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NANOPLASMONIC COLORIMETRIC SENSORS FOR DETECTION OF AMMONIA FROM
WATER AND URINE

by

ZURIEL CARIBE COUVERTIER
B.S. University of Central Florida, 2016

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Nanotechnology
in the College of Sciences
at the University of Central Florida
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ABSTRACT

Motivated by the need for inexpensive, simple, and portable devices for aqueous chemical analysis, we developed a nanoplasmonic colorimetric sensor capable of direct detection of wide range of chemicals. This novel sensor exploits the plasmonic resonance of metallic nanostructures with natural light to transduce changes in the chemical environment to changes in color, thus offering a simple route for real-time, in-situ, and low-cost analysis of aqueous chemical species.

Due to its environmental and medical relevance, we chose aqueous ammonia to analyze and determine the efficacy and limit of detection of this sensing platform. For the metallic nanostructures we selected aluminium for its well established high reactivity with ammonia. However, the nanoparticle's metal can be chosen based on its reactivity with any given target analyte, therefore creating a tailorable sensor.

The work here sets the foundations for a comprehensive analysis which aims to establish how various nanoparticle materials can be used to make a selective biosensor for chemical analysis in aqueous matrices such as environmental water samples, urine, blood serum, and saliva. In this thesis, we discuss the physics behind the sensors structural color, and the analytical techniques developed for ammonia quantification from aqueous solutions.

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INTRODUCTION

Motivation

Biomedical

There have been widespread demands for low-cost, easy-to-use and portable point-of-care testing devices, especially those that can be utilized within the comfort and convenience of one's home. Point-of-care sensors are relevant to an extensive spectrum of clinical applications such as blood pressure monitoring, glucose sensing, viral infection sensing, pregnancy tests and drug/alcohol testing

In addition, ammonia is considered to be an extremely neurotoxicological metabolite found within the human body. Concentrations of ammonia must be maintained under homeostatic conditions as abnormal concentrations can cause and are indicative of various health complications. These complications include but are not limited to brain edema, coma, and severe kidney/liver damage. Therefore, the early detection and quantification of ammonia is significant (Continuous, 2019). Approximately 2% of urine's chemical composition is ammonia in the form of urea (Self-screening, 2016). Many degenerative diseases, such as congestive heart failure, gastrointestinal bleeding and urinary tract obstruction, are attributed to abnormal concentrations of ammonia within the body (Pandey, 2019 pulled). Ammonia and urea in human urine and blood are critical biomarkers, as they are indicative to the onset and progression of many diseases such as those

associated with the kidneys, liver, and brain. In a healthy human, proteins are first metabolized as toxic ammonia and then converted into a non-toxic form, urea, which is excreted from the body within urine. It is by this mechanism that the body is able to metabolize and safely remove nitrogen. Kidney diseases and enzymatic disorders are relative to the onset of hyperammonemia or endogenous ammonia intoxication, as well as renal failure and amino acid deficiency (Middya, 2014). Hyperammonemia is a condition characterized by high mortality rates and occurs in people with hematologic conditions or those who have had hematopoietic stem or solid organ transplant (Smith, 2019 pulled). Patients with these conditions may suffer from hyperammonemia even in the absence of liver disease (Smith, 2019 pulled). Severe hyperammonemia is associated with blood ammonia levels in excess of 1600 micro molar (Smith, 2019 pulled). Ammonia in urine may also be indicative of a bacterial infection (Pandey, 2019 pulled). Ureaplasma species of bacteria can be responsible for heightened concentrations of ammonia, as they typically hydrolyze urea for energy production. As the result of an invasive infection, an immunocompromised individual is likely to acquire toxic levels of ammonia in the bloodstream. A clinical case study reported a young female patient which was placed on a high dose continuous venovenous hemodiafiltration to remove excess ammonia after an infection from a ureaplasma bacterial species. Her symptoms included confusion, excessive sleepiness, and difficulty in stimulation, which are correlative to excess ammonia in the blood. Excess ammonia by ureaplasma species has also been associated with adverse pregnancy outcomes, in addition to chronic lung disease, and septic arthritis (Smith, 2019 pulled). High ammonia concentrations have been associated with patients suffering from type 2 diabetes mellitus. Therefore, ammonia sensing may be implemented in correlation to blood glucose levels and ammonia in urine (Self-screening, 2019). A study approved by the Mayo Clinic

and Brigham, reported that genetic abnormalities involved in ammonia metabolism can contribute significantly to the pH of urine which in turn leads to the solubility of many minerals found in urine, which is relative to the onset of kidney stone disease. The constituents of urine, such as ammonia, contribute to the pH of urine. Urine pH is linked directly to the transport of ammonia and ammonium out of the body (Benjamin, 2019).

In life-threatening conditions where ammonia has reached toxic levels on the blood, the ammonia concentration should be reduced as soon as possible to prevent and limit further complications. Prolonged exposure to toxic ammonia levels leads to neurological conditions. Therefore, early treatment and diagnosis is essential in order to prevent neurotoxicity and even death. Peritoneal dialysis and continuous renal replacement therapy are the most extreme but effective and widely used treatment for acute kidney injuries, especially in patients who have high blood plasma ammonia levels. Similar technology for removing blood plasma ammonia are; continuous venovenous hemofiltration, and continuous venovenous hemodialysis which have been used in critically ill children since the 1970s. Continuous Renal Replacement Therapy is considered for ammonia concentrations of over 400 μM in the blood and can successfully reduce ammonia concentrations to average levels to approximately 50 μM in patients with renal diseases. Medical conditions associated with excess nitrogen in the body typically require extreme treatment methods such as the use of catheters which are inserted and left permanently. Patients with conditions such as hyperammonemia must continue to undergo hemodialysis indefinitely (Continuous, 2019). Other methods that are effective in treating ammonia levels within the body include the use of harsh chemical drugs such as Arginine or intravenous sodium benzoate phenyl butyrate which can

reduce blood pressure (Continuous, 2019). These traditional and invasive treatment methods may be avoided with early at home/point of care detection of ammonia from urine and/or blood.

Environmental

Portable devices for environmental monitoring have been developed as well (Middya 2014). Environmental pollution, due to heightened concentrations of ammonia, has negatively impacted ecosystems and the lives of organisms that inhabit them (Middya, 2014). In particular, these have been utilized for the characterization of environmental samples by pH and quantification of relevant analyte such as chlorine, ammonia, and toxins within water. Ammonia is found thoroughly throughout the environment and is active in biological and ecological processes. The compound is constantly at the ready in both gaseous and molecular states. While ammonia is unionized it can be related to its positively charged ionized form ammonium (NH_4^+) (EPA: Ammonia, n.d.). Both positive and negative effects have been associated with ammonia and over the years it has become easier to produce and utilize, but more difficult to control. Being able to sense and control ammonia is key to prevent negative impacts from toxicity associated with ammonia levels.

Ammonia is a highly volatile organic compound and one of the most harmful environmental contaminants, having caused considerable, yet preventable damage to a vast array of ecosystems

(Self-screening, 2016). Concentration in the environment alters between different mediums including air, soil, and water. As temperature increases, ammonia is also known to increase (EPA: Ammonia, 2021). Surface water both freshwater and saltwater have ammonia at a level from 0.2 mg/L to 12 mg/L in comparison to groundwater which only reaches levels of 0.2 mg/L (Edition, 2011). Ammonia can be detected in drinking water at 1.5 mg/L by smell and tasted at around 35 mg/L (Edition, 2011). Unionized ammonia in water is said to not have any direct form of health concerns at lower concentrations, but its reaction with other chemical could create concern (Aquatic Life Criteria: Ammonia, 2013). Aquatic life is found to live best at levels below 1.9 mg/L according to the EPA (Aquatic Life Criteria: Ammonia, 2013).

