

Fluorescent probe detection of metals in corrosive environments

Senior Thesis

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11/18/2010

Abstract:

Corrosion of metal surfaces is slowed down with the addition of protective coatings. Two studies that have explored monitoring corrosion of pigmented and non-pigmented coatings, using metal sensitive fluorescent probes will be discussed. The Rawlins group at the University of Southern Mississippi investigated the use of KMG-20-AM, a magnesium sensitive probe, to predict the lifetime of a magnesium coating on an aluminum alloy panel. An increase in fluorescence intensity of KMG-20-AM doped magnesium coating suggested an interaction with the free magnesium in the coating. Sibi and Zong focused on fluorescence of samples of the corrosion solution for an aluminum alloy panel with the use of three metal ion selective probes. The fluorescence intensity of the corrosion solution samples, of the aluminum panel, suggested different leaching progressions of metal ions from the panel. Based on the results of these studies, the data suggest that, there is a high potential for the use of fluorescent probes as a technique to monitor metal corrosion.

Introduction:

The Eiffel tower is painted and treated for corrosion every seven years. The process of repainting and buffing down corroded spots to apply an anticorrosive coating takes 15 months.¹ The prevention of corrosion is what allows for this historical landmark to remain open for people to visit yearly. The study of anticorrosive coatings is important not only to landmarks but to vehicles, buildings, planes, bridges and pipes.² It is not infrequent for any of the previously mentioned structures to suffer failures from corrosion. When structures fail the result can have severe consequences for whole cities. Commercially, anticorrosive coatings are

developed and rated based on toxicity, efficiency, versatility, and price.³ Monitoring corrosion of materials and developing lifetime prediction models for anticorrosive coatings to elongate the lifetime of structures is an important field of research not only financially but for human safety. A novel way to monitor corrosion was investigated by the Rawlins group and Sibi and Zong with fluorescent probes.

Fluorescence spectroscopy is a common technique used for monitoring fluorescence intensity (aka emission intensity) and wavelength shifts during chemical processes. When a photon of light is absorbed in the lowest energy state it promotes an electron to an excited state which. When this energy is released, it results in a process known as fluorescence. The release of the energy to bring the electron down to the lowest energy level produces a photon, which is seen as a color. This color is then quantified by an emission intensity processed by the detector, given off at a specific wavelength. Factors that allow for a molecule to be fluorescent are low energy π to π^* transitions, n to π^* transitions, conjugated pi-bonds, and structural rigidity.⁴

Fluorescence can be used as a quantitative tool by employing dyes whose fluorescence emission can be precisely calibrated at known concentrations. Many fluorescent dyes are made for specific functions such as having sensitivity for one specific molecule or ion: these are called fluorescent probes. Such probes provide a glimpse into the environment of cells, coatings, composites, etc. when used to measure analytes at very low concentrations.^{5,6} Some of these probes can cost as much as \$100 per milligram of probe, which is often not cost efficient for

multiple trials or expanded studies. On occasion it is more cost effective to make a probe than to purchase it commercially when possible.

Specifically discussed in this thesis, are four metal ion selective probes that chelate metal ions and can be used to monitor corrosion. Three of the probes were commercially purchased and the other is made from a synthesized dye, which was more cost effective than purchasing the premade dye. The three commercially available probes are Lumogallion, Phen GreenTM, and SABF (N,N''-bis-(salicylidene)-2,3-diaminobenzofuran). Lumogallion is selective to Al³⁺, Phen GreenTM is selective to Cu²⁺, and SABF is selective to Mg²⁺.⁵ The synthesized dye in the second study is ((13-aza-3-oxa-4-oxotetracyclo[7.7.1.0-<2,7>.0<13,17>] heptadeca-1(17),2(7),5,8-tetraen-5-ylcarbonyloxy)-methyl acetate) known as KMG-20-AM, and it is selective to Mg²⁺.^{6,7} The structures of the probes used in these studies can be seen in figure 1.

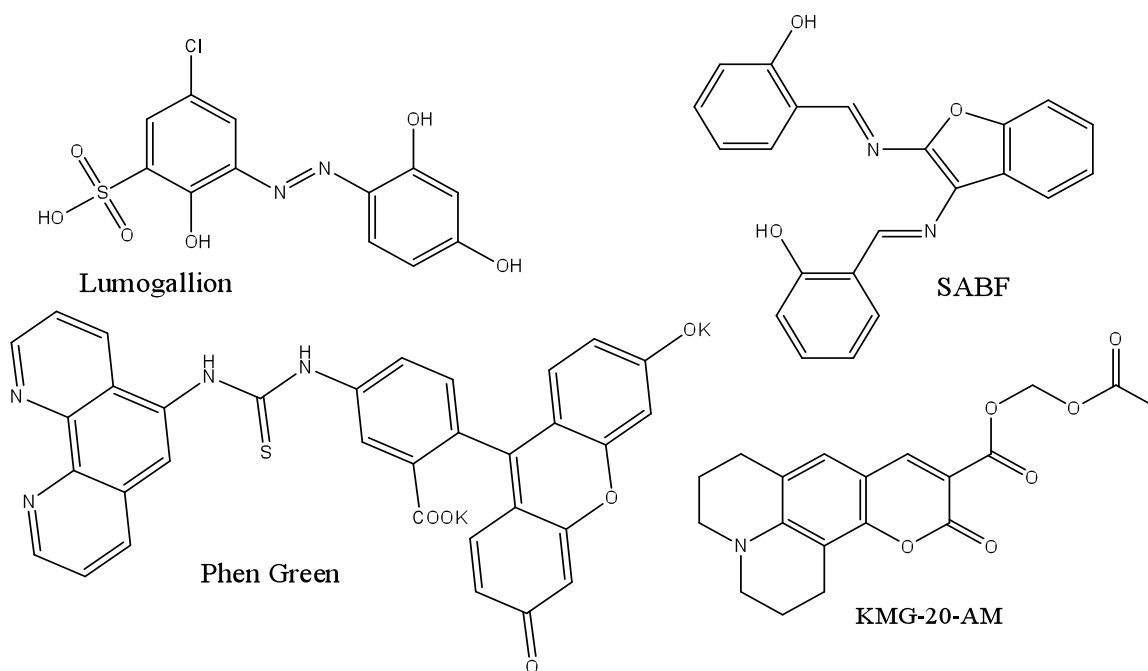


Figure 1. Chemical structures of fluorescent probes

The process through which the fluorescent probes bind to their metal ions is known as chelation. During chelation, ligands bind to a central atom.⁸ Ligands in this case are Lumogallion, Phen Green™, SABF, and KMG-20-AM. All these ligands are organic electron donors. The functional groups that make these molecules electron donors are the ketones, alcohols, and amines. The classic example of a chelation ligand is EDTA (Ethylenediaminetetraacetic acid), which is a polydentate ligand that wraps its self around a metal ion as a central atom in the complex (Figure 2).⁸ When EDTA binds to the metal ion, it displaces the metal ions positive charge.

