1. Synthesis of Acrylamide Phosphonate Monomers and Polymers for Binding Bioimaging Agents and Inorganic Drugs

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A new acrylamide phosphonate monomer was synthesized via a two-step reaction. N-Butylacrylamide diethylphosphonate was prepared through an aza-Michael addition of n-butylamine across the vinyl bond of vinyldiethylphosphonate. This was followed by reaction with acryloyl chloride to form the acrylamide monomer. Free radical polymerization of the monomer followed by quantitative deprotection of the phosphonate ethyl ester groups yielded an anionic phosphonic acid polyacrylamide. Solubility studies showed that the anionic polymer was soluble in multiple solvents, including water (pH 7), DMSO, and methanol. This is important for binding the polymers to a variety of imaging agents and drugs. The new phosphonate-containing polyacrylamides will be bound to inorganic particles (e.g., magnetite) and salts (e.g., manganese cations) to serve as MRI imaging agents and to organoplatinum drugs for chelating and delivering platinum-based chemotherapy drugs.

2. Synthesis of Melt Processable Poly (Acrylonitrile-co-Methyl Acrylate) Statistical Copolymers via RAFT Polymerization

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Poly(acrylonitrile-co-methyl acrylate) copolymers are solution processed industrially to produce acrylic fibers, which requires the use of solvents that make it a less economically desirable alternative to melt spinning. Melt extruded polyacrylonitrile (PAN) copolymer fibers and films could have many useful properties; however, there is no way to melt process these materials at this time. Our research used reversible addition-fragmentation chain transfer (RAFT) polymerization to synthesize PAN copolymers, and focused on changing synthetic parameters such as initiator, chain transfer agent, and temperature to maximize monomer conversion. Our goal was to design a copolymer with high enough acrylonitrile content to retain the desirable properties of polyacrylonitrile such as thermal stability, chemical resistance, and low permeability, as well as to synthesize high strength and melt processable copolymers. The results showed that the combination of cyanomethyl dodecyl trithiocarbonate as the RAFT agent and 1,1'-azobis(cyclohexanecarbonitrile) as the initiator with polymerization at 100°C yielded the highest monomer conversion. 1,1'-Azobis(cyclohexanecarbonitrile) provided a more controlled conversion rate and growth of molecular weight as a function of time. Size exclusion chromatography (SEC) and nuclear magnetic resonance (NMR) molecular weight analysis were found to yield similar results using SEC universal calibration calculations, but not SEC light scattering analysis. Further investigation of polymer architecture is necessary to better understand the discrepancy between the light scattering and NMR analysis.

3. Novel Monomer Syntheses for Polymeric Membrane Separations

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Polymeric membranes are a versatile material platform for producing potable water, cleaner air, and for bioseparations including kidney dialysis and purification of blood. Membranes have wide scale application in reverse osmosis, which is important because it can produce drinkable water from seawater.

Gas separation membranes are used to help preserve food and prevent fires inside of aircraft. Novel monomers are the key to synthesize novel polymeric membranes, which may reduce the industrial costs of these separations. Synthesis of monomers like tetramethyl bisphenol A can be cost effective and the resultant materials can be crosslinked to improve performance in gas separations. Our goal was to synthesis novel monomers to make poly(arylene ether sulfone) copolymers for membrane applications. Electrophilic aromatic substitution of 2,6 xylenol was utilized with a variety of ketones as a highly versatile route to novel monomers. Tetramethyl bisphenol A and a novel monomer 4,4'-(2,2,2-trifluoro-1-phenylethane-1,1-diyl)bis(2,6-dimethylphenol) were synthesized and purified with acceptable yields. Efforts to increase the yield of the synthesis of tetramethyl bisphenol A consisted of varying reaction time, temperature and the relative concentrations of reagents. Proton NMR and melting points were used to confirm the structures and purity of these monomers. Further confirmation of the purity of the tetramethyl bisphenol A was obtained through the synthesis of a high molecular weight poly(arylene ether sulfone) using 4,4'-dichlorodiphenylsulfone as the activated dihalide.