Within the atmosphere, ammonia is found to be the largest quantity of basic gas (Behera et al. 2013), Levels of 0.6-3.0 parts per billion (ppb) tend to be found in both urban and rural environment but in areas of animal husbandry and agriculture levels are found to be higher from manure and fertilizer (Ryer-Powder, 1991). Emergency level of acute toxicity is listed to be at 300 mg/L within the air, but odor begins to provide warning at around 20 mg/L with irritation at 50 mg/L (Department of Labor: Ammonia Refrigeration, n.d.). Soil ammonia concentration varies based on organisms living in the area, temperature, pH and other environmental factors. Some values were found to be as high as 360 mg/L and would decrease slowly overtime, but increase if an external force or process was applied (such as fertilization, etc.) (Du, 2015).

Ammonia is toxic and can cause negative impacts on the environment. As its vaporization point is low, it mainly disperses to the atmosphere (volatilization) but can also remain in an aqueous state and damage the water system in the form of runoff from agricultural and industrial usage (Agricultural ammonia emissions, n.d.). Over the past few years, ammonia has slowly increased in level and as it is made of nitrogen and hydrogen it is causing nitrogen pollution (Misselbrook, 2019).

Nitrogen Pollution from ammonia causes eutrophication and soil acidification, which works to both destroy life and plant habitats. With eutrophication, growth of certain plant life such as mosses and algae are supported which decreases the amount of applicable oxygen for competing organisms, and some plants are also destroyed by the high concentration (Berg, 2017). Ammonia that enters the atmosphere can condensate with moisture to produce acid rain and in turn increase soil acidification. Soil acidification causes there to be a high acidity of the soil which damages some plants and other organisms (Berg, 2017).

Overfertilization, excessive farming and industrialization can be the cause of this occurrence and is typically seen downstream of farms and factories (Berg, 2017). Ammonia emissions from vehicles can also be a cause of pollution but is subjected to vehicles with catalytic converters. Apart from vehicles, ammonia is made from many different industrial processes, and wastewater contains high amounts of ammonia (Sutton, 2008). Unionized ammonia at lethal doses (both acute and chronic), can cause harm to organisms at high levels and particularly affects breathing and

respiratory along with tissue becoming damaged and pH drop (Sutton, 2008). With the compound's interaction with other constituents in the atmosphere and organisms can produce other complications such as particulates and irritation respectively (Ammonia SDS, 2014). If effective and efficient sensory and control is not established, ammonia can have many negative impacts on the environment.

Synthesis of ammonia is completed naturally and with manmade innovations. Naturally, ammonia is a part of the nitrogen cycle and an imbalance will disrupt the homeostasis between plants and organisms making it more difficult to sustain viable relationships. Nitrogen fixation by bacteria creates unionized ammonia and ionized ammonium (Schaechter, 2009). Breakdown of urea and other decomposing waste matter can cause an increase in ammonia levels (Wang, 2021). Gas exchange between molecules of the atmosphere can pull nitrogen and hydrogen to make new unionized ammonia (Service, 2018). Manmade forms of ammonia include both agricultural and industrial processes. Agriculture yields excess waste from animals that are raised, and fertilizers with ammonia are used to aid in crop growth. Machinery/ manufacturing, destruction of natural gases/ fossil fuels, forest fires, and vehicles contribute to the introduction of excess ammonia to the environment (Sutton, 2008).

Sensing ammonia is important to the first step in eliminating toxic amounts of ammonia in the environment to prevent the different negative impacts from occurring. With large agricultural and industrial operations generating more ammonia each year, it has become apparent that levels are growing, and sensing has become regularly completed to ensure safe qualities of health and living

for all organisms. Lowering levels of ammonia in all forms of medium (air, water, and soil) could prevent eutrophication, acid rain, soil acidification, and other forms of pollution caused directly and indirectly. Enabling the ability to sense both gaseous and molecular states at a low cost and efficient way would favor better conditions and signal where removal of ammonia is appropriately needed. Creating a form of equipment with an easy to read sensor and quick, at the ready results is ideal to determining levels and toxicity of ammonia.

Current Methods in Ammonia Detection

Early treatment is crucial in order to prevent neurotoxicity, coma, or death and therefore early detection is key (Aygün, 2019). Current methods for detecting ammonia in the bloodstream are costly, require sophisticated equipment and capable personnel to operate, collect the data, and interpret the results (Pandey). Common methods include; conductimetry, amperometry, potentiometry, fiber optic sensors colorimetric, photometric, mass spectrometry, crystal microbalance, gas chromatography, and field effect transistor (Middya, 2014, Pandey, 2019 pulled).

In recent times, research for ammonia detection are usually photocatalytic or electrocatalytic based. Scientist commonly use Nessler's reagent, the Indophenol Blue method, Ion Chromatography, Fluorescence, Ammonia Selective Electrodes, and H NMR Spectroscopy (Zhao

et. al, 2019). The results from each of these methods can be qualitative and quantitative, but present a higher margin of error. Nessler's Reagent proved to be more accurate, but produces less of a color change than the Indophenol Blue Method.

Maity and Ghosh show an thin film based ammonia gas sensor using a paper based model that utilizes perovskite halide (MAPI) to detect ammonia at room temperature in parts per million. This system shows a color change from carbon black to a bright yellow in the presence of ammonia. It has not yet been shown to be quantifiable, but is a good concept for dealing with environmental samples. This system is not as accurate because it is a qualitative perception of color change and produces little data. The paper model has been tested at different concentrations of ammonia, however the paper only transitions from black to yellow without much change in color intensity. (Maity, Ghosh 2018).

Recently, various research groups are trying to develop newer techniques. A perfect ammonia detection device would be one that is sensitive, label free and selective (Middya, 2014). Padney et al. have developed a fast and highly sensitive chemiresistive nanocomposite made of polysaccharide and gold nanoparticles which has claimed to detect and quantify ammonia concentrations at room temperature in the range of .1 parts per quadrillion to 75000 parts per million from urine and blood serum (Pandey, 2019 pulled). This sensor correlates change in conduction to the ammonia concentration. However, its limitations lie in that it can only detect ammonia in vapor form. This detection method does not require any additional reagents such as urease to facilitate the measurements of ammonia from urea, and can produce a quantifiable

response in 3 minutes per sample (Pandey, 2019 pulled). Other ammonia sensors such Nano-PANI and gold composite chemiresistive sensors also correlate ammonia concentration to change in current (Pandey, 2019 pulled). Backgate field-effect transistors have been developed to detect ammonia and urea using a p-type silicon substrate with n-type channel and a cadmium sulfide titanium oxide Nano composite material as a dielectric layer. This is a reusable sensor which measures ammonia concentration as a change in drain current as ammonia levels increase so does the drain current. However, is this sensor can only sense is ammonia in its gaseous state, and requires the use of enzyme catalysis in order to detect urea as ammonia gas. Its developers claim an ammonia sensing limit of detection at approximately 0.85 parts per million, with results within 30 seconds (Middya, 2014). The proposed back gate Field Effect transistor device developed by the Indian Institute of Technology is non selective and will react to produce a change in current in the presence of sulfur dioxide or dihydrogen sulfide. However, the change in current was different in the case of ammonia and therefore can be distinguishable from the current changes when in the presence of other molecules which may elicit a sensory response. Furthermore, such molecules are usually either absent or present in negligible amounts in urine samples and therefore the response can be well correlated to ammonia detection (Middya, 2014).