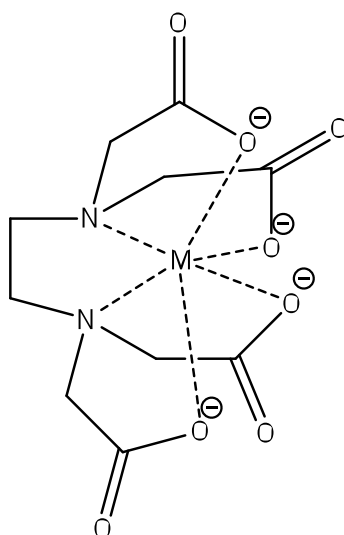


Figure 2. Ethylenediaminetetraacetic acid chelation with a metal (M)

In fluorescence, when a metal is bound to a probe there can be different responses. These responses were investigated in two separate experiments, each of which will be reviewed. The main focus of both studies is corrosion of metals. Corrosion is the irreversible degradation of a material such as metal, ceramic, or polymer.⁸ As metals corrode they produce metal ions that break off the main solid of the metal. A significant amount of money is invested

in anticorrosive coatings yearly in an effort to make materials last longer.² The lifetime afforded by an anticorrosive coating is very important in judging how often repainting may be necessary to ensure continued protection of the substrate. There are three main types of anticorrosive coatings: inhibitors, sacrificial, and barrier.⁹ Inhibitors protect through the release of soluble species that promote the formation of a surface layer.⁹ Sacrificial coatings protect by corroding before the material it is protecting. This is done through the coating acting as the anode and the material acting as the cathode when in electrical contact. This process allows for the anode to protect the cathode from corrosion.⁹ Barrier coatings are the most common, they come in the form of top coats and primers because they do not react with the metal that they are protecting.⁹ Anticorrosive coatings are tested by constant immersion in corrosive environments. The main types of corrosive environments are salt water, burial in soil, atmosphere, and ultraviolet light.² A common technique for testing the lifetime and integrity of the coating is salt fog.² Salt fog is a technique where the panels treated with the protective coating are placed into a chamber and are blasted for a period of time with hot salt water. Salt fog testing simulates environments such as the ocean or the salty road for cars during winter.

The most common anticorrosive coating for aerospace aluminum is hexavalent chromium, which can act as an anode or cathode when inhibiting corrosion.¹⁰ This unique property makes chromium a very effective protective coating. However, hexavalent chromium is toxic to the body and may cause cancer.⁹ Mg-rich primers are gaining interest as a viable alternative to hexavalent chromium because they can anodically protect a metallic substrate without harmful side effects on the environment.⁹ Presently, there is no technique to predict

the lifetime of Mg-rich primers. It is also well known that Mg-rich primers perform well during natural weathering but fail rapidly when placed in an accelerated weathering salt fog chamber.^{11,12} In addition to magnesium coatings not having a lifetime prediction model there are not many techniques, other than microscopy, that allow preservation of the entire sample to perform corrosion studies over time.

In the present study, in an effort to preserve the sample throughout the corrosion process while collecting data, fluorescent probes will be added to anticorrosive coatings and to corrosion solution samples to measure the metal ion concentration.

As previously stated, Magnesium coatings are an interesting alternative to chromium coatings because of the development of a magnesium sensitive fluorescent probe by Suzuki et al., called KMG-20-AM. KMG-20-AM is a Coumarin 343 derivative that has a diketone functional group which was added to make the probe more selective to magnesium ions and to allow for easier penetration of the PC12 cells (structures of KMG-20-AM and Coumarin 343 can be seen in Figure 3).⁶ With a fluorescent probe that can penetrate the PC12 cells it was possible to quantify the concentration of intercellular Mg^{2+} . This measurement is biologically relevant because Mg^{2+} plays an important role in ATP synthesis. KMG-20-AM was originally designed to quantify the concentration of intracellular magnesium; however this probe has been predicted to be valuable in magnesium coatings, through quantifying the amount of magnesium lost over time. To test this prediction, the Rawlins group at the University of Southern Mississippi doped a magnesium coating with KMG-20-AM and tested the fluorescence potential of the coating.

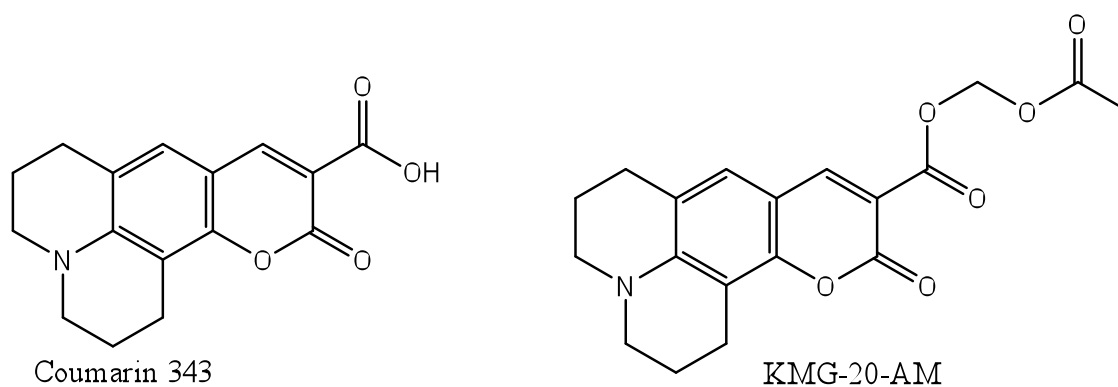


Figure 3. Structure of Coumarin 343 and KMG-20-AM (((13-aza-3-oxa-4-oxotetracyclo[7.7.1.0-
<2,7>.0<13,17>] heptadeca-1(17),2(7),5,8-tetraen-5-ylcarbonyloxy)-methyl acetate))

Sibi and Zong wanted to answer a similar question to that of the Rawlins group in regards to the monitoring of corrosion with fluorescent probes. Sibi and Zong utilized three fluorescent probes that are selective to metals that make up an aluminum alloy. The aluminum alloy that Sibi and Zong used is 1.6% Mg, 4.7% Cu, 92.71% Al, and 0.99% other metals. The fluorescent probes Lumogallion, SABF, and Phen GreenTM were used to detect the metal ions in solution to then quantify the amount of metal lost over time. Sibi and Zong focused more on the metal ions in the corrosion solution to determine which metals corrode off the alloy panel in a 0.50 M NaCl corrosion solution.

