March 23, 2011

Dear Students, Colleagues and Guests:

The National Society of Black Engineers (NSBE) serves as a sounding board and resource for minority engineering students around the world, and we are proud that you decided to showcase your research in the 2011 Technical Research Exhibition. With more than 35,000 members globally, our mission is “to increase the number of culturally responsible Black engineers who excel academically, succeed professionally, and positively impact the community.” Academic excellence is our first priority.

As National Academic Excellence Chair my goal is to promote research and technical excellence, and to understand the academic needs of our students. These proceedings are proof that NSBE is a one-stop-shop for academically gifted and technically savvy students. I invite you to attend the oral presentations and poster session to learn about the research your fellow members are conducting around the world.

Thank you for helping us see our mission realized.

Yours in NSBE

K.L Jordan

K.L. Jordan
National Society of Black Engineers (NSBE)
National Academic Excellence Chair
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www.nsbe.org
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ENGINEERING AND TECHNOLOGY:

Abstracts
Tayloria Adams
Adding conductive carbon fillers to insulating thermoplastic resins increases composite electrical and thermal conductivity. Often, as much of a single type of carbon filler is added to achieve the desired conductivity, while still allowing the material to be molded into a bipolar plate for a fuel cell. In this study, varying amounts of three different carbons (carbon black, synthetic graphite particles, and carbon fiber) were added to Vectra A950RX Liquid Crystal Polymer. The in-plane thermal conductivity of the resulting single filler composites were tested. The results showed that adding synthetic graphite particles caused the largest increase in the in-plane thermal conductivity of the composite.

The composites were modeled using ellipsoidal inclusion problems to predict the effective in-plane thermal conductivities at varying volume fractions with only physical property data of constituents. The synthetic graphite and carbon black were modeled using the average field approximation with ellipsoidal inclusions and the model showed good agreement with the experimental data. The carbon fiber polymer composite was modeled using an assemblage of coated ellipsoids and the model showed good agreement with the experimental data.

Adetayo Adesanya
This is a project based off my own idea to improve the convenience of electronics in a potential startup company. My project is devoted to allowing consumers to get through their day with that extra energy needed to keep their handheld devices in full operation for an extended period of time. Students need to be able to charge their devices while on the go. A solar-powered backpack would be the primary object of choice since most college students carry it while doing normal day-to-day activities. Students in college typically have a fast-paced lifestyle, spending most of their day on campus either in class, studying, doing campus activities or just socializing. The appeal factors for this product would include both mobility and versatility for multiple devices such as an iPad, cellphone and mp3 player to be charged simultaneously. PowerSavvy is a spin-off of my design that I created primarily for my senior design project at Purdue University.

The solar powered backpack charger uses a 4400 mAh lithium polymer battery pack to power all of the devices. Solar panels are used to harvest direct energy from sunlight to charge the main supply up to 12 watts. Other ways to charge the battery include wall A/C outlet, car charging outlet, and laptop usb (special feature). The backpack has 3 output’s that can be used simultaneously (USB, Mini-USB and 12V adaptor) to charge handheld devices. In order to charge the main battery above 80%, about 6 hours of direct sunlight or 1.5 hours of charge is required from the A/C wall outlet or car charger. The entire product weighs less than five pounds and will feature padded mesh straps for increased comfort.

Joshua Anquandah
The wireless FM bug is a sensitive miniature very high FM wireless microphone transmitter of the type “Wide Band Frequency Modulation Wireless Microphone (WBFMWM)”. Basically, it serves as a detective device to secretly monitor conversation and can be used to detect lies. We can call it a surveillance device. It is a hardware device which involves Radio Frequency (RF) circuit design. What makes the FM bug different from other devices is its sensitive nature and wireless property. In telecommunication, a ‘bug’ is described as a hidden listening device.

The FM bug receives sound waves preferably human voice of a frequency 300-3000Hz through a microphone. In view of that, there are 3 stages (modules) involved in the implementation of the bug. They are the audio amplifier, FM oscillator and the RF buffer/Power amplifier stages. The sound wave received through the microphone is amplified at the audio amplifier stage to a level that can be modulated over electromagnetic wave. The electromagnetic wave which is constantly being generated at the FM oscillator stage is buffered...
with adequate power to be transmitted strongly through the antenna. Finally, the audio signal is retrieved from a broadcast radio of a calculated FM band example could be a free band of 101.7MHz within the FM range 88MHz-108MHz. The free band, 101.7MHz is deduced from calculations.

The wireless FM bug is made to camouflage its location of instalment. Assuming conditions are favourable; broadcasting within the range of 50-500meters of transmission is phenomenal. Favourable conditions will involve wireless communication where there are no obstacles such as bad weather, trees and metallic structures to hinder electromagnetic wave flow. This miniature FM transmitter being deployed is of a simplex protocol with respect to communication. The security service, police department and military service should add the wireless FM bug to their shopping carts.

Idrissu Awudu
The recent discovery of oil in Ghana has received prominent attention and equal expectations of delivery from both the citizens and donor countries. As the citizens welcome this ‘black gold’ and waiting to cash in on the revenue, it is critical to examine the current global trends, and for that matter suggesting alternative energies for future consideration. Biofuel energy as an alternative and additive form of energy to fossil fuel especially in the transportation sector, has gained much attention in recent times. In order to sustain such a vision, a strong network on which the supply chain is built on is of extreme importance. This work focuses on the supply chain of ethanol and the incorporation of uncertainty models as the basis for a robust and reliable supply chain. Firstly, a deterministic model is developed. Secondly, a stochastic extension is developed for the multistage, multiperiod stochastic refinery problem. Uncertainties from price and demand are modeled using the Geometric Brownian Motion (GBM) and Mean Reverting Jump Diffusion Model respectively. The resulting stochastic problem is solved using Benders Decomposition. To verify the stochastic case, a simulation model based on Monte Carlo is employed. The deterministic method shows an expected profit that is high, while the stochastic results show that employing the uncertain case realizes a decrease in the profit margin of the plant, thereby emphasizing the importance of incorporating uncertainties in most models. A case study which is based on an ethanol plant in the United States is utilized.

Rachel Bonas
The objective of information security includes the protection of information from theft and or corruption, while allowing said information to still be accessible to its intended users. There are various factors that can contribute to breaches in computer security, one of the major causes can be found in latent vulnerabilities, such as buffer overflows. Attackers can use buffer overflow vulnerabilities to execute arbitrary code and therefore buffer overflows are considered to be an extremely dangerous exploit that can result in system compromises and failures that can be catastrophic. Security problems caused by buffer overflows are often responsible for damages totaling in the millions and in some more disastrous cases the loss of life. Buffer overflow attacks may be classified as today’s single most important security threat and is an important and persistent security problem. Buffer overflows account for approximately half of all security vulnerabilities. Protection approaches against buffer overflow have been categorized into three main categories: Isolation, Dynamic and Static approaches. In this paper static analysis is the approach that will be discussed. Static analysis is the technology which analyzes source code for the purpose of detecting defects such are buffer overflows. Static analysis analyses computer software in order to locate the solve the problem before releasing the code. The static analysis option does not offer any protection but it is able to point out vulnerabilities that may be present in the code being analyzed before it is released for use. The earlier an error is determined, the lower is the cost of its correction.

Joshua Chukwuka
Chomp is a two-player combinatorial game of strategy like Nim, Hackendoth, Subset-Takeaway and others. This research aims to design and implement a winning strategy for any chomp grid size. The research started with a grid
of two rows. The take-away game Chomp is played on a rectangular grid with 'm' number of rows and 'n' number of columns. The grid is divided into squares/blocks. Two players alternate removing pieces from the grid. The lower right hand piece is a 'poison' and the player who removes this piece loses the game. By expanding the standard version of Chomp of 2 rows and 5 columns to 3 rows and indefinitely many columns, we investigated if we can come up with a way to find all the losing positions of a chomp grid when there are 3 rows and 0, 1, 2, or 3 pieces in the top row. Mathematically, the game is played on a partially ordered set S. A move is made by selecting an element x \in S and removing x and all other elements greater than x, that is all elements n \geq x. The player who chooses the element 0 in the set loses. Winning positions are in the set W and losing positions in the set L. From this, we extended the research to any grid size. If a player can “force” the game to alternate from a position in 'W' to a position in 'L' and back to a position in W making the game go "W, L, W, L", the other player will lose. We developed theorems for common conditions in chomp so that the player can manipulate the game so he wins. Other important proofs and principles like the principle of mathematical induction were used to validate the results. A computer program which simulates actual gameplay and winning moves was also developed.

**Mouhamadou Diop**
The steady laminar flow of a second grade fluid over a radially stretching sheet is considered. The axisymmetric flow of a second grade fluid is induced due to linear stretching of a sheet. The developed non-linear differential equations are solved analytically using homotopy perturbation method (HPM) and variational iteration method (VIM). The series solutions are developed and the convergence of these solutions is explicitly discussed. The analytical expressions for velocity is constructed and shown graphically. Finally, a comparison between the HPM and VIM solutions is also included and it is found that the results of the two methods are in excellent agreement.

**Keywords:** Homotopy Perturbation Method (HPM); Variational Iteration Method (VIM); Fluid flow; Second grade fluid; Axisymmetric flow.

**Ian Duncan Jr.**
Motion based driving simulation is a safe and expedient way to examine research questions that can be performed in a real vehicle. Supplementing automotive simulation with practical peripherals such as an automotive instrument cluster can provide realism to the simulation environment, immersing the user. The use of mechatronics in such a system is a useful tool for adding to the authentication of the simulation environment while adding functionality for the designer. Mechatronics is a multidisciplinary field that incorporates mechanical, electrical, computer, and control systems engineering to implement embedded designs. Through this synergy, the incorporation of an actual automotive instrument cluster can be integrated with the motion based simulator in the New York State for Engineering Design and Industrial Innovation’s (NYSCEDII) lab. This design will lay a foundation for future instrument cluster design and simulation analysis tools used within NYSCEDII, and add significant realism and capability for future vehicle and driver studies.
Shadrach Elechi
Abstract: This paper discusses the design of a 6GHz high directivity dual tapered slotline antenna (DTSA) fabricated on a thin dielectric substrate. The antenna is designed by introducing two linearly tapered slotlines on same side of a substrate and feeding them from one feed port via a single microstrip line. Antenna impedance, return loss, and principle plane radiation patterns are presented. Return loss is far below -10 dB at both resonant points. The DTSA design produces a fan-beam that is suitable for situational awareness interrogation, with a half-power beam width, HPBW= 13 degrees in the E-plane and a HPBW=52 degrees in the H-plane, and a feed structure that lends itself to beam steering in the E-plane. A peak gain of 19 is reported.

Piaget Francois
Pelvic organ prolapse is a disorder caused by the failure of the vaginal support mechanism, which is composed of the levator ani muscles and connective tissue attachments between the vagina and pelvic sidewall. This disorder affects a large number of women each year and results in the distension of the pelvic organs into the vagina and often through the genital hiatus. It is believed that hormonal status may play a role in prolapse development and progression because most prolapsed women are post-menopausal. Therefore it is important to evaluate the effects of hormones on connective tissue in the vagina, specifically collagen because this protein provides the main structural support to the vagina. In my research, I evaluated collagen alignment in the vagina with varying levels of hormones present, by studying the estrous cycle of the rat as an animal model. We have preliminary evidence to suggest that prolapse is correlated with disorganized collagen in the vaginal support mechanism.
I designed a protocol to assess the stages of the rat estrous cycle using wet vaginal smears and trichrome staining post-mortem. Using a picrosirius red stain to collect qualitative data on collagen alignment, we came to the conclusion that, at least in the short term, hormonal status does not play a significant role in collagen alignment. However since extreme changes can be observed in the epithelial tissue of the vagina, a further study would be to track the long term effects of hormonal status on collagen alignment in post-menopausal or ovarirectomized rats.

Lauren Frazier
Nodes in distributed systems can fail for numerous reasons. Hardware failures, malicious users, and other things can cause nodes to behave arbitrarily, rather than simply crashing. These arbitrary behaviors are best described by the Byzantine fault model, and can sometimes be detected in distributed systems. Though there is much work on crash fault detection, and Byzantine fault tolerance, there is relatively little work on Byzantine fault detection. Haeberlen and Kuznetsov [4] first defined the fault detection problem for fully connected networks, showing that it can be solved for commission and omission faults.

Deneen Gaynor
Elevated levels of 3-nitrotyrosine (3NT)-modified proteins have been observed in various neurodegenerative diseases and age-related pathologies. In previous studies, identification and quantification of 3NT-modified proteins has involved measuring free nitrotyrosine in plasma. Alternative methods also include hydrolysis of proteins using various detection methods such as liquid chromatography (LC) coupled with UV-Vis detection or mass spectrometry (MS) and gas chromatography-MS. While these methods provide an overall level of nitration they do not provide information on the identification of nitrated proteins or specific sites of nitration. We are developing methods to identify specific sites of 3NT-modifications in individual proteins. As an example, β-lactoglobulin was 1) nitrated in vitro using peroxynitrite, (2) digested with trypsin, and (3) tryptic peptides were analyzed using nano-LC-MS/MS on an Orbitrap MS. Preliminary results from these experiments are presented.

Key Words – nitrotyrosine, mass spectrometry, peroxynitrite, tyrosine modification

Hani Ghazi
Failure of many orthopedic procedures can often be attributed to loosening of the metal components of total joint replacement implants
as a result of insufficient bone ongrowth and, therefore, weak fixation. A novel coating of self-assembled monolayer of phosphonate molecules (SAMP) that chemically enhances metallic surfaces by covalently bonding to their naturally occurring oxidized surfaces has been developed. The SAMP coating is chemically stable, and its in-vitro results showed that it promotes cell proliferation, osteoblast adhesion and enhances biological attachment to metal surfaces. The purpose of this study is to establish the efficacy of this novel chemical surface treatment in improving the fixation of metallic implants in both cortical and cancellous bone over a conventional untreated implant. We hypothesize that a SAMP monolayer coating would lead to greater in-vivo bone ongrowth and fixation of SAMP-coated stainless steel (SS) implants when compared against uncoated ones. An in vivo model was used and the outcomes used to establish efficacy were the histological appearance of the interface between implant and bone and the static mechanical strength of the interface. Cylindrical 316L SS implants, 25mm long and 5mm in diameter, were machined and highly polished and half of these implants were randomly chosen to be coated with the phosphonate-terminated SAMP. 43 male, skeletally mature New Zealand White rabbits, weighing between 3.5 and 5kg, were implanted bilaterally in the distal femoral intramedullary canals where each animal received one coated and one uncoated implant in each leg. The rabbits were randomized to one of four time points: 4, 8, 12 and 16 weeks. Histological evaluation proved that SAMP treatment resulted in the appearance of higher amount of bone growth and apposition onto the bone-implant interface. The biomechanical test showed that the resistive pull-out force was higher in SAMP-coated implants than uncoated ones at higher time points.

Theodore Glave
Dye-sensitized solar cells (DSSCs) have garnered interest recently since they present a relatively low cost means of energy generation. Single wall CNTs, used as conducting scaffolds in a TiO2 based cell, were capable of doubling the photoconversion efficiency of a photoelectrochemical cell. The work proposed here aims at developing solid-state dye-sensitized solar cells using TiO2/CNT/polymer nanocomposites.

The focus of this research is to:
• Increase the power conversion efficiency and the overall performance characteristics of dye sensitized solar cells (DSSCs) through the development of novel polymer-titania nanocomposites.
• Improve conventional TiO2 DSSCs by employing blends of carbon nanotubes (SWCNTs) with novel polymer electrolytes.
• Develop a model to characterize the interfacial behavior at the nanotube polymer surface.

The modeling to be performed is based on the Scheutjens-Fleer Method. This method is based on the Flory Cell Model of Polymers. The method can be used to obtained detailed information about the composition, surface energy, and structure of polymer films. Thus far, work is being carried out using the Scheutjers-Fleer Model to: • Develop a program that can be used to model stiff polymer chains that adsorb onto a cylindrical surface. • Use the program to obtain detailed information about the surface energies and the conformation of chains that are adsorbed onto a carbon nanotube that will be treated as an infinitely long cylinder.

Pierce Gordon
In-Situ Resource Utilization technology developed for lunar operation use processes which require thermal energy on the scale of tens of thousands of watts for extended periods of time to chemically break down lunar regolith into oxygen. Solar concentrators are a well researched and developed technology which harnesses the energy from the sun to heat the regolith to the temperatures of 1273-1800 K required for the chemical processes. As such, the many different types of concentrator technologies, their advantages, disadvantages, and technology opportunities have as of yet been discussed in one encompassing overview. This trade study compares and contrasts different solar concentrator designs that have been developed, such as Cassegrains, offset parabolas, compound parabolic concentrators, and secondary concentrators. The trade study
also compares differences between thermally based concentrators made from lenses and mirrors, and differences between rigid and flexible concentrators. Possible substrate elements for a rigid mirror concentrator are chosen and discussed, with criteria being a low CTE, a high modulus of elasticity, and a low density. Possible lunar locations for concentrator technology are also compared and contrasted. Options for energy transfer technology and future plans for concentrator research are also discussed.

Shakir James
Internet Service Providers (ISPs) have failed to reduce the cost of peer-to-peer (P2P) traffic. Traffic throttling devices increase user download times, and caches store content that may infringe copyright. We propose ISP-Managed P2P (IMP): a transparent peer-discovery service that returns peers favorable to ISPs. Unlike similar services, IMP does not require the direct support of developers who have no incentive to cooperate. This paper covers the design, implementation, and experimental evaluation of our IMP prototype, which reduces costly, cross-ISP traffic by eight times without significantly increasing user download times.

Natee’ Johnson
In effort to make harvesting solar energy more affordable, and given the high processing costs associated with silicon-wafer solar devices, researchers are driven to explore alternative materials that are cheaper to process and equally available. Copper (I) Sulfide has been identified as a viable solution, as it has a bulk band gap of 1.2eV, within the range yielding the Shockley theoretical maximum efficiency. The motivation of this work is to synthesize Cu25 nanocrystals and optimize its performance for use in photovoltaic devices. The procedure for making crystals is done in accordance with that detailed by Paul Alivisato’s group at UC Berkeley, where it was speculated that ligands surviving the cleaning process reduced electron mobility. We believed these ligands to be carbon chains, and performed an annealing study to address this issue. Heat treatments proved significantly reduce the carbon content, but simultaneously changed the crystal size, and potentially, the phase of the nanocrystals. The crystals were characterized using x-ray diffraction techniques, x-ray photospectroscopy, photoaluminescence, and conductivity tests. Longer and higher heat treatments proved to increase the conductivity and dize of the crystals, but it is not clear if that correlation is direct, as the phase of the crystals may have changed as well. Further investigation can be done with higher resolution XRD to identify composition changes.

Renee Oats (Poster)
To explore the ethical and societal implications of internet-based engineering education the authors conducted focus groups with undergraduate and graduate engineering students and personal meetings with engineering faculty in Mechanical, Civil, Electrical, Chemical, and Environmental Engineering. The responses from these meetings were analyzed and discussed in this poster.

Internet-based engineering education has been in existence since the early 1990s in several forms. Through internet-based learning students are able to pace themselves, interact with instructors and other classmates, and participate in live feedback sessions. Internet-based learning also accounts for various types of learning styles and physical disabilities. There are many positive implications of internet-based learning such as a means to interactively present and disseminate curricula through coursework management tools such as Blackboard. It also opens doors for collaboration and continuing education for full time employees, i.e. “learning anywhere, anytime.” Students are encouraged to expand their knowledge of the material being taught through media, images, animation and streaming audio/video.