Ions are defined as any chemical species that carries an electrical charge. These chemical entities are soluble in water and exhibit conductive nature. The quantification of ions dissolved within matrices is relevant to a broad spectrum of applications, such as medical diagnostics(Hibbard, Crowley et al. 2013, Cuartero, Parrilla et al. 2019) and environmental monitoring(Nakatani, Kozaki et al. 2011, Varden, Smith et al. 2017). Currently, the most common and high-performing

methodologies known for separating ions in solution include ion-exchange chromatography(IEC)(Cummins, Rochfort et al. 2017) and capillary zone electrophoresis (CZE)(Zemann, Rohregger et al. 2008, Voeten, Ventouri et al. 2018). Both ion-exchange chromatography and capillary zone electrophoresis are characterized by a critical separation process where analyte/ions of interest induce the transposition of their co-ions (ions of the same charge as the analyte and are present in the same sample as analyte ion) when passed through a detector. The detection signal produced when using these methods is dependent on differences among the analyte ion and its co-ions regarding their electrochemical properties, such as charge. The measurements collected by the use of these methods are quantifiable by the transfer ratio(TR), which is the ratio of charges between the analyte ion and co-ion during the mobile phase. If the ion of interest and its co-ions possess similar charge, the TR may read as a value of 0, or close to 0. In these cases, IEC and CZP are disadvantaged and fail to separate the ionic analyte from its co-ions effectively. Typical IEC instrumentation is extensive and consists of a pump to control stable flow rate, injectors depending on the nature of the sample, columns that facilitate separation, a detector and recorder.

Once ions of interest are separated using methods like IEC and CZE, techniques that contribute to the further separation and quantification of these ions include conductivity, amperometric /potentiometric detection, indirect UV-Vis detection, mass spectroscopy and post-separation reaction detection(Buchberger 2000).

For instance, amperometric detection can take place after ions are separated from either CZE and IEC methodologies. Amperometric detection(Gil, Amorim et al. 2019, Zavolskova, Nikitina et al. 2019) following CZE relies on the redox reaction of the ionic analyte at an electrode where a potential(particular to the analyte of interest) must be applied to initiate this reaction. The electrical current that results from the redox of the ionic analyte serves as the detection signal and is directly proportional to the ionic analyte. Amperometric detection can also be done with constant potential following IEC. Apart from the typical carbon, platinum and silver electrodes, there has also been use of conductive polymers, such as polyaniline(PANI)(John, Ongarato et al. 1996, Hibbard, Crowley et al. 2013, Rahimi, Ochoa et al. 2017) and polypyrrole (PPy) (Sadrolhosseini, Noor et al. 2014). In general, amperometric detection is not a very streamlined approach. In many applications, the electric field that is used for further separation is decoupled and then further characterization must be carried out using detection cells.(Buchberger 2000)

Indirect UV-Vis detection requires that the co-ions possesses high molar absorptivity, as well as similar mobility of the analyte ions. This is important to ensure optimum peak shapes. This method works well with slowly migrating or viscous analyte, opposed to fast migrating analyte. On the other hand, techniques such as conductivity are known to achieve high sensitivity with analyte that travel faster, rather than slower(Buchberger 2000).

After reviewing all presently practiced and few relevant emerging technologies, it became evident that there is a much need for a simple, low cost and high throughput ammonia detection technique.

In this context we propose a unique self-assembled structural color-based substrate for colorimetric detection of ammonia both from urine and blood for different cases.

Aluminum Nanoparticle Based Sensor

The plasmonic system consists of a dense array of aluminum nanoparticles formed atop an oxide coated aluminum backplane, an illustration of which is shown in Figure 1. Incident light excites resonances within the structure which are confined to the gaps between particles and the mirror. These resonances demonstrate a high degree of angle-independence and their spectral location is primarily a function of the size distribution of the aluminum particles, and the optical distance from each other and the mirror. Light which is not absorbed by the surface is reflected to result in a perceived color.

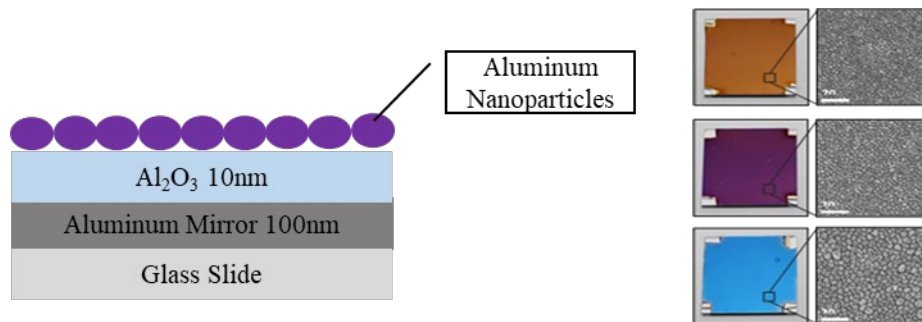


Figure 1: Aluminum Nanoparticle Substrate

(Left) Schematic of self-assembled aluminum particles atop an oxide spacer and aluminum mirror. Wavelength of plasmon resonance scales with particle size. **(Right)** Scanning electron microscope images of three sampled surfaces corresponding to yellow, magenta and cyan. Insets are optical microscope images of the respective regions.

The particles form through a temperature and pressure dependent thin film growth mechanism in an ultra-high vacuum electron beam evaporator and understanding these growth mechanisms is vital in designing and controlling attributes of the surface. Aluminum exhibits the Volmer-Weber growth mode which is initiated by the adsorption of atoms to the surface of the substrate. The energetic molecule is free to diffuse along the surface until it settles on a site with a local energy minimum. Subsequent aluminum atoms adsorb, diffuse and condense about these low energy sites in a process termed nucleation. As this continues, particles anchored at adjacent nucleation sites and form grain boundaries, or if energetically favorable, coalesce to form larger particles. The shape of combined particles depends on the equilibrium reached between the aluminum's free energy and interfacial stress with the substrate. The accumulative result of this process at three deposition thicknesses (5 nm, 8.5 nm and 10 nm) can be seen in Figure 1 (right). Scanning electron micrographs (SEMs) are taken of the yellow, purple and blue samples which show an increase in particle size as a function of material deposited. Insets depict microscope camera images taken

from the respective surfaces. Interestingly, the fill fraction of particles on the surface remains near unity for all samples which indicates a high number of nucleation sites in the initial phase of deposition. From the SEM images we obtain histograms of particle size using the Gwydian software package and approximate the area of each particle with an equivalent circular area. The distributions indicate that the spread of particle diameters increases as within a sample as deposition continues. This is also seen qualitatively from the SEM images that the rather circular particles of the yellow sample become more irregular as lateral particle size increases. We attribute this to the strong bonding of aluminum to the aluminum oxide substrate at odds with the aluminum particle surface energy, preventing the aluminum from reforming into symmetric oblate ellipsoids.