4. Synthesis of Regioselective Cellulose Acetate Propionate Polysaccharides

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Randomly substituted cellulose acetate propionate (CAP) has been found to express extraordinary wavelength dispersion, an important property of retardation films that improves the viewing angle and contrast of liquid crystal displays. Regioselectivity has been shown to improve the optical properties of CAP, but common regioselective synthesis methods involving protection/deprotection groups are difficult and inefficient as they are time-consuming, expensive, can reduce the reactivity of cellulose, and produce low yields that are only partially regioselective. Our research focused on the synthesis of completely regioselectively-substituted CAP via deacylation with tetrabutylammonium fluoride (TBAF), a relatively new and efficient method for regioselective synthesis developed by Edgar et al.[1] Randomly substituted CAP was produced via peracetylation and/or perpropionylation for comparison. The goal was to produce four different polymers for optical testing: A) regioselective CAP with an acetate group at O-6 with degree of substitution (DS) equal to one and propionate groups at O-2/3 with DS equal to two, B) random CAP with DS(Ac)=1 and DS(Pr)=2, C) regioselective CAP with a propionate group at O-6 with DS=1 and acetate groups at O-2/3 with DS=2, and D) random CAP with DS(Pr)=1 and DS(Ac)=2. ^{1}H NMR spectroscopy was used to determine the DS values for the substituents on each product. DS values close to those desired were obtained by modifying the reaction parameters of TBAF deacylation and peracetylation and/or perpropionylation, including amount of reactants, temperature, and reaction duration.

1. Xu, D.; Edgar, K. J., TBAF and cellulose esters: unexpected deacylation with unexpected regioselectivity. Biomacromolecules 2012, 13(2), 299-303.

5. Structure-Property Relationships of Segmented Phosphonium Ionenes for Treating Genetic Diseases

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Gene delivery vectors for treatment of genetic diseases have been studied in recent decades. Efficiency and low cytotoxicity can be improved with low cation to anion charge binding ratios and introduction of biocompatible polyethylene glycol (PEG) segments. Using step growth polymerization, well defined novel phosphonium ionenes were synthesized for use as gene therapy vectors and thermoplastic elastomers. Three different classes of phosphonium ionenes were synthesized: non-segmented alkyl ionenes, PEG-based ionenes, and segmented ionenes. ^{1}H NMR spectroscopy and

aqueous size exclusion chromatography (SEC) were used to verify the expected compositions and measure the molecular weights. With the exception of the 3P,2-ionene, all polymers exhibited high molecular weights (M_w = 8.6 - 23.7 kg/mol). The 3P,2-ionene likely formed cyclic compounds due to the thermodynamic stability of the 7-membered ring generated. Phosphonium ionenes possessed high thermal stabilities (>300 °C). Non-segmented phosphonium ionenes displayed amorphous behavior while segmented polymers were semicrystalline. They exhibited microphase separation between the hard phosphonium ionene segments and soft PEG segments. Crystalline PEG segments melted at 35°C. Glass transition temperatures of the amorphous phases depended on the cationic charge density within the polymer. Higher charge densities resulted in higher glass transition temperatures, ranging from 130 to 190°C. DNA binding assays showed that both PEG-based ionenes and segmented ionenes successfully bound DNA with a low charge ratio of 1. The microphase separation combined with the electrostatic properties of the segmented phosphonium ionenes may make this new class of materials important for many adhesive and thermoplastic elastomer applications in addition to their potential to advance the field of gene therapy.

6. Bio-inspired Histamine-Containing Elastomers

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The imidazole ring has very important features in biological systems such as superior buffering capacity and the formation of key catalytic sites within proteases. Histamine is a naturally occurring biocompatible compound that contains an imidazole ring. Elastomers created using this compound are expected to have features similar to the imidazole ring, including self-complementary hydrogen bonding and π-π stacking. In this study, histamine was incorporated into an acrylamide monomer and copolymerized with *n*-butyl acrylate to form an elastomeric copolymer. The monomer and copolymer synthesis were confirmed through NMR. The properties of the copolymer were measured with thermal gravimetric analysis (TGA) and dynamic mechanical analysis (DMA). The TGA revealed that the copolymer had a 5 wt% loss at 257 °C under nitrogen atmosphere. The DMA curves showed broad glass transitions believed to be from the disruption of hydrogen bonds within the copolymer. In addition, a novel methacrylamide monomer was synthesized and crystallized. The expected structure of the crystals was confirmed through NMR and x-ray crystallography.

7. Polysaccharide-Polymer Nanoplexes for Wound Healing Applications

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Hyaluronic acid is a naturally produced polysaccharide integral in tissue regeneration. As an anionic molecule, it is capable of electrostatically interacting with cationic polyelectrolytes to form nanoplexes. The objective of this research was to deliver nanoplexes consisting of hyaluronic acid and the cationic polymer poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) to stimulate proliferation of HeLa cells. Formation of HA-PDMAEMA nanoplexes was studied in various aqueous media, at different charge mixing ratios, and with differing PDMAEMA molecular weights. Charge mixing ratios ranged from 0.1-10, and PDMAEMA weight average molecular weights were 83, 104, 148, 282, and 346 kDa. Aqueous solutions included purified water, acetate buffer, PBS buffer, and water adjusted to pH 5. Long term dispersion stability was observed for nanoplexes at pH 5 with charge ratios ranging from 0.1-1 cation-to-anion. The stable nanoplexes were used in a MTT assay to confirm increased cell proliferation with the hyaluronic acid nanoplexes. Preliminary results indicate that lower cation-to-anion ratios were nontoxic, while higher charge ratios were cytotoxic as expected. This demonstrates the wound healing potential for these semi-synthetic hyaluronic acid nanoplexes.