Although internet-based engineering education is an accepted practice across the United States and abroad, there are obvious ethical and societal consequences that should be addressed. Do students feel they are gaining the proper knowledge in their courses and labs? Do their expectations of what will be asked of them on homework and exams reflect reality? Are students prone to cheating and plagiarism?
I will discuss the ethical and societal implications of internet-based engineering education, specifically focusing on the submission of internet-based homework and exams.

**Renee Oats (Oral)**

The condition of transportation infrastructure, specifically bridges, has received a great deal of attention in recent years as a result of catastrophic failures, deteriorating conditions, and even political pressure. Bridge transportation authorities attempt to establish maintenance priorities for an aging infrastructure, but the challenges of deteriorating infrastructure continues to be in the forefront of bridge inspection practices. The foundations of structural health monitoring attempts to monitor infrastructure’s condition, assess in-service performance, detect deterioration, and estimate remaining service life. Current practices for condition assessment include: visual evaluation, measurement of bridge response to known loading, and the use of specialized sensor technologies for specific effects; however to date, no single solution exists that is capable of Completely determining structural condition, with the likely solution being a combination of multiple techniques.

The focus of this project is to evaluate the applicability of commercial remote sensing technologies for bridge condition assessment as well as developing a decision support system (DSS) to integrate the results from different techniques in order to support and expand the current bridge inspection practice. This presentation explores the feasibility of using commercial remote sensing technologies such as 3D Photogrammetry, Digital Image Correlation and Thermal Infrared for condition assessment. Remote sensing technologies will be correlated with conventional in-place sensors to obtain bridge condition assessment data without the need to place heavy instrumentation on the structure. This information will then be analyzed by a computer DSS to develop unique signatures of bridge condition. A concluding project assessment will explore how these techniques can be potentially combined with current practices to assess current bridge condition and health.

**Dennis A Oriero**

Tyrosinase encapsulating ultrafine silicate-polymer bio-composite fiber matrix were synthesized by the combination of sol-gel chemistry and electrospinning. These ultrafine enzyme carrying fibers with average diameters of less than one micron, were further studied by their incorporation into a small-scale, flow-through device to demonstrate phenol detection and bio-catalysis feasibility. Bio-catalytic activity of the electrospun enzyme (tyrosinase) was evaluated using the integrated form of the Michealis-Menten equation, having the advantage of requiring fewer experimental runs than other approaches. Micro-Raman spectra analysis was also used to confirm bio-catalysis and the bio-composite nature of the enzyme carrying fibers. Bio-catalytic activity of the electrospun enzyme was retained at phenol concentrations of 0-5 mM which are usually typical phenol concentrations found in waters contaminated with phenol. These fiber matrix are promising candidates for immobilization of enzymes due to their relatively high surface area. This research demonstrates the potential of this approach as a cost effective sensing material for bio-sensing, bio-catalysis and bio-detection applicable in environmental and bio-industrial analysis.

**Sampson Owusu**

Goldfields Ghana Limited, a subsidiary of Goldfields South Africa, holds a license to the Tarkwa Mine Concession. It is located to the north and northeast of Tarkwa in the Wassa West District of the Western Region of Ghana. Tarkwa is about 90 km north of Takoradi and 278 km south of Kumasi which it connects both by rail and road, and to Accra directly by rail (approximately 315 km).

This Thesis provides a comparative study of Ordinary Kriging (OK) and Inverse Distance Weighting (IDW) methods of grade estimation to the deposit at Teberebie Pit which is part of Goldfields Ghana Limited concession. Surpac was used in the resource estimation. The study is aimed at evaluating the reliability and
applicability of IDW method as an alternative resource estimation method for Goldfields Ghana Limited Tarkwa Mine. The application of OK and IDW methods of resource estimation has proven to be successful. It is therefore recommended that Goldfields Ghana Limited, Tarkwa Mine should use IDW method as an alternative resource estimation method when there is much pressure and time constraint to produce a model.

Maziar Rostamian
The steady laminar flow of a second grade fluid over a radially stretching sheet is considered. The axisymmetric flow of a second grade fluid is induced due to linear stretching of a sheet. The developed non-linear differential equations are solved analytically using homotopy perturbation method (HPM) and variational iteration method (VIM). The series solutions are developed and the convergence of these solutions is explicitly discussed. The analytical expressions for velocity is constructed and shown graphically. Finally, a comparison between the HPM and VIM solutions is also included and it is found that the results of the two methods are in excellent agreement.

Keywords: Homotopy Perturbation Method (HPM); Variational Iteration Method (VIM); Fluid flow; Second grade fluid; Axisymmetric flow.

John Shelton
Using molecular dynamics simulations, an analysis of the thermal conductivity enhancement found in copper/argon nanofluid is performed. First, a verification of an increase of as much as ~30% in the thermal conductivity of the theoretical nanofluid over the corresponding base fluid due to increasing nanoparticle concentration is presented. Thermal energy transport is then decomposed into potential, kinetic, and virial components based on the Green-Kubo autocorrelation function used to calculate thermal conductivity from the microscopic properties of the system. An analysis of these components showed that as the concentration of the nanoparticle increases, the energy that is transported through the system due to collisions within the fluid decreases by as much as 80% and that the nanofluid system increasingly shows the characteristics of an amorphous-like material. The decrease in energy exchange due to collisions suggests another physical mechanism is present for thermal energy transport. Therefore, it is proposed that thermal diffusion is this physical mechanism that plays the more significant role in thermal energy transport within a nanofluid than had been previously suggested.

Amber C Wingfield
The complementary metal oxide field effect transistor (CMOSFET) continues to be an impacting device within the semiconductor industry. Known for its amplification and switch like capabilities, the CMOSFET has great significance in logic circuits. Miniaturizing such technology to the “nanolevel” has been of great interest to the industry. We will demonstrate the use of a gallium nitride (GaN) nanoFET as a long term solution to the physical limits performed by silicon CMOS technology. Our process of fabrication is as follows; a highly doped- low resistant silicon wafer was thermally oxidized on one side to form silicon dioxide (SiO2), which acts as the gate dielectric. Photolithography was then performed to define the drain and source contacts. The source and drain are formed by a lift-off process using aluminum. Evaporation of aluminum on the reverse side of substrate forms the back gate electrode. GaN nanowires grown by chemical vapor deposition (CVD) are then suspended in toluene. Using a syringe, the suspension was then dropped on to the surface containing the source and drain. Nanowires lying across a source and drain complete the nanoFET. I-V characterization at different temperatures is performed using a probe station. Essentially, oxidation rates for SiO2 were determined through ellipsometry and gate oxides were successfully grown. I-V measurements confirmed the existence of a source-drain channel in the nanowire. Overall, success in fabricating GaN nanoFETs using conventional fabrication processes was achieved. Although the method of depositing nanowires by dropping them in solution needs improving, nanowires were deposited this way. Perhaps a different solvent would help. To insure nanowires-oxide contact in the future, the source and drain...
spacing could be widened to allow the nanowires to sag downward more towards the oxide and the thickness of the source-drain contacts could be made thinner.

**Velencia Witherspoon**

Particle based membranes gives one the ability to control pore sizes and permeabilities with a wider range than commercial membranes. When coupled with biological species (proteins) to produce composite membranes with required stability and performance characteristics. In this work, composite latex membranes were synthesized using soft latex nanoparticles with the average diameters of 200 nm, 650 nm, and 950 nm and composite protein membrane were synthesized using the protein ABeta(1-40) and base supports of Supor polyethersulfone membranes. The latex composite membranes were characterized in terms of their pore size distribution, water flux, and average pore size. The protein composite membranes were characterized through SEM imaging and IV characteristics. It was found that the membrane pore diameters were 14% of the particle diameter. This trend included the 200 nm particles where the measured permeabilities were much lower than that of packed bed of spheres. Various modification were made to the methodology of synthesis (as developed by Erin Holley), no changes made altered the resistance of the composite membranes. Understanding of fouling mechanisms was found in the literature and the high resistance was related to a change in the diameter of the pore to particle size ratio. Methodology was developed that successfully deposited the protein ABeta (1-40) onto the porous Supor 100 support. This was confirmed through SEM imaging and decrease in the volumetric flow rate of the solution. IV characteristic after protein deposition did not result in lower resistance (ohms), but indicated that the protein increased the resistance and was acting as an insulator. Therefore the IV-characteristics results were inconclusive. Future plans include understanding the effect of solution concentration on the deposition and conductivity and trying to deposit the commercial available protein, Rada(14-1) on the Supor support, and to make more accurate IV measurements by making adjustments to the current protocol.

**Maynard G Yates II**

With the continued growth of the mobile device market, the possibility of their use in criminal activity will only continue to increase. While the mobile device market provides a great variety of manufactures and models causing a strong diversity. It increases the difficulty for choosing proper forensics tools for seizing internal data from mobile devices. Through this thesis, I will give a comprehensive perspective of each popular digital forensic tool and offer an inside view for investigator to choose their free sources or commercial tools via a practical investigation. In addition, a summary for the future direction for forensics tools in mobile devices will be discussed.

**Frank Agyei-Ntim**

Implantable Medical Device such as the Total Artificial Heart (TAH) requires high bandwidth systems capable of producing simultaneously large force and displacement (stroke) to drive its actuators. Conventional hydraulic pumps used in TAH applications, despite its simple concept and large displacement, are bulky, have low bandwidth and require many moving parts. This research work proposes the design of a 2-Stage muscle-powered piezoelectric energy source which converts mechanical energy of a muscle specifically the lattisimus dorsi into electrical energy to drive the actuators of the TAH. The design incorporates piezoelectric element and ultra-capacitors and classical linear motor to convert the high force and low stroke to low force but high stroke needed in TAH application. This work describes a bi-directional power flow: from the piezoelectric element to the TAH during systole and from the TAH to the ultra-capacitor field of the piezoelectric element during diastole. Power and efficiency analysis of the design would be incorporated to determine the feasibility of replacing the conventional hydraulic pump used to drive the actuators of TAH with this system. Finally based on the design, the physical forces and physiological flow pattern of the pumping of blood would be analyzed to eliminate the likelihood of hemostasis and pathologic thrombosis usually
associated with blood clotting. Keywords: Implantable Medical Devices, Total Artificial Heart, Piezoelectric element, blood clotting

Charlee Bennett, PhD

CuIn_{1-x}Ga_{x}Se_{2} (CIGS) as an absorber material in solar cells is already known to present a less toxic alternative to current solar cells based on toxic elements such as CdTe. CIGS developed as nanoparticles in solution are ideal for non-vacuum deposition methods which help achieve lower-cost solar cells, compared to high vacuum deposition methods. This study investigates deposition and characterization of aqueous-based CIGS nanoparticles synthesized via Nanofermentation process, and deposited with sono-spray deposition. Sono-Spray deposition is a non-vacuum technology which is versatile, inexpensive, is used for deposition of uniform coatings on various substrates. This technique is also adaptable for larger scale development with areas of up to 12 sq in. In this study, CIGS nanoparticles are dispersed in aqueous and organic solvents. The nanoparticles vary from 10-20nm in diameter. They are then deposited via sono-spray deposition method onto various substrates including Moly/Glass, FTO/Glass, and Moly/Quartz. The thickness of the thin films range from 0.5 to 1 micron. The thin films are characterized via X-Ray Diffraction (XRD), Optical Microscope, and Scanning Electron Microscopy (SEM, and STEM). XRD shows the crystal structure of CIGS and the buffer layers, and SEM and STEM cross sections imaging show thickness of the thin film layers.

Jasmine Davenport

Biotinylated microtubule filaments partially coated with streptavidin and gliding on surface-adhered kinesin motor proteins converge to form linear “nanowire” and circular “nanospool” structures. We present a cellular automaton simulation tool that models the dynamics of microtubule gliding and interactions. In this method, each microtubule is composed of head, body, and tail segments. The microtubule surface density, lengths, trajectory persistence length, and modes of interaction are dictated by the user. The microtubules are randomly arranged and move across a hexagonal lattice surface with the direction of motion of the head segment being determined probabilistically: the body and tail segments follow the path of the head. The analysis of the motion and interactions allow statistically meaningful data to be obtained regarding the number of generated spools, the radial distribution in the distance between spools, and the average spool circumference which can be compared to experimental results. This technique will aid in predictions of the formation process of nanowires and nanospools. This tool may also be of use in the simulation of other systems exhibiting transport and aggregation.

Mykel Green

The in vitro fabrication of functional tissue mimics may provide unique perspective into in vivo cellular behavior and interactions. In order to fabricate these tissue mimics, one must fabricate scaffolds with multiple cell types in complex designs. In this work, poly(ethylene glycol) diacrylate (PEGDA) was used as the scaffold material, due to its tunable bioactivity and tissue-like mechanical properties. An inverted fluorescent microscope, equipped with a metal halide light source, was used to precisely align photomasks to guide photopolymerization of PEGDA hydrogel structures within a microfluidic device. The manner in which photomasks are projected and aligned in our fabrication system allows us to fabricate complex multicellular structures that mimic in vivo tissue architecture. As proof of concept, we chose to first fabricate an in vitro system that mimics the liver acinus, the smallest functional unit of the liver. In order to determine an ideal environment for hepatocyte function, we evaluated hepatocyte (HepG2) bioactivity when cultured with supporting cell types. Existing literature indicates the benefit of co-culturing hepatocytes with fibroblasts. We hypothesized that co-culturing hepatocytes with cells that spontaneously form microvascular networks may better mimic the in vivo liver microenvironment. Hepatocyte bioactivity was evaluated by measuring the fluorescence due to metabolism of 7-ethoxy-4-trifluoromethylcoumarin (EFC) by cytochrome P450 enzymes within studies progressing from two dimensional well-plates to three dimensional hydrogels. The results validate that
a co-culture metabolizes more efficiently than a single culture of HepG2s alone. Future studies include fabricating a tissue mimic within a microfluidic device and measuring EFC metabolism by fluorometric assay of the culture medium used to perfuse the system. The following results theoretically should be similar to previous studies, thus providing evidence of a functional tissue mimic. The ability to successfully fabricate and study a hydrogel mimic of a native tissue significantly furthers the knowledge and resources in the field of tissue engineering.

**Brett Harris**

UI designers use a variety of prototyping tools, from paper and pencil sketching, to drag-and-drop mock-up tools (e.g., Balsamiq Mockups), to sophisticated suites of modeling tools and toolkits (e.g., iRise or dijit, the dojo GUI toolkit). Many projects would benefit from quickly analyzing prototypes at an early stage without the effort of bringing in users for empirical tests. Most analysis tools, however (e.g., AutoCWW [1], Bloodhound [2], and CogTool [4]), require prototypes to be in their own format, which forces the designer to re-do the prototypes in order to analyze them. Our work is a step toward allowing the CogTool analysis tools to import from many different prototyping tools, so designers will have a path to quick usability analysis without changing the way they currently express their preliminary designs.

**Bakari Hassan**

Soil Moisture Active & Passive (SMAP) is a 3-year, in-house earth science mission headed by NASA’s Jet Propulsion Laboratory (JPL). SMAP, initiated in 2008 and set to launch in 2014, will allow scientists and engineers to gain a better understanding of how the earth’s climate change will affect future water and food supplies. However, SMAP must endure an eclipse season once a year in which it will endure an eclipse of approximately 20 minutes every orbit. Since the solar array is not operational during this time, it is important that SMAP will be able to endure each eclipse and restore the battery to full state of charge before the next eclipse. Eric Wood of the Mars Science Laboratory (MSL) has created an advanced Microsoft Excel sheet to calculate various power statistics. The Multi-Mission Power And Thermal Analysis Tool (MMPAT) has been used on many top missions at JPL, and is to be implemented on SMAP. It is believed that the battery will survive the launch and post-launch procedures. However, once MMPAT is integrated with SMAP mission scenarios, the tool will be of great use to the Flight Systems team, and the final battery state will be determined. Results thus far have shown that SMAP will successfully endure each eclipse season, but as the flight system design is maturing with better estimates of the power loads and its usage, the depth of discharge margin will be better determined once calculated and validated using MMPAT.

**Ryan Leon**

The interaction of an intense laser pulse with near-critical density plasma makes a channel both in electron and then in ion density. The propagation of a laser pulse through such a channel is connected with the acceleration of electrons in the wake of a laser pulse and generation of strong moving electric and magnetic fields in the propagation channel. Upon exiting the plasma the magnetic field generates a quasi-static electric field that accelerates and collimates ions from a thin filament formed in the propagation channel. Two-dimensional Particle-in-Cell simulations show that a 100 TW laser pulse tightly focused on a near-critical density target is able to accelerate protons up to an energy of 250 MeV [1]. We present the experimental results on ion acceleration from 150 micron thick silica aerogel targets with density of 40 to 100 mg/cm^3 using 100 TW, 30 fs laser pulses from HERCULES laser at normal incidence, producing intensities of up to 1022 W/cm2. The experimental data show protons of 1.5 MeV.

**Alex Macharia**

Though Renal Arteries occur in roughly 2% of the American population, little is known regarding their effects on flow dynamics within the human circulation system. Part of the
challenge results from the difficulty of verifying flow patterns with and without aneurysms. Our project seeks to investigate this condition, by simulating flow through replicas of human renal arteries with and without aneurysms. We seek to investigate the relation between the aneurysms and hypertension and will make use of Computational Fluid Dynamics to verify the results.

Ane Muvadgah
University of Central Oklahoma is a metropolitan university in Edmond, Oklahoma founded in 1890. It is Oklahoma’s oldest institution of higher learning. It has a population of 17,101 students as of fall 2010. Following the fall 2010 statistics, 1,623 African American students were enrolled, which was 9.5% of the total enrollment excluding undeclared ethnicity status. We had a total of 380 students enrolled in the Engineering and Physics department and only 25 students were of African American/African descent. This represented only about 6.6% of the total number of students in the Engineering and Physics program at UCO. However, this was a significant increase from only 5 African American/African students in the spring of 2008 when I was college freshman. We have seen a gradual increase in the number of African American/African students in the UCO Engineering Physics Department in the past three years. UCO has over 200 student organizations with only 7 organizations in which African Americans are a majority. None of these 7 African American student associations have the promotion of science and engineering among African Americans and other minorities as their major objective.

Priscilla Paul
The goal of this project is to develop assays for the chemoenzymatic synthesis of heparin. Heparin currently comes from animal sources, mainly porcine intestine, and synthesizing heparin would increase availability as well as improve manufacturing safety. Heparin has been synthesized on the milligram scale thus far and the process is being scaled up to reach a goal of 1 kg. In the biosynthesis, N-sulfated heparosan, produced by E. coli fermentation, is modified by several O-sulfotransferases (OST) to produce heparin. 2-OST, 6-OST-1, and 3-OST-1 transfer sulfo groups from 3’phosphoadenosine-5’phosphosulfate (PAPS) to various positions of glucosamine respectively. Assay development is critical to the scale up process in order to minimize cost and maximize efficiency. In the past, OSTs have been radiometrically assayed for their activities including 2-OST, 3-OST-1, 6-OST-1, and 6-OST-3, however radiolabel assays are expensive and unsafe on an industrial scale. Therefore, alternative methods to determine sulfotransferase activity via colorimetric methods, Liquid Chromatography-Mass Spectroscopy, and Nuclear Magnetic Resonance are preferred and were explored in this project.

Deonte ’Thomas
The first excited state of 229 Th has the lowest energy of any known nuclear excitation, which has been indirectly determined to be 7.6 eV ± 0.5 eV. 229Th is produced by the alpha decay of 233U; this was discovered by the Kroger and Reich in using high resolution germanium detectors. My group, which studies low energy physics, has taken an exotic approach to search for the half life of 229Th. The setup involves a vacuum chamber pumped down to the order of 4 – 6 × 10^{-6} Torr. The chamber contains a 233U source electroplated on an aluminum plate and allows 229Th recoils to escape following the alpha decay of the source. The 233U source faces two parabolic shaped wire mesh grids, one behind the other. The back grid is biased positive to +5000 V to allow alpha particles of heavy ions to travel behind the grid and deflect only the low energy ions backward through a collimator towards a micro channel plate (MCP) detector, which can amplify a signal from a single internal conversion electron by a factor of 10^7. Usually, MCPs have an efficiency that is proportional to the open area of the exposed surface of the detector ~ 60%. The MCP was biased to about 3300 volts to attract the low energy electrons reflected off the catcher plate, that we want to observe. Utilizing state of the art data acquisition systems we are able to gate the detector signal and graphically visualize the decay curve of the electrons. The half life of the 229Th has not yet been observed, but there would be many life changing applications to the discovery. One of the most important
discoveries would be a nuclear optical clock and the enabling to search for a new fine structure changing in time.