Figure 1 (right) shows the optical images of a one square inch area of uniform nano-structured substrate of various colors and their corresponding SEM images. The apparent color of the metallic, nanoparticle sensing devices is determined by the size of the self-assembled nanoparticle system. This can be tailored by controlling the temperature and duration of the growth process. Altering this will contribute to the alteration of the effective layer's thickness and therefore, the color of the substrate. The thin-film preparation techniques that have been utilized are based on the physical and chemical deposition of thin films from the vapor phase. On a thoroughly cleaned glass substrate, the composition of the colorimetric surface underlies three primary layers consisting of a 100 nm aluminum mirror, 10nm aluminum oxide spacer layer, and aluminum nanoparticles of varying sizes.

Utilizing our metallic nanoparticle-based substrate, we have been able to demonstrate promising detection schemes of various biologically relevant entities such as the formation of antigen-antibody complexes and biohazardous compounds, such as ammonia. The apparent surface color of our device is primarily determined by the morphology and size of the self-assembled aluminum nanoparticle system, which composes the superficial layer of the substrate. This property of the substrate can be tailored by altering parameters such as the temperature and duration of the growth process during deposition of the most superficial layer. Such phenomena can be observed in Fig. 1, where 5 nm, 8 nm and 10 nm Al NPs correspond to the apparent surface color: yellow, purple and blue, respectively.

As discussed, the colorimetric property of our device is inherently structural. Therefore, upon structural alteration of the nanoparticle based surface, there is correlative change in color, which can be quantitatively determined by the aid of a spectrophotometer or cell phone images. It is the change in color derived from structural change of the superficial layer that primarily underlies our detection scheme.

Our assay exploits the reactive nature of the aluminum nanoparticle-based surface with analyte of interest that possess oxidative chemistry. Unlike the ligand based assay, this method does not require extensive sample preparation or functionalization of the surface. Aluminum is typically reactive and reacts spontaneously with surrounding water/air to form aluminum oxide. Aluminum oxide form a stable passive layer that protects the aluminum from corrosion. As long as this

approximately 4nm thick oxide layer is stable, it serves as corrosion protection from typical environmental attributes. However, this protective layer of aluminum oxide will become unstable or deteriorate when exposed to a pH that deviates from 7.0 to 9.0. It is within the pH range of 7.0-9.0 that the oxide layer is most stable. At pH values below 7 or above 9, the rate of surface corrosion seems to increase at an increasing rate.

We have been able to demonstrate that samples containing an analyte of interest that is either highly acidic, basic or oxidative in nature are able to produce changes in reflection of the colorimetric surface as a result of this corrosion effect. This is generally in correspondence with change in analyte concentration within the sample of interest. We have been able to demonstrate this extensively with biologically relevant compounds such as ammonia. Experimentally we show that the aluminum nanoparticle substrate, changes color due to the physical deformation or removal of the nanoparticles. This phenomenon can be exploited to detect wide range of biologically and environmentally relevant molecules

METHODS & MATERIALS

Standard Operating Procedure

We prepared solutions of aqueous ammonia of varying concentrations from a commercially available 25% ammonia solution purchased from Supelco, and deionized water. 50uL of aqueous ammonia solution is brought to contact over the sensor contained within a PDMS well and sealed with a microscope slip to avoid ammonia depletion due to evaporation, as depicted in figure 2. Each reactive site within the PDMS well is only used once, and upon completion of experiment the contents of the wells are removed and disposed of.

The sensor is placed under a spectrometer set on reflective mode and calibrated with a silver mirror standard. Reflectivity measurements are taken in triplicate and averaged, within the visible range from 400λ to 800λ , at desired time intervals for a predetermined period of time. To calculate and quantify the percent change in reflectivity over the sensor surface as a function of concentration overtime, equation 1 is used. Subsequently the percent change in reflectivity is plotted as a function of wavelength, and the area under the change in reflectivity curve is calculated using Origin software, and plotted as a function of ammonia concentration, see figure 6.

Equation 1:

$$\% \Delta \text{Reflection}_{\lambda} = | \% \text{Reflection}_{B\lambda} - \% \text{Reflection}_{A\lambda} |$$

Where,

λ = Any given wavelength

$\text{Reflection}_{B\lambda}$ = % reflection before chemical reaction at a given wavelength

$\text{Reflection}_{A\lambda}$ = % reflection after chemical reaction is permitted to occur for an amount of time at a given wavelength

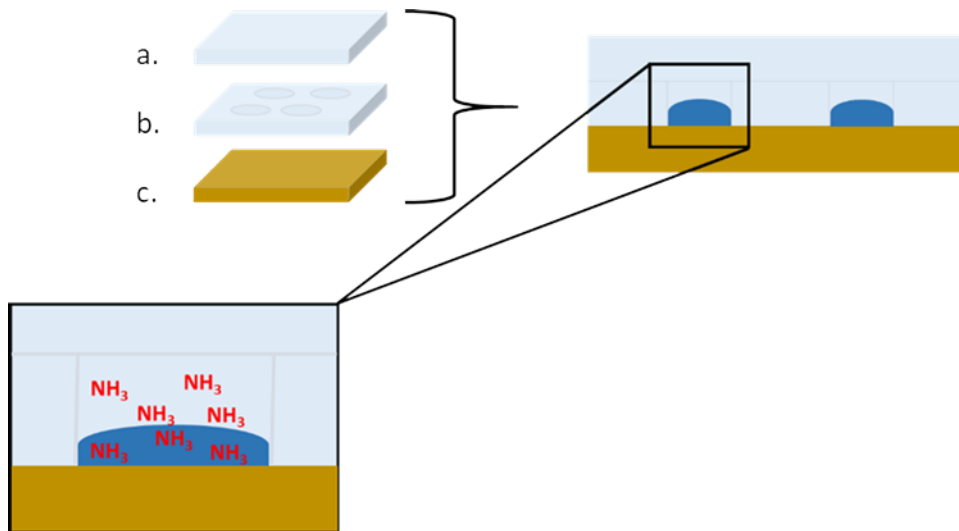


Figure 2 Sensor Schematic

(a) A PDMS slab is cut to fit sensor area. A hole puncher is used to make wells onto the PDMS. The PDMS wells are adhered onto the **(b)** sensor surface to confine liquid analyte with, and allowing analyte to contact the sensor surface. As ammonia is extremely volatile, a **(c)** glass slip is used to seal the well and confine our analyte within, while allowing visible light to transmit for spectral analysis.

Analytical Software and Instrumentation

The OceanView Software was utilized throughout the duration of the research. OceanView is an analytical spectrometer software that provides a visual graph of the absorbance spectrum, collection of data, and contains various types of methods of analysis of the spectrum. For this research, reflectivity analysis was utilized. When calibrating the software for reflectivity, an average of 10 scans and a boxcar width of 20 were used to configure the software. Followed by placing a mirror under the spectrometer and adjusting the platform, on which the mirror was placed, to obtain maximum reflectivity. Then a black matted box was placed under the spectrometer to absorb the light that is being sent out. Once the data of both ranges are collected, the calibration of the OceanView software is complete.

The nano spectrometer is built with a light source whose beam is sent inside and broken into a variety of wavelengths for analysis. The beam of light then interacts with the sample and the spectrometer's detector measures the transmittance, which is the amount of light that passes completely through the sample, the absorbance, which is the measurement of light absorbed by the sample, and the reflectivity, which is the measurement of light that is reflected into the detector, then the measurements are processed by the OceanView software and were manifested onto the graph that shows the change of reflectivity over time. Using the COPY action within the software and then using the PASTE action in the excel sheet to transfer the data of the spectrometer onto the excel sheet for analysis.

