350	7.40	127576
32	0.0025	0.01400	7.40	103845
33	0.0025	0.01450	7.40	129344
34	0.0025	0.01500	7.42	115031
35	0.0025	0.01550	7.39	111557

36	0.0025	0.01600	7.39	116499
37	0.0025	0.01650	7.40	114272
38	0.0025	0.01700	7.40	127590
39	0.0025	0.01750	7.40	111208
40	0.0025	0.01800	7.38	119427
41	0.0025	0.01850	7.38	120572
42	0.0025	0.01900	7.41	144093
43	0.0025	0.01950	7.42	133157
44	0.0025	0.02000	7.38	138615
45	0.0025	0.02100	7.38	151257
46	0.0025	0.02200	7.41	144205
47	0.0025	0.02300	7.41	148912
48	0.0025	0.02400	7.41	134986
49	0.0025	0.02500	7.40	144823