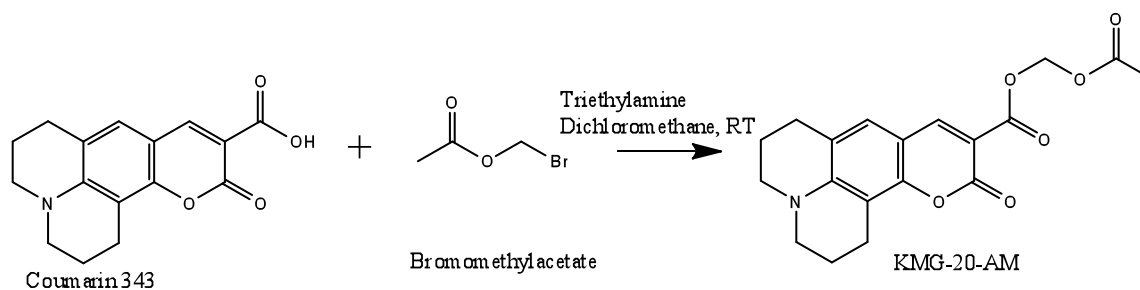
2. Results and Discussion:

2.1 Synthesis of KMG-20-AM

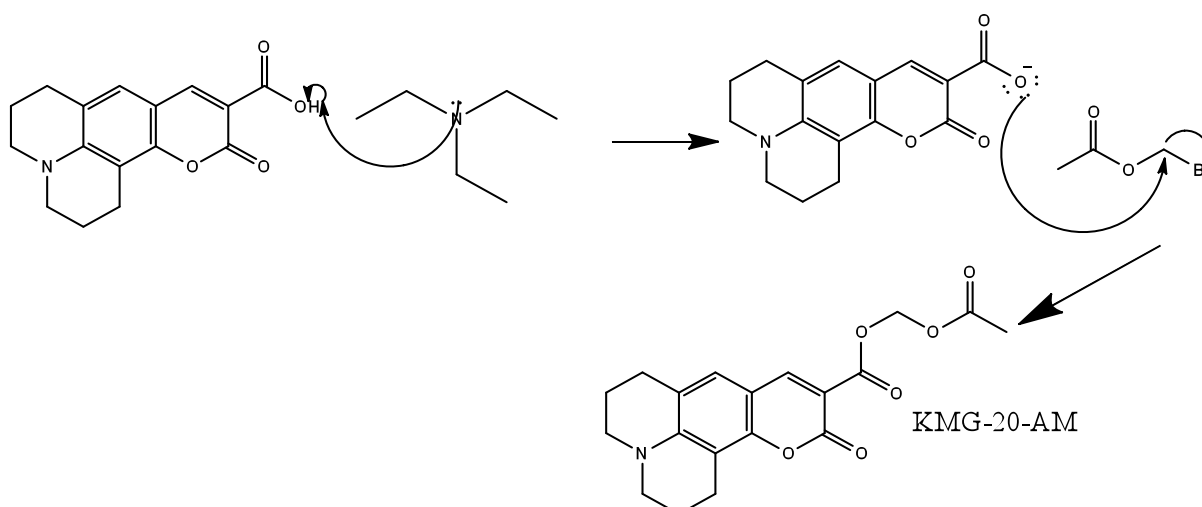
Herein the chelating effect of four fluorescent probes on Al³⁺, Mg²⁺, and Cu²⁺ are discussed. As was noted previously, three of the fluorescent probes are commercially available: lumogallion (selective to Al³⁺), N,N'-bis-(salicydene)-2,3-diaminobenzofuran (SABF) (selective to Mg²⁺), and Phen GreenTM (selective to Cu²⁺) (Figure 1). The fluorescent probes have electron

donating groups that bind with the metal ions. Al^{3+} and Mg^{2+} have coordination complex of four or six. Cu^{2+} has a coordination complex of four. The coordination complex number states the number of potential binding spots on the metal. Since there is more than one binding site per metal ion, it is likely that multiple electron rich groups on the probe participate in the complex or that multiple probes participate with one ion.

The fourth probe, ((13-aza-3-oxa-4-oxotetracyclo[7.7.1.0-<2,7>.0<13,17>] heptadeca-1(17),2(7),5,8-tetraen-5-ylcarbonyloxy)-methyl acetate), previously defined as KMG-20-AM, is another Mg^{2+} sensitive dye that was synthesized and tested by Suzuki et. al (Figure 1). In an effort to recreate the results reported by Suzuki et al., KMG-20-AM was synthesized with their reported procedure (Scheme 1). KMG-20-AM is synthesized through an SN2 reaction where triethylamine (TEA) acts as a proton acceptor of the carboxylic acid of Coumarin 343 in the first step (Mechanism 1). The deprotonated acid then binds with the carbon that bromine is bonded to resulting in bromine leaving; this forms KMG-20-AM (Mechanism 1).

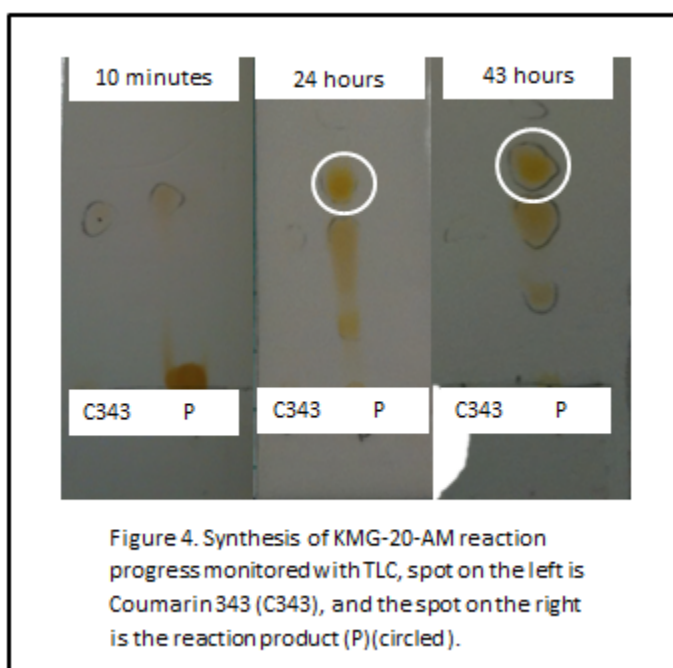


Scheme 1. Synthesis of KMG-20-AM



Mechanism 1. Synthesis of KMG-20-AM

The reaction was monitored by thin layer chromatography (TLC). Presented in figure 4 are three TLC plates from 10 minutes, 24 hours, and 43 hours of reaction time. In all three TLC



plates there are two spots, the one on the left is the reactant, Coumarin 343. In order to monitor a reaction through TLC the reactants and products must be UV active which results in the molecules involved having conjugated pi bonds. Thus the only reactant used to monitor the reaction is Coumarin 343. The spot

on the right is the product at the given time. In figure 4 there are circles around the product that later through NMR spectroscopy was identified as KMG-20-AM. Ten minutes into the reaction there is no product formed, the spot is Coumarin 343 because it has the same R_f value

as Coumarin 343. After 24 hours, there is separation in the product with a clear difference between left over reactants and one main product. After 43 hours there are three side products in addition to the left over reactants. At this point the reaction was stopped and column chromatography was used to purify the product.