For the experiments, the sensors were placed under the spectrometer, with the light being sent into a specific well on the sensor. Data were collected thrice before the wells filled with the substrate and stored in the excel sheet, then data was collected within a repeated interval over a specific time frame where at each interval data was collected thrice again and placed onto an excel sheet to record the change over time. Ensuring that once time has begun, a lid was placed over the well to prevent evaporation of the substrate, to ensure accurate data collection.

RESULTS

Aluminum Nanoparticle Reactions with Aqueous Chemicals

To compare the effect of various aqueous chemicals on our sensor we tested how ammonia, sodium hydroxide, sodium chloride, and sodium bicarbonate aqueous solutions would affect reflectivity of the sensors surface, as reflectivity is to be the analytical tool which correlates to concentration. Experiment was conducted as mentioned in the “Standard Operating Procedure” section. All experimental parameters were equal for each of the chemical solutions tested, such as temperature (room temperature), analyte volume (50uL), and reaction time (30 minute). Results are depicted in figure 3, where percent reflectivity is plotted at 500λ against varying concentrations of ammonia, sodium hydroxide, sodium chloride, and sodium bicarbonate aqueous solutions. Each chemical solution produces a unique change in reflectivity over the sensors surface at any given wave length within the visible range. Spectral analysis results indicate that ammonia is the most reactive with aluminum and produces the greatest change in reflectivity when compared to the other analytes.

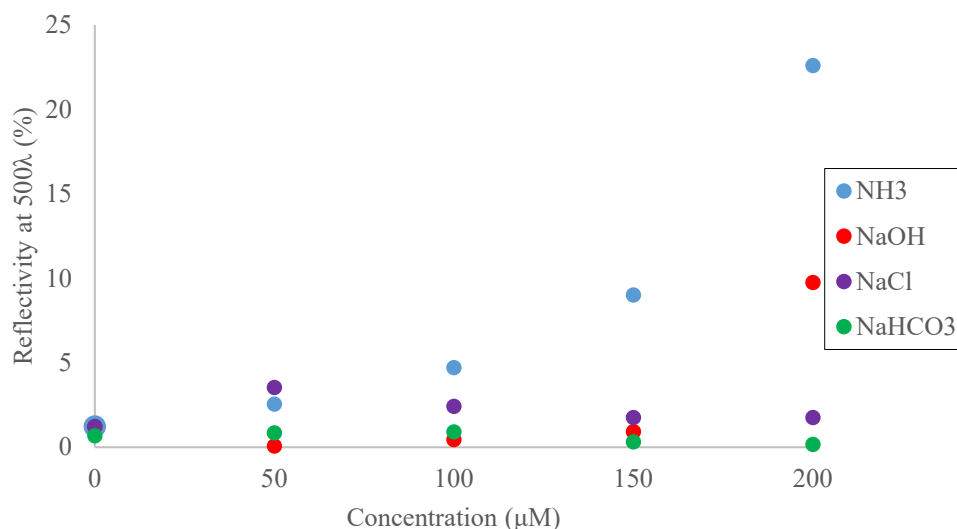


Figure 3: Change in Reflectivity Induced by Chemical Solutions

Ammonia, sodium hydroxide, sodium chloride, and sodium bicarbonate each react differently when in contact with aluminum. As a result, our sensor exhibits a unique trend in reflectivity at any given wavelength for each of the before mentioned analytes. In this graph, percent reflectivity is plotted at 500λ against varying concentrations of ammonia, sodium hydroxide, sodium chloride, and sodium bicarbonate aqueous solutions.

Varying Nanoparticle Size

The sensitivity of this proposed colorimetric sensing depends on the interactions between light and the nanostructured substrate. Hence, the size of the nanoparticles plays an important role for a given assay. This property can be further exploited to impose selectivity to this otherwise blind process. A set of substrates can be used to form a unique set of color change for a specific assay. Three sensors of varying aluminum nanoparticle size were fabricated to analyze if nanoparticle size has an effect on sensitivity. The three sensors are described as follows; 5nm diameter aluminum nanoparticles of apparent color yellow (yellow sensor), 8.5nm diameter aluminum

nanoparticles of apparent color purple (purple sensor), and 10nm diameter aluminum nanoparticles of apparent color blue (blue sensor). All other build parameters as described in the “Aluminum Nanoparticle Based Sensor” section, remain the same for all sensors. We hypothesized that smaller nanoparticles would be more reactive due to greater contact surface area with the aqueous analyte. To test this hypothesis experimentally we prepared varying concentrations of ammonium hydroxide solution and allowed it to react over each of the sensors for 30 minutes, all experimental parameters were maintained for each of the different sensors and the experiment was conducted as stipulated in the “Standard Operating Procedure” section. The results are presented in figure 4, where the intensity in change in reflectivity is depicted by color map where red is higher intensity and correlates with higher change in reflectivity from 400λ to 700λ corresponding to each ammonia concentration. The yellow sensor with the smallest nanoparticle diameter of 5nm, is the most sensitive when compared to the purple and blue sensor with nanoparticle diameters of 8.5nm and 10nm respectively, as the reflectivity over the yellow sensor is affected by ammonia concentrations as low as 100uM. We conclude that decreasing nanoparticle size increases sensitivity.

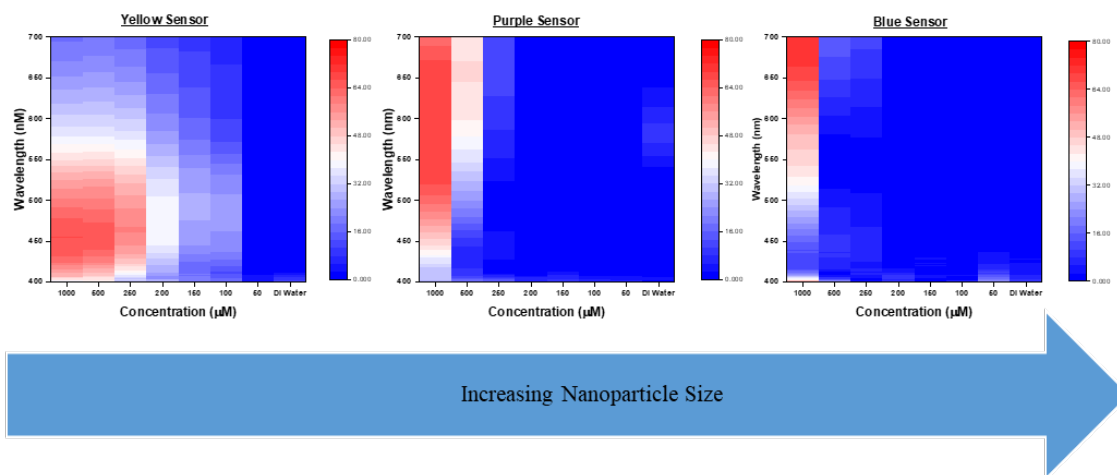


Figure 4: Nanoparticle Size Effect

Graphs depict intensity in change in reflection from 400-700 λ for various concentrations of aqueous ammonia, after a 30-minute reaction time. The yellow sensor is more sensitive and able to quantify aqueous ammonia from 100-500 μ M, with the greatest shift in reflectivity from 400-550 lambda.

In these studies, the only parameter that was adjusted was the size of the aluminum nanoparticles that comprise the superficial surface of the colorimetric substrate. Reflectance spectrum was taken before and after the chemical reaction, and the difference in reflection was calculated using equation 1. Different concentration ranges may require varying nanoparticle size for more reliable quantification. For ammonia we found that blue substrate is suited for higher concentrations of ammonia of over 500 μ M, and the yellow sensor is capable of quantifying lower concentrations of ammonia, from approximately 100 μ M to 250 μ M, where the other sensors reflectivity is unaffected by such low concentrations due to their larger nanoparticle size.