**D’Anthony Ward**

Control of component life and reliability has continued to be an endeavor for NASA’s team of aerospace and materials engineers. Though complete control over any process or component is unlikely, striving for control will certainly be acceptable. In the design of a turbine disk, the process that is most acceptable is powder metallurgy. For this creates a more dense material, tightening the interstitial gaps in the planes of the material. This causes the microstructure to achieve higher levels of mechanical properties that would not have been possible using other forms of processes such as casting or hot forging. With this knowledge, NASA is creating a new disk with low solvus high refractory properties (LSHR). This super alloy is intended to withstand high temperature environments that exceed 600° C. Before this material can be used for its temperature applications, it must first prove that its normal and service fatigue life is acceptable. To gain knowledge of the fatigue life due to stress, NASA’s team of aerospace engineers use computational probabilistic software developed jointly by NASA and the Southwest Research Institute in San Antonio, Texas known as Numerical Evaluation of Stochastic Structures under Stress, or simply, NESSUS. NESSUS is used to evaluate the sensitivity that is most important to the outcome of a product. NESSUS is governed by rules of basic statistics and calculus and is used to simulate uncertainties in loads, geometry, material behavior, and any other user defined variables. For this study, it was used to determine the sensitive’s of stress given four variables that directly affect stress. The material properties of LSHR are similar to a titanium alloy. Using a general purpose FEA package ANSYS, allows the deconstructing of a complex system into elements. ANSYS implements equations that explain the behaviour of these elements and displays the results graphically. This creates a comprehensive explanation of how the system acts as a whole e.g. the effects of stress on a turbine engine disk. The material properties can be customized to include this LSHR material. After observing the results, the necessary steps can be made to make a judgment about of the reliability. The effort has been to identify four important design variables affecting this LSHR superalloy that would have the most influence on the stress that occurs on the disk. The variables are the density, modulus of elasticity, rotational velocity and Poisson ratio. Design simulator ANSYS, in partner with NESSUS, will capture uncertainties regarding stress levels and design factor significance and ultimately proving that the most critical factors are the rotational speed and the material density. The objectives of this report are to highlight the efforts in the reliability study of a gas turbine engine disk and determine the sensitivities of the stress to the design variables as it relates to the central tendencies, and the standard deviation.

**Dwight Williams**

In today’s society, people are falling ill to more diseases than ever before. We have to produce more efficient technology not to reduce the number of ill people, but to keep up with the rapid growth of people becoming affected by diseases. Many of these diseases, such as HIV and cancer, are detectable in microfluidics. Microfluidics is the category of the behavior of nanoscale fluids. DNA falls under this category. One of the most effective ways of analyzing DNA is through a technique called Polymerase Chain Reaction (PCR). The Sanger method of utilizing PCR has an extraordinary ability to produce numerous copies of accurate DNA sequences. A key element of Sanger DNA sequencing is thermal cycling. This is when a series of heating and cooling takes place for
DNA melting and enzymatic replication of the DNA. We hypothesize that using printed electronics instead of lithography, the PCR process can be more time and cost efficient. To test this, we used a Dimatix Inkjet Printer to create a resistive temperature detector (RTD). The purpose of the RTD on the bioprocessor is to constantly monitor the temperature of the reactor since the biological processes that take place there is highly temperature sensitive. The material that was chosen to construct the RTD with was gold. Gold is used instead of the traditional platinum or silver because gold is resistive to corrosion. Gold is also very malleable so it can be formed into any shape that we need it to. Gold also possesses a linear relationship between resistance and temperature which is needed to know the temperature that the reactor is at after possible heat dissipation. Multiple RTD’s were created using different parameters such as print height, print speed, and drop spacing. Varying these different parameters allowed us to analyze the best route in order to optimize the RTD. Our results yielded fully functional RTD’s. All of which had extremely similar coefficients of resistance.

**Megan Barnum**

Three Anterior Cruciate Ligament (ACL) bioscaffold prototypes are being investigated for use in tissue scaffolds for musculoskeletal tissue repair. The objective of this study is to design a bioscaffold prototype that is capable of withstanding extreme temperatures, 100 newtons of force and supporting chondrogenic cell differentiation. An initial prototype was constructed using a 3-D replica of an ACL made out of acrylonitrilbutadiene styrene (ABS). The prototype was then cast into a polydimethyl siloxane (PDMS) mold. The PDMS mold’s impact on the bioscaffold material was tested by injecting the bioscaffold material into the mold and observing it after freezing. Revisions were then made to the design of the prototype to include prototype B having an air channel and prototype C having an air channel and a pathway for scaffold materials to be injected. The 7%, 7mL bioscaffolds are composed of a 36:24:24:16 polymer blend ratio of poly l-lactic acid (PLLA): polyethylene glycol (PEG): hydroxyapatite (HA):beta-tricalcium phosphate (B-TCP). The scaffold material was analyzed according to optimal cell growth, pore size and durability; revisions are currently being processed.

**Kamal Ibrahim**

Stochastic processes are random systems that unfold over time. They are used extensively in diverse fields such as queuing theory and quantum mechanics. Both the time dependent behaviors of stochastic quantities like the average number of customers waiting in a queue or the wave function for a quantum well can be analyzed by using dynamical systems. The latter are deterministic models whose time dependent evolution is governed by an explicit set of differential equations. One problem in visualizing these dynamics is using a two dimensional screen to represent higher dimensional behavior. For our specific queuing and quantum examples we want to develop visual graphics that give a full sense of their dynamical behavior. Using a Java based graphical user interface; we can represent higher dimensional quantities such as time dependent behavior and complex valued functions through animation and color.

In our queuing model, we animate its trajectory through phase space. For periodic input, the notion of a static equilibrium point is replaced by a limit cycle. When dealing with our quantum well problem, we animate the square of the wave function amplitude. We simultaneously use color to identify the complex phase of the wave function at any given position or time.
ENGINEERING AND TECHNOLOGY:
Selected Manuscripts
Fabrication of a Gallium Nitride (GaN) Nano-Field Effect Transistor (nanoFET)

Amber C. Wingfield

Introduction
The complementary metal oxide field effect transistor (CMOSFET) continues to be an impacting device within the semiconductor industry. Known for its amplification and switch like capabilities, the MOSFET has great significance in logic circuits, which is central to the operation of computing systems. Components forming the MOSFET include a source, drain, channel, and gate, Figure 1. Miniaturizing such technology to the “nanolevel” has been of great interest to the industry. Since the beginning of the twenty first century, and even before, there has been a push in the semiconductor industry towards the miniaturization of devices. This miniaturization would lead to the compacting of more devices on a single chip, decreased chip size, increased speed and decrease overall component and finished product size. However using the prediction of Moore’s law with the present technology by the year 2020 the steadfast approach to miniaturization will reach its limitations. Nanotechnology, which some view as the new era in technology presents us with the possibility to surmount this barrier. Hence the focus of this research is to demonstrate the use of gallium nitride (GaN) nanowires to fabricate a C-MOSFET using the nanowire as the conducting channel.

Methodology
Fabrication of the nanoFET was initiated with a low resistant, highly doped silicon wafer, which was cut into 1 in² squares. These silicon wafers were then thermally oxidized in a quartz furnace at 1100°C. To form a layer of silicon dioxide (SiO₂) which will be used as the gate dielectric. The thickness of the grown SiO₂ layer was measured by ellipsometry.

For this process we used SiO₂ layer of thickness between 275-300nm. To form the gate contact a 150nm thick aluminum (Al) ohmic contact layer was deposited onto the backside of the silicon wafer by electron beam evaporation. Prior to this evaporation the backside of the wafer was cleaned with hydrogen peroxide to remove any extraneous oxide from the surface. To protect this layer during subsequent fabrication processes, a layer of photoresist was spun onto the surface and baked for 30mins. Employing photolithography, the source and drain electrodes for the nanoFET were defined atop the SiO₂ layer. We then evaporated 150nm of aluminum, or gold, over this surface. A lift off process was then used to form the source and drain contacts. The mask used in this work was specifically designed to maximize the probability of getting nanowires across the source and drain.
The GaN nanowires used were grown in house and measured roughly 50nm in diameter and 10-100µm in length. The GaN nanowires were suspended in toluene to aid in their dispersion across the wafer when applied. Using a syringe, the solution was dropped onto the wafer surface where many source-drain contacts were. The solvent was allowed to evaporate, and the sample was then examined under an optical microscope and a scanning electron microscope to confirm nanowire placement on source-drain contacts.

**Results:**

With many source-drain contacts on each wafer, only a few nanowires landed correctly across the source and drain. Of those which did, electrical contact was not made by testing with an I-V curve tracer. After applying a large voltage across the contacts, some devices began to conduct current which served to burn-in the metal-nanowire interface which may have been contaminated by metal and/or nanowire oxides or toluene residue. This is the main reason why gold was substituted for aluminum.

**Conclusion:**

Oxidation rates for SiO₂ were determined and gate oxides were successfully grown. I-V measurements confirmed the existence of a source-drain channel in the nanowire. Although we were unable to modulate the nanowire channel in our devices, we were able to fabricate GaN nanoFETs using conventional fabrication processes. Although the method of depositing nanowires by dropping them in solution needs improving, nanowire were deposited this way. Perhaps a different solvent would help. To insure nanowires-oxide contact in the future, the source and drain spacing could be widened to allow the nanowires to hang down and the thickness of the source-drain contacts could be made thinner.

**Acknowledgements**

Being a part of this research experience has been like no other. Thus, I would like to thank Dr. Gary Harris, Dr. William Rose, and Mr. James Griffin for all their help, support, and advice. I as well would like to spread gratitude.
to the National Science Foundation (NSF) and the National Nanotechnology Infrastructure Network (NNIN). A last, but certainly not least thanks goes to the entire HNF staff for all their support.

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Detecting Sites of Modification in 3-Nitrotyrosine-Modified Proteins
Deneen Gaynor

INTRODUCTION

The immune system has a unique mechanism of defense against pathogens; however, there is a body of evidence that the immune system of the aged is eroded, a phenomenon termed immunosenescence. There is a growing interest in immunosenescence and how it may contribute to increased number of pathologies such as: Parkinson’s disease, Alzheimer’s disease, and fragile X syndrome with aging. Oxidative stress is thought to be implicated in immunosenescence [1].

Release of reactive oxygen species that consist of oxygen free radicals and other chemical entities can result in the development of oxidative stress in the body, though the exact mechanism is still unknown. Oxygen free radicals can be generated in elevated quantities during the reduction of oxygen and lead to cell injury. Reactive oxygen species (ROS) can involve superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite [2].

This research focuses on reactive nitrogen species and in particular peroxynitrite (ONOO−). These reactive species readily react with tyrosine residues in proteins (in vivo) to form 3-nitrotyrosine (see figure 1.)

\[
\text{HOO} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{O}}{\text{R}} \quad \overset{\text{RO}}{\text{O}} 
\]

\[
\text{HOO}_2 \quad \overset{\text{O}}{\text{O}} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{H}}{\text{O}} 
\]

\[
\text{RO} \quad \overset{\text{H}}{\text{O}} \quad \overset{\text{OH}}{\text{RO}} 
\]

Nitrotyrosine has been suggested and used as a biomarker for diagnosis of oxidative stress-induced pathological conditions [4]. In previous studies, identification and quantification of nitrotyrosine has involved measuring free nitrotyrosine in plasma or through protein nitration and applying various detection methods, such as, (LC-UV), 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE), matrix-assisted laser desorption/ionization (MALDI) peptide mass mapping, electrospray ionization (ESI) mass spectrometry and gas chromatography mass spectrometry (GC-MS) [5-16]. However, these methods only provide an overall level of nitration and no specific protein identification can be obtained.

In this study, a protocol was developed to successfully nitrate β-lactoglobulin using peroxynitrite. The method was based on one developed by Abello et al [14]. LC-MS/MS along with manual data analysis techniques were used to quantify nitration success.

MATERIALS AND METHODS

Synthesis of Peroxynitrite

Peroxynitrite was synthesized using a modified protocol from the paper by Uppu et al [3]. A solution of 30% (~9 M) H₂O₂ (22ml) was diluted to a final volume of 50 ml with water and chilled to about 4°C in an ice/water mixture. The above solution was then added to 40ml of 5M NaOH and 5ml of 0.04M DTPA in 0.05M NaOH, mixed gently, and diluted to a final volume of 100ml with water. The final concentration of H₂O₂ in the solution was approximately 2 M with a pH of 12.54. An equimolar amount of isoamyl nitrite (0.02 mol, 27ml) was added to the solution and stirred vigorously for 3 hours at room temperature. Once the yield of peroxynitrite reached a maximum, the aqueous phase was washed 6 x 2 vol of hexane to remove the contaminating isoamyl
nitrite and isoamyl alcohol. Unreacted H$_2$O$_2$ was then removed by passing the aqueous phase through a 1.5 x 10 cm column filled with 25g of 99% pure granular MnO$_2$. The MnO$_2$ column was washed with 20ml each of water and 0.1-0.5M NaOH before use. To minimize dilution of the peroxynitrite in the aqueous phase, the first few milliliters eluted from the column were discarded.

**Sample Preparation**

Bovine β-lactoglobulin (1mg), from Sigma-Aldrich, in 0.675ml of phosphate buffer saline was nitrated using 0.125ml, a final concentration of 250 mM of peroxynitrite. After quick vortexing, the sample was incubated for one hour at room temperature and then snap-frozen in liquid nitrogen until further use.

**Protein Digestion**

The protein was denatured by first adding 2.42 µl of 30.47mM dithiothreitol (DTT), from Fisher Scientific, to break the disulfide bonds within the folded protein conformation. This was incubated for 2 hours in a 37°C warm water bath. Next, 4.84 µl of 51.9 mM of iodoacetamide, from Acros Organics, was added to bond to the free thiols for 2 hours, on ice, in the dark. To quench the reaction, 2.42µl of 31.36 mM L-cysteine from Sigma-Aldrich, was added to react with any excess iodoacetamide for 30 minutes at room temperature. Lastly 2% (w/w) trypsin/protein was added and incubated at 37°C. Trypsin was used to cleave the protein into smaller peptides at the arginine and lysine residues and after 24 hour incubation period the samples were snap frozen in liquid nitrogen to stop reaction.

**Sample Clean up**

Samples were thawed to room temperature and C18 30 mg HBL-cartridges were used to clean the samples. The column was first conditioned using 1ml of acetonitrile 0.1% formic acid then 1.5-2ml of 0.1% formic acid in water. Protein samples were then eluted from the column at a rate of about 1ml/min. Next, 1ml of 60:40 acetonitrile/water solution was eluted through the column as well as 250µl of 100% acetonitrile. Both were collected into a small eppendorf with a total product volume of approximately 1250µl.

**Sample Preparation for Analysis**

After clean up, all samples were dried using the SPD1010 speedvac system and suspended in 0.1% FA/water. All samples were diluted to a concentration suitable for the ORBI Trap Velos with total protein mass of approximately 250 ng in 5 µl.

**Instrumentation**

Samples were loaded onto the NanoLC with a flow rate of 3ul/min injection. The samples had a volume of 5ul of a 50ng/µl sample, making 250ng total protein loaded onto the mass spectrometer. Samples were run on the LTQ Orbitrap Velos (pictured below) with a 75 minute gradient where buffer A, a 0.1% FA/H$_2$O solution and buffer B, a 97% ACN/0.1%FA/ H$_2$O which was eluted through the liquid chromatography column at different concentrations described in table 1.
Table 1. 75 minute gradient run

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Flow rate</th>
<th>%B</th>
<th>%A</th>
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<td>0</td>
<td>100</td>
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<td>75</td>
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<tr>
<td>75</td>
<td>300nl/min</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Data Analysis

A hypothetical digest of the β-lactoglobulin was done, *in silico*, using the Protein Prospector program developed by the University of California, San Francisco. The hypothetical digest was then compared to the actual fragmentation of the peptides done and the MS/MS data obtained from proteome discoverer, a mass informatics software.

Results and Discussion

The protein sequence of bovine β-lactoglobulin is shown below in figure 2 highlighting tryptic digest cleavage and tyrosine residues.

![Protein Sequence](image)

Fig. 2. Highlighted Y’s are tyrosine residues that can readily be nitrated, underlined K’s and R’s are lysine and arginine residues, respectively, where trypsin cleaves the protein.

After the hypothetical digest and comparison with the actual digest, a list of nitrated peptides was made. The list includes nitrosylation; addition of an NO group, nitro addition (NO₂), and amino (NH₂) nitration all on the tyrosine residue. However, the focus of the research was on the nitro addition. Each item on the list was within a ±10ppm error range, a form of nitration, and had a probability of 80 or more; this list can be seen in table 2. Once theoretical digestions were compared the real sample data was analyzed using LC-MS/MS data. Below in figure 3 is an example of the chromatograph of all peptides being eluted from the liquid chromatography column. MS data was then analyzed to confirm peptide nitration has been achieved. An example of this form of analysis is shown in figure 4 where the base peak chromatograph of the nitrated peptide “VYVEELKPTPGEDEILLQK,” with a theoretical m/z value of 590.31684, being eluted into the ORBI trap over a 70min span showing that at 20.20 minutes the most amount of this specific peptide is being eluted. This chromatograph is a representative of the fact that the m/z value 590.31 is confirmed as an actual peak, proving that the nitrated peptide VYVEELKPTPGEDEILLQK was identified.

To further confirm that nitration occurred, the parent ions were fragmented, *in silico*, using protein prospector, and mass to charge (m/z) values were calculated. Once calculated, these m/z values were matched with peaks taken from the corresponding chromatographs to confirm that the peptide was correctly identified. An example of this is shown in table 3 with the peptide VLVLDTDYYKK. Here an m/z value for each amino acid in the sequence was identified, giving full sequence coverage of the peptide. This confirmation process was done for all 3NT modified peptides.

Conclusion

The goal for this experiment was to effectively nitrate the protein β-lactoglobulin through the use of peroxynitrite. Results were compared to database searching techniques, LC-MS/MS and manually going through MS/MS data, which confirmed nitration occurred.

For the future, other proteins such as Bovine Serum Albumin or Cytochrome C can be nitrated to improve the effectiveness of the nitration technique. Other experiments for the future are to use a chemical labeling approach designed to allow
enrichment of tyrosine-nitrated peptides using the protocol developed by Abello et al.

ACKNOWLEDGMENTS

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Practical Investigations of Digital Forensics Tools for Mobile Devices
Maynard Yates

ABSTRACT
With the continued growth of the mobile device market, the possibility of their use in criminal activity will only continue to increase. While the mobile device market provides a great variety of manufactures and models causing a strong diversity. It becomes difficult for a professional investigator to choose the proper forensics tools for seizing internal data from mobile devices. Through this paper, we will give a comprehensive perspective of each popular digital forensic tool and offer an inside view for investigators to choose their free sources or commercial tools. In addition, a summary for the future direction for forensics tools in mobile devices.

