Analyzing the Formation of Nanoscale Complexes of DNA and Polyimidazoles for use in Gene Transfection

Sarah Bruner, Scott Forbey, Daniel Schoonover and Robert Moore
Macromolecules and Interfaces Institute and Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Abstract

Polyplexes formed by electrostatic interactions between cationic polymers and DNA offer a non-viral means of gene therapy. A promising technology still in its early stages, gene therapy introduces genetic material into cells in order to treat or prevent diseases such as cancer and HIV. Although the most common types of carriers used in gene therapy are altered viruses, non-viral vectors offer the advantages of being easier to create and eliciting none of the immune responses typical of viruses. Cationic polymers are being explored as non-viral vectors since they form condensed nanoparticle complexes with negatively charged DNA and protect the DNA from degradation and inactivation *in vivo*. In addition, these cationic polymers can be tailored for controlled efficient delivery of therapeutic genes due to the numerous potential combinations of composition and structural parameters.

This work furthers the study of structure-property relationships leading to stable polyplex formation by analyzing the formation and binding affinity between calf-thymus DNA and polyimidazoles of varying structures: poly(1-ethyl-3-vinylimidazolium) and poly(1-butyl-3-vinylimidazolium). Dynamic light scattering and isothermal titration calorimetry were employed to examine the sizes of the polyplex structures and to determine the associated binding thermodynamics, respectively. Experimental methods and trial analyses were first developed using model polymers. Once a basic comprehension of the techniques was established, they were implemented to analyze the desired polyimidazole/DNA polyplexes. Dynamic light scattering showed that increasing the length of the alkyl chain attached to the imidazole results in increased polyplex dimensions. Size changes over various charge ratios indicated that polyplex formation did not begin instantly; condensation occurred at a certain charge ratio, different for each polyimidazole, and then aggregation followed. Isothermal titration calorimetry demonstrated an increase in the binding affinity associated with an increase in the attached alkyl chain length.

Synthesis and Functionalization of Heterobifunctional Polyethers for the Stabilization of Magnetite

Alfred Chen, P. P. Huffstetler and J. S. Riffle
Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061

Abstract

Long-term stabilities of the bonds that hold polymers on magnetite nanoparticle surfaces have been a concern when these magnetite-polymer complexes are exposed to physiological media, particularly media that contain phosphate salts. We herein describe how ammonium and zwitterionic ammonium phosphonate functionalities on polymers affect the stability of the polymer-magnetite bond. Trivinylsilyl-functional poly(propylene oxide-*b*-ethylene oxide) diblock copolymers were functionalized via ene-thiol free-radical chemistry to afford triammonium functionality on the hydrophobic end of the copolymer. These triammonium-functional copolymers were further functionalized with diethylvinyl phosphonate via Michael addition, then the ethyl groups were removed with trimethylsilyl bromide/MeOH to yield tri-zwitterionic phosphonate-functional polyethers. The polymers were adsorbed onto the surfaces of magnetite nanoparticles and the stabilities of the polymer-magnetite composition and sizes in water and phosphate buffered saline (PBS) were studied. Both the triammonium-functional and the triammonium phosphonate-functional polyether-magnetite complexes were stable upon aging in water, but only the triammonium phosphonate-functional polyether-magnetite complexes were stable upon aging in PBS. Thermogravimetric analyses were used to determine the polymer loadings before and after aging in water and in PBS. Dynamic light scattering was utilized to establish whether the sizes of the nanoparticles remained constant over 24 hours. In agreement with the TGA results, only the polymer-magnetite nanoparticles that were bound through the zwitterionic phosphonates maintained their sizes after exposure to PBS.

Adsorption Studies of Phospholipids onto Cellulose and other Surfaces

Heejun Choi, Zelin Liu, Qiongdan Xie, Xiaosong Du, and Alan R. Esker
Department of Chemistry and the Macromolecules and Interfaces Institute
Virginia Tech, Blacksburg, VA 24061

Abstract

The construction of model cell membranes on biosensors is of great interest for investigating interactions between biomolecules and membranes. In this study, the adsorption of 1,2-dioleoyl-*sn*-glycero-phosphocholine (DOPC) was examined via quartz crystal microbalance with dissipation (QCM-D) and atomic force microscopy (AFM) experiments. The adsorption studies of DOPC on silica, gold, trimethylsilyl cellulose (TMSC), and regenerated cellulose surfaces demonstrated that adsorption of DOPC required a concentration above a critical aggregation concentration (CAC). For concentrations higher than the CAC, adsorption depended on surface structure. A hydrated DOPC bilayer was formed on silica with a thickness of 4.2 (Voigt-based viscoelastic modeling) to 4.4 nm (AFM cross-section analysis). Uniform vesicle adsorption was also observed on gold and regenerated cellulose surfaces with average thicknesses of 22.3 nm and 10.4 nm, respectively. AFM studies revealed a non-uniform layer of bilayer fragments and vesicles formed on TMSC with an average thickness of 5.1 nm (from Voigt-based viscoelastic modeling).