Reaction Time for Ammonia Detection

There are two main parameters affecting the structural color change over our substrate, concentration of analyte and the reaction time in which the analyte is allowed to come into contact with our substrate surface and react. In practical applications the analyte concentration is an uncontrollable variable, however reaction time may be set by the person running the analysis. Reaction time is related to surface area, as we observe that nanoparticles of smaller surface area produce a quicker color change when compared to larger nanoparticles under equal experimental parameters. We must determine how nanoparticles of various sizes relate to the reaction time, therefore improving reaction time and producing a product capable of quick analysis. First, we wanted to determine at which concentrations the color change over the substrate is visible to the human eye, any color change not apparent to the human eye would be analyzed via spectrometry. We tested varying concentrations over our aluminum nanoparticles substrate, and observed that in high concentrations of ammonia greater than 1mM, the color change over the substrate occurs almost immediately within seconds and is visible to the human eye, see figure 5. However, at low concentrations of ammonia under 1mM, the apparent color change is nonapparent to the naked eye even after a 60-minute reaction, and therefore spectral analysis is necessary.

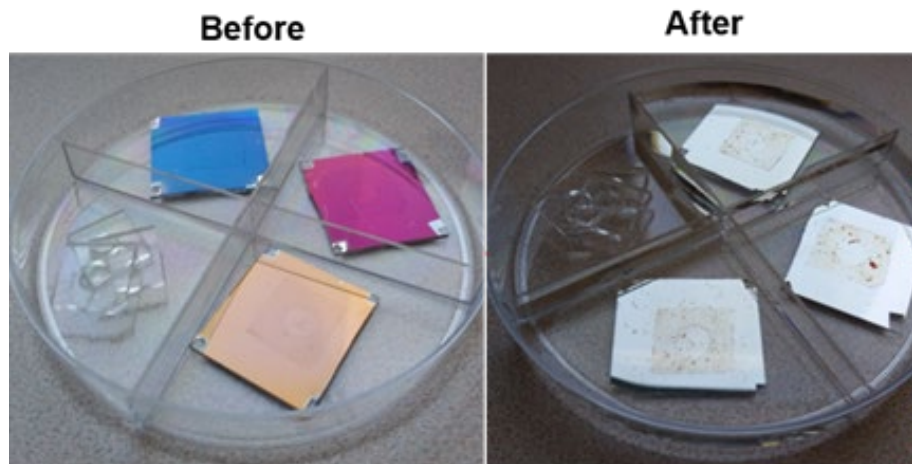


Figure 5: Visible Color Change

Exposure to aqueous ammonia corrodes the nanoparticles responsible for the structural color visible over the sensor. At high concentrations change in color is visible to the naked eye, at lower concentrations color change may only be measured via spectrometry.

To determine the minimum time required for the chemical reaction over the sensor to produce a measurable shift in reflectance, the reflection spectra was measured from 400-700 lambda for one hour in ten-minute intervals using the lowest detectable concentration of aqueous ammonia of 100uM. The graph of figure 6 depicts percent change in reflectivity over the visible spectrum from 400λ to 700λ for the yellow 5nm Al NP sensor when in contact with 100uM ammonia hydroxide solution. The yellow sensor shows a measurable change in reflectivity when the reaction is allowed to take place for a minimum of 20 minutes. As our goal is to measure concentration from an unknown from a sample of unknown concentration we first needed to standardize the reaction time this is the time which we were allowed or analyze solution to being contact where the nanoparticle surface of the sensor. Therefore, after acquiring this information we decided to set the reaction time at 30 minutes for all subsequent experiments.

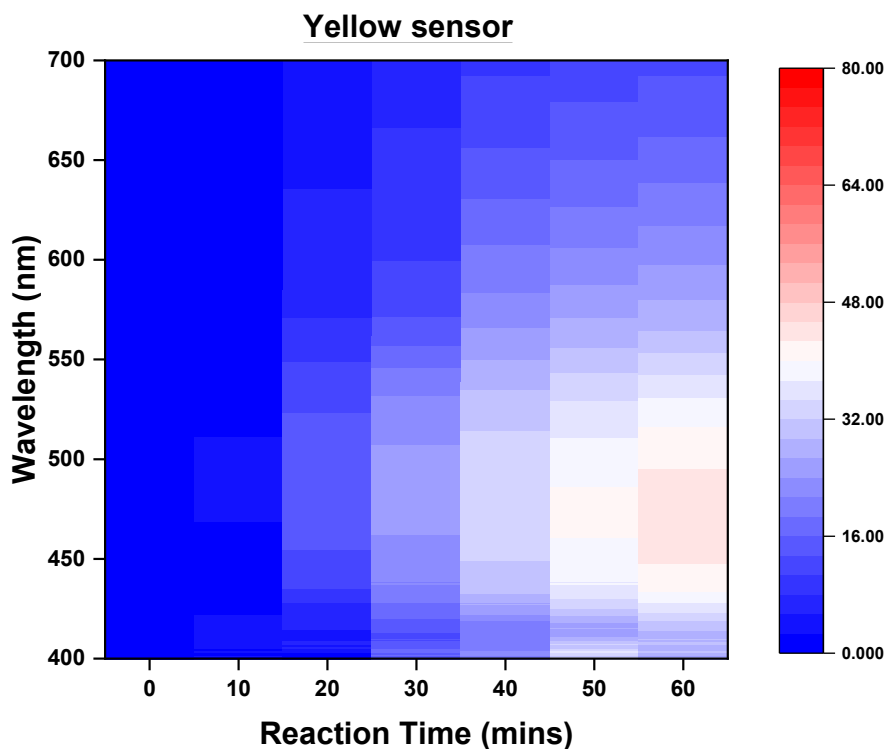


Figure 6: Determining Reaction Time

Change in reflectivity intensity as colormap over the yellow sensor plotted against time after sensor has come into contact with 100uM ammonium hydroxide solution. Measurable change in reflectivity occurs after the reaction is allowed to take place for a minimum of 20 minutes.

Limits of Detection for Aqueous Ammonia

We are measuring a change in reflection caused by the structural changes of the nanoparticles responsible for the structural color of our substrate induced by varying concentrations of ammonia.

We considered two factors which influence the rate of deterioration of the nano particles, time and

concentration. We define the upper and lower limits of detection as follows; upper limit of detection would be the concentration at which at under a specified concentration there is no longer an increase of change in reflection over any given wavelength. The lower limit of detection is defined as the concentration of analyte where the percent change in reflection is equal to or less than the matrix without the analyte, for our purpose we established the lower limits of detection at those concentrations of ammonia in which the percent change in reflection was less than or equal to that of the deionized water.

Using the yellow sensor and allowing a 30-minute reaction time we experimentally analyzed the limits of detection by plotting the integrated value under the percent change in reflectivity curve from 400λ to 700λ , against concentration of ammonium hydroxide from 0 to 1000uM, see figure 7. With the information provided from these experiments we concluded that limits of detection for aqueous ammonia under the current experimental parameters are from 100uM to 250uM, as concentrations of 50uM or less produce comparable results to that of the negative control (deionized water), and concentrations over 250uM produce comparable results and are indistinguishable from one another.