INTRODUCTION
Advancements in technology over the last 20 years have drastically altered the way we live and do business. The continued evolution and development of mobile device technology will increase the need for security protocols and forensics of these devices. Technology has permeated almost every aspect of society from the way we communicate to the way information is discovered about a particular subject. A few examples of these changes are:

- **Correspondence**: Postal mail → Electronic mail (E-mail) → SMS messages (text messages)
- **Telecommunications**: Telephones → car powered cell phones→ battery powered cell phones
- **Calendar**: Secretary → Day Planner→ Personal Data Assistant (PDA)→Smartphone’

As technology continues to permeate society and mobile computing becomes more prevalent, people will more heavily depend on applications such as e-mail, SMS (Short Message Service), MMS (Multimedia Messaging Service) and online transactions (i.e. bank, ins, etc); such devices provide a good source of evidence for forensic investigators to prove or disprove the commitment of crimes or location of suspects/victims [6].

Digital forensics for handheld devices is starting now. Unlike traditional computers, two important factors that must be accounted for in a forensic investigation are the state of the device at the time of acquisition and radio isolation. Traditional digital forensics with personal computers allows an investigator to perform a dead forensic data acquisition simply by disconnecting the power source to preserve the current state of the computer. That option is not available with mobile forensics for fear of loss of evidence or security mechanisms, such as device locks or passwords, being activated [15]. The fact that various operating systems are used for different mobile devices in current markets makes development of digital forensics tools for mobile devices more complicated.

This paper is being proposed to survey available digital forensics tools for capturing e-evidence from mobile devices and meet the demand of e-evidence for current and future’s crimes. This paper focuses on practical investigations for digital forensics tools that will help investigators or students obtain first-hand experiences in digital forensics for mobile devices. Investigators should be able to perform their job more informed as a result of this case study.

This paper is organized as follows: section 2 will discuss the popular operating systems for mobile devices, while section 3 will discuss tools available for forensics of mobile devices. Section 4 will discuss related work; section 5 will discuss how this case study will be carried out, followed by conclusion in section 6.
I. OPERATING SYSTEMS
Compatibility with a tool is based upon the mobile device’s operating system, but how to determine compatibility with rapidly developing technology is a challenge. There are open-source operating systems as well as proprietary, each with own unique features. This paper will examine four of the most popular mobile device operating systems.

A. Android
Android OS [3] relies on the Linux 2.6 kernel, which acts as an abstraction layer between the hardware and the rest of the hardware stack. The Linux kernel provides access to core services such as security, memory management, process management, network stack, and driver model. It also provides support for the Dalvik virtual machine’s functionality, such as threading and low-level memory management.

Libraries are the next layer up, and are divided into the Android Runtime library and application libraries. Written in JAVA, the Android Runtime Libraries consists of the Dalvik Virtual Machine (VM) and the core libraries that provide the available functionality for the applications. Each time an Android application is launched, it runs as a separate process and instance of the VM. Android can run multiple instances of the VM efficiently.

The Applications Framework layer builds on the advantages that the Android operating system is open source and open platform. This framework was designed to simplify the reuse of components as developers are given full access to the same framework APIs used by core applications. Any application can publish its capabilities and any other application may then make use of those capabilities (subject to security constraints enforced by the framework). Listed below are the core set of services and systems that support open development:
- A rich and extensible set of Views that can be used to build an application, including lists, grids, text boxes, buttons, and even an embeddable web browser
- Content Providers that enable applications to access data from other applications (such as Contacts), or to share their own data
- A Resource Manager, providing access to non-code resources such as localized strings, graphics, and layout files
- A Notification Manager that enables all applications to display custom alerts in the status bar
- An Activity Manager that manages the lifecycle of applications and provides a common navigation backstack

The top layer, Applications, consists of email client, SMS program, calendar, maps, browser, contacts, and other JAVA applications as depicted by Figure 1.

Figure 1 Android OS Model

B. iPhone
The iPhone operating system derives from Mac OS X desktop operating system with the 3 base layers being ported over from the OS X architecture to the iPhone OS. iPhone OS [4] is a UNIX based operating system by virtue of sharing the Darwin Foundation from OS X. The iPhone OS has four layers: the core OS, core services, media, and Cocoa Touch, a variation of OS X Cocoa layer with added multi-touch functionality for the iPhone, depicted by Figure 2. The bottom two layers, Core OS and Core Services, contain the fundamental interfaces for iPhone OS, including those used for accessing files, low-level data types, network sockets, as well as access to POSIX and UNIX sockets among others.
The next layer, Media, contains the fundamental technologies used to support 2D and 3D drawing, audio, and video such as Open GL, Quick Time, an audio & image viewer, Core Audio and Video. The top layer, Cocoa Touch, provides the fundamental infrastructure used by iPhone OS. Figure 3 shows that the Cocoa Touch layer has been divided into an application and application framework layers.

Two major components of Cocoa Touch are the Foundation framework in the Core services layer and the UIKit in the Application Frameworks division of the Cocoa Touch layer. The Foundation framework provides support for file management, network operations, collections, and more. The UIKit framework provides the visual infrastructure for your application, including classes for windows, views, controls, and the controllers that manage those objects. However, there are other frameworks available at this level that gives you access to user’s contact and photo information and other features of hardware for an iPhone.

C. Blackberry
Canadian company, Research in Motion (RIM), created the Blackberry phone that was originally geared towards business professionals as a way to stay connected while traveling. The Blackberry OS [9] that powers Blackberry phones is a proprietary system, with little information about it publicly. What is known, as depicted by Figure 4, is that like the Android, the Blackberry runs through a JAVA virtual machine.

The hardware level is accessed through the RIM JVM through standard JavaME and Mobile Data Service (MDS) applications. There are 2 runtime environments in the operating system: Proprietary and MDS. The proprietary runtime environment contains the main RIM APIs (memo, calendar, Bluetooth, etc.) as well as the JAVA applications that contain profiles, configurations and optional packages for specific functionality, and services such as the Blackberry Desktop Manager. Mobile Data Service (MDS) focuses mainly on web and enterprise services. MDS is the runtime container for processing pushed data, such as email as depicted below in Figure 5.

D. Windows Mobile
Conceptually similar to the iPhone OS, Windows Mobile [11] is a Windows OS for mobile devices. They are structured similarly, with some of the same protocols in regards to user info and activities such as registry entries, files, and web activities (web browsing, recently connected computers, WiFi access points), but there are substantial differences that distinguish Windows Mobile from Windows OS. While Windows has 2 diff types of file systems, NTFS & FAT, Windows Mobile uses a variation of the FAT file system called Transaction-Safe FAT, which has some recovery features in the event of sudden shutdown.

There are currently four different family types of processor cores in Windows Mobile, ARM (most
common), MIPS, and SH4 and x86. There are 2 different types of flash memory, NOR and NAND. NOR has a RAM-like interface; it has a data bus, an address bus and control lines. NOR flash is mapped in the processor’s memory map and processor code can be executed directly from it (this is called ‘execute in place’; XIP). NOR flash can also be used as storage location for user data. NAND flash can be regarded as the solid state equivalent of a hard disk. It has an interface with an I/O bus and control lines connecting the memory chip to the processor. Over this I/O bus, commands, addresses and data are sent. As NAND flash memory is not mapped in the memory space of the processor, code stored in a NAND flash chip cannot be executed directly, but has to be loaded into RAM first, again much like a hard disk. [12]

**E. Symbian**

The Symbian system [10] architecture has three layers, but each layer contains packages, which consist of collections of components as depicted by

<table>
<thead>
<tr>
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<th>1:n</th>
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<tbody>
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</tr>
<tr>
<td>Collection</td>
<td>1:n</td>
</tr>
<tr>
<td>Component</td>
<td></td>
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![Figure 6. Symbian Decomposition Hierarchy](image)

Figure 5 Decomposition Hierarchy for the Symbian OS

- Layers contain packages with no static upward dependencies between layers. A package may depend on other packages in the same layer or in any lower layer.
- Packages are modular collections of components, owned and maintained by a single organization (although contributed to more widely).
- Component collections are used to organize the components within a package. All components are aggregated into component collections. A collection should be formed even if there is a single collection in the package.
- Components contain the files needed to build and test at least one target file. Components implement programming interfaces.

The 3 layers of the Symbian OS device platform:
- Application
- Middleware
- OS

The Application layer primarily implements interactive UI applications, such as the organizer application suite, multimedia applications, network applications, device settings, etc. Many of the applications provide interfaces to allow their functionality to be accessed by another application program, or to support extensibility or customization.

The Middleware layer provides APIs that are typically useful for multiple programs in the application layer. A middleware layer component is independent of the hardware platform and its APIs are not used by the operating system (OS) layer. It provides access to services, such as messaging, multimedia, and web & IP services.

The OS layer abstracts the hardware platform and contains lower-level APIs that are used within the OS layer. This layer defines plug-in interfaces (HAIs) for components that implement hardware adaptations. The OS layer device driver framework includes the API that is available to kernel-mode software (which mostly consists of device drivers).

**II. FORENSIC ANALYSIS**

Unlike traditional computers, two of the most important factors in a forensic investigation are the state of the device at the time of acquisition and radio isolation.[13] Legally, you can establish the chain of custody and document the entire investigation; but in order to perform an acquisition an investigator must take into account the state of the device whether it is On/Off. Regardless of device, an investigator should be aware of these issues.

**A. Mobile Device Analysis**

There are issues to consider about each operating system as an investigator prepares to forensically analyze a mobile device. There are two types of cell phone networks: code division multiple access (CDMA) and time division multiple access
CDMA uses a spread spectrum technology using a special coding scheme thereby allowing multiple digital signals on the same channel, making CDMA less costly to implement, more efficient, and more secure than other cell phone network technologies. CDMA devices operate on variants of the 2G network standards, CDMA 1X, CDMA1XEV-DO (evolution data optimized), or CDMA1X EV-DV (evolution data voice). They also possess the following characteristics:

- **Electronic Serial Number (ESN):** This number is found on the compliance plate located under the phone battery and can be displayed as ESN DEC, ESN HEX, ESN or D. The ESN is a unique 32 bit number assigned to each mobile phone on a network. You will note that the ESN in its decimal format contains only decimal numbers, distinguishing it from its ESN HEX equivalent which will contain both decimal and alpha characters.

- **Mobile Equipment ID (MEID):** This number is 56 bits long, replacing the originally used ESN, because of the limited availability of the 32 bit ESN numbers.

- **May or may not use a Subscriber Identity Module (SIM)**

TDMA, which is better known by its variants Global System for Mobile Communication (GSM) & Integrated Digital Enhanced Network (iDEN), uses an allotted radio channel divided into time slots; allowing each time slot to handle one call. Devices that operate on a TDMA network require a SIM card, or just SIM, for the device to operate. GSM is the largest mobile phone network in the world operating on 5 different bandwidths on most continents. GSM devices originally derived from 2G network standards, like CDMA, but now operate on these variants GPRS (General Packet Radio Service), EDGE (Enhanced Data Rates for GSM Evolution), GSM and HSPA (High Speed Packet Access) with the following characteristics:

- **International Mobile Equipment Identifier (IMEI)** – this is a unique 15 digit code and used to identify a GSM cell phone to its network and is found on the compliance plate. This code also code identifies manufacturer, model type, and country of approval of a handset.

- **Subscriber Identity Module (SIM):** There will be at least one slot for this card usually found under the battery panel. The face of this card may also contain the name of the network to which the SIM is registered to.

- **Integrated Circuit Card Identification (ICCID):** This is a 18 - 20 digit number (10 bytes) imprinted on the face of the SIM. This number uniquely identifies each SIM. This number is tied to the IMSI which is associated to the IMEI when a handset is registered to a GSM network.

- **International Mobile Subscriber Identity (IMSI):** This number is typically a 15 digit number (56 bits) that consists of three parts, stored electronically in the SIM:
  - Mobile Country Code (MCC)
  - Mobile Network Code (MNC)
  - Mobile Station Identification Number (MSIN)

iDEN is a proprietary variant of TDMA/GSM network developed by Motorola. iDEN devices have the same characteristics of a GSM device with the additional ability of push-to-Talk, a two-way radio system.

- **Direct Connect Number /Radio-Private ID/MOTOTalk ID/iDEN Number:** iDEN use a number based on the following format for communicating device-to-device: 012*345*67890.
  - The first three digits (012) make up the Area ID (region of your home carrier’s network).
  - The next three digits (345) define the Network ID (specific iDEN Carrier such as Nextel, SouthernLink, Nii, MIKE/Telus, etc.)
  - Last five digits determine the Subscriber’s ID (personal number from home carrier’s network, sometimes the last five of the phone number).
  - The asterisk (*) is also part of this Direct Connect Number used as a separator to divide each of the aforementioned parts

- **Subscriber Identity Module (SIM) is required by most GSM to successfully boot the phone.**

SIM is a smart card for all intents and purposes
containing a 16-128KB electronically erasable programmable read-only memory (EEPROM).
The SIM is assigned the cell phone number from the network which is tied to its ICCID, IMSI number as well as the IMEI number of the handset. [ # ]

Currently there are currently two types of data acquisition: logical and physical. Logical acquisitions are the most common type of acquisition and are performed with software such as Encase, FTK, etc. while physical acquisition allows you low level access to the device bypassing the device’s security mechanisms.

1. **Android**

   Android OS is relatively new compared to other operating systems, initially released in the latter part of 2008. Over the next two and one-half years, there would be six releases/updates to the Android OS which makes it harder to develop standards for data acquisition or forensic tools. In order to perform an acquisition an investigator must gain access to the OS memory through a process termed rooting. What rooting means is gaining access to the root directory (/) and having the appropriate permissions to take root actions within the device’s OS. Some devices that run Android OS may require a third party application to be installed on the mobile device to root the device. However, this would make any data recovered inadmissible in court making an alternative way a priority. Once you have gained root access, the Android SDK shell will need to again be launched. Once in the shell, the dd command can be used to image the memory files using a command line interface. Although it may vary on the device, but the file system is stored within the /dev/mtd directory in the following are of interest:
   - mtd0 handles miscellaneous tasks
   - mtd1 holds a recovery image
   - mtd2 contains the boot partition
   - mtd3 contains system files
   - mtd4 holds cache
   - mtd5 holds user data

2. **iPhone**

   iPhone OS has 3 primary domains: System, Local, & User; which correspond to the Core OS, Core Services, and the Application sub layer of the Cocoa Touch layer. The System domain contains all Apple installed software while on the contrary the User domain consists of all user files. The local domain includes the libraries and applications directories as well as OS specific applications. An investigator has to consider any possible options, which may include checking the iPhone firmware, mounting the iPhone in a Linux environment, or using the Apple Filing Protocol (AFP) to access the file system among other options.[13]

3. **Blackberry**

   Unlike other operating systems, Blackberry has an extra option for messaging: PIN to PIN. Each Blackberry device is uniquely identified by an eight alphanumeric combination, known as a PIN. These messages are encrypted and can only be decrypted by another Blackberry device. PIN messages are not attached or associated with an email address resulting in different communications protocol from SMS and MMS messages which use TCP/IP and are not encrypted. Examining a Blackberry device, an investigator is required to create an interactive pager backup (IPD) file that contains a backup of the Blackberry device database. Any Blackberry device can be analyzed if not pin-protected. [13]

4. **Windows Mobile**

   There isn’t a preferred or standard method to forensically acquire data from a device with Windows Mobile OS.[10] Neither type of acquisition, logical or physical, will recover all relevant information from the mobile device. While a logical acquisition is better when acquiring active data, there is still a risk because RAM and flash memory is overwritten to establish an ActiveSync connection. ActiveSync is developed by Microsoft used to forensically acquire data from Windows Mobile O/S. It requires a dedicated .dll file that can be loaded onto the device under investigation. Logical acquisitions only provide a file system view only allowing an investigator to image the unallocated clusters that reside in active flash pages. Other disadvantages are that deleted data could be erased beyond recovery, expired flash blocks that still contain data won’t be imaged, Active Wear Leveling, and Garbage Collection in the RAM. However, in some instances when a device is broken beyond repair or there is no standard interface for forensics, a logical
acquisition is not possible and a physical acquisition would be the next best option.

5. **Symbian**

Version 9.1 of Symbian’s S60 platform [18] implemented a new security model that made it impossible to preserve the present state of a device running Symbian OS during forensic acquisition. This made it harder for an investigator to get a full memory image of the device and have to resort to methods similar to forensic acquisition on Windows Mobile Devices. Similar to Windows Mobile, it uses a connection agent but only if it is certified and properly signed. No open source tools are allowed due to the new security model.

B. **Logical Acquisition**

Logical acquisitions are those where forensic tools, commercial or open-source, are used and nothing is physically removed from the mobile device. The amount of extracted data varies with each individual device, but will usually include SMS, MMS, call registers, videos, pictures, audio files and calendar entries and tasks. Some forensic software will also include information that properly identifies the mobile device, such as make, model, ESN number off of the compliance plate, etc.

1. **Internal Memory**

What actually happens during a logical acquisition is that the forensic tool being used requests data from the phone, to which the phone responds and returns the requested data when it is available. [ref #] Once a device that’s part of an investigation is properly seized, the device’s state determines the next step. Whether the device is on or off is very important because if it is still on, then an investigator can perform an acquisition with their tool of choice. However, if the device is off, it needs to stay off to prevent any changes to the device’s memory and also prevent the device’s security mechanisms to activate.

Each OS [#] has different things to consider or require extra software when performing acquisitions for investigations:

- Android OS devices will need to be rooted
  - permissions will need to be given to the investigator for them to be able to take actions in the root directory of a device’s memory
- iPhone OS
  - which may include checking the iPhone firmware
  - mounting the iPhone in a Linux environment
  - using the Apple Filing Protocol (AFP)
- Blackberry OS requires an investigator is required to create an interactive pager backup (IPD) file that contains a backup of the Blackberry device database. It also recommends the having the following at an investigator’s disposal
  - Blackberry Desktop Manager - this tool is used to create the IPD file as well as restoring the IPD file into a BlackBerry simulator
  - Blackberry Simulator - specific to the model you are examining; allows the evidence IPD file to be viewed in a virtual environment
  - Software that can parse the IPD file and allow export to various formats
  - Forensic Analysis Tool – Device Seizure, Encase, FTK, etc

When forensically analyzing a CDMA phone the internal memory is the main priority because unlike a GSM phone where SIM cards are required, it may or may not use a SIM card.

2. **SIM**

SIM [ # ] analysis is pertinent in the investigation of GSM/idEN/hybrid CDMA/GSM devices because they can be placed in any unlocked device on the GSM network and can contain the following information that could be treated as evidence:

- Last Number Dialed (LDN)
- Phonebook/Contacts (ADN)
- Text Messages (SMS), including deleted text messages
- Location information (LOCI) from position of last usage
- Service Related Information

However the device state determines whether the SIM is analyzed first or last. If the device is on, SIM is analyzed after internal memory; otherwise, it is analyzed first. Ideally the originally SIM should be cloned and use the cloned SIM inside the
device during forensic analysis. The cloned SIM will not allow network access but will act similar to the original SIM. It is important to note that deleted SMS messages can only be extracted from SIM when it is not in a mobile device.

Taking these things into consideration will help the investigator to get the most out of each acquisition because these considerations cater to each individual operating system.

C. Physical Acquisition

Physical acquisition is considered as a last resort because it involves a greater risk of damaging evidence on the device. Information retrieval can be achieved by either physically removing the flash memory chip, using a bootloader, or JTAG to extract data. [10] These methods all bypass any device security mechanism which gives you direct access to a device’s memory. Through physical acquisitions, you view memory as hardware components view it, in its rawest form. Although you view the memory in its rawest form, the advantage is that you have limitless access to the phone’s memory and not bounded by operating system restraints.

However to perform a physical acquisition, it requires special hardware outside of the forensic software an investigator may already have and then that investigator needs to be properly train doing such acquisitions. The following methods can be used:

1. Removing the memory chip from the device’s circuit board
2. Access the JTAG access points, if they are any, to access the data.