Synthesis of (+) and (-) tartrate-based linear and branched poly(glycoamidoamine)s for structure and property relationship studies

Theresa Cutler, Antons Sizovs, Karina Kizjakina, Theresa M. Reineke
Macromolecules and Interface Institute, Virginia Tech, Blacksburg, VA 24061

Abstract

Nucleic acid-based therapeutics such as genes and siRNA have potential for treating genetic diseases such as cystic fibrosis, leukocyte adherence deficiency, canavan disease, and multiple sclerosis as well as many others. Nucleic acid-based therapeutics must be carried into cells to be effective, and polycationic polymers are of interest for condensing with the polyanionic nucleic acids to achieve these objectives. Polymeric vectors have some advantages over viral vectors including the ease of modifying their chemical and physical properties, thus affording a means of lowering toxicity. Our group has developed a series of polyglycoamidoamines (PGAAs) that have high transfection efficiencies and low toxicities. To date, no study has been attempted to reveal the influence of hydroxyl group stereochemistry in tartrate based PGAAs. Herein the (+) and (-) forms were synthesized for the purpose of comparing the effect of the stereochemistry of the hydroxyl groups on cellular uptake.

Synthesis of *n*-Butyl Acrylate and 1-Vinylimidazole-containing Copolymers

Joe M. Dennis, M. D. Green, T. E. Long
Macromolecules the Interfaces Institute, Virginia Tech, Blacksburg, VA 24061

Abstract

Ionic liquids are salts in the liquid state, and the temperature range of interest is up to ~100^{o}C. They are under intense study because of their negligible vapor pressure and easily tuned physicochemical properties. Imidazole-containing polymers have been well documented for their ease of anion exchange of their counterions associated with the quaternary nitrogen. This exchange can result in major changes in the thermophysical properties of the polymer. Tuning these polymers shows promise for use as liquid electrolytes in solar cells, lithium batteries, conductive-polymer-based superconductors, artificial muscles and electrochromic devices. This investigation involved the synthesis 1-vinylimidazole-*n*-butyl acrylate and effects of different counteranions on the copolymers. Our approach was to synthesize a systematic series of copolymers and to determine effects of copolymer composition and counteranion on conductivities. Random copolymers of *n*-butyl acrylate and 1-vinylimidazole were prepared and analyzed. Co-polymerizations of these monomers through conventional free radical copolymerization was not ideal and feed ratios differed from copolymer compositions. However, alkylation of the copolymer chains and anion exchange were accomplished. Synthesis of a diblock polymer from these two monomers was also explored.

Design and Synthesis of Drug-Polymer Complexes for Drug Delivery

Meagan Douple,[1,2] R. Mejia-Ariza,[1] W.C. Miles,[1,2] Nikorn Pothayee,[1,3] Nipon Pothayee,[1,3] T. Vadala,[1,3] N. Geter,[1] J.S. Riffle[1,3] and R.M. Davis[1,2]

[1]Macromolecules and Interfaces Institute, and the Depts of [2]Chemical Engineering and [3]Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Abstract