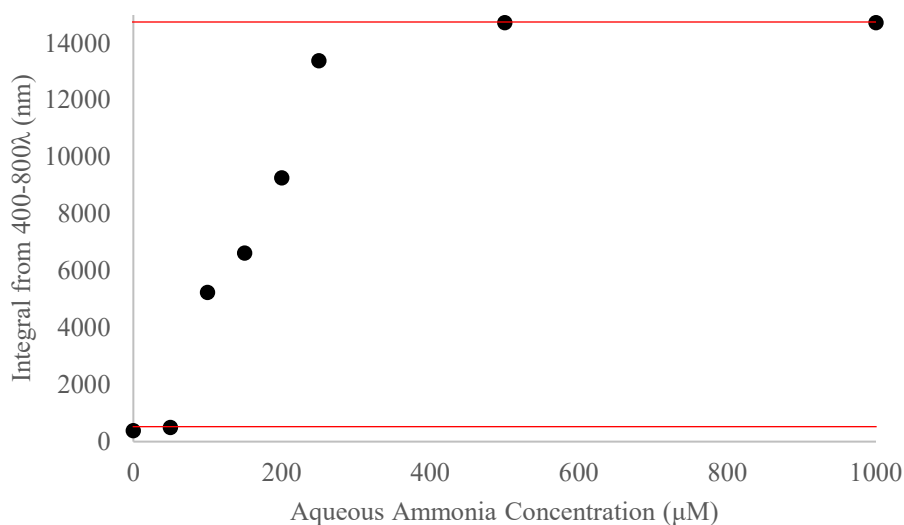


Figure 7: Limits of Detection

Upper and lower limits of detection for the Yellow 5nm Al NP Sensor are marked by a red line. Graph shows linear relationship between increasing aqueous ammonia concentration and change in reflectivity, from 0 to 250µM.

Ammonia in Urine

Using the experimental parameters established in detection of ammonia in water, we moved onto synthetic urine as a matrix. Synthetic urine was purchased from Spectrum Labs, and spiked with aqueous ammonia to desired concentrations. The yellow sensor was selected due to its sensitivity to ammonia when compared to the purple and blue sensors. A reaction time of 30-minutes was selected based on the reaction time experiments conducted using water as a matrix, refer to figure 6. Experiment was conducted as described in the “Standard Operating Procedures” section. Results indicate a change in reflectivity trend as ammonia concentrations vary. However, results were

inconclusive with our preliminary experiments possibly due to the unknown constituents present in the synthetic urine. Figure 8 shows the plotted values of the integrated area under the change in reflectivity curve, for three experimental trials and the averaged results of the three trials for varying concentrations of ammonia in synthetic urine.

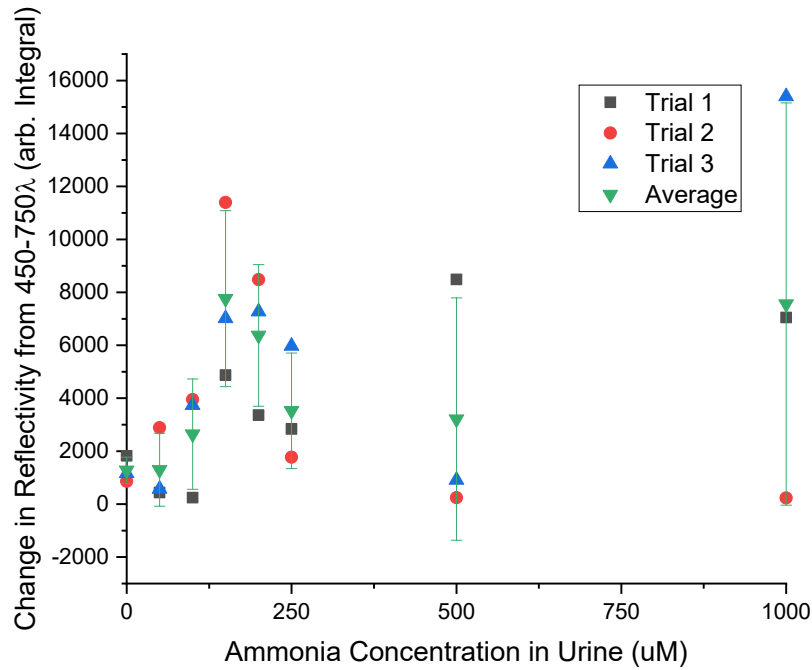


Figure 8: Ammonia in Synthetic Urine

Ammonia in synthetic urine was exposed to the sensor and allowed to react for 30 minutes. Reflectivity over the sensor surface was measured before and after the chemical reaction and the difference in reflectivity was calculated as per equation 1.

DISCUSSION

Reaction Mechanism

Ammonia gas in the presence of water produces an ammonium hydroxide solution, also known as aqueous ammonia or ammonia solution. The aluminum nanoparticle naturally reacts with the ambient oxygen in the air to form a thin aluminium oxide layer over the nanoparticle. The aluminium oxide layer will react with the ammonia solution and produce aluminum hydroxide as a precipitant. Once the aluminum oxide layer dissolves, and pure aluminum metal of the nanoparticles is exposed to the ammonia hydroxide solution which oxidizes aluminum to produce aluminum oxide, which reacts with ammonia hydroxide to produce aluminium hydroxide precipitate, therefore consuming the nanoparticles and converting them into aluminum hydroxide, an inorganic salt in the form of a white solid, insoluble in water. This chemical process alters the physical properties responsible for producing the structural color of the substrate.

If left to react for infinite time all of the sensor's aluminum nanoparticles will be consumed completely and converted to aluminum hydroxide, at this point all structural color will be lost and change in reflectivity will have reached its maxima. The proposed reaction mechanism illustrated in figure 9 was concluded upon XPS analysis of the sensor surface before and after 20, 40, and 60

minutes of the sensor being in contact with aqueous ammonia solution. XPS analysis shows the integrated area under the peaks corresponding to aluminum metal and aluminum oxide to decrease over time, while the integrated area under the peak corresponding to aluminum hydroxide increases over time.