After the data has been retrieved, a binary file known as a permanent memory (PM) file that would need to be processed to a easier, more recognized, and readable format so that an investigator may be able to recover all data, viewable and deleted.

From an investigative point of view, a physical acquisition is more relevant to those mobile operating systems where a connection agent is used for a logical acquisition, such as Windows Mobile and Symbian, because it maybe more advantageous to perform a physical acquisition instead. Using a connection agent to perform a logical acquisition will limit the amount of data you retrieve from the device because of the operating system constraints. Windows Mobile uses Microsoft ActiveSync, which requires a dedicated .dll file that can be loaded onto the device under investigation. Logical acquisitions only provide a file system view only allowing an investigator to image the unallocated clusters that reside in active flash pages. Other disadvantages are that deleted data could be erased beyond recovery, expired flash blocks that still contain data won’t be imaged, Active Wear Leveling, and Garbage Collection in the RAM. Unlike Windows Mobile OS, Symbian OS uses a connection agent also but only if it is certified and properly signed; no open source tools are allowed due to the new security model.

While this is a last resort for other operating system, it may be a viable option for those types of systems because you would have to perform both logical and physical acquisitions to get as much information as you can. However, in some instances when a device is broken beyond repair or there is no standard interface for forensics, a logical acquisition is not possible and a physical acquisition would be the next best option.

D. Summary

Considering these issues and taking them into preliminary consideration will help the investigator determine what needs to be done on site at the crime scene and what needs to be taken back to their labs for analyzing. Understanding how each operating system interacts with each tool will benefit an investigator to conduct a more efficient forensic analysis.

III. DIGITAL FORENSICS TOOLS

The convenience of mobile computing has become frustrating for the forensic community because it is harder to build tools that can be considered industry standard. Unlike computers, technologies for mobile devices are constantly advancing faster than any other technology. Device are advancing so quickly, that development for tools are not able to keep up because there are some drastic differences between forensics of computers and mobile devices as describe in the table below.

A. Digital Forensics tools for Computers

Forensics for computers is easier and less complex in comparison to mobile devices. Computers have
two types of memory: Random Access Memory (RAM), or secondary or volatile memory, and Read Only Memory (ROM), or primary memory. A mobile device only has one, RAM, unless a SIM card is present then the SIM card functions as ROM. The most popular operating systems for personal computers are: Windows, Mac, and UNIX, but there is a variety of manufacturers that produce mobile devices: RIM, Apple, Symbian, Palm, etc. just to name a few. Table 1 shows some of the differences [6]:

Table 1 Forensics of Computers versus Forensics of Mobile Devices

<table>
<thead>
<tr>
<th>Issues</th>
<th>Forensics of Computers</th>
<th>Forensics of Handheld Devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>On/off dilemma</td>
<td>Less problematic</td>
<td>More problematic</td>
</tr>
<tr>
<td>Evidence volatility</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Imaging process</td>
<td>Less tricky</td>
<td>More tricky</td>
</tr>
<tr>
<td>Size of evidence</td>
<td>Larger</td>
<td>Smaller</td>
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<tr>
<td>Technological development</td>
<td>Slower</td>
<td>Faster</td>
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<tr>
<td>Operating systems</td>
<td>Less problematic</td>
<td>More problematic</td>
</tr>
<tr>
<td>Training</td>
<td>Clear</td>
<td>Unclear</td>
</tr>
<tr>
<td>Forensic tools</td>
<td>More proprietary tools</td>
<td>More open source tools</td>
</tr>
</tbody>
</table>

B. FTK Mobile Phone Examiner

FTK Mobile Phone Examiner (MPE) [2] is the most commonly used forensic tool for mobile devices in the US, a distinction shared with Guidance’s Encase Forensic suite. Mobile Phone Examiner can be used as a standalone application or as a fully integrated part of Forensic Toolkit (FTK) interface. Using MPE affords the investigator the option of a quick and easy field acquisition via cable, Infrared, or Bluetooth connection without altering data on the device, which is essential in establishing court admissible evidence. When integrated with FTK, MPE can take advantage of leading technology validated by courts and organizations such as Securities & Exchange Commission (SEC), Federal Bureau of Investigations (FBI), and the Internal Revenue Service (IRS) just to name a few. This integration would allow MPE to perform forensic analysis on multiple phones simultaneously within the same FTK interface as well as manipulate that data for easy interpretation. Reports produced by the integrated suite, which are instantly ready to be used as evidence in court, include both phone and computer analysis which allows an investigator to easily correlate data from a mobile phone to evidentiary data from a computer or another phone.

C. Oxygen Forensic Suite

Oxygen Forensic Suite [7] is the tool of choice for many agencies in Europe, serving law enforcement, tax and customs, government authorities in Great Britain, Germany, Australia, Sweden, and Finland among others. Oxygen prides itself on its reputation of being able to extract unique information from a smartphone such as phone basic information and SIM-card data, contacts list, caller groups, speed dials, missed/outgoing/incoming calls, standard SMS/MMS/E-mail folders, custom SMS/MMS/E-mail folders, calendar events schedule, tasks, and text notes. However the features are not truly unique as all three tools can extract this information. However Oxygen’s ability to tap into the LifeBlog and geotagging in Symbian OS in nokia phones gives it an advantage over its competition. Unlike MPE or Device Seizure, a special agent application is used to perform forensic analysis combining the advantages of both logical and physical data acquisitions.

D. EnCase Neutrino

Guidance Software has become an industry leader on the strength of its product EnCase Forensic software, aside from AccessData’s Forensic ToolKit (FTK), and has over 30,000 licensed users of EnCase®. Its customer base includes more than 100 of the Fortune 500 and over half of the top 50, including: Allstate, Chevron, Ford, General Electric, Honeywell, Mattel, Northrop Grumman, Pfizer, UnitedHealth Group, Viacom and Wachovia. As a complement to their award winning, industry leading forensic solution, EnCase Neutrino [5] is designed to provide the same technology and foundation for forensic investigations for mobile devices. Amidst all the
wireless signal blocking technologies, EnCase boasts a claim that the WaveShield technology used in EnCase Neutrino is the only extensively tested technology, including within close proximity of cell towers, to ensure integrity of evidence and reliability for field acquisitions. When performing data acquisition, a phone wizard is launched that identifies the device and determines the correct USB cable for a forensically sound acquisition. Unlike other tools, data acquisition and analysis starts with the device’s SIM, if present, and then continues to the device. Neutrino’s ability to obtain the device’s serial number, cell tower location, manufacturer information among other information, shows why it is considered the de facto standard for forensic solutions.

E. Paraben’s Device Seizure

Device Seizure has low minimum system requirements so it can run on any computer, new, old, or ancient. It can also add support and perform forensic analysis on unsupported phones if they come from supported manufacturers. Similar to MPE, but unlike Oxygen, Paraben’s device seizure [8] can search through a phone’s memory dump for crucial evidence. Device Seizure focuses on the physical level of acquisition because you can acquire more information with physical acquisition than logical.

F. Other Tools

There is many other free source or commercial tools that are available for use in forensic investigations such as:

- **Palm dd (pdd)** [16], which is a spin off the UNIX dd, is a windows based command-line tool that allows an investigator to complete a physical data acquisition from Palm OS handhelds. PDD creates two files; one file has device specific information and the other file contains the bit by bit image. These files can then be exported to different forensic tools, such as EnCase or Autopsy. However since this is a command-line tool, graphic libraries, report generation, and search facilities are not included in these files.

- **Pilot-Link** [16] can be used to retrieve an image of the RAM of a PDA device. Pilot Link is open source software developed within the Linux community to provide a communication bridge between a Linux host and Palm OS digital devices. It uses the HotSync protocol which allows Pilot-Link to logically acquire the devices contents that can then be analyzed by EnCase, HEX editor, or Palm OS Emulator. Unfortunately, it doesn’t support hashing algorithms, making it harder to compare acquisitions for data integrity.

- **TULP2G** is short for Telefoon Uitlees Programma, 2e Generatie and used to recover evidence from handheld devices. Currently, available plug-ins are mainly targeted towards GSM phone examinations.

IV. RELATED WORK

Forensic tools for handheld devices are relatively fewer than those available for personal computers, and of those available, their application is generally limited to the popular operating systems – Palm and Pocket PC [6]. Most previous publications [11, 12] are concentrated on forensics concerns either a particular operating system (i.e. Symbian, iPod, PDA) or a comprehensive analysis of most smartphone features and performance.

This paper [12] introduces the forensic application of freely available tools and describes how known methods of Physical Acquisition can be applied to Windows CE devices. Casey et al [12] provided an overview of Windows Mobile Forensics, describing various methods of acquiring and examining data on Windows Mobile devices. Mislan wrote a similar paper [15] concerning Blackberry and iPhone forensics. NIST has an excellent paper on PDA Forensics Tools that discusses the different procedures and techniques when performing Mobile forensics [14].

However, there lacks an overview research paper that does a comprehensive study of forensics tools for mobile devices within more operating systems or from the perspective of a digital forensics investigator.

This overview will lay out the foundation of digital forensic tools for mobile devices as we endeavor to provide an avenue for discussion regarding mobile forensics.
V. PROPOSED WORK

There are many free sources and commercial digital forensics tools for mobile devices. However, there are few comparisons and benchmarks are available to guide investigator or students to choose those tools for their practical needs. The section will address those issues.

During the experimentation of this case study, we will use SIMfill [14], a tool created by the National Institute of Standards and Technology (NIST), to automatically generate the test data for this case study which will then be placed on each mobile device via USB cable connection. After the data has been transferred to each device, each forensic tool will perform a forensic data acquisition and the data acquired documented. This process will be repeated two more times to ensure consistency and accuracy of the data being acquired and to satisfy the Federal Rules of Evidence [1]. Once the process is complete, we will compare the results based upon the following:

- Time it takes to acquire data
- The type of data acquired against the test set
- Categorically
  - By device model
  - By forensics tool
- How admissible is it as evidence

Inconsistencies with the forensic tool and with the particular carrier (Verizon, AT&T, Sprint, etc.) of the phone will be recorded and how the results were affected by the inconsistencies.

Through this exploratory experimentation, we will be able to give substantial detail to back up a claim of which investigation tool is optimal for various mobile devices. In addition, we will build a set of benchmarks for robust comparisons of all digital tools for mobile/handheld devices in different operating system environments.

VI. CONCLUSIONS

With the increase in research and practical use towards mobile devices, we hope to not just follow the trend but to supply investigators/practitioners a more interactive, convenient, efficient way of capturing e-evidences via choosing reliable and suitable digital forensics tools. We make the set of benchmarks available for any researcher who wants to compare the new tools with other tools for different operating systems.

In the future we hope to include more tools and create more benchmarks that exploit the features of many different handheld devices and concur with the design variations we want. In addition, we will improve on existing benchmarks and continuously retrieve various feedback to make benchmarks more effective and easy to use. Future research will be conducted to formalize the abstract design discussed in this paper that will eventually lead to implementation and testing.

REFERENCES


A Potential Enzyme-Encapsulating, Ultrafine Fiber for Phenol Detection

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Introduction

Phenol ranks 23rd among the top 50 chemicals produced in the world [Chang, 2002, Seetharam, 2003]. It is an important starting material used in a broad range of chemical manufacturing processes such as for resins, polymers, pharmaceuticals, dyes, pulp and paper, textiles, metal coatings, coal conversion, and petroleum refining [Lee, 1996, Karam, 1996, Bevilaqua et al, 2002]. Despite its commercial importance, phenol and many of its derivatives are considered to be, not only toxic chemicals, but also highly hazardous materials. This is due to the fact that it can be easily absorbed by animals and humans, through the skin and mucous membrane [Freire, 2002]. In addition, phenol is also known for its persistency in the environment and propensity for bio-concentration and bio-magnification [Freire, 2002]. Phenol concentrations greater than 50 ppb are harmful to some aquatic life, while ingestion of 1 gram can be fatal in humans [Girelli, 2006, Ibrahim, 2001, Atlow, 1983]. Therefore, the selective and sensitive detection of phenol from industrial aqueous effluents or water contaminated with phenol, followed by remediation, is of practical importance.

In the past three decades, several techniques have been developed for the detection and monitoring of phenol in aqueous environment. These include both non-enzymatic and enzymatic methods. The non-enzymatic methods are mostly analytical techniques such as chromatography, spectrophotometry, flow injection analysis and mass spectrometry [Ni, 2010]. Most of these analytical techniques are expensive, time-consuming, may generate other hazardous by-products, require skilled operators, and sometimes require pre-concentration and extraction steps that increase the risk of sample loss [Freire, 2002, Ibrahim, 2001, Atlow, 1983]. Recent advances in biotechnology have promoted the use of enzymes for the detection and monitoring of phenols. Enzymatic methods possess promising potentials in comparison to non-enzymatic techniques due to their specificity, selectivity, speed, simplicity, reliability, predictability and low cost [Aitken, 1993, Karam, 1996], in addition to the benefit of eliminating the target species in the process—remediation by default.

Horseradish peroxidase (HRP, EC 1.11.1.7) and tyrosinase (polyphenol oxidase, EC 1.14.18.1) are undoubtedly the most studied enzymes that have been used for phenol detection [Karam, 1996, Ikehata, 2000]. HRP catalyzes the oxidation of a wide variety of toxic aromatic compounds including phenols, bisphenols, anilines, benzidines, and related hetero-aromatic compounds over broad pH and temperature ranges [Karam, 1996]. The reaction products undergo polymerization via non-enzymatic processes, resulting in the formation of water insoluble precipitates that can be easily removed [Karam, 1996]. HRP requires the presence of hydrogen peroxide that not only acts as a co-reactant but also activates the enzyme, which in turn oxidizes the substrate; however, the drawbacks associated with the use of HRP include the high cost of both the enzyme and hydrogen peroxide in addition to the toxicity of the reaction products [Ikehata, 2000, Wada, 1993, Atlow, 1983].

By comparison, tyrosinase catalyzes the ortho-hydroxylation of phenol in the presence of molecular oxygen, to catechols (diphenols) and their subsequent oxidation to o-quinones [Jang, 2010, Selinheimo, 2008]. The fact that tyrosinase is ubiquitous in nature, abundant in
a wide variety of sources such as vegetables, fruits, seafood, etc., and also requires oxygen as a co-reactant presents its use as a less expensive alternative with respect to HRP and other non-enzymatic methods for phenol detection [Ikehata, 2000] and potential remediation.

Despite the fact that enzymatic methods, in contrast non-enzymatic methods, possess immense potential for hazardous pollutant detection, challenges remain due to their complexity and practical difficulty in implementation, such as successful enzyme immobilization and the related problems of poor stability in terms of suitable reaction conditions, non-reusability, surface area restrictions, enzyme leakage (loss) and complex functionalization chemistries [Patel et al, 2006]. However, advancements in nanomaterials have facilitated some promising strategies that could overcome some or perhaps all of these challenges.

This research presents the potential of one aspect of the multidisciplinary field of nanotechnology by demonstrating the synthesis of ultrafine silicate-polymer bio-composite fiber matrix that immobilize enzymes due to their relatively large and accessible surface area. The ultrafine fibers, having average diameters of less than one micron, are produced by the combination of sol-gel chemistry and electrospinning. This simple, highly versatile and relatively inexpensive method allows for simultaneous pore formation (for increased surface area and loading) and enzyme encapsulation to reduce leakage during use. To date, only two enzymes—HRP and now tyrosinase—have been successfully immobilized with electrospun fibers in a sol-gel process; the HRP-fibers were used for glucose detection and sensing [Patel et al, 2006]. The resulting bio-composite fiber mats supporting tyrosinase are further studied by incorporating them into a small-scale, flow-through device to demonstrate phenol detection and biocatalysis feasibility. Bio-catalytic activity of the electrospun enzyme (tyrosinase) has been evaluated using the integrated form of the Michealis-Menten equation, having the advantage of requiring fewer experimental runs than by other approaches [Bisswanger, 2008].

Materials and Methods

Reagents

Mushroom tyrosinase (polyphenol oxidase C. 1.14. 18.1) with a specific activity of 1460 units/mg was obtained from Worthington Biochemical Corporation (Lakewood, NJ). L-tyrosine, phenol, 4-aminoantipyrine (4-AAP, 98% pure), and poly(vinyl alcohol) (130,000 mol. wt.) were purchased from Sigma Aldrich Chemical Company, Inc. Tetramethyl orthosilicate (TMOS, 97%) was supplied by Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ). D-fructose was obtained from Fisher Scientific (Fairlawn, NJ). Sodium phosphate monobasic monohydrate crystals and sodium phosphate dibasic anhydrous (for preparation of sodium phosphate buffer, pH 6.8) and acetic acid were purchased from Mallinckrodt (Baker, NJ). The buffer solution pH was determined with a Dwyer PH0-1 pH meter (Dwyer Instruments, Inc., Michigan City, IN).

Indium-tin oxide (ITO) glass plates (coated on one surface) were obtained from Delta Technologies (Stillwater, MN). Aluminum, neoprene rubber and acrylic glass sheets were obtained from MacMaster-Carr (NJ) and cut to design specifications for the small-scale flow-through sensor/reactor.

Preparation of Solution for Sol-gel Electrospinning

The preparation of a silicate-fructose-PVA-tyrosinase sol-gel precursor solution for electrospinning was accomplished by an approach similar to that of Patel et al. (2006) with some modifications to make electrospinning of a sol-gel simpler. A mixture of TMOS (0.76g silica precursor), water (0.18g for a H₂O:TMOS mole ratio of 2) and HCl (30 μL of 40mM stock as catalyst to
speed up the hydrolysis reaction) were added with continuous stirring to form the hydrolyzed silica sol. Thereafter, the reaction mixture was heated to a temperature of 60°C for 30 minutes to evaporate the methanol formed during the hydrolysis stage of the sol-gel process. The resulting sol was allowed to cool and the pore-forming material (500 µL of 50% w/v aqueous D-fructose) was added with continuous stirring. PVA (700 µL of 15% w/v) was added next to the mixture with continuous stirring. The addition of both PVA and D-fructose made the sol highly viscous, which is a desirable property modification to facilitate electrospinning into fibers rather than electrospaying into droplets.

At this stage, the sol had a strong tendency to gel due to the condensation reactions already occurring. This was exacerbated by the addition of enzyme solution in sodium phosphate buffer, which caused rapid gelation inside the syringe, making the electrospinning process impossible. Patel et al. (2006) also observed this behavior while preparing their horseradish peroxidase sol-gel electrospinning mixture. As part of their approach in solving this problem, they suggested cooling the sol mixture to low temperatures (e.g., 0°C) before electrospinning, slowing the condensation reaction rate. Since this was impractical with the electrospinning system in the present study, the introduction of acetic acid (15 µL of 100 mM) was used as the alternative to counteract early gelation and provide a short but feasible window for electrospinning. The actual mechanism for the reduction in condensation reaction by acetic acid is unclear; however, there is the possibility of this acid inhibiting the formation of some of the Si-O-Si bonds which are responsible for the formation of the three-dimensional network that builds into the gel.

After the addition of acetic acid, 300 µL of the tyrosinase enzyme in buffer solution (6 mg/mL) were introduced into the sol with continuous stirring for about 4-6 minutes before the mixture was electrospun into fibers. A second electrospinning sol was similarly prepared, adding 15 µL of 100 mM of acetic acid, without the enzyme, viz., silicate-fructose-PVA. The delay in gelation is expected to be the same as with the enzyme, since it is not believed to participate or interfere directly in the sol-gel reaction, other than the simple dilution effect of the additional enzyme buffer.