The efficacy of antibiotic treatments used to reach intracellular pathogens can be enhanced through specific design of polymers for drug encapsulation. This study uses a Multi-Inlet Vortex Mixer (MIVM) to synthesize nanoparticle complexes, with the ultimate goal of generating particles with well-defined particle size distributions and compositions and colloidal stability in physiological environments. In the first part of this work, complexes of the antibiotic doxycycline were prepared with a pentablock poly(caprolactone-*b*-ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide-*b*-caprolactone) copolymer. The doxycycline was first converted to either a cholate or deoxycholate salt to make their solubility in water very low. Next, a solution of the doxycycline salt and the pentablock copolymer were dissolved in tetrahydrofuran were this was mixed with an aqueous antisolvent in the MIVM under turbulent conditions. Nucleation and growth of core-shell polymer-drug nanoparticles occurred in the mixer, with the hydrophobic doxycycline salt and the polycaprolactone blocks of the copolymer forming the cores and the amphiphilic poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) comprising the shells. The effect of supersaturation in the MIVM as the two solutions rapidly mixed on particle size was examined. In the second part of this work, the antibiotic gentamicin (a water-soluble, cationic compound) was complexed with a blend of a pentablock poly(sodium acrylate-*b*-ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide-*b*-sodium acrylate) copolymer and a diblock poly(ethylene oxide-*b*-sodium acrylate) copolymer. The electrostatic interaction between the positively charged gentamicin and the negatively charged polyacrylate blocks facilitated the formation of a polymer-drug complex consisting of a gentamicin-polyacrylate core attached to a poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) and poly(ethylene oxide) shell. For both the doxycycline and gentamicin complexes, particle size decreased with an increase in supersaturation, which was adjusted by changing the inlet concentration of the drug as well as the solvent to anti-solvent ratio within the mixer. Additionally, the molecular weight of the polyacrylate blocks in the gentamicin complexes proved critical to the stabilities of these nanoparticle complexes. This indicated that an interaction between the hydrophobic poly(propylene oxide) blocks with the cores may be important for future drug-polymer core-shell nanoparticle design.

Modeling *Listeria monocytogenes* Contamination in Ready-To-Eat Deli Meats to Establish Risk Assessment Metrics.

Owen Gallagher[1] and Daniel Gallagher[2]

[1]Department of Computer and Electrical Engineering, University of Virginia, Charlottesville, VA 22904. [2]Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24060.

Abstract: *Listeria monocytogenes* is a foodborne pathogen responsible for over 500 deaths annually, primarily from ready–to–eat deli meats. In order to determine effective regulations for *L. monocytogenes* that reduce the level of risk to the consumer, risk assessment models are needed that link concentrations in food to public health outcomes. Thus, a computer model was written to determine current levels of protection for the consumer as well as to evaluate the potential benefits of stricter regulations. The model used a second order Monte-Carlo simulation designed to account for both variability within the process and uncertainty about the input parameters, and followed the meat from preparation in the plant, through the retail environment to its consumption in the home environment. A dose-response model then estimated the number of resulting illnesses. The model additionally evaluated different forms of industry response to new regulations on *L. monocytogenes*. Initial runs with the model have begun, with a calculated median level of consumer protection of approximately 3.9e-7 risk of illness per serving. Additionally, the impact of specified performance objectives regarding *L. monocytogenes* contamination appears to be measurable, and further work on the model is attempting to refine these measurements.

Polymer-Antibiotic Nanoplexes as Delivery Vehicles to Intracellular Bacteria. N. Geter, Dept. of Chemistry, University of South Carolina Upstate, Spartanburg, SC and T. P. Vadala, N. Pothayee, C. Van Duyn, A. Ranjan, M. Douple, W. C. Miles, R. Mejia-Ariza, N. Sriranganathan, R. M. Davis and J. S. Riffle, Macromolecules and Interfaces Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Food-borne pathogens such as *Salmonella* and *Listeria* reside partially in immune cells and replicate in them. Eradication is difficult since the recommended antibiotics cannot enter the cells in sufficient concentrations to adequately treat the intracellular population of bacteria. Core-shell polymeric delivery vehicles that contain high concentrations of gentamicin have been developed in efforts to overcome this challenge. Pentablock and diblock copolymers comprised of nonionic polyethers and ionic poly(sodium acrylate) have been complexed with the cationic aminoglycoside, gentamicin. The anionic polyacrylate blocks bind electrostatically to the cationic antibiotic to form the cores of the delivery systems while the nonionic polyether blocks form the outer shells that interact with cells and the physiological medium. The amphiphilic nature of the polyethers that comprise the outer shells can be fine-tuned to be sufficiently hydrophobic to transport across cell membranes and enter the cells where the bacteria reside, yet remain sufficiently hydrophilic to disperse in physiological media. We are investigating the critical parameters governing the properties of these nanoplexes for treating intracellular *Salmonella* and *Listeria* including the molecular weights of the copolymer blocks, the media in which the nanoplexes were created, and their colloidal properties at 4 and 37 ºC. Results indicate that poly(sodium acrylate) ($PAA^{-}{}^{+}Na$) segments with an average of 20-30 repeat units on the ends of an amphiphilic polyether copolymer can be combined with PEO-*b*-$PAA^{-}Na^{+}$ diblock copolymers with 50-100 units of the $PAA^{-}Na^{+}$ to efficiently encapsulate the antibiotic. In vitro studies with mouse macrophages demonstrated excellent efficacy against intracellular *Listeria* and some improvements in eradicating *Salmonella*.