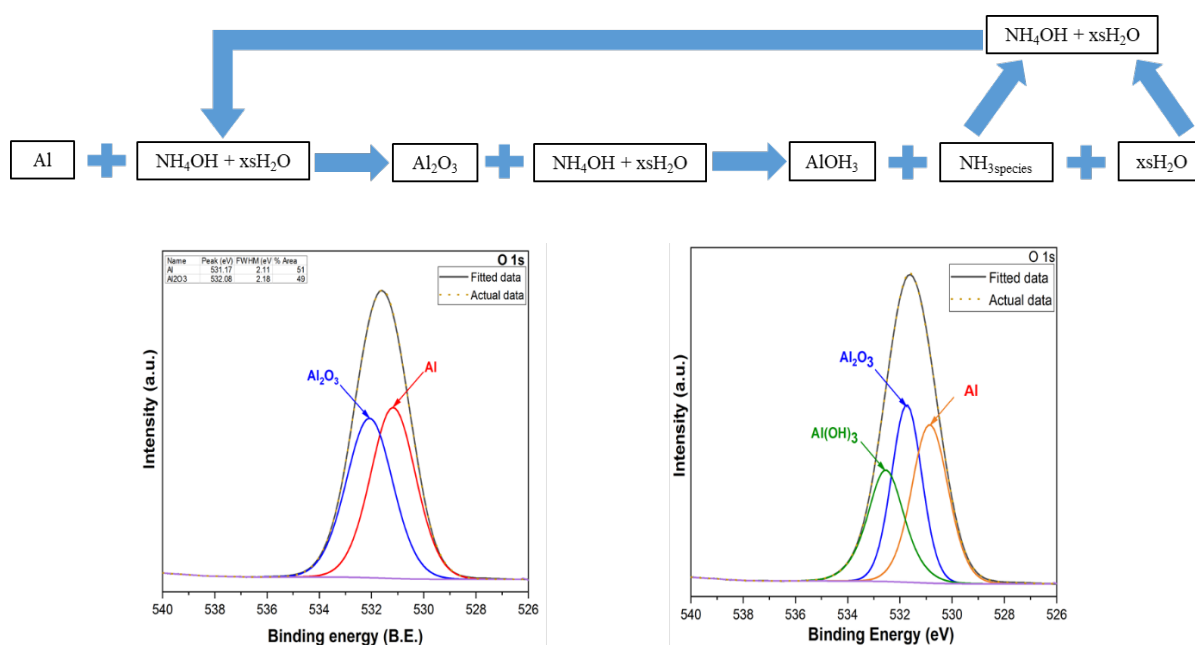


Figure 9: Proposed Reaction Mechanism Based on XPS Analysis

(top) Ammonium hydroxide in solution with excess water reacts with aluminum metal to produce aluminum oxide, and subsequently ammonium hydroxide reacts with aluminum oxide to produce aluminum hydroxide, ammonia species and excess water. Next, the ammonia species and excess water react to form ammonium hydroxide which is recycled back into the solution. (bottom) The proposed reaction mechanism was concluded upon XPS analysis of the sensor surface before (left), and after 60 minutes (right) of the sensor being in contact with aqueous ammonia solution. XPS analysis shows the peaks corresponding to aluminum metal and aluminum oxide to decrease over time, while the peak corresponding to aluminum hydroxide increases over time.

Aqueous Ammonia Colorimetric Sensor

We propose to develop a low cost, portable, disposable, easy to use, label free, metallic nanoparticle substrate for colorimetric ammonia detection, which may be used at home or in clinical point of care settings. The proposed sensor will detect ammonia directly from water or urine, without the need of additional reagents or sample preparation. The sensor's nanoparticle material may be chosen based on its reactivity to the target analyte, providing some level of tailorable selectivity without the need for sensor functionalization or use of reagents, allowing for direct detection from source. We conclude that decreasing nanoparticle size increases sensitivity, by providing greater surface area for the chemical reaction between aluminum and ammonium hydroxide to take place. Therefore nanoparticle size may be tailored to correspond with the desired concentration range of interest, with larger nanoparticles being more resilient to higher concentrations and therefore more adequate to quantify in high ranges and smaller nanoparticles being more sensitive to low concentrations. Limits of detection for aqueous ammonia under the current experimental parameters are from 100uM to 250uM using the yellow sensor. The range of detection for the yellow sensor is within biologically relevant concentrations and potentially suitable for the detection of ammonia from human and animal fluids as well as water monitoring.

Future Direction

Preliminary results have demonstrated that the proposed sensor is sensitive to medically relevant concentrations of aqueous ammonia. Future experiments will focus on using this sensing platform to detect and quantify ammonia in human urine, saliva and blood. We will determine analytical parameters for ammonia detection from these matrices, in order to quantify as well as narrow the limits of detections of each matrix. We also must consider and therefore study the effects of pH and temperature on the substrate as well as on the analyte and the matrix being analyzed, as well as testing for any other interfering ions which may be present in these matrices, and study how these may affect the substrate surface and the surface and the overall chemical reaction. We must perform sensitivity and selectivity assays and consider the incorporation of reagents, catalyst, and other metal or alloy nanoparticles.

We propose to integrate artificial intelligence to develop a colorimetric sensing platform to which detects a wide range of molecules where a cell phone image, or a portable spectrometer coupled with an AI enabled application will be able to perform the diagnosis for a wide range of applications, including; biomedicine, industrial quality control, and environmental monitoring. The goal is to take advantage of the mobile device camera in the detection of any given compound where the human eye fails to notice the subtle changes in color reflection caused by low concentrations of analyte.

In order to obtain information on the surface chemistry, chemical reactions, compounds, and byproducts present before and after we have introduced our analyte to the substrate, and in order to observe how the subsequent reaction is occurring we will implement the use of sophisticated instruments available to us, such as XPS. This information will allow us to further optimize or product by understand the chemical reactions taking place over our substrate. With the aid of instruments and techniques such as scanning electron microscopy (SEM) to visualize the substrate nano-surface before and after analysis in a resolvable image, X Ray photoelectron spectroscopy to analyze the elemental composition of the substrate surface, as well as spectrometry and spectrophotometry as our analytical tool to relate reflection on our substrate surface in relation to concentration analyte in solution.

Furthermore, the sensor may be coupled to plasma separator microfluidics for blood analysis, and functionalized for applications in detecting patient specific biomarkers such as those for diabetes, pregnancy, or vitamin deficiencies, in the form of and inexpensive and disposable at point of care device. We have conducted preliminary research into the functionalization of our substrate with single stranded DNA complementary to viral RNA. As aluminum is very reactive to the reagents necessary for functionalization of the ssDNA, a 5nm layer of TiO₂ was deposited over the aluminum nanoparticles. The TiO₂ layer serves two purposes, first it protects the aluminum nanoparticles without affecting the plasmonic resonance responsible for the structural color of the sensor, and secondly it allows for a platform for which the ssDNA may be attached onto the sensor surface. In our preliminary work we report a measurable difference in reflectivity over the ssDNA functionalized sensor when hybridization occurs with viral RNA complementary to the ssDNA.

Figure 10 graphs the area under change in reflectivity curve when the functionalized sensor detects a virus. Future work will focus on quantifying viral load from saliva.

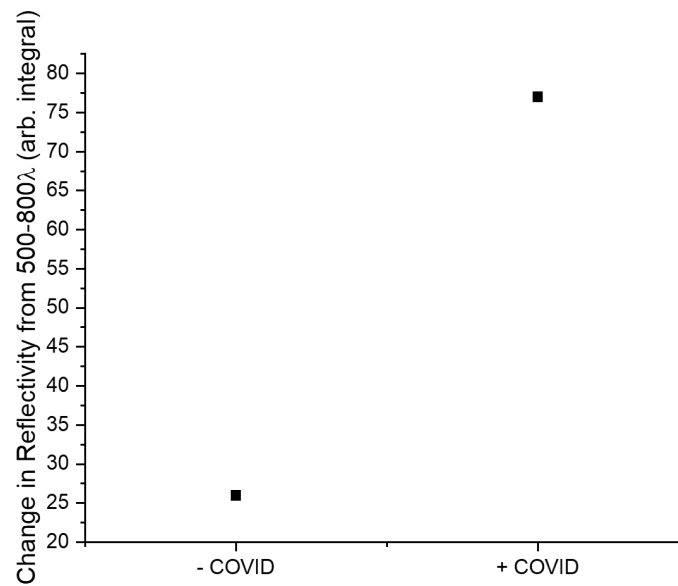


Figure 10 Virus Detection

COVID RNA was detected by functionalizing the sensor surface with ssDNA complementary to the viral RNA. The yellow sensor was used for this experiment. The surface of the sensor was analyzed spectrally before and after hybridization between ssDNA and viral RNA, and the change in reflectivity was calculated using equation 1. (-COVID) indicates a negative result where no viral RNA is present, and (+COVID) indicates the detection of the virus and a positive result.

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