Nuclear magnetic resonance spectroscopy (NMR) was run on the four products of the reaction. The second eluent off the column was identified as KMG-20-AM (Figure 5). The other side products were not characterized. There are three areas of the proton NMR spectra that indicate the synthesis of KMG-20-AM. The first is the absence of a peak at about 12 ppm; a peak in this general region would represent the carboxylic acid proton which was a functional group on Coumarin 343. The absence of this peak indicates that the carboxylic acid has reacted to form KMG-20-AM. The next peak of importance is at 5.9 ppm, this peak represents the methylene carbon originally bonded to bromine and the oxygen of the deprotonated carboxylic acid of Coumarin 343.

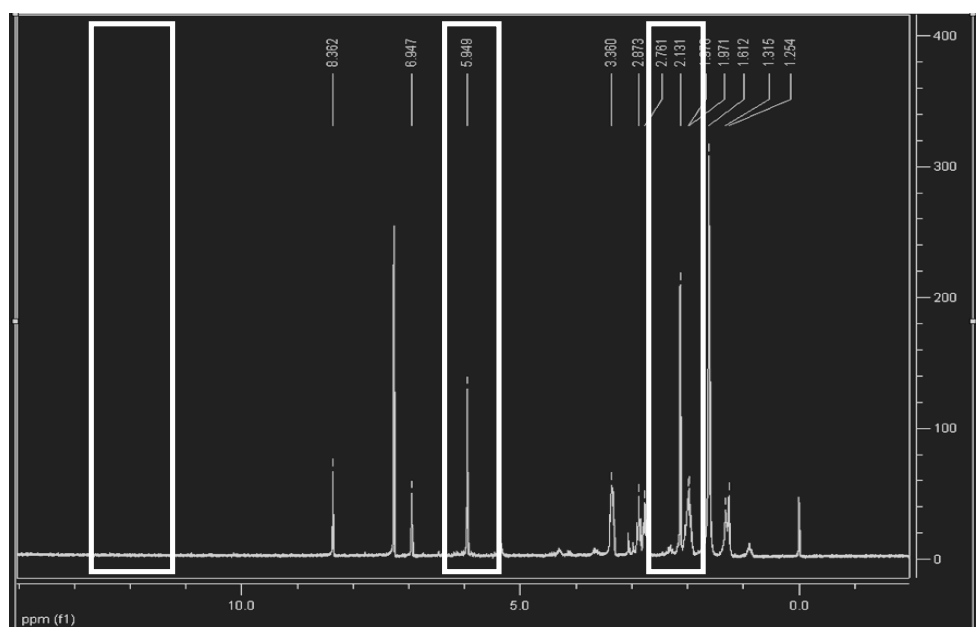
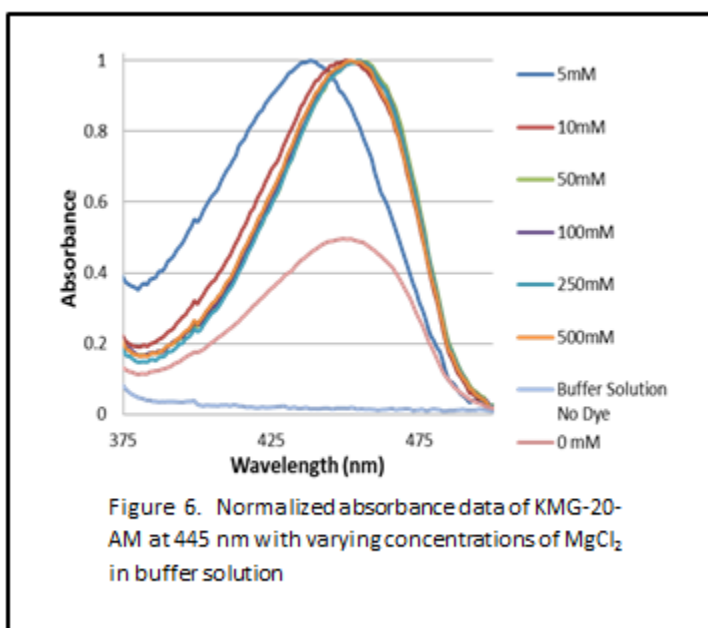


Figure 5. NMR Spectra of KMG-20-AM

The last peak that indicates the formation of KMG-20-AM is at 2.131 ppm, this peak represents the methyl protons at the end of bromomethylacetate. This peak is important because it shows that bromomethylacetate bound to Coumarin 343. This value is where primary carbons are generally found in the spectra. If bromomethylacetate had not bound to Coumarin 343 the peak would also be present, but the presence of the peak at 5.9 ppm further supports the bond of bromomethylacetate to Coumarin 343. In summary, KMG-20-AM was successfully synthesized via an SN2 reaction that was monitored through TLC and identified through NMR. The peaks at 5.949 and 2.131 ppm show the binding site of bromomethylacetate to Coumarin 343 and the end of bromomethylacetate. The absence of a peak at 12 ppm shows that the carboxylic acid of Coumarin 343 is where the reaction took place supporting mechanism 1.

2.2 Chelating of Mg^{2+} with KMG-20-AM

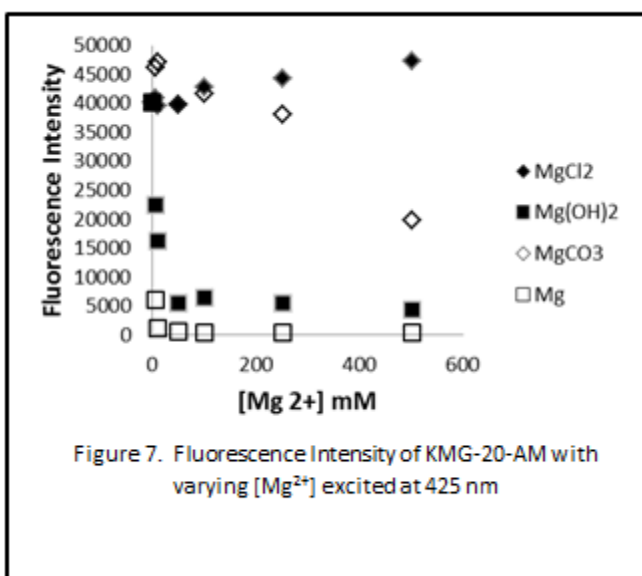
Suzuki et al. tested the absorbance and fluorescence of KMG-20-AM with $MgCl_2$, and found an increase in emission intensity with an increase in concentration of Mg^{2+} .⁶ To test the