Investigating Polyplex Stability and Thermodynamics for Gene Delivery using Gel Electrophoresis

Alec S. Good, Matthew D. Green, Mike H. Allen, Eveline van Der Aa and T. E. Long
Department of Chemistry and the Macromolecules and Interfaces Institute
Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Abstract

The concept of structure versus function is a very important model in the realm of life sciences. For nonviral gene therapy, it is imperative that the structure and properties of synthetic vectors for binding with potential nucleic acid therapeutics be tailored to improve interactions among DNA and cell components. In this study, we determined the influence of partially alkylated poly(1-vinylimidazole) (PVIM) on DNA binding, cytotoxicity, and *in vitro* plasmid DNA delivery efficiency in African Green Monkey kidney endothelial cells (Cos-7) as a function of the percentage of alkylation. The vinylimidazole monomer is considered to be a structural mimic of the amino acid histidine, which has an aromatic ring thought to be important in the active sites of enzymes involved in cell proliferation, inflammatory response, and the transmission of nerve impulses. Electrophoretic gel shift assays revealed that an increase in alkylation percentage on PVIM increased the binding affinity toward plasmid DNA, with lower percentages (9%) showing minimal attraction, even as N/P ratios were increased above 10. Heparin competitive binding experiments revealed that higher N/P ratios of PVIM bound more strongly to DNA, and that heparin (with its 3-sulfate groups per unit), has a strong affinity for the cationic polymer even at low concentrations. In cell viability assays, PVIM was shown to be negligibly cytotoxic to the Cos-7 cells, even at high concentrations of 200 ug/mL. Only when PVIM with higher alkylation percentages (83%, 88%) was introduced to the cells did cytotoxicity become evident. The sizes and zeta potentials of heparin/PVIM complexes were measured via dynamic light scattering and the results of each experiment were compared.

Design of Magnetic Field Flux Concentrator for an AC Magnetic Field-Triggered Drug Delivery Application. Stefan Green, Nursing Program, New River Community College, Dublin, VA and V. Soghomonian and J. J. Heremans, Dept. of Physics, Virginia Tech, Blacksburg, VA

AC magnetic fields couple to superparamagnetic nanoparticles, and the energy transferred is dissipated as heat throughout the particle and into the surroundings. The goal here is to exploit this idea for local, efficient and gentle means for delivering drugs, where drugs are tethered to iron oxide superparamagnetic nanoparticles and placed in an AC magnetic field. We increase the strength of the magnetic field by building a flux concentrator to be used in conjunction with an AC magnetic field generation assembly. We design a magnetic field concentrator to boost the magnetic field strength experienced by the sample. The magnetic field is generated by a solenoid wrapped around a C-shaped iron core. The iron concentrates the magnetic field. The ends of the C-shaped core are tapered, to further increase the magnetic flux experienced by the sample with a volume of 1 cm^3. After the sample is exposed to an AC magnetic field, the resulting rise in temperature is measured by a thermocouple immersed the sample solution. Initially, we test our set-up and design by recording the rise in temperature of iron oxide nanoparticles dispersed in different solvents. From these results, we aim to optimize AC magnetic field parameters - frequency, duration and strength. For drug delivery applications, the local heating at the surface of the nanoparticles will be used to release the drugs or biomolcules attached to the particles.

Enzymatic Degradation of Regenerated Cellulose Cinnamate with Varying Degrees of Cinnamate Substitution

Liz Huh[a], Heejun Choi[a], Ezra Yohannes[a], Zelin Liu[a], Andreas Koschella[b], Thomas Heinze[b], and Alan R. Esker[a]

[a] Virginia Tech, Department of Chemistry and the Macromolecules and Interfaces Institute, Blacksburg, VA 24061

[b] Center of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstraße 10, Jena, 07743 Germany

Abstract

Enzymatic degradation of regenerated cellulose cinnamates (CC) was tested. Trimethylsilyl cellulose (TMSC) was synthesized from Avicel with a 2:1 and 1.63:1 molar ratio of hexamethyldisilazane (HMDS) to anhydroglucose units (AGU). Trimethylsilyl cellulose cinnamate (TMSCC) was synthesized from TMSC samples thought a reaction with cinnamoyl chloride in the presence of an acid scavenger, pyridine. Ultraviolet-visible (UV-vis) spectroscopy was used to analyze the degrees of substitution (DS) of cinnamate, which were found to be 0.17 and 1.30 for the samples made from the 2:1 and 1.63:1 HMDS: AGU, respectively. TMS groups lost during cinnamate formation were then replaced through a second HMDS reaction. The resulting TMSCC was then spin-coated onto gold crystal sensors for quartz crystal microbalance with dissipation monitoring (QCM-D) and hydrolyzed for analysis of enzyme catalyzed degradation. The results showed that the presence of cinnamate groups promoted irreversible enzyme adsorption and inhibited enzyme activity, even at a DS that corresponded to one cinnamate group per six AGUs.