8. Facile Synthesis of Novel S-benzoyl Thiooximes for H_2S Delivery

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Recently, H_2S has been identified as the third gasotransmitter employed in mammals. Alterations in metabolism of H_2S have been linked to diabetes, neurodegenerative and cardiovascular disease states, as well as inflammation in the GI tract. These recent discoveries have shown a need for compounds capable of controlled, sustained H_2S release under specific physiological conditions. Research in Dr. John Matson's laboratory has led to the facile synthesis of a novel H_2S donor. This H_2S releasing functional group can be synthesized in two steps from commercial aldehydes and ketones with yields >90%. The resulting H_2S donor showed selective reactivity with the amino acid cysteine to induce H_2S release. Both aliphatic and aromatic donors were synthesized, with H_2S release half-lives being approximately 60 min for the aliphatic compounds and ranging from 108-190 min for the aromatic compounds. H_2S release half-lives were modulated via substitution on the nitrogen side of the S-N bond of the H_2S donor. Early hydrolysis kinetics showed hydrolysis half-lives on the timescale of weeks at physiological pH.

9. Enzymatic Degradation of Lichenan Layers Adsorbed onto Regenerated Cellulose Surfaces

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Mixed linkage glucans (MLGs), hemicellulose components of cell walls, are mainly confined to cereals, grasses, and lichens. Quartz crystal microbalance with dissipation monitoring (QCM-D) was utilized to measure the adsorption of MLG lichenans onto cellulose surfaces regenerated from spin-coated trimethylsilyl cellulose films (RC). The lichenan layers were subsequently degraded by lichenase, an endo-1,3(4)-beta-Glucanase from Bacillus subtilis, and this was studied using QCM-D. Lichenase cleaves beta-1-4 bonds adjacent to beta 1-3 bonds in lichenan and has been used in successful conversion of lichenan to bioethanol. The degradation of lichenan layers was studied as a function of enzyme activity and pH while keeping temperature, substrate surface properties, and the ionic concentration of buffer constant. The RC surface layers had a thickness of 10 nm and a root-mean-square surface roughness of 1 nm. The adsorption of lichenan was also studied as a function of temperature and concentration. The lichenan layer was adsorbed onto the surface at 50 °C with a concentration of 0.05 percent by mass for one hour. A Voigt-based viscoelastic model was used to fit the time dependent adsorption profiles for the soft MLG layer. The first adsorbed layer was found to be 19±1 nm thick with an areal density of 21±1 mg m^{-2}. The layer had an elastic shear modulus of 0.32± 0.04 N m^{-2} and a shear viscosity of 0.98± 0.03 Ns m^{-2}. Further studies will be required to characterize the lichenan layers after degradation to gain insight into how the enzyme changes the physical parameters of the lichenan layer and into how pH and enzyme activity affect these changes.

10. Polymer Coated Mn^{2+} MOFs for Theranostic Applications

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Metal organic frameworks (MOFs) have recently attracted considerable attention for use in biomedical imaging and theranostic applications. Mn^{2+} nano-MOFs used in this study featured paramagnetic metal centers that make them ideal as positive contrast agents for MRI imaging. The goal of

this study was to evaluate the use of polymeric shells for suspension and covalent functionalization of nano-MOFs under simulated physiological conditions of the brain. A 0.6% agarose gel was used to mimic the mechanical properties of the brain while a mock cerebrospinal fluid was used to mimic the chemical environment of the brain. Degradation of the nano-MOF was determined as a percentage of organic 4,4-azobenzenedicarboxylic acid (ABDA) linker released and was measured at 330 nm by UV-Vis spectroscopy. A poly(ethylene glycol-b-caprolactone) (PEG:PCL) block copolymer shell was efficient in suspending the nano-MOFs and protecting them from degradation. Increasing the molecular weight of the PCL from 1000 to 1500 in the PEG:PCL block copolymer further increased suspension. To demonstrate that primary amines on the termini of the polymer were on the outside shell of the nano-MOF for further functionalization, carboxy-fluorescein was covalently coupled to the polymer-coated nano-MOFs.