**Ultrafine Fibers Electrospun with Tyrosinase**

Approximately 1 mL of the silicate-fructose-PVA-tyrosinase sol was introduced into a 3 mL syringe and placed in a syringe pump. The electrospinning setup is similar to the one used by Jabal et al. (2009), Jabal et al. (2010); an Advance Infusion System series 1200 syringe pump (Cellpoint Scientific Inc, Gaithersburg) was used to control the flow rate of the sol and thus the droplet size undergoing electrodynamic distortion from a 10-kV source during the process. Syringes and needles (1 mL and 3 mL with 27G 1 ¼-in. metallic needle) were obtained from Becton, Dickinson (Franklin Lakes, NJ).

The syringe containing the enzyme-sol mixture was subjected to an electric field of 100 kV/m from the tip of the needle to the collecting electrode. The grounded collector plate (ITO glass plate 25×18×1.1 mm³, surface conductivity 0.010-0.014 S/m) was placed at a distance of 10 cm from the needle tip. The ITO was centered on a piece of aluminum foil, which helps to attract the electrospun fibers onto the surface of the plate. At a controlled (pump) flow rate of 10 µL/min, a droplet of the enzyme-sol solution first extends from the needle due to interfacial electrostatic forces generated by the applied voltage, resulting in the progressive transformation from hemispherical to conical (i.e., the Taylor cone). Finally, a continuous fluid jet results in the formation of ultrafine fibers, which were randomly deposited onto the ITO plate as white, nonwoven mats containing the enzyme tyrosinase [Li, 2004]. It took approximately 3 minutes to obtain fiber mat thicknesses of 10 µm to 15 µm thick for roughly 6 mg of fiber
material per ITO plate. In order to achieve high biocatalyst/enzyme loading, and hence enhance bio-catalysis, very dense fiber mats were desired. Fiber mats without enzyme were produced in identical fashion.

Characterization of Electrospun, Enzyme-encapsulated Fibers

Morphology

The morphology of the electrospun fibers and mats, with and without enzymes, were analyzed with both optical dark-field microscopy (BX51, Olympus America Inc., Melville, NY) and scanning electron microscopy (Zeiss Supra 35 VP field emission-SEM, Center for Electron Microscopy and Microanalysis, University of Idaho). The optical microscope was used with a 100x BF/DF objective to evaluate the morphology of the fiber mats immediately after electrospinning, in addition to estimating the overall thickness of the mat. Dark-field microscopy is capable of distinguishing shapes and optical features from the scattering light of objects significantly smaller than the classic diffraction limit (<200 nm), and thus gleaning information on nanomaterials beyond the true optical resolution of far-field optics. After optical examination for overall sample quality, the fiber mats on ITO plates were mounted on SEM sample holders and sputtered with a thin layering of gold. High-resolution micrographs of fibers were obtained to determine diameter distributions and to investigate the success and extent of pore formation or other mesoscopic structures induced during the sol-gel electrospinning process.

Raman Spectra Analysis of Electrospun Fibers

An alpha 300 scanning confocal Raman microscope (WITec GmbH, Ulm, Germany) equipped with a 100x objective using 532-nm excitation at an incident power of 19.6 mW was used to verify the local chemical constituency of individual fibers. The Raman spectra of the fibers fabricated from both the silicate-fructose-PVA sol and the silicate-fructose-PVA-enzyme (tyrosinase) sol were collected using confocal microscopy near the diffraction limit. Raman spectra were obtained by focusing the excitation laser on individual fibers in each sample type without further preparation, using a spectral integration time of 3 s to measure the Raman emission range from 200 to 3800 cm\(^{-1}\) (relative) and average 10 accumulations per location.

Viscoelastic Measurements of the Silicate-Fructose-PVA-Enzyme Sol

The viscoelasticity of the spinning solution (silicate-fructose-PVA-enzyme sol) with varying fructose concentration was performed using a Böhlin Instruments rheometer (East Brunswick, NJ) in order to study the relationship between the viscosity of the mixture and the final diameter of the spun fibers. Viscoelastic response was measured based on the oscillatory testing method using the parallel plate configuration, which involved the application of an oscillating stress as an input to the silicate-fructose-PVA-enzyme- sol solution and the resulting output was measured over time with the dynamic viscosity extrapolated from the data that was generated.

Catalytic Assay of Free and Electrospun Tyrosinase

Colorimetric assays for phenol and the absorbance of the samples were monitored using a HP8453 UV-VIS spectrophotometer at 25°C with a 1.5 mL glass cuvette and an optical path length of 1 cm (Environmental Biotechnology Institute, University of Idaho). The 500 µL of phenol (concentrations of 0-5 mM), 350 µL of 0.1M sodium phosphate buffer (pH 6.8), and 100 µL of 20.8 mM 4-AAP were mixed with 30 µL of the enzyme (6 mg/mL), and the initial reaction rate was based on the absorbance change measured with time (over 300 seconds) at a wavelength of 510 nm. The phenol concentration plot and double reciprocal plot were generated from the data obtained.

Evaluation of the kinetic parameters of both free and electrospun tyrosinase was based on
the modification of the colorimetric method used by Ikehata (2000). Oxidation of phenol by tyrosinase liberates quinones (o-quinones) that can be monitored with spectrophotometry. The quinone liberated from this reaction is highly unstable and polymerizes to yield a variety of products, making it difficult to determine the actual concentration. However, quinones form a very stable complex with 4-aminoantipyrine (4-AAP), known as quinoneimide, that absorbs light at a wavelength of 510 nm; hence, the catalytic activities of both free and electrospun tyrosinase were determined based on monitoring quinoneimide. The catalytic activity of free (soluble) tyrosinase involved initial rate measurements of the formation of quinoneimide, due to the absorbance increase resulting from the intensity of the colored product. The higher the intensity of the colored product formed, the higher the absorbance.

Estimations of retained catalytic activity and of kinetic parameters for immobilized (electrospun) tyrosinase were based on data acquired through the use of a small-scale flow-through reactor designed for this purpose. The electrospun enzyme-encapsulated fibers supported on ITO plates were assembled into a small-scale flow-through reactor (Figure 3), which was inspired by a slightly more complex construction used by Schilke (2009).

These modifications include the use of acrylic glass plates instead of just glass plates and the complete removal of a Teflon™ spacer. Spacing between the acrylic glass plate and the ITO plate containing the electrospun enzymes was achieved by drilling a specific distance on the inner surface of the acrylic glass plate. The substrate solution (phenol, sodium phosphate buffer and 4-aminoantipyrine, 500 µL of phenol (0-5 mM), 350 µL of 0.1M sodium phosphate buffer (pH 6.8), and 100 µL of 20.8 mM 4-AAP) flows from a syringe (3 ml) in a syringe pump, at a controlled flow rate (2.5 µL/min), via rubber tubing through the drilled aluminum and acrylic plates and flows across the randomly electrospun non-woven mat of the enzyme encapsulated fibers. The flow path of the fluid, through the reactor is made possible, due to the presence of a channel formed by drilling a depth of 0.4 mm on the inner surface of the acrylic glass plate, which is 1.5 mm from the length and 2 mm from the width of the acrylic glass plate. Based on these dimensions, the volume of the small-scale flow-through sensor/reactor is approximately 12.32 µL. Reaction between the electrospun enzymes and substrate, flowing through resulted in the formation of the product, which flows out through the outlet, via rubber tubing connected to the small-scale flow-through reactor, into a cuvette. The absorbance of the solution is recorded at a wavelength of 510 nm and the concentration of the product (quinoneimide) was estimated using Beer’s Lambert law, with a molar extinction coefficient or molar absorptivity value of the product to be 0.1591 L·mM⁻¹·cm⁻¹ or 159.1 L·M⁻¹·cm⁻¹. This value was determined experimentally by assuming a 1:1 ratio between the dye product and the substrate phenol. A 1:1 series of dilutions of a certain concentration of the product (8 mM quinoneimide) was prepared and the molar extinction coefficient was obtained from a plot of absorbance vs. concentration of the data generated via regression analysis. The following assumptions were observed while performing the experiments:

1. The enzyme reactions followed Michealis-Menten kinetics
2. The reactions occurred at steady state.
3. The reaction is irreversible
4. No axial mixing occurs in the reactor

Results and Discussion

Optical Images of Enzyme Encapsulating Fibers

The surface morphology of the electrospun fiber mats were initially observed using the optical microscope (100× objective, dark-field mode). This was to ensure that the appropriate amount/volume/ratio of the components that
make up the electrospinning mixture, produced fibers, and not other structures such as beads on the ITO glass surface.

Surface morphology of the electrospun fiber mats were observed based on comparisons between two different sets of electrospinning mixtures which had varying D-fructose concentrations (responsible for pore formation in the fibers). They are silicate-fructose-PVA and silicate-fructose-PVA-tyrosinase sol mixtures. Both types were prepared with D-fructose concentrations ranging from 0-50% (w/v), while the silicate and PVA components of the mixture were kept constant (0.76g per batch and 15% w/v, respectively). Optical images of the fibers with enzymes electrospun with D-fructose concentrations of 10-40% (w/v) displayed similar morphology (non-woven fiber mats), with the exception of those obtained from the silicate-fructose-PVA-tyrosinase sol (50% w/v D-fructose) (Figure 4), which displayed some structures on their surface, that were better revealed using SEM. In dark-field, or ultramicroscopy mode, they do not appear as smooth, where light-scattering effects are able to contrast morphological features much smaller than the classic diffraction limit of the light. The 50× and 100× objectives of the optical microscope were also used to estimate the thickness of the fiber mats, which are mostly dependent on the electrospinning time and collection distance. Average thickness of the fiber mats were between the range 10-15 (µm) and it took an average of 3 minutes to obtain such fiber mat thickness.

**SEM Images of Enzyme Encapsulated Fiber Mats**

Scanning electron microscopy (SEM) was able to reveal smaller details of the physical morphology of the fibers, electrospun from both sets of solutions: (silicate-fructose-PVA and silicate-fructose-PVA-tyrosinase sol). Table 1 gives the average size of the fibers, obtained from the electrospinning, of both sets of sol mixtures.

A summary of the results displayed in Table 1, shows that the fiber diameter were in the range of 250-850 nm. The average diameter of the fibers were obtained by measuring about 25 fibers of a single sample from the SEM micrographs and hence a total of 300 fibers. There seem to be an increase in the diameter of the fibers with and without enzymes electrospun from the sol mixture with fructose concentration ranging from 20% to 50% w/v. The 10% w/v concentration does not fit this trend and there is no statistical difference between each group or sets of fibers. This observed trend may be due to the combined effects of PVA, and fructose, towards enhancing the viscosity of the sol solution [Patel, 2006]. High solution viscosity results in an increase in the resistance of the solution to be stretched into smaller capillary jets by the electrostatic and surface forces and hence the relative increase in the diameter of the fibers produced [Ramakrishna, 2005]. Furthermore, increasing the concentration of an electrospinning solution or mixture and hence the concentration of the total dissolved solids in such solutions/mixtures, has a direct relationship with the diameter of the electrospun fibers obtained from such mixtures [Ramakrishna, 2005]. This increase could be attributed to the higher degree of entanglement of the fructose and PVA on the fibers [Patel, 2006, Ramakrishna, 2005]. The diameters of fibers obtained from silicate-fructose-PVA-sol solution with 0% w/v fructose (silicate-PVA-sol) were quite high compared to the fibers obtained from both silicate-fructose-PVA-sol with increasing fructose concentrations (10% to 50% w/v) and silicate-fructose-PVA-enzyme-sol solutions. This could be attributed to the highly viscous nature of one of the component of the silicate-PVA-sol mixture (15% w/v PVA). The high concentration of PVA (15% w/v) in addition to its high molecular weight (130,000) significantly contributed to the increased viscosity of the solution. The silicate component in the silicate-PVA-sol mixture had little or no effect on the overall viscosity of the mixture. The fructose and enzyme-in-
buffer solution components possibly contributed a “dilution effect” on the overall viscosity of the silicate-fructose-PVA and silicate-fructose-PVA-enzyme-sol solutions, hence the lower diameters of the fibers obtained from such sol solutions.

SEM micrographs of fibers with enzymes that were electrospun using 50% w/v D-fructose as pore forming material, displayed pores/pore-like or crater-like structures on the fibers as shown in Figure 5. These structures, visible in the SEM are mostly covered with a thin film of gold sputtered onto the fiber samples to improve image contrast. This was also observed in the fibers produced with 30% w/v and 40% w/v fructose that had enzymes. However these structures were highly pronounced in the fibers with enzymes, electrospun with 50% w/v fructose, hence they were used for bio-catalysis, since these highly pronounced pore-like.crater-like structures could facilitate substrate/product diffusion and increase surface area. Higher resolution SEM images showed these pores/pore-like or crater structures on the fibers with average diameters of about 111.7 nm. Fibers that were electrospun with D-fructose concentrations of < 30% w/v did not show these structures. There is the possibility that 30% w/v D-Fructose could be the critical concentration, or very near it for the formation of such structures on the fibers.

Raman Spectra Analysis of Electrospun Enzyme Encapsulating Ultrafine Fibers

Results of Raman spectra generated from electrospun fibers with enzyme produced from 50% D-fructose and the precursor components (source materials) used for the synthesis of the fibers are presented in this section for the purpose of distinguishing chemical signature deviations that may be caused by concentration changes in the initial solutions. Generally all the sample spectra exhibited prominent peaks at 450-550 cm\(^{-1}\) that shows the presence of silica or silica networks in the fibers. This wavenumber range is synonymous with Si-O-Si symmetric vibrational modes [Schrader, 1995]. Also, there were strong peaks at 2800-3000 cm\(^{-1}\) that corresponds to the stretching vibrational modes of C-H and symmetric and antisymmetric stretches of CH\(_2\) bonds (sp\(^2\) and sp\(^3\) carbon), synonymous with alkane chains indicating the presence of the polymer PVA and also the pore forming template fructose [Schrader, 1995; Colthup, 1990]. In addition, the area under the spectral bands were quite broad, indicating the presence of aliphatic (flexible) compounds in the fibers. Both PVA and fructose are aliphatic compounds.

Detailed sectional plots of the Raman spectra generated comparing the major precursors for electrospinning of these fibers; 50% w/v fructose solution, 15% PVA pellets, fructose crystals powders and 15% PVA gel solution respectively, revealed more details, showing the composite nature of the fibers and how they are similar or different from their environment in the composite (Figures 7-9). First of all, the spectra bands of all the component materials displayed broad peaks/wide areas indicating that they were aliphatic in nature. At wavenumber range of 250-425 cm\(^{-1}\) and 600-1300 cm\(^{-1}\), (Figures 7 and 8) there are very high peaks of the fructose powder, 50% w/v fructose solution and the 15% w/v PVA solution. These peaks are also observed in the spectral band of the fibers with enzyme (tyrosinase). The peaks in the wavenumber region of 250-425 cm\(^{-1}\) (Figures 7 and 8) represent bending vibration (symmetric deformation) \(\delta_{s}(CC)\) C-C sigma bond) of the carbon atoms in the aliphatic hydrocarbon chain of both PVA and fructose. The wavenumber range of 600-1300 cm\(^{-1}\) (Figure 8) corresponds to stretching vibrations of C-C bonds (v(CC), C-C sigma bond) in the aliphatic chains of both PVA and fructose, which are also present in the spectra band of the fibers with enzyme (tyrosinase). At wavenumber of 1450 cm\(^{-1}\), all the spectra had peaks, which represent CH\(_2\) bending vibrational modes (CH\(_2\) scissoring). This also reflects the presence of both PVA and fructose in the fibers. The wavenumber range of 2800-
3100 cm\(^{-1}\), was common in all the spectra confirming stretching vibrational modes of C-H and, symmetric and antisymmetric stretches of CH\(_2\) bonds (sp\(^2\) and sp\(^3\) carbon) as observed in the spectra of the fibers with different fructose concentrations.

Raman spectra analysis was also used to confirm bio-catalysis and the bio-composite nature of the fibers with enzyme. Fibers electrospun from the silicate-fructose-PVA-tyrosinase sol solutions, (50% w/v D-fructose) were used for bio-catalysis and the spectra of the fibers before and after the reaction are presented in Figure 10. Spectra bands of the fibers before and after reaction, displayed similar peaks, which actually indicates that the fibers were still intact. However, the spectra bands of the fibers after reaction displayed broad spectrum florescence, which is a characteristic signature of raman spectra bands of dyes or dye-containing products. This was due to the presence of the product (Quinoneimide) (a dye-containing product) formed from the reaction catalyzed by tyrosinase as shown is Figure 10. The conversion of phenol by the enzyme tyrosinase results in the formation of quinone, which forms a stable complex (quinoneimide) with the dye 4-aminoantipyrine. The spectra bands displayed in Figure 10 was both confirmation that the enzyme was actually encapsulated in the fibers (bio-composite nature) and was involved in catalyzing the reaction (bio-catalysis).

**Viscoelastic Measurements of the Silicate-Fructose-PVA-Enzyme Sol**

The approximate values of dynamic viscosity (\(\eta\)) of the silicate-fructose-PVA-enzyme sol solution with varying fructose concentrations were extrapolated from the plots of dynamic viscosity (\(\eta\)) against time (s). The approximate dynamic viscosity (\(\eta\)) values shows a decrease in viscosity (12 to 5.9 Pas), with corresponding decrease in fructose concentrations (50% w/v to 10% w/v). Silicate-fructose-PVA-enzyme sol with 0% w/v fructose concentration had a very high viscosity value as compared to the others (39.9 Pas). This could be attributed to the viscous nature of the high concentration of PVA (15% w/v), which had a higher degree of entanglement of polymer chain molecules and increased polymer length [Teo, 2006; Ramakrishna, 2005]. Increasing the concentration of fructose for both silicate-PVA-fructose and silicate-PVA-fructose-enzyme sol contributed to an overall increase in the concentration of the dissolved solute in the sol and a corresponding increase in the viscosity of the sol. These results display a relationship between viscosity, concentration of the electrospinning sol and fiber diameter.

As the sol begins to gel the viscosity curve increases with time which is indicative of the setting or curing of the sol gel solution. At these long times it is difficult, if not impossible, to electrospin the solution, because of gel formation; a phenomena of cross-linking reactions of the siloxane bonds formed, from the condensation reactions, resulting into a three dimensional silica matrix network.

**Catalytic Measurements of the Enzyme-Encapsulating Fibers**

Catalytic activity of both soluble and electrospun tyrosinase were based on the determination of the kinetic parameters \(K_M\) and \(r_{max}\) values. The \(K_M\) is the Michaelis-Menten constant, which is the substrate concentration at which the rate of reaction (initially) is half of its maximum value [Grunwald, 2009]. \(K_M\) indicates the affinity of the enzyme to its substrate. A higher \(K_M\) value indicates lower affinity for its substrate and vice-versa. \(r_{max}\) is the maximum (initial) rate of an enzyme catalyzed reaction. It virtually means that all of the enzyme present in the reaction mixture is in the form of the Enzyme-Substrate (E-S) complex, hence it is a measure of how fast the enzyme catalyzes the reaction [Kuby, 1991; Lee, 1992; Shuler, 2002]. The results of the kinetic parameters (\(K_M\) and \(r_{max}\)) for electrospun and soluble tyrosinase are presented in Table 2. Both parameters (\(K_M\) and \(r_{max}\)) for soluble tyrosinase were obtained
from the initial rate measurements based, on the formation of the reaction product, quinoneimide. This was used to plot the concentration curve (Figure 11) and the double reciprocal plots (Figure 12) and the $K_M$ and $r_{max}$ for free (soluble) tyrosinase, were extrapolated.

The integrated form of the Michaelis-Menten equation was used to evaluate the catalytic activity of the electrospun tyrosinase enzyme. The ITO glass plate containing the electrospun enzyme encapsulating fibers (which had white colored fiber mat) were inserted inside the small scale flow-through reactor, and removed after the reaction process. Upon removal, the color of the fiber mat had changed to dark red, which was indicative of the formation of the dye product quinoneimide. Figures 13 and 14 display the photographs of the fiber mats before and after the reaction, while Figures 15 and 16 display the pictures of the substrate mixture, before and after the reaction (the substrate mixture was colorless before the reaction and developed a red colour in the product after the reaction).