A New Delivery System for Mitomycin C

Barbara Lawson, T. Horseman, J. Logsdail, B. Zhang, S. R. Turner and A. W. Morgan
Macromolecules and Interfaces Institute and Depts of Materials Science and Engineering and Chemistry Virginia Tech, Blacksburg, VA 24061

Abstract

Mitomycin C is an anticancer drug that binds to the DNA in cells, and causes death to the cells. The effectiveness of mitomycin C in inhibiting growth in cancer cells has been demonstrated, but due to the toxicity of the drug, novel delivery methods need to be evaluated to reduce the effective dose. Gelatin microspheres were prepared and crosslinked with glutaraldehyde. The crosslinked microspheres were swelled in a mitomycin C/PBS solution to give 1 ng drug/mg microspheres-μL of PBS. *In vitro* release was measured on fibroblasts in Dulbecco's Modified Eagles Medium. Rhodamine 123 was also used as a model drug due to the similarity in chemical structure with mitomycin C to measure *in vitro* release. Results showed that free rhodamine 123 diffused quickly through the dialysis tubing. The drug-loaded microspheres in the absence of collogenase type I released rhodamine 123 at a slower rate than microspheres incubated with the enzyme. MTT cytotoxicity assays showed that the mitomycin C released *in vitro* halted cell proliferation. In collaboration with Julia Logsdail (another SURP student), an alternative crosslinking reagent to glutaraldehyde was developed that contained an acid-labile acetal group and a maleimide that could be reacted with pendent amino groups on the gelatin matrix. A model compound was prepared and MTT assays were run on it to determine whether tethering of the crosslinking agent to the mitomycin C would destroy its toxicity to cancer cells. Importantly, the mitomycin C tethered to the new crosslinking agent maintained its ability to halt cell proliferation to the same extent as free mitomycin C. This suggests that even if the new crosslinking agent reacted with the drug, its activity against cancer cells will be preserved.

Synthesis of *Bis*-Maleimide with a Degradable pH Sensitive Acetal Linkage for Drug Delivery Systems

J.K. Logsdail,[†] B. Zhang,* S.R. Turner,* B. Lawson,* T. Horseman,* A. Morgan*
[†]*Florida Institute of Technology,*Department of Chemistry, Department of Material Science and Engineering and the Macromolecular Interfaces Institute, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060*

Abstract

There is significant interest in developing therapeutic delivery systems to target cancer sites and tumors. Such materials have potential to lower harmful side effects of powerful anticancer drugs. A popular recent strategy has been to couple the anticancer drug to a macromolecular carrier with a pH sensitive (acid degradable) linkage. In the present work, a bis-maleimide containing a pH sensitive ketal (1) has been prepared and studies have been initiated to understand its efficiency in enhancing the delivery of Mitomycin C, which is a well known chemotherapeutic agent. The degradation product, N-(hydroxyethyl)maleimide, was synthesized for characterized, and interactions with Mitomycin C and gelatin were measured. The synthetic approach was initiated using both a furan-protected and unprotected N-(hydroxyethyl)maleimide to synthesize the bis-maleimide. Strategies involving the use of (1) to form acid degradable crosslinked gelatin microspheres containing Mitomycin C, and the coupling of Mitomycin C to gelatin via Michael addition with (1) will be further investigated.

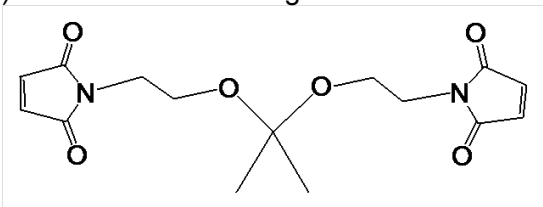

Figure 1. Compound 1. pH sensitive-bis-maleimide.