functionality of KMG-20-AM that was synthesized, the fluorescence and absorbance testing that Suzuki et al. did was repeated and expanded through additional absorbance and fluorescence testing with $Mg(OH)_2$, $MgCO_3$, and Mg

powder. $\text{Mg}(\text{OH})_2$, MgCO_3 , and Mg powder emission intensity and absorbance were not tested by Suzuki et al., however they were included in the repeated study to determine the effects KMG-20-AM could have in a coating. $\text{Mg}(\text{OH})_2$ is one of the products a Mg coating produces when it comes in contact with water, MgCO_3 is a product of a Mg coating when interacting with air, and Mg powder is what the coating is made out of. MgCl_2 , $\text{Mg}(\text{OH})_2$, MgCO_3 , and Mg powder were dissolved in a buffer solution (10 mM HEPES(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mM EGTA(ethylene glycol tetraacetic acid), 130 mM KCl, 20 mM NaCl) at pH 7 with 10 μM KMG-20-AM.

The solutions were mixed for 24 hours and added to a 96 well plate for fluorescence and absorbance spectroscopy via a Safire Tecan plate reader. Figure 6 shows the absorbance of KMG-20-AM, versus the concentration of MgCl_2 . KMG-20-AM forms a complex with Mg^{2+} which causes the absorbance to shift right. KMG-20-AM has a λ_{max} of 445 nm but when



complexed with Mg^{2+} the λ_{max} increases to 445 nm. These results correlated directly with those reported by Suzuki et al., who reported that there was a shift in absorbance to the right when KMG-20-AM complexed with Mg^{2+} , caused by the lone pair of electrons from the diketone functionality.⁶

Fluorescence testing was also done with KMG-20-AM and MgCl_2 as well as with Mg(OH)_2 , MgCO_3 , and Mg powder. All solutions were excited at 425 nm and emission intensity was collected at 450 nm. MgCl_2 showed an increase in emission intensity as the concentration of Mg^{2+} increased as reported by Suzuki et al. (Figure 7). As previously stated this is because KMG-20-AM acts as a Mg^{2+} sensitive probe and chelates the Mg^{2+} through the diketone functionality. The two partial negative charges of the diketone is what complexes with Mg^{2+} . The electrons from the oxygen aid in the delocalization of the positive charge of the metal ion which stabilizes the complex.

However, the solutions containing Mg(OH)_2 , MgCO_3 , and Mg powder exhibited an unexpected decrease in fluorescence intensity (Figure 7). It was predicted that Mg(OH)_2 , MgCO_3 , and Mg powder would exhibit an increase in fluorescence intensity because they dissociate in to Mg^{2+} and their respective counter ions. The Mg^{2+} complexes with KMG-20-AM, therefore all the Mg compounds in solution should show an increase in fluorescence intensity. The unexpected decrease in emission intensity prompted further testing and observations to explore why the other Mg compounds did not exhibit the predicted response.

It was noted that MgCl_2 solutions were light green in color before and after shaking. Solutions of Mg(OH)_2 , MgCO_3 , and Mg powder were initially light green in color but became colorless when spectra were taken after 24 hours of shaking. The colorless solution was an indication there was a problem with the dye. Previous experience with fluorescein based dyes, which can become colored and colorless depending on the resonance form from the open to closed position. Fluorescein changes between the open and closed form due to pH, this

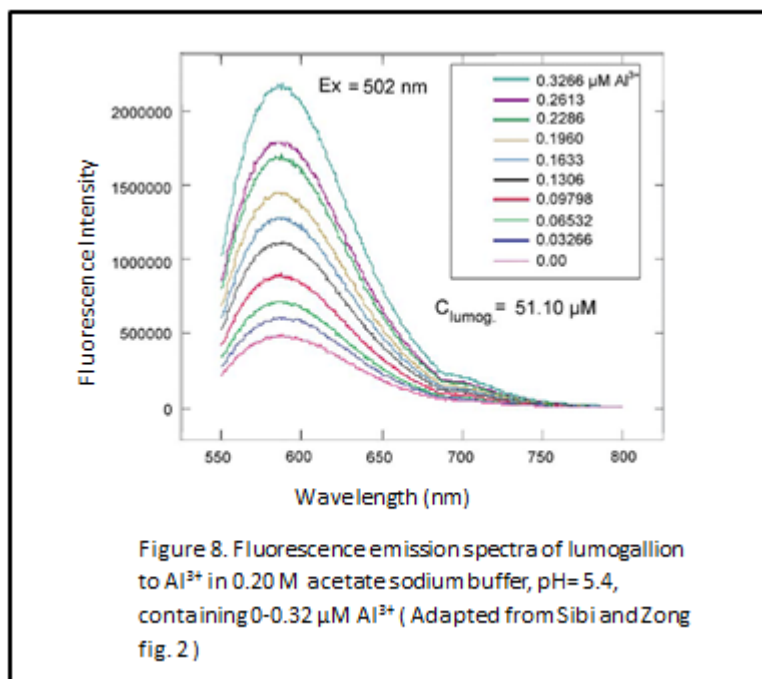
concept promoted the investigation of a pH change in the buffer solution. It was determined that there was an increase in pH in the colorless solutions causing the probe to be inactive. The irreversibility of the pH change was determined by a pH titration. It was found that the solution never regained color or fluorescence when it was returned to a lower pH. This showed the degradation of KMG-20-AM at high pH values. Thus the decrease in fluorescence is due to KMG-20-AM's sensitivity to pH. When the pH is greater than 7, the dye loses its ability to fluoresce and no longer operates as a fluorescent probe.

In summary, KMG-20-AM experiences a shift in lambda max in response to complexation of Mg^{2+} , and also experiences an increase in fluorescence intensity with Mg^{2+} . The exception to the increase in fluorescence intensity is due to an increase in pH which renders KMG-20-AM inactive as a fluorescent probe for Mg^{2+} ions. The increase in fluorescence intensity and shift of absorbance are support for KMG-20-AM as a more cost effective Mg^{2+} sensitive fluorescent probe.