Kinetic parameters obtained from the integrated form of the michaelis-menten equation were extrapolated from the plot in Figure 17 and displayed in Table 2, respectively.

Results of the initial rate measurements for soluble tyrosinase (Figure 11) displayed a characteristic behavior similar to that observed for Michaelis-Menten enzyme kinetics at low substrate concentrations. At low substrate concentrations, the rate of formation of quinoneimide, was a fairly linear curve, which increased (with corresponding increase in substrate concentration) and became relatively flat at concentrations of about 3.5 mM. Estimated $K_M$ and $r_{max}$ values for free (soluble) tyrosinase are 0.23 mM and 27.03 mM/min. In comparisons with values obtained from literature, Yang (2006) reported $K_M$ and $r_{max}$ values for free (soluble) tyrosinase by Yang were based on a different substrate Tyrosine (a monophenol) which was converted to Dopachrome (a Quinone) by tyrosinase in the presence of oxygen, which absorbs light at a different wavelength, however if there are high variations in terms of order of magnitude in these values, then they may not be acceptable. Others include different values of the Molar Extinction coefficient for these products, which absorb at different wavelengths. Estimated $K_M$ values for soluble tyrosinase (0.23 mM) were lower than the estimated $K_{M,app}$ values for electrospun tyrosinase (0.62 mM). This result display immobilization effect of the enzymes, due to the confined nature of the enzyme in its electrospun state which restricts or limits substrate interaction with enzyme, unlike the free or soluble form [Lee, 1992; Bisswanger, 2008]. The lower $K_M$ values for soluble tyrosinase indicates the stronger affinity of the enzyme (in its free form) to its substrate. Estimated $r_{max}$ values for free (soluble) tyrosinase were higher than $r_{max,app}$ values for electrospun tyrosinase which also indicates faster reaction rate for soluble tyrosinase. Apart from the fact that the enzyme in its electrospun state, is subject to mass transfer resistance and spatial or morphological accessibility to substrates and products, the addition of acetic acid, which was used to delay gelation during the electrospinning of the sol mixture, could possibly have some inhibitory effect on the electrospun enzyme. Irrespective of these challenges, bio-catalytic activity of the electrospun tyrosinase was retained by the conversion of phenol at such low concentrations (0.5 mM).

Conclusion
For the first time, tyrosinase encapsulating ultrafine silicate-polymer bio-composite fiber matrix were fabricated using the combination of electrospinning and sol-gel chemistry. Bio-
catalytic activity of the electrospun enzyme was retained at phenol concentrations of 0-5 mM, which are usually typical phenol concentrations found in waters contaminated with phenol. These fiber matrix are promising candidates for immobilization of enzymes due to their relatively high surface area. This research demonstrates the potential of this approach as a cost effective sensing material for bio-sensing, bio-catalysis and bio-detection applicable in environmental and bio-industrial analysis.

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References
THE EFFECT OF AIR SPARGING ON MFC PERFORMANCE
Oyinkansola Adewamidele Dina

INTRODUCTION

There are over six billion people on the planet with 9.4 billion projected for 2050 [1]. With our energy needs on the rise, it is important that we meet the increasing demand for sustainable energy. Microbial fuel cells (MFCs) are promising technologies that provide waste water treatment while also capturing energy as electricity. MFCs can be used to simultaneously treat wastewaters and generate power. These devices work by transferring electrons from biodegradable substrates to terminal electron acceptors via a simple wire. MFCs were actually discovered in 1911 (Potter, 1911), but little was done to advance the technology until 1993, when Kim et. al., 1999 discovered that expensive chemicals were not needed for proper operation.

MFCs consist of two chambers – an anode exposed to an electron donor, and a cathode exposed to a terminal electron acceptor. The two chambers are usually separated by a permeable membrane. Bacteria grow on the anode forming a biofilm, oxidize organic compounds, and produce electrons that are transported exogenously to the electrode. The electrons then travel through a wire (and across an external load, presumably the appliance being powered) to the cathode, where the terminal electron acceptor is reduced. Protons migrate through the substrate in the anode across the membrane to the cathode where they combine with oxygen and electrons to form water [2].

The resulting power output is known to be limited by the internal resistance, which in turn depends on a number of factors, including electrode spacing, the presence of the permeable membrane, and the overall configuration of the device.

The cathode is an important factor in the performance of a MFC due to the poor kinetics of oxygen reduction reaction in a neutral pH medium [3, 4]. Catalysts – iron (II) phthalocyanine and CoTMPP [5], air cathodes [6] and pressurized cathode chambers [7] have been used to improve oxygen reduction at the cathode. There is preliminary evidence that shearing forces may reduce internal resistance at the cathode. Angenant, 2005 found that rotating the cathode of a two chamber MFC increased the power output by 30%. Thus, there is potential to increase power output if shear forces can be introduced at the cathode surface.

In this paper, I will explain the different batch test experiments I conducted to determine the optimum sparging rate at the cathode of a two chamber MFC treating synthetic wastewater.

METHODS AND MATERIALS

Two MFCs were used in the sparging experiments. The first MFC (MFC-I) consisted of two chambers, both rectangular flasks with a volume of 800cm³. Both electrodes were made of perforated graphite, the anode with the larger surface area. The anode was semi-filled with 500mL of substrate (650mg/L) collected from a reactor currently in operation. The two chambers were separated by a proton exchange membrane (PEM).

The second MFC (MFC-II) was a dual chamber, H-shaped device constructed using PVC, and was operated as a sequencing batch reactor. The initial inocula was a mixed microbial consortia originating from a MFC currently in operation. Both chambers had a volume of 130cm³. Viton rubber was used to seal the anode to prevent leaking air or water, and the anode and cathode chamber was separated by a cation exchange membrane (CEM) (Nafion 111, Sigma Aldrich).
Synthetic wastewater (same as in MFC-I) was introduced into the anode.

FIGURE 1
THE H-TYPE MFC USED AS MFC-II

OPERATION

MFC-I was operated for a period of six days, with an air stone attached underneath the cathode. Air was supplied by house air available in the laboratory where the MFCs were set-up. The air stone was connected to an air meter that used to gauge the gas flow. MFC-II was operated for the next five days and the same protocol was observed using the air stone in the cathode. For both MFCs, the voltage and current were intermittently recorded for 24 hours across a 1 kΩ resistor using a multimeter.

I measured the DO (Dissolved oxygen) in the cathode intermittently and I confirmed that sparging the cathode chamber with air increased the concentration of oxygen.

RESULTS AND DISCUSSION

The objective of this paper is to find the optimum sparging rate that generates the highest power. Despite the fact that two MFCs that were operated differ in volume, the results were in no way impinged. The first half of the batch tests was conducted using MFC-I while the second half using MFC-II. Preceding each batch test, 1mL of acetate (from 50mg/L of acetate) was added to the anode chamber. All voltages recorded were in OCV (Open circuit voltage) as there was no external resistor attached to systems.

FIGURE 2
Microbial Fuel Cell Batch Tests, 2L/min

Figure 2 shows the results from the MFC batch tests with a sparging rate of 2 L/min. Current (mA) and Power (mW) are on the secondary vertical axis while the Voltage (V) is situated on the primary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a maximum value of 0.01 mA, and it remained at this value over the course of the 24 hour test. The voltage increased to 0.46V and remained constant for the course of the batch test. These data resulted in a power output of approximately 0.00457 mW. These data show relatively stable current, voltage, and power output over the course of the batch test.
FIGURE 3

Figure 3 shows the results from the MFC batch tests with a sparging rate of 3 L/min. Current (mA) and Power (mW) are on the primary vertical axis while the Voltage (V) is situated on the secondary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a maximum value of 0.01 mA, and it remained at this value over the course of the 24 hour test. The voltage increased to 0.41V and decreased to 0.35V and steadily to 0.39 V before finally decreasing. These data resulted in a power output that decreased from the initial 0.00408 mW to 0.00350 mW. The graph of the power can be described as almost stable owing mostly to experimental errors. Data show relatively stable current, voltage, and power output over the course of the batch test.

FIGURE 4

Figure 4 shows the results from the MFC batch tests with a sparging rate of 1 L/min. Current (mA) and Power (mW) are on the secondary vertical axis while the Voltage (V) is situated on the primary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a value of 0.05 mA, which increased steadily till it reached 0.10 mA over the course of the 24 hour test. The voltage increased from 0.29V to 0.34V. The increase in current greatly affected the power output which ranged from 0.014 mW to 0.034 mW. These data resulted in a power output that decreased from the initial 0.00408 mW to 0.00350 mW. The graph of the power can be described as non-linear but progressively increasing throughout the course of the batch test.

FIGURE 5

Figure 5 shows the results from the MFC batch tests with a sparging rate of 1.5 L/min. Current (mA) and Power (mW) are on the secondary vertical axis while the Voltage (V) is situated on the primary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a maximum value of 0.11 mA, and remained the same over the course of the 24 hour test. However, the voltage continuously decreased from an initial 0.41V to 0.36V. The decrease in voltage was compensated by the constant high current which enhanced power.
generation, power output ranged from 0.04532 mW to 0.03949 mW. The graph of the power can be described as dramatic as it decreases and sometimes increases mildly throughout the course of the batch test.

Figure 6 shows the results from the MFC batch tests with a sparging rate of 0 L/min. Current (mA) and Power (mW) are on the secondary vertical axis while the Voltage (V) is situated on the primary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a value of 0.09 mA, which increased steadily till it reached 0.11 mA over the course of the 24 hour test. The voltage increased from 0.31V to 0.37V. The increase in current greatly affected the power output which ranged from 0.02790 mW to 0.04070 mW. The graph of the power can be described as fairly constant throughout the course of the batch test.

Figure 7 shows the results from the MFC batch tests with a sparging rate of 0.5 L/min. Current (mA) and Power (mW) are on the secondary vertical axis while the Voltage (V) is situated on the primary vertical axis. They are all plotted in relation to Time (min). After injection of acetate, the current reached a value of 0.11 mA, which increased to 0.12 mA over the course of the 24 hour test. The voltage increased from 0.36V to 0.39V. The increase in current greatly affected the power output which ranged from 0.03905 mW to 0.04632 mW. These data resulted in a power output that decreased from the initial 0.00408 mW to 0.00350 mW. The graph of the power can be described as non-linear but progressively increasing throughout the course of the batch test.

**CONCLUSION**

The maximum power generated at each sparging rate is as follows:

- At 0 L/min, it was 0.04279 mW
- At 0.5 L/min, it was 0.04704 mW
- At 1 L/min, it was 0.03410 mW
- At 1.5 L/min, it was 0.04037 mW
- At 2 L/min, it was 0.00458 mW
- At 3 L/min, it was 0.00408 mW

Therefore, from the above results, the optimum sparging rate was found to be at 0.5 L/min as shown in Figure 8.
REFERENCES


Novel Nanocomposite Materials for Solar Cell Fabrication
Theodore Glave

Introduction
Dye-sensitized solar cells (DSSCs) have garnered interest recently since they present a relatively low cost means of energy generation. DSSCs can be fabricated relatively cheaply and are suitable for incorporation in flexible substrates using roll-to-roll processing. With Ru-complex sensitizers DSSCs have power conversion efficiencies of up to 11% which is indicative of a cost effective alternative to conventional crystalline silicon cells.

In a typical DSSC, titanium dioxide nanoparticles are deposited onto a conductive glass substrate and heated to form a nanoporous coating. The coated substrate is then dipped into a solution of ruthenium containing dye, in order to adsorb the dye molecules on the TiO2 surface. The pores of the TiO2 coating are further infused with a liquid electrolyte that contains iodide salts. The device fabrication is completed by placing a counter electrode of platinum-coated conductive glass on top of this nanoporous TiO2 coating. Upon absorption of light, the sensitizer molecules (S) inject electrons into the TiO2, and are oxidized. The iodide, I−, in the electrolyte supplies electrons to the oxidized dye, and regenerates the dye. The iodide oxidizes to triiodide, I3 −, during this step. Then the electrons passing through the external load arrive at the counter electrode and reduce the triiodide back to iodide. It is evident that the photo-current density, and the power output of the solar cell, will increase with an increase in the mobility of the redox mediators (I3 − /I−) in the electrolyte solution.

High power conversion efficiencies in DSSCs have been obtained using liquid electrolytes, such as acetonitrile, that support high ionic conductivities. Volatile liquid electrolytes limit the long-term stability and high temperature operation of conventional DSSCs. Thermal expansion results in leakage of liquid electrolytes in DSSCs operated at elevated temperatures. Further problems include desorption of dye molecules from the TiO2 surface, and chemical degradation of the catalytic features of the TiO2/Pt electrodes. The development of DSSCs for commercial applications largely relies on successful resolution of these limitations. The proposed research focuses on these specific material-aspects of DSSCs, with the goal of developing solid-state/quasi-solid-state cells based on novel polymer electrolytes.

The performance of a DSSC is strongly governed by the efficiency of charge separation (current collection) in the cell. Because of their superior electron-accepting ability, semiconducting carbon nanotubes (CNTs) can facilitate electron transport, and increase the photoconversion efficiency of TiO2 solar cells. The formation of a CNT–TiO2 Schottky barrier junction will increase the recombination time of electron–hole pairs, which will increase the current collection efficiency in a D SSC constructed using these materials. Promising results in support of this strategy have been reported, where single wall CNTs, used as conducting scaffolds in a TiO2 based cell, were capable of doubling the photoconversion efficiency of a photo-electrochemical cell. This earlier work, however, used a Pt-foil counter electrode with aqueous KOH solution as the electrolyte in an electrochemical DSSC. The work proposed here aims at developing solid-state dye-sensitized solar cells using TiO2/CNT/polymer nanocomposites.

Objective
The primary goal of the proposed research is:

1. To increase the power conversion efficiency and the overall performance characteristics of dye sensitized solar cells (DSSCs) through the development of novel polymer-titania nanocomposites.
2. To improve conventional TiO2 DSSCs by employing blends of carbon nanotubes (SWCNTs) with novel polymer electrolytes.
3. To develop a model to characterize the interfacial behavior at the nanotube polymer surface.

**Literature Review – Polymer Film Theory**

Polymer surface technology is widely applied today in order to generate engineering solutions. Multi-component polymer systems are capable of producing adhesive and release surfaces, and even smart surfaces with selective adhesion. When functional groups are incorporated surfaces can be further refined to accomplish more specific applications, including surface interactions and surface tension. [3]

Polymer surface interactions aim to either increase polymer surface interaction (adhesion) or to decrease that surface interaction with a particular material (antifouling). Low-surface tension groups at a polymers surface encourage release, while high surface tension groups encourage adhesion. [3]

There are three design principles are used for polymer surface models: surface segregation, surface structure and surface reorganization. In describing the behavior of model end functional polymers, the general behavior of all possible linear functional polymer architectures can be determine.

Surface segregation, refers to how the multi-component polymer will arrange itself in a film and the distribution at the surface. Depending on whether adhesion or antifouling is desired, the model will represent the constituent of polymer that allows the particular characteristic to be dominant at the surface. This is accomplished by evaluating the free energies in the bulk of the film as well as at the surface. The Gibbs-Duhem equation illustrates the degree to which one constituent segregates preferentially to the surface is determined by a balance of bulk and surface free energy changes.

\[
\sum d(n_r \mu_r) + d(n_f \mu_f) = -A \partial \gamma = A(\gamma_r - \gamma_f) \partial \phi_{s,f} \tag{1}
\]

The subscripts \(r\) and \(f\) refer to the repeat unit (i.e., the polymer backbone) and the functional group, respectively; \(\mu\) is the chemical potential, and \(n\) is the number of moles. The surface area is \(A\), the surface fraction of the functional group is \(Q_{s,f}\) and the interfacial tensions are \(\gamma_r\) and \(\gamma_f\). [4]

Surface structure, refers to the density of the polymer surface. It is important to control the distribution of constituents at the surface in order to engineer specific surface properties. Thus, surface segregation alone is inadequate to create a comprehensive model. The theoretical framework to model surface structure is the lattice treatment developed by Scheutjens and Fleer [5] to describe polymer adsorption. It requires three parameters: the normalized chain length, equal to the volume of the functional polymer molecule divided by a reference volume (\(v_{\text{REF}}\)); the Flory interaction parameter (\(\chi\)) between the polymer repeat unit and functional group segments; and a surface interaction parameter (\(\chi_s\)) reflecting the driving force for surface segregation defined by the Gibbs-Duhem equation.

Surface reorganization, occurs when the environment contacting the polymer surface at fabrication changes. Most polymers are fabricated in a hydrophobic environment. Post fabrication, if the environment changes to hydrophilic the polymer will attempt to reorganize itself at the interacting surface. Thus, the Gibbs-Duhem equation that characterized the initial interaction would have changed and would need to be adjusted in order have an accurate model. [6]

The theoretical framework to model surface structure is the lattice treatment developed by Scheutjens and Fleer [5][10] to describe polymer adsorption. It requires three parameters: the normalized chain length, equal to the volume of the functional polymer molecule divided by a reference volume (\(V_{\text{REF}}\)); the Flory interaction parameter (\(\chi\)) between the polymer repeat unit and functional group segments; and a surface interaction parameter (\(\chi_s\)) reflecting the driving force for surface segregation defined by the Gibbs-Duhem equation. To estimate the parameters without the use of experimentation, the solution theory for \(\chi\) may be used:
\[ \chi = \frac{\nu_{REF}(\delta f - \delta_f)}{kT} \]  
(2)

Where \( k \) is the Boltzmann’s constant and \( T \) is the temperature.

This expression is used because the solubility parameters \( (\delta_f) \) can be estimated directly based on the polymer repeat unit or functional group by group-contribution methods. \[11\]

Thus, the chi value (\( \chi \)) can be estimated without the need for experimentation. The value of \( \chi \) dictates the magnitude of the bulk free energy cost, the left hand side of the Gibbs-Duhem equation (1), associated with the segregation of one constituent to the surface.

The right hand side of the Gibbs-Duhem equation (1) represents the reduction in the surface free energy associated with surface segregation. The dimensionless form of this term \( (\chi_s) \) is defined as follows:

\[ \chi_s = \frac{\Delta(\gamma_f - \gamma_f)}{kT} \]  
(3)

With this definition the convention is that \( \chi_s \) is negative when functional groups have the lower surface tension, that is, when functional groups are attracted to the surface. It is positive surface tension when the repeat unit is lower and the functional group is repelled form the surface.

The surface area of a segment \( (A) \) is equal to \( v_{REF}^{2/3} \) because a cubic lattice is assumed, and the surface tensions \( (\gamma_i) \) are again estimated by group-contribution methods \[7\] based on the chemical structures of the polymer repeat unit and functional group.