Cellulose Nanocrystals for Targeted Drug Delivery Applications

S. Oh,[1] S. Dong,[1] Y. W. Lee,[2] M. Roman[1]
[1]*Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061*
[2]*VT–WFU School of Biomedical Engineering and Sciences, Blacksburg, VA 24061*

Abstract

Cellulose nanocrystals (CNCs) are rod-like nanoparticles of cellulose with average lengths between 100 and 150 nm and average diameters between 3 and 5 nm. CNCs have attractive properties for applications as drug carriers in cancer therapy. They are biocompatible, nontoxic, and are produced from a renewable resource. This study evaluates the potential of CNCs in targeted drug delivery applications. CNCs were prepared by sulfuric acid hydrolysis of bleached wood pulp. The surface of the CNCs was decorated with amino groups through step-wise reaction with epichlorohydrin followed by aqueous ammonia. The aminated CNCs were functionalized with fluorescein isothiocyanate (FITC) as fluorescent tracing agent and folic acid (FA) as a targeting agent. The number of covalently attached FITC and FA moieties was measured by UV-visible spectroscopy. The shape and size of the functionalized CNCs was analyzed by atomic force microscopy. The uptake of untargeted and targeted CNCs by KB cells was analyzed by laser scanning confocal microscopy. Cell uptake was not observed for the untargeted CNCs. However, cellular uptake of FA conjugated CNC-FITC was confirmed.

Design of an AC magnetic field assembly for localized and on-demand drug delivery through interaction of an AC magnetic field and its coupling to superparamagnetic nanoparticles. Carlton Parker, Dept. of Physics, Daytona Beach, FL and Bethune-Cookman University, Department of Physics, V. Soghomonian and J. J. Heremans, Dept. of Physics, Virginia Tech, Blacksburg, VA

AC magnetic fields couple with magnetic materials, and this coupling may be exploited in the case of superparamagnetic nanoparticles for drug delivery purposes. Upon exposure to the AC magnetic field, a superparamagnetic nanoparticle will experience a fluctuation and relaxation of its magnetic dipole vector. As a consequence, it will dissipate energy as heat that can be used to release conjugated biomolecules or drugs. To achieve this, a Helmholtz coil, which is known for the extremely uniform magnetic field it produces, is being designed and constructed. The uniformity arises from the specific distance between the two coils which is equal to their radius. These coils are to be equal in their radii, and also in their number of turns, length, and current. In this specific case, the goal is to have the Helmholtz coil produce an AC magnetic field with a magnitude of 50mT and 300 kH frequency. A pick-up coil is being constructed to measure the strength and frequency of the AC field. To accomplish drug release from superparamagnetic iron oxide nanoparticles, several factors including magnetic field strength, frequency, and duration of magnetic field exposure, will be optimized. While various relaxation mechanisms may be at play, we choose 8-nm diameter magnetite nanoparticles, decorated with a polymer brush shell that favors the Néel relaxation regime.

Abstract

The preparation of doxycycline-bile acid salt complexes was reported. The structure and composition of the products were characterized by ^{1}H NMR and UV-Vis Spectroscopy. The salt of doxycycline with bile acid is much less soluble in water relative to doxycycline precursor. The simple technique of ionic exchange followed by complex salt formation between antibiotic and bile acid sodium salt was demonstrated to be useful to increase hydrophobicity of hydrophilic drugs. For the first time, the direct ionic modifications of bile acid and antimicrobial agents have been reported. The dissociation of doxycycline from the complex salt is pH-dependent which could be utilized for developing controlled release drug delivery system. The attempts to encapsulate complex salt into various amphiphilic biodegradable polyesters were also demonstrated.

Characterization of Random and Block Copolymers for Use in Reverse Osmosis Applications. Elvin Santiago, Dept. of Chemical Engineering, University of Puerto Rico - Mayagüez Campus, Mayagüez, PR 00681 and Desmond VanHouten and James E. McGrath, Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061