2.3 Fluorescence Emission Response of fluorescent probes to Al^{3+} , Mg^{2+} , and Cu^{2+}

A study of fluorescence emission by Sibi and Zong investigated the emission response of fluorescent probes that are sensitive to one of the following metal ions in solution: Al^{3+} , Mg^{2+} , and Cu^{2+} . In the study varying concentrations of Al^{3+} from 0 to 0.3266 μM were added to a solution containing 51.10 μM lumogallion in 0.20 M sodium acetate buffer (pH= 5.2) and the fluorescence emission intensity was recorded (Figure 8). Each aluminum ion sample was excited at a wavelength of 502 ± 1 nm and emission intensity was measured at a λ_{max} of 588 ± 1 nm. There was no spectral shift of wavelength with an increase in concentration of Al^{3+} . As

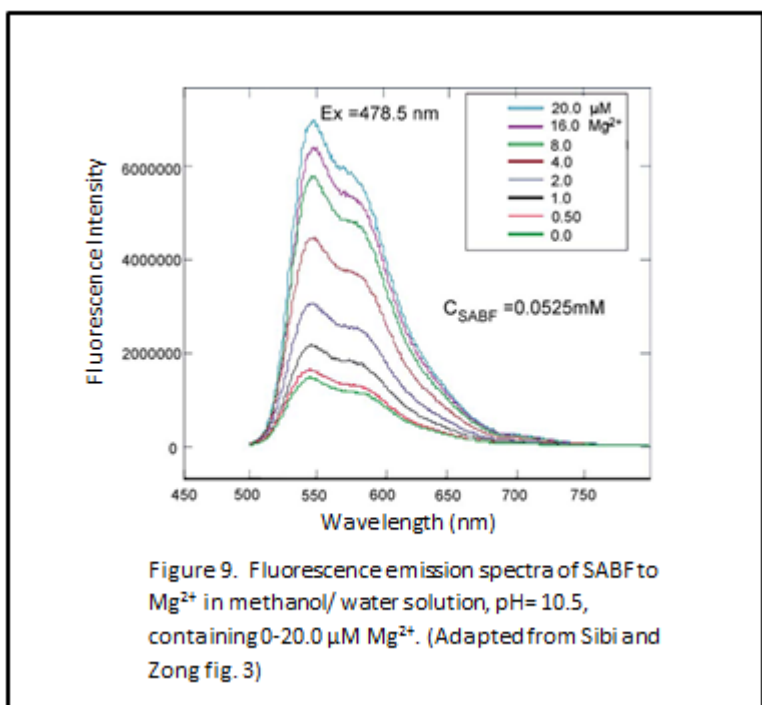
the concentration of Al^{3+} increased so did the fluorescence intensity. This is because more



metal ions in solution complex with lumogallion.

The fluorescence intensity of SABF in the presence of Mg^{2+} with concentrations ranging from 0 to 20 μM in a solution of 0.525 mM SABF in a diethylamine buffer (pH= 10.5) was measured (Figure

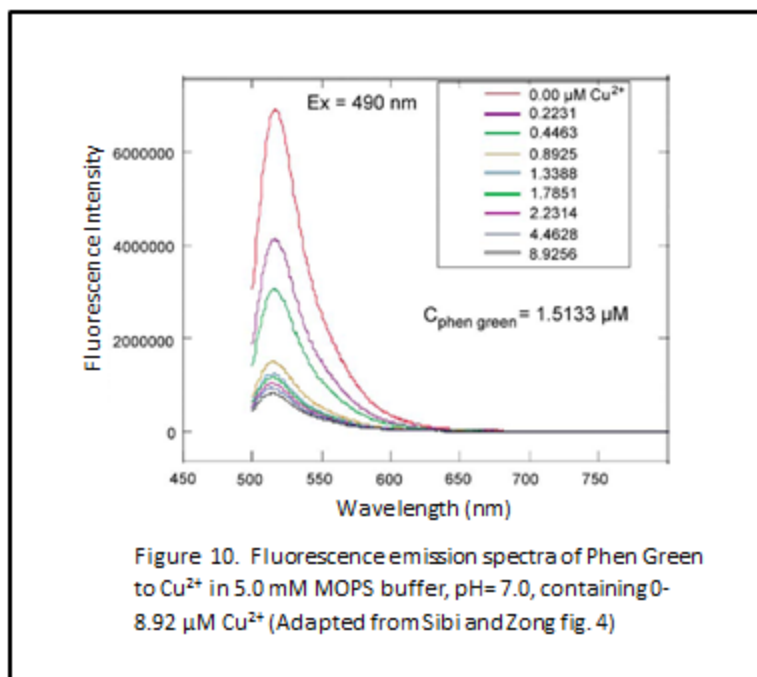
9). SABF has an excitation wavelength of 478 ± 1 nm and an emission λ_{max} of 546 ± 1 nm.



There was a shift in the maximum absorption when Mg^{2+} bound with SABF, this shift moved the maximum from 478 ± 1 nm to 485 ± 1 nm. Although there was a shift in the absorption maximum, there was no change in the emission wavelength. However, there was an increase in fluorescence intensity as the concentration of

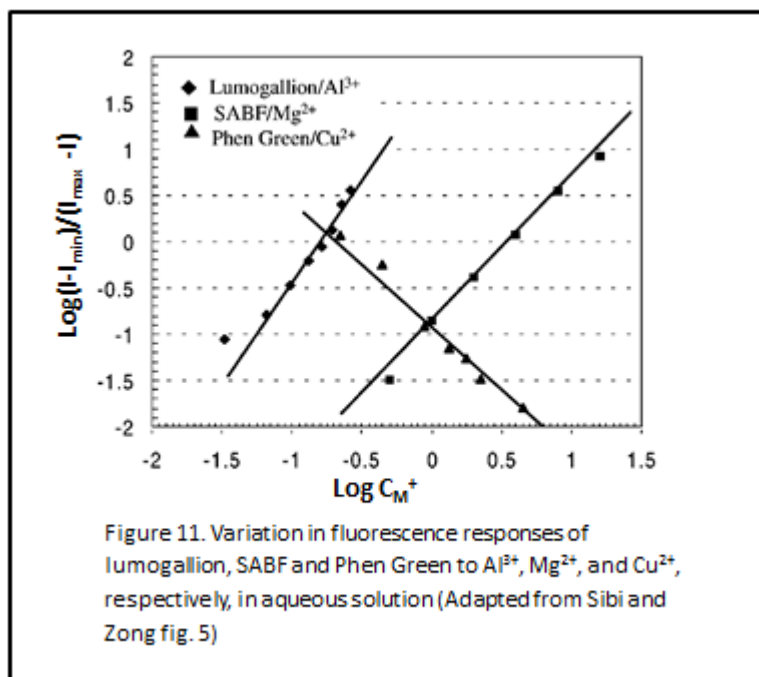
Mg^{2+} increased. Like in the case of lumogallion this because of the increase of metal ions available to bind with SABF.

The fluorescence response of Phen Green™ was tested with varying concentrations of



Cu^{2+} ranging from 0 to 8.926 μM in a solution of Phen Green™ at a concentration of 1.5133 μM in a 5.0 mM MOPS buffer (pH= 7.0) (Figure 10). The samples were excited at a wavelength of 490 ± 1 nm and an emission λ_{max} of 515 ± 1 nm. Unlike the previous fluorescent probes and metal ions

studied, when the concentration of Cu^{2+} increased the fluorescence intensity decreased. The process of binding and losing fluorescence is a type of quenching. Quenching is the process whereby emission from an excited molecule is decreased by energy transfer to another



molecule.¹³ In the case of Cu^{2+} and Phen Green™, Cu^{2+} acts as the quenching agent, absorbing the energy from the excited state of Phen Green™ decreasing the fluorescence intensity of the probe.