11. Cancer Cell Protrusion Dynamics on Nanofibers

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Cellular cytoplasmic protrusions have been shown to degrade extracellular matrix (ECM), aid in cell migration, and increase invasiveness of cancer cells. Little characterization in a 3D environment has been done. In cancerous cells, filopodia-like projections known as invadopodia break down the surrounding ECM and infiltrate the proximate blood vessels. Previous studies used chemical gradients to force cell protrusions across a porous membrane. In this study, nanofibers were aligned using a Spinneret based Tunable Engineered Parameter (STEP) fiber manufacturing technique and these aligned fibers were used to elicit cell protrusions. In brain and breast cancer cells, the maximum length of protrusions was found to increase at a constant rate with decrease in fiber stiffness. Cell protrusion retraction rates for both cell types increased with an increase in fiber diameter. Curcumin, a natural anti-cancer agent, was incorporated into the nanofibers and was shown to affect cell strength. Primary data showed that the force exerted by the cell on the curcumin-loaded nanofibers was 10.4 nN, nearly double the force of 5.9 nN that was exerted on the non-curcumin loaded fibers.

12. 3T3 Mammalian Cell Chemotaxis on Nanoscaffolds in a Flow-Based Microfluidic Device

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Mammalian cell chemotaxis in flow-based microfluidic devices has been thoroughly studied and has yielded several useful results including hydrodynamic effects and chemical gradient analyses. However, cellular movements on different substrates in the chemotactic environment have not been explored. We investigated 3T3 cell migration on three substrates; glass, polystyrene fibers aligned parallel to the chemical gradient, and polystyrene fibers aligned perpendicular to the gradient. The scaffolds were prepared using a spinneret based tunable engineered parameter (STEP) method to wrap polystyrene fibers around cellophane films. The scaffolds were then placed in the main microchannel of a serpentine flow-based microfluidic device at a location where a 50 (μg/mL) mm^{-1} chemical gradient of fibronectin had developed. Cells were tracked using a manual tracking plugin in ImageJ, which stores x-y coordinates from mouse clicks made on cells over each video frame of the experiment. These coordinates were then referenced to the origin of the main microchannel before tracking parameters such as total distance traveled, average speed, and chemotactic index were calculated. Initial results from cell tracking indicate that cells on fibers parallel to the gradient moved up the gradient 85% of the time while cells on glass and with the gradient perpendicular to the fibers only moved up the gradient 46% and 40% of the time respectively. Another interesting result was that on average, cells attached with the gradient perpendicular to the fibers traveled 660 μm over a 21.5 hour time frame while cells on glass and parallel gradient fibers only traveled approximately 300 μm. These preliminary results could indicate that chemotactic migrations

are aided by gradient parallel fibers and that gradient perpendicular fibers cause cells to travel farther thus consuming more energy trying to find a route towards the chemical gradient.

13. Optimization of DNA Bisulfite Conversion for Microfluidic Chip Applications

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Significant information regarding the presence of cancer can be revealed by analyzing epigenetic patterns in DNA. The cytosines in 5'CpG islands in a DNA sequence can contain methyl groups which affect DNA transcription and translation and can be altered by the presence of a disease. These epigenetic methylation patterns can be studied through bisulfite conversion. Bisulfite conversion is the process by which non-methylated cytosines are converted to uracil and methylated cytosines are left unconverted. After conversion highly specific primers are used to amplify the DNA for analysis.[1] This study used genomic DNA taken from GM-12878 lymphocytes and primers specific for the promoter region of the p15 tumor suppressor gene. If abnormal methylation patterns exist in this region of the DNA and therefore do not match the selected primers, the DNA will not be fully amplified and the likelihood of cancer is high. Polymeric microfluidic chips are being designed for fast, simple, and affordable disease prognosis. The chip assay requires the conversion process to be highly efficient, repeatable, and accurate. The known process of bisulfite conversion was optimized from an average 50% conversion efficiency to an average 80% efficiency through the addition of catalysts and protecting buffers and by varying the incubation times and temperatures. The added agents included trolox, TAC, TETRAEN, and guanidine hydrochloride. The optimized conversion process was designed for application on the PDMS microfluidic chip with novel diffusion design. This chip design allows for all steps in bisulfite conversion including cell lysis, denaturation, conversion, DNA clean-up, and PCR to exist on a single chip.

1. Susan Clark, A. S., Clare Stirzaker, Peter Molloy, Marianne Frommer, DNA methylation: Bisulphite modification and analysis. Nature Protocols 2006, 1 (5), 2353-2364.