Potable water shortage is a growing global concern. Because of the increased demand and short supply, membrane based desalination of water has become the principal methodology. Reverse osmosis (RO) is the leading method of water purification because it is economically viable and able to remove monovalent ions and other solutes. Polyamide interfacial composite membranes are the most widely used commercial membranes for RO. While polyamides yield high flux and salt rejection, they are not resistant to critical chlorinated disinfectants which are used in 98% of drinking water systems. These materials also have relatively poor membrane fouling characteristics, partially due to the rough surface of the crosslinked polyamides. Our strategy is to develop a new chlorine resistant membrane for RO application so we can desalinate water without dechlorinating the water, and thus minimize the operational cost. Focus has been on the characterization of thermal and mechanical properties of novel random and block copolymers for both the selective and the porous support layers that are potential candidates for our desired composite RO membrane. Disulfonated poly(arylene ether sulfone) random and multiblock copolymers derived from disulfonated 4,4'-dichlorodiphenylsulfone and 4,4'-biphenol or bisphenol-A have been investigated. These polymers exhibit high temperature stability (up to ~450 °C in a nitrogen atmosphere) and ductile tensile properties at various levels of sulfonation. Novel poly(ethylene oxide)-*b*-polysulfone-*b*-poly(ethylene oxide) (PEO-BisAPsf-PEO) triblock copolymers for use in the porous support layer exhibited good thermal stability as well. From differential scanning calorimetry, it was concluded that the PEO is compatible with the polysulfone because of the depression of the glass transition temperature of the polysulfone. Research is underway to produce a porous support from these novel triblock copolymers.

Design, Fabrication, and Testing of Bismuth Hall Biosensors Capable of Detecting Superparamagnetic Nanoparticles

A. Sigillito*, M. Rudolph+, R. Kallaher+, V. Soghomonian+, J. J. Heremans+
**Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061 and Department of Physics, University of Dallas, Irving, TX 75062*
+Department of Physics, Virginia Tech, Blacksburg, VA 24061

Abstract

The efficacy of treatments for many diseases depends on early diagnosis. One promising diagnostic technique is the use of Hall biosensors because of their high sensitivity, low cost, and accuracy. Hall biosensors work by outputting a voltage proportional to the strength of the magnetic field created by a magnetically labeled biomolecule attached to the surface of the sensor. These sensors were fabricated using bismuth thin films because of bismuth's low toxicity, low cost, and large Hall coefficient, making it an ideal material for medical applications. Hall bars were characterized by making magnetoresistance measurements and were found to have good qualities for detecting superparamagnetic nanoparticles that could be attached to biomolecules. One sensor was exposed to nanoparticles as a test of the sensor's efficacy with positive results. This research suggests that bismuth Hall biosensors are a promising alternative to traditional diagnostic techniques.

Application of GC/MS and DSC for Characterization of New and Extracted Polyethylene Pipe

Timothy Smiley and A. M. Dietrich

Abstract:

Polyethylene has become a popular material used for drinking water pipes, but these pipes have been known to cause off flavors and odors in the water. These pipes contain antioxidants that tend to migrate to the surface that is in contact with the water running through the pipe. This causes reactions between chlorine and possibly other ions that are put in drinking water. This project focuses on the interaction between chlorine and the pipe by placing pieces of polyethylene pipe in water with a chlorine concentration of 250 mg/L at 37 ^{o}C, with the water being changed every 3 days. The water and the solid are being analyzed at different stages to determine the rate at which the antioxidants leech into the water. This is being done by running soxhlet extractions on the solid, liquid-liquid extractions on the water after it has been changed, and performing oxidation induction time measurements (OIT). The results have shown an increase, over an 18 day period, of chlorinated compounds in the water, along with other compounds associated with antioxidant degradation, and a decrease in the remaining antioxidants in the pipe verified by OIT of a piece of pipe and GC/MS analysis of the solid extract.

Unconventional Esterification of Cellulose in Different Homogeneous Media

C. Tate, B. Li, and K. Edgar, *Macromolecules and Interfaces Institute and Critical Technology and Applied Science, Virginia Tech, Blacksburg, VA 24061*

Abstract

Structure-property relationships of three different cellulose esters were compared in ionic liquid and binary solvent homogeneous media. The poor solubility of cellulose in common solvents severely limits strategies for its chemical modification. This difficulty is compounded by effects that solvents have on the selectivity and degree of substitution (DS) of the hydroxyl groups on the glucose monomer. Various methods have been developed for dissolution and esterification of cellulose in homogenous media. The present study has compared unconventional syntheses of 2,4,6-trimethylbenzoyl (TMB), adamantoyl (Ad), and trimethylacetyl (pivaloyl) celluloses in the ionic liquid 1-allyl-3-methylimidazolium chloride ([Amim]Cl) and in the binary solvents *N,N*-dimethylacetamide/lithium chloride (DMAc/LiCl) and tetrabutylammonium fluoride trihydrate/dimethylsulfoxide (TBAF/DMSO). The temperature and reaction time were held constant and the molar ratio of reagent to cellulose was varied from 1.0 to 3.0 for each esterification reaction. DS values of the cellulose esters ranging from 1.00 to 3.00 were obtained and their respective solubilities in tetrahydrofuran (THF), chloroform, DMSO, DMAc, acetone, water, and methanol were compared.