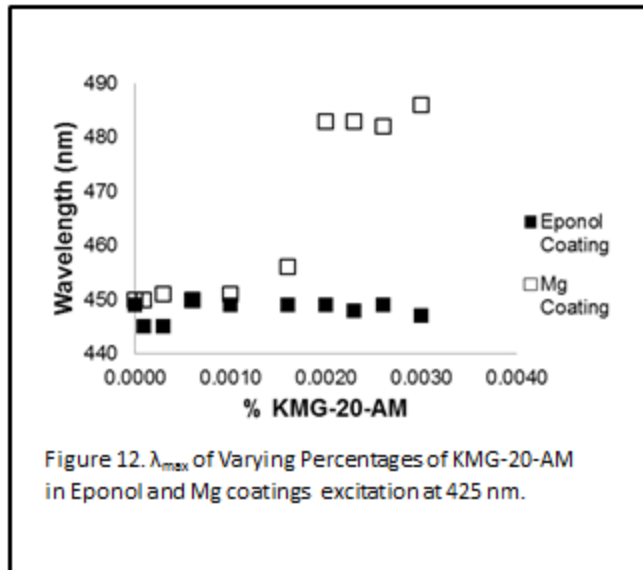
With the varying emission

responses of the fluorescent probes to their respective metals, the data were plotted to relate the fluorescence intensity to the concentration of the metal ions in solution. To account for variations in fluorescence, the $\log(I - I_{\min}) / (I_{\max} - I)$ was plotted. I_{\min} accounts for the emission intensity when the metal ion concentration is zero, I_{\max} is the emission intensity at the maximum ion concentration, and I is the emission intensity for the sample plotted. The emission intensity was plotted against ion concentration in the form of $(\log C_{Mn}^+)$ (Figure 11). As seen in Figure 11 there is a linear relationship between emission intensity and ion concentration. The slope of this plot is represented by the variable K and is a measurement of probe sensitivity to ion concentration. This allows for the plot to act as a calibration curve, because a linear regression equation would enable the calculation of the concentration of a metal ion of interest in an unknown aqueous solution, based on emission intensity of the sample.

In summary, the respective fluorescent probes for Al^{3+} and Mg^{2+} experienced an increase in emission intensity as the concentration of each of the metal ions increased. This shows the binding of the metals to the probes in a way that amplifies the excited state of the probe. Cu^{2+} exhibited the opposite response with Phen GreenTM. When the concentration of Cu^{2+} increased there was a decrease in emission intensity, showing quenching. Quenching shows the binding of Cu^{2+} to Phen GreenTM but instead of the probe absorbing energy, it transfers the excited state energy to Cu^{2+} upon binding, which decreases the emission intensity. The relationship of the emission intensity is dependent on the concentration of the metal ions which will allow for the calculation of free metal ions in aqueous solutions.

2.4 Corrosion Study

In a corrosion study by Konecki et al., the fluorescence intensity of KMG-20-AM was

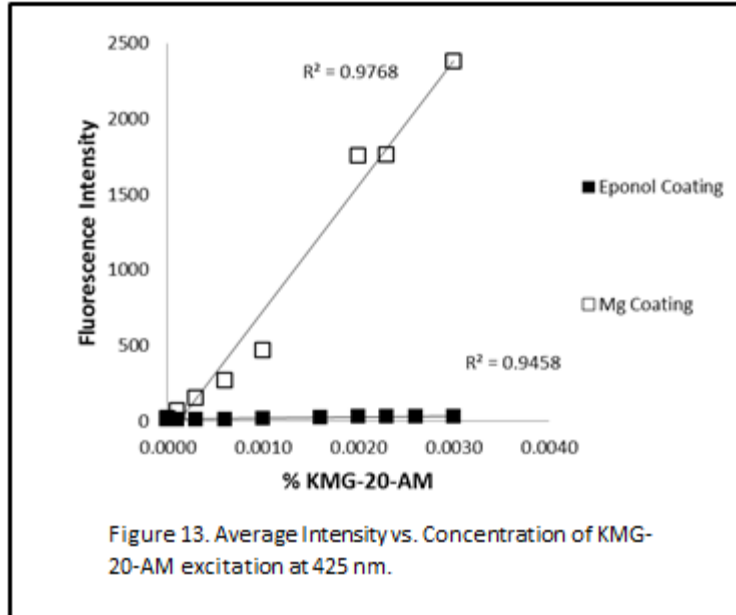


monitored in two different coatings and the λ_{max} was monitored for both. A second study, by Sibi and Zong, reflects the monitoring of metal ions in corrosion solution with fluorescent probes that are sensitive to the metal ions expected to be in solution.

In the study by Konecki et al., KMG-20-AM was added to an eponol coating and a Mg coating by weight percent ranging from 0 to 0.0030%. The coatings were drawn on aluminum alloy panels and the fluorescence response was monitored with the use of a TECAN Safire plate reader. The panels were excited at 425 nm, and the emission λ_{max} of 450 nm was monitored for the eponol coating with KMG-20-AM. The emission λ_{max} for the Mg coatings varied based on percentage of KMG-20-AM from 450 to 490 nm (see Figure 12 for values). There was a shift in λ_{max} in the Mg coating at 0.0020% KMG-20-AM, from 450 nm to 480 nm. This shift in λ_{max} indicates the binding of Mg to KMG-20-AM. It is suggested that the magnesium binds to KMG-20-AM in the coating because with the eponol coating (which is magnesium free) there is no shift in λ_{max} in relation to the percentage of KMG-20-AM.