Determining \( v_{REF} \) presents the most difficult aspect of the modeling. There are two logical alternatives in doing so: the volume of the repeat unit and the volume of the functional group. When it is assumed that \( v_{REF} \) is the repeat unit volume the chain stiffness and entropy are correctly accounted for. However, this is problematic if it leads to a fractional number of functional groups. Thus, at times an approximation or a compromised definition that best corresponds to the functional polymer chain under consideration must be employed. This approximation can then used in the lattice model to provide a prediction of the volume fraction of functional groups \( (O_f) \) in each lattice layer. \[7][8\]

One of the most important properties in describing the character of a polymer surface is the surface tension. The lattice model provides a rigorous route to calculating the surface tension. It may also be estimated to a degree of accuracy however, from knowledge of only the fraction of functional groups in the first lattice layer \( (O_{f,1}) \). If additivity by the surface area is assumed, the surface tension can be calculated in a group – contribution manner as follows:

\[ \gamma = \gamma_f O_{f,1} + \gamma_r (1 - O_{f,1}) \]  
(4)

This simple relationship successfully reproduces the molecular weight dependences of the surface tension for various polymers. The \( \chi_s \) values and constituent surface tensions are calculated from group-contribution methods. The equation is extremely useful in calculating surface tensions because \( O_{f,1} \) values calculated from the lattice model can be put in a tabular format along with interpolation formulas, so that it is not necessary to redo the calculation each time for a new polymer structure. Also, for \( \omega \)-functional or \( \alpha, \omega \)-dysfunctional polymers the \( O_{f,1} \) can be found based on the chain length and the \( \chi_s \) value. \[8][9\]

This model can essentially be extended for all possible functional chain architectures and to examine the effects of functional group adjacency and the inclusion of high and low energy functional groups on the same chain. The calculations and approximations applied to developing simplified strategies for the estimation of the material parameters provide an accurate means of calculating both surface concentration and tension of functional polymers. \[3][12\]

**Methodology**

The interfacial behavior at the carbon nanotube (CNT) surface of the polymers of interest may be modeled using the self consistent mean field (SCMF) lattice method. This method offers information about
composite depth profiles and lateral variation that arise due to phase segregation. Initially, this method will be applied to polymers chains in contact with a flat graphite surface, before being extended to curved surfaces as present with carbon nanotubes. A program is already developed that can be used for this study. Currently, work is being carried out on the model for the flat surface, in which alterations are being made to code for stiffer polymer chains.

The SCMF lattice method is based on the notion that one and only one mer can occupy a lattice site. Each mer interacts with a certain number of neighboring mers. The number of neighbors depends on the type of lattice. For a simple cubic lattice, it is assumed that there are four neighbors in the same layer as the given mer, one mer above it, and one mer below it. This arrangement is described by weighing factors: $\lambda_0 = \frac{2}{3}$ while $\lambda_{-1}$ and $\lambda_1$ is $\frac{1}{6}$.

For a cylindrical surface, the layers are concentric annuli. Layers that are farther from the cylinder have larger volumes. Thus, for curved surfaces, the weighting factors have to be altered in order to model the differences in the radial direction. By considering the volume bounded by the layer and setting one layer as a reference the weighting factors can be adjusted.

The number of cells can be found based on the layer they are in. For example, in the $n$th, $n+1$ and $n-1$ layers:

$$n \text{ layer} = \pi((n + 1)^2 - n^2)l^2$$  
(5)

$$n - 1 \text{ layer} = \pi(n^2 - (n + 1)^2)l^2$$  
(6)

$$n + 1 \text{ layer} = \pi((n + 2)^2 - (n + 1)^2)l^2$$  
(7)

where $l$ is the unit length of a cell’s edge.

Using the $n$th layer as the reference the weighting fractions may be defined as follows:

$$\lambda_1 = \frac{1}{6} \left( \frac{n+2}{n+1} \right)^2 - \frac{(n+1)^2}{n^2}$$  
(8)

$$\lambda_{-1} = \frac{1}{6} \left( \frac{n^2}{n+1} - (n-1)^2 \right)$$  
(9)

$$\lambda_0 = \frac{2}{3}$$  
(10)

From these proportions it can be seen that as $n$ tends to infinity this model will approach a flat surface. Notably also, the sum of $\lambda_0$, $\lambda_{-1}$ and $\lambda_1$ is 1. Based on the size of the carbon nanotubes it is expected that the model for the cured surface will still closely approximate a flat surface.

The SCMF lattice method provides detailed information about the polymer chains contacting with the surface. This includes statistical information about the probability that a mer at a given distance from the interface is part of an adsorbed chain. One can even determine the probability that a mer is part of a chain that is adsorbed on the interface at more than one point along the chain backbone. This information helps in the understanding of the orientation of chains that are adsorbed at the surface.

For relatively small, flexible chains that are adsorbed on carbon nanotubes (CNTs) having large diameters, the above study is directly useful. That is, multi-walled CNTs having diameters of 60 – 100 nm in diameter. If the radii of gyration are small compared to the size range, for polymer molecules, the approximation of a flat interface may be acceptable. In order to accomplish these conditions, the axis of the CNT will be assumed to be orthogonal to the periodic directions, and the interface between the two components will be faceted, with local sections that are parallel to either of the two period Cartesian geometries. This type of discretization of the interface is consistent with underlying lattice assumption of the SCMF lattice method. Recent work by Van de Grampel et al. using the SCMF method to study phase segregated systems suggests that it may be possible to create a smoother interface by permitting lattice cells in the interfacial region to have non-zero volume fractions of both solid phases.
**Design Method**

- Flory-Huggins interaction parameters for ionic mers with CNT’s and non-ionic mers were estimated.
- The behavior of CNT-polymer mixtures were determined with the self-consistent mean field lattice model using the sfbox (“Houston”) program.

**Results**

![Figure 1. Volume fraction of monomer A in the polymer lattice.](image1)

![Figure 2. Volume fraction of monomer B in the lattice.](image2)

![Figure 3. Volume fraction of monomer C in the lattice.](image3)

![Figure 4. Volume fraction of Iodide ions in the lattice.](image4)

![Figure 5. Volume fraction of charged mer (on 10 mer polymer chain) in the lattice.](image5)

![Figure 6. Volume fraction of charged mer (on 20 mer polymer chain) in the lattice.](image6)
In order to conduct the stiffness investigation between the surface and the copolymer the lattice conditions as well as the copolymer structure had to be defined. The copolymer was given a structure that contained three different types of mers; a charged head, a 4 mer long body and a 8 mer long tail, the ―A‖, ―B‖ and ―C‖ monomers respectively.

The polymer-surface system that was analyzed has the following parameters:

- **Phi bulk value = 0.9**
- **Charge of Mer = +1**
- **Charge of Iodide = -1**
- **Surface Geometry = flat**
- **Polymer Composition = 1(A)4(B)8(C)**
- **Surface Energy Potential (ua) = 0 - (-90)**
- **Χ Values = 0 – 1.0**

Similarly, to investigate the relationship between the polymer and the surface energy another copolymer system was defined. Two copolymers were defined one with one charged head and a tail with nine uncharged mers and another with one charged head and a tail with nineteen uncharged mers. The following parameters were used:

- **Phi bulk value = 0.99**
- **Charge of Mer = +1**
- **Surface Geometry = flat**
- **Polymer Composition = 1(A)9(B) and 1(A)19(B)**
- **Surface Energy Potential (ua) = 0 - (-90)**
- **Χ Values = 0.33**

**Stiffness**

Stiffness is a relevant parameter to the experiment since stiff chain will create more loops/chains along the surface of the carbon nanotubes. Electrons will be greater propagated in the solar cell and thus increase the power conversion. The Flory Huggins Interaction parameter, χ describes the interaction between the mers within a copolymer and between mers and surfaces. So, by investigating the effect of the χ value on the copolymer, it will become clearer how stiff copolymers can be produced.

Figure 1 showed that the ―A‖ monomers were not significantly impacted by the increased repulsion characterized by higher chi values. A slight increase in the attraction to the surface was noticed. In examining figure 2, a 15% increase in the number of ―B‖ mers was observed in the third layer. This was indicative that at higher χ values there was increased repulsion which was resulting in an increase in the chain stiffness. Another feature of the graph was a 10% drop in the number of ―B‖ mers in the fifth layer. This observation was due to the presence of the ―C‖ mers at the end of the copolymer chain. Further down the lattice, they were occupying most of the space.

The examination of figure 3, lead to similar inferences as figure 2. In layer 5 there was a 10% increase in the number of ―C‖ mers which was due to the increase in the χ value.
This feature further supported the conclusion that the increased repulsion due to the higher χ values was producing stiffer polymer chains.

**Iodide**

Iodide acts as the dye in the solar cell and it also acts as a counterbalance. Since iodide is an ion it will have an attraction to the carbon nanotube surface. It is important that the surface is coated with polymer and not iodide, however. Therefore, it became necessary to investigate how the iodide was binding to the surface. In figure 4, the iodide appeared to be relatively unaffected by the changes in χ; except that between layers 3 and 5, at high χ values, less iodide are present.

**Surface Energy**

In order to investigate the influence of the surface energy the second copolymer lattice system was used. The influence of the surface energy on the charged mer was consistent regardless of the polymer chain length (Figures 5-6). For high surface interactions the charged mer was found closer to the polymer-surface interface than for lower surface interactions. Another observation was that a region was formed in the layer with no charged mers. This was due to the charged mers migrate to the surface of the layer and thus the uncharged mers occupy the subsequent layers. Further down in the layer the charged mers that were unable to reach the surface can be found.

When the surface interaction is high and negative the likelihood of finding trains and loops close to the surface will be great (Figure 7-8). Notably, trains and loops can be found only in the first 2-3 layers. It was also observed that there is little change in this finding when the polymer length changes.

**Conclusion**

To increase power conversion it would be recommended to use material with high surface energy and strong repulsive χ interactions. In the future the model needs to be refined to account for the size of each type of mer as well as include real world χ values for the polymer. It is also important for the project to model the linearization of CNT’s and the interaction with the electrode terminals.

**References**

Synthesis and Characterization of Composite Membranes
Velencia Witherspoon

Introduction
The main goal of this work is to use particle membrane technology to synthesize composite membranes of controlled pore size, permeability and enhanced properties such as conductivity. The enhanced conductivity can be achieved by forming composite membranes with proteins. The particles when coupled with proteins provide the necessary structural rigidity for the proteins, thus resulting in a more stable membrane. The plan for the semester of Fall 2010 was to synthesize membranes from colloidal particles, to show that one can control pore sizes and permeabilities and then to combine this technology with proteins to synthesize membranes with enhanced conductivity. Packed beds of colloidal particles were deposited in layers onto porous support to narrow the pore size distribution. The proteins used are understood by Dr. Paravastu’s group and are thought to be conductive by an ion hoping mechanism. It is believed that the proteins trap water within the molecule, creating a structure of channels that can conduct or transfer protons. Results from this semesters work are given below.

Polystyrene Latex Composite Membranes
Latex membranes were synthesized using the method developed by Erin Holley by depositing latex particles onto a porous support. The membranes were characterized in terms of pore size distribution and water permeabilities. In this work, 200nm particles were deposited on a base polyethersulfone membrane with a rated pore size of 100 nm. The basic methodology for deposition of nanoparticle includes pressurization of a dilute solution through a membrane and the annealing for 30 minutes to help stabilize the membrane.

Figure 1a. Normalized relative flow as a function of pore size for Supor 100 (base membrane) and for six layers of 200nm particle on Supor 100. As can be seen from this graph the average pore size gets shifted to lower sizes as particles are deposited.

Figure 1b. Flow rate as a function of applied pressure for the base membrane of Supor 100 and different amounts of 200 nm particles.
The main conclusions that can be drawn from these results are that 1) A narrower pore size distribution results when particles are deposited on commercial membranes 2) pore sizes are 14% of the particle diameter and 3) permeabilities can be controlled by controlling the number of layers deposited. From Figure 1c, the resistance of the packed bed can be calculated by multiplying the slope of this line by the ratio of the effective area to the viscosity of water. As can be seen in Table 1, the measured resistances were very high. It was hypothesized that the increase in resistance was due to fusion of the particles together during thermal annealing. This would create a non-porous solid layer of fused particles instead of a packed bed with pores. Modifications were made to the synthesizing process such as decreasing the time in the oven to 20, 10, 5 and 0 minutes in order stop the particles from fusing together. This modification did not result in lower resistances. It was then hypothesized that the smaller particles were not packing in the same manner as the large particles when the particle solution was pressurized through. It is possible that the dispersion of the particle in the deposition solution was not even and could be improved by changing the weight percent of the solution used. In order to test this hypothesis the nanoparticle solution was diluted further and then deposited onto membranes. However, the final weight percent of the solution did not affect the resistance of the composite membranes. The pressure used when depositing the nanoparticle was varied from 5psi-20psi. This modification did not result in any changes to the measured resistances.

None of these modification resulted in the desired increase in permeability of the membrane. It was concluded that this composite membrane was fouling. Different types of fouling schematics of membranes were researched in order to better understand the high resistance of the smaller particles packed beds. The fouling mechanisms were found in the literature and are best represented by the following diagram.

Figure 2. Depicts different fouling schematics that could be occurring. Where \( d_p \) is the diameter of the pore and \( d \) is the diameter of the particle.

In synthesizing the composite membranes we have controlled the pore size by packing the
nanoparticles on top of a porous support. This packing is best represented by the schematic for Case C, where a thin layer of particles have formed on top of the membrane or porous support. We have been able to narrow the pore size distribution through the packing of particles on top of the membrane. In the case of 950 nm particles on Supor 450 and 600 nm particles on Supor 200, the particle layer is formed without any major fouling that would affect the resistance of the composite membrane. This is because the average diameter of the large particles is much larger than the largest pore size of the support. However, when the 200 nm particles are deposited onto Supor 100, the most common pore size diameter of the membrane is 166 nm, which is very close to the actual size of the particle. Thus, fouling occurred because the nanoparticles being deposited are being absorbed into the membrane, as demonstrated by Case A, Figure 2. The particles have the potential to fuse with the membrane and cause a decrease in the permeability of the membrane while in turn increasing the resistance of the membrane. There is also a possibility that the particles are plugging the large pores in the membrane. The scenario is best represented by the Case B fouling schematic. Both Case A and Case B fouling would result in a significant reduction in the permeability and an increase in the resistance of the membrane.

In conclusion the composite membranes resulted in narrower pore size distributions that were on average at least 14% of the particle diameter. The resistance of the composite membrane involving 950 nm, and 600 nm diameter were reasonable. However, fouling occurred when depositing 200 nm onto the Supor 100 support because the diameter of the particles was very close to the large diameter of the pores. This study shows that pore size distribution can successfully be controlled by the particle size deposited onto the membrane. It shows that in order to prevent fouling when synthesizing the composite membrane, the particle size must be significantly larger than the largest pore size.

**Protein Composite Membranes**

An opportunity was presented by Dr. Paravastu’s research group to re-apply the deposition techniques gained during latex nanoparticle composite membrane synthesis by incorporating nano-fibrils, proteins into the membranes. The advantages of forming nanocomposite membranes are 1) enhanced functionality, 2) improved stability, and 3) enhanced conductivity. The proteins being used are well understood by Dr. Paravastu’s research group. They can enhance the functionality of a membrane because they have the potential to be used as biosensor. The functionality of a membrane can be improved by the proteins, because in biological systems, proteins can bind a specific molecule and can also perform this task when attached to a membrane. The binding of a molecule will alter the conductivity of the membrane and allow the membrane to signal the presence of a selected molecule. This can allow the membrane to act as a biosensor. The proteins being used in this study are self-healing, regenerative, proteins. This regenerative property can ameliorate the one time use of most bio sensing technology. Dr. Paravastu’s group’s research shows that the proteins are highly conductive by trapping moisture within their molecule structure. This allows for the proteins to form ion/proton conducting channels. These conducting channels enhance the conductivity of the membrane and the network of fibrils result in decrease permeability of the membrane. Enhanced conductivity and low permeability are properties desired in fuel cell membranes. The composite membranes formed this semester have the potential to improve the current conductivity and reliability of current fuel cell membrane. The regenerative property of the proteins will make the membrane more economical if they do not have to be completely replaced when damaged. One goal for this semester was to develop a protocol for deposition of proteins onto porous supports.
and for characterization of the membranes. The protocols that were developed to deposit the protein relied on pressurizing and recycling a dilute solution of proteins through a porous support. The membranes were then characterized using SEM imaging and I-V measurement. Protocols for characterization are still being improved, however a basic method for deposition has been discovered. A brief report of the protocol and results are given below.

The ideal deposition of the proteins on to the membrane is represented by the figure below. In this figure the proteins are deposited around the inner pores of the membrane and form a thin layer of protein fibril on top of the membrane. If the proteins were to surround the inner core of the support, it would significantly lower the permeability and increase the resistance to fluid flow. Securing a continue matrix of protein fibrils to a membrane could potentially increase the membrane conductivity and improve its functionality as a fuel cell membrane.

Figure 3. Demonstrates the ideal deposition of protein nano-fibrils around the pore of a Supor Membrane.

Methodology

![Methodology Diagram]

Figure 4. Illustrates step by step the methodology for synthesis of the protein composite membranes.

It was hypothesize that with the above procedure one would be able to coat the core of the membrane with protein fibrils. The experiments were carried out using the above methodology. In order to confirm the presence of proteins onto the membrane the following test were place.

Figure 5. Illustrates the step taken to characterize the composite membrane and to confirm the deposition of protein onto the porous support.

Results

The membrane were characterise through IV test and SEM imaging. The results of the experiment place are given below.

Figure 6. Is a SEM Image of a blank Supor 100 membrane.
should enhance the conductivity of the composite membrane. Recall Ohm’s law, \( R = \frac{V}{I} \), where \( R \) is the resistance of the medium, \( V \) and the voltage drop of the medium, and \( I \) is the current. The more conducting a substance is the lower the resistance value. Logically the addition of conductive proteins to the substrate should lower the resistance. In order to test for the change in resistance Voltage was applied across a section of the membrane and the current through that section was measured. The electrodes were placed approximate 2 centimeters apart on the membrane and the voltage was varied from 4 V to -4V. If the composite membrane was more conductive the resistance value measured should decrease. The range of applied voltage was limited to ensure that the protein did not suffer degradation during testing, these value were previously determined by Dr. Paravastu’s Group.

Figure 7. Illustrates that the fibrils have deposited on top of the membrane created a solid layer.

Figure 8. Illustrates the fibrils of the A\textbeta \textsubscript{(1-40)} distributed on the Supor 100 membrane. It also illustrates that the fibrils may be absorbing into and aggregating on the top layer of the membrane.

These SEM images confirm the successful deposition of protein fibril onto a Supor 100 porous supports. The string-like matrix of fibers is the protein and has successfully been attached to the membrane. The fibrils have formed a caked layer or coating over the surface of the membrane, Figure 7. Figure 8 shows that the fibrils have been somewhat absorbed into the matrix of the Supor 100 membrane. The thickness of the layer of protein fibrils is due to the fact that the protein solution was recycled five times to try and deposit as many fibrils as possible. The protein, A\textbeta \textsubscript{(1-40)}, is conducting and

Table 1. Summary of Resistance for the Membranes
<table>
<thead>
<tr>
<th>Membrane</th>
<th>Resistance (Ohms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supor 100 Dry</td>
<td>$12.92 \times 10^9$</td>
</tr>
<tr>
<td>Supor 100 Wet</td>
<td>$13.85 \times 10^7$</td>
</tr>
<tr>
<td>Supor 100 + Protein Dry</td>
<td>$2.64 \times 10^9$</td>
</tr>
<tr>
<td>Supor 100 + Protein Wet</td>
<td>$18.97 \times 10^{10}$</td>
</tr>
</tbody>
</table>

These IV-Characteristics results were inconclusive. Because the protein is considered conductive, deposition of the protein onto the Supor support should decrease the overall resistance. However, the results shows a slight increases in resistance and indicates that the protein are acting as an insulator which deviated from the Paravastu’s group experimental findings. This could be attributed to whether or not the machine can accurately measure current on a very small scale.

Plan For Spring 2011
Next semester will involve a systematic set of experiments in which proteins will be incorporated in commercial membranes and their IV characteristics measured. The goal is to tune the protein properties and membrane characteristics such that one can get a good control of the conductivity of the resulting membrane. In this regard there are many self healing proteins available that are conductive and these will be used in our experiments. Another protein that could be used is Rada(14-I), which is commercially produced and also well understood by Dr. Paravastu’s research group. The advantage with using these proteins are that the microstructure can be controlled by solution conditions (pH, stirring, temperature, concentration etc..) Hence we expect to get good control over conductivity of the composite membranes.
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