Determination of hydrophobic interactions between polymer-magnetite complexes

Pedrolando Valdez, W.C. Miles, P.P. Huffstetler, Alfred Chen, R.M. Davis, Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061.

Polymer-magnetite nanoparticles are exceptional tools in the field of therapeutic imaging and drug delivery. Magnetite (iron oxide) nanoparticles make excellent contrast agents for magnetic resonance imaging (MRI) due to their size and high magnetic susceptibility. Adsorbing a polymer coating on the exterior of the nanoparticle may enable specific cell targeting as well as significant cell-complex interactions. By tuning the polymer composition to target certain types of cells, this would not only allow for a higher contrast image, but would also mean that specific organs could be targeted for imaging. During our current work, magnetite nanoparticles were synthesized and coated with an amphiphillic poly(propylene oxide)-*b*-poly(ethylene oxide) (PPO-*b*-PEO) copolymer at varying diblock polymer ratios and polymer loadings. These complexes consisted of a magnetite core surrounded by an inner hydrophobic PPO layer followed by an outer hydrophilic PEO brush. Octadecyltrichlorosilane (OTS) was used to hydrophobize both silica slides and silica crystals for Quartz Crystal Microbalance with Dissipation (QCM-D) measurements. Contact angle measurements were used to characterize a hydrophobic surface while QCM-D measured the mass of complex adsorbed onto the OTS functionalized silica crystal. Lipid bilayers were also used as an alternative substrate for adsorption measurements.

Elimination of Intracellular *Listeria monocytogenes* and *Salmonella typhimurium* from J774A.1 Mouse Macrophage Cells by Polymeric Nanoplexes Encapsulating Gentamicin

Christine VanDuyn,[1] A. Ranjan,[2] N. Geter,[1] T. Vadala,[1] M. Douple,[1] J. S. Riffle,[1] N. Sriranganathan[1]

Virginia Polytechnic and State University, Blacksburg, Virginia 24061,[1] Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia 24061[2]

Abstract

Pathogens such as *Listeria* and *Salmonella* that can enter phagocytic cells have proven very difficult to treat. They have a high capacity to occupy host cells and find a comfortable intracellular niche where they can avoid host defenses. With the low efficiency of many antimicrobial drugs to pass through the host cell membranes, the host organism's immune capabilities falter and intracellular pathogens are difficult to eliminate efficiently. With hopes of fabricating improved methods of drug delivery for the treatment of chronic infections related to *Salmonella* and *Listeria*, polymeric nanoplexes encapsulating aminoglycoside antibiotics, specifically gentamicin, were developed to pass through the membrane and enable the drug to reach therapeutic levels inside the cells. J774 monocyte-macrophages were infected with two strains of bacteria, *Listeria monocytogenes* (wild type) and *Salmonella typhimurium* LT2 strains, and treated with 50 μg of free gentamicin or nanoplexes encapsulating gentamicin over a 6 h period, and tested for bactericidal efficacy. A comparative analysis was made of different methods for developing nanoplexes, and the bactericidal efficacy varied, depending on the method used, with promising results in some.

Adsorption of polysaccharides on pullulan model surfaces. Ezra Yohannes, Zelin Liu, Heejun Choi and Alan R. Esker, Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA 24061.

Wood-plastic composites are widely used materials due to their excellent properties, such as light weight and a high resistance against rotting, cracking, and splintering. Derivatives of cellulose with hydrophobic units can enhance bonding between cellulose and thermoplastics. However, derivatization of natural cellulose degrades and weakens its structure, which is extremely dependent on hydrogen bonding between its hydrophilic groups. Surface modification with self-assembling polysaccharides and their derivatives is one promising method to tailor properties of material surfaces. Our primary objective was to hydrophobically modify pullulan, a water soluble polysaccharide, with just enough cinnamate groups to ensure that the polysaccharide was insoluble in water. Thus, derivatized pullulan model surfaces were synthesized and analyzed using a quartz crystal microbalance with dissipation monitoring (QCM-D). The rate that the model surface degraded when exposed to the enzyme cellulase was measured relative to the rate of degredation of a cellulose surface exposed to the same enzyme. We discovered that there was very little adsorption of cellulase onto the model surface and no there was no degradation associated with the process. On the contrary, there was very high adsorption of the enzyme onto the cellulose surface and the surface degraded significantly.