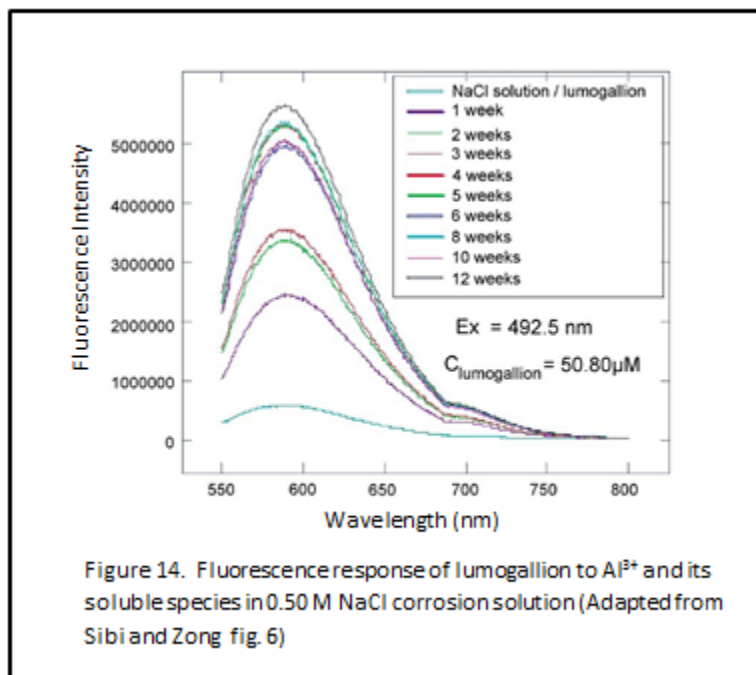
When the emission intensity was plotted against the concentration of KMG-20-AM in both coatings at each samples λ_{max} , it was found that both had a linear relationship (Figure 13).

Based on preliminary data, there is an increase of fluorescence intensity in the Mg coating



compared to the eponol coating. This shows that there is an interaction between the free Mg in the coating and the fluorescent probe. The binding of Mg to KMG-20-AM gives evidence that as the coating corrodes away it will be possible to monitor corrosion

through fluorescence intensity. The linearity of the preliminary data suggests that more trials are needed to determine if the same linear trend can be used as a method to determine

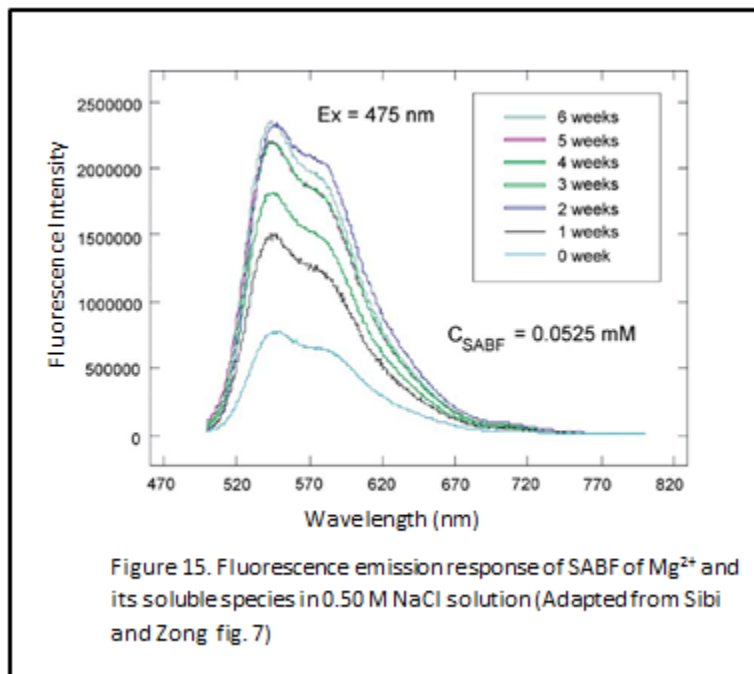


concentration of KMG-20-AM in relation to the emission intensity of the sample. With this, it would be possible to see if KMG-20-AM is leaching out of the coating throughout corrosion.

In the second study Sibi and Zong cross cut aluminum

alloy panels that are composed of 1.6% Mg, 92.71 % Al, 4.7% Cu, and 0.99% other metals. The cross cut panels were submerged in a 0.50 M NaCl corrosion solution. With the use of the fluorescent probes, lumogallion, SABF, and Phen Green™ it was possible to detect Al^{3+} , Mg^{2+} , and Cu^{2+} , respectively. The fluorescence intensity of lumogallion increased over time, indicating that more Al^{3+} is leaching out of the panel as corrosion proceeds (Figure 14). In this study with lumogallion the concentration was 50.80 μM and the solution was excited at 492.5 ± 1 nm and the emission was recorded at 588 ± 1 nm. Towards the beginning, there were significant increases in Al^{3+} shown by large jumps in fluorescence intensity. After week four, the increases became less significant. This could be due to a quick initial leaching of aluminum from the panel and then a slower corrosion process after four weeks.

The corrosion solution was also tested with SABF for Mg^{2+} with a concentration of



0.0525 mM SABF in the 0.50 M NaCl solution. Over time it was possible to track Mg^{2+} leaching out of coatings. The samples were excited at 475 ± 1 nm and the emission was recorded at 545 ± 1 nm. There was an increase in fluorescence intensity over time. However, it leveled out after six

weeks, compared to the 12 weeks of Al^{3+} , which suggests that Mg^{2+} leaches out faster than Al^{3+} (Figure 15).

With the same method, there was an attempt made to measure a decrease in fluorescence intensity with Phen GreenTM and Cu²⁺. There was no measurable decrease in fluorescence which supports Cu²⁺ not being in the corrosion solution. This implies that Cu²⁺ remains in the metal state throughout the corrosion process. Another reason that there may not have been a measurable decrease in fluorescence is that the amount of copper in solution may be lower than the detection limit of Phen GreenTM, or that it is corroded much later than magnesium and aluminum.

In summary, in the first study it was found that there was an increase in the fluorescence intensity in a Mg coating compared to an eponol coating with varying percentages of KMG-20-AM which is a Mg²⁺ sensitive probe. There was a shift in the λ_{\max} of the Mg coating once the concentration of KMG-20-AM was 0.0020% or higher. This shift in wavelength expressed a correlation in the binding of free Mg with KMG-20-AM in the Mg coating. In the second study it was found that in the corrosion process of an aluminum alloy panel, Mg²⁺ and Al³⁺ were measurable in a 0.50 M corrosion solution over time with their respective fluorescent probes. It was found that Copper in the aluminum alloy remains in the metal stage and does not leach into solution in the form of Cu²⁺.

Conclusion:

KMG-20-AM is a known fluoro-magnesium probe that exhibits fluorescence proportional to the magnesium concentration. KMG-20-AM has been shown to be an effective monitor of fluorescence in Mg-free and Mg-rich coatings with a suggested increase in fluorescence intensity in Mg-rich coatings over Mg-free coatings. This increase arises from KMG-20-AM complexing with the Mg in the coating exhibited by a λ_{\max} shift at a concentration

of KMG-20-AM 0.0020% and higher in Mg coatings. In addition lumogallion, SABF, and Phen Green™ have exhibited potential to determine the concentration of metal ions in a corrosion solution over time without degrading the aluminum panel. Lumogallion and SABF showed an increase in fluorescence intensity when bound to Al³⁺ and Mg²⁺, respectively. Phen Green™ had a quenching effect when bound with Cu²⁺. Both sets of studies exhibited the use of fluorescent probes as a viable option to the monitoring of corrosion in solution of a panel and of a coating with further testing.

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