FINAL REPORT
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CONTINUATION OF: BIODEGRADABLE LACTIDE NANOPARTICLES FOR THE DESTRUCTION OF MALODOROUS ORGANICS

Principal Investigator(s): Daniel C. Whitehead, Assistant Professor
dwhiteh@clemson.edu
Department of Chemistry
467 Hunter Laboratories
Clemson University
Clemson, SC 29634
(864) 656-5765/(864) 656-6613 FAX

Collaborators: Frank Alexis, Assistant Professor
falexis@clemson.edu
Department of Bioengineering
203 Rhodes Annex
Clemson University
Clemson, SC 29634
(864) 656-5003

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Lay Summary: This project focuses on the development of new technologies to reduce odor emissions from the rendering industry. The main target of this work is to develop engineered, biodegradable poly(lactic acid) nanoparticles whose surface is decorated with appropriate reactive sites (*i.e.* functional groups) that are poised to sequester or destroy malodorous volatile organic byproducts of rendering processes. Briefly, by decorating the nanoparticles with appropriate reactive sites, offending volatile byproducts will be absorbed onto the particles by the formation of either ionic or covalent bonds. Alternatively, the offending odors will be chemically oxidized on the particle surface in order to generate less odorous byproducts. The malodorants will be chemically modified so as to both harness them onto the biodegradable particles for disposal, and to render them less odorous. This study will serve as a proof-of concept to demonstrate that functionalized biodegradable poly(lactic acid) nanoparticles have the potential to become a next-generation strategy for odor remediation in the rendering industry. Long term goals include the evaluation of our materials in on-site at a rendering facility, as well as the development of a scalable, commercializable product.

The technology being developed has the potential to be very beneficial to the rendering industry in terms of providing a next-generation alternative to established methods for odor remediation. The particles described herein might present several uses in an industrial setting. The particles could be employed as a rapid-use odor adsorbent in an emergency or spill situation. Further, the particles could, in principle, be incorporated into existing odor remediation equipment as an added means for odor elimination. The obvious long-term goal would be to develop an appropriate formulation of functionalized PLA nanoparticles that would compete with or ideally supplant existing odor elimination measures. One advantage of such an outcome includes the ability to land-fill or perhaps regenerate the spent scrubbing material. Indeed, PLA polymers are biodegradable and non-toxic. Additionally, since their means of neutralizing malodorants is based on chemical reactivity instead of thermal degradation, the implementation of this strategy should return significant cost savings in terms of reduced energy consumption and reduced greenhouse gas emissions. Finally, the general strategy of surface decoration of polymer matrices with reactive sites is not limited to PLA polymers. One could envision the development of similar strategies for the preparation of “smart” clothing or other materials that are equipped with an odor elimination mechanism.

Objective (s): The Objectives of this proposal were:

1. To assay the ability of amino-functionalized engineered poly(lactic acid) nanomaterials to sequester and destroy malodorous compounds.

2. To prepare and assay an ammonium peroxysulfate functionalized poly(lactic acid) nanomaterial for the oxidative destruction of malodorants.

3. To prepare and assay a hybrid poly(lactic acid) nanomaterial functionalized with amino and ammonium peroxysulfate functional groups for the destruction of malodorants.

Project Overview:

1.1. Introduction: The Rendering Process and Malodorant, Environmentally Harmful Pollutants

Rendering is inextricably linked to the success and efficiency of the broader agricultural enterprise in the US. Roughly one third to one half of the live weight of livestock is not typically consumed by humans. Specifically, 49% of the live weight of cattle, 44% of the live weight of hogs, 37% of the live weight of broiler chickens, and 57% of the live weight of fish are not consumed by humans, and must therefore be processed by rendering methods. Indeed, 35 million cattle, 100 million hogs, and approximately 8 billion chickens are slaughtered per year in the US alone. The so-called co-products (or by-products) of slaughterhouse and butchering operations include hides, skins, hair, feathers, hooves, horns, feet, heads, bones, toenails, blood, organs, glands, intestines, extraneous muscle and fat tissues, egg shells, and whole carcasses (*i.e.* dead stock and slaughterhouse rejects). The total output of these materials from slaughterhouse operations exceeds 54 billion pounds per year. Rendering processes cook down these co-products (along with used restaurant cooking oils and expired grocery products) in order to drive off moisture, separate the animal fats, and concentrate the protein-rich material into various dry meals including meat and bone meal, meat meal, poultry meal, hydrolyzed feather meal, blood meal, and fish meal.[1]

The products of the rendering process, protein rich meals (11.2 billion pounds per year) and rendered animal fats (10.9 billion pounds per year), typically reenter the agricultural enterprise as feeds for livestock, poultry, aquaculture, and companion animals. Additionally, some rendering products including edible tallow (4.2 million pounds per year), lard (2.35 million pounds per year), and gelatin are suitable for human consumption.[1]

The contribution of the rendering process to the sustainability of agriculture and food processes is two-fold. First, the process allows for the efficient removal, decontamination, and repurposing of the very large by-product stream from livestock and slaughterhouse operations. The cooking process serves to reduce the volume of the by-product stream by removing moisture (60% of gross weight) as well as to neutralize or deactivate biological contaminants including bacteria, viruses, protozoa, and parasites that would otherwise be deposited into the environment. Secondly, the products of the rendering process provide a nutrient-rich, high-energy food source for livestock, poultry, and aquaculuture. In the absence of rendering operations, the alternative disposal strategies for slaughterhouse offal including landfilling or incineration, pose serious environmental concerns in terms of the potential for water and soil contamination and the venting of noxious gaseous emissions.[1]

Significant efforts and capital are invested into controlling odor emissions that result from processing unused organic matter from livestock production, meat and food processing, and the food service industry. The positive environmental impact of the rendering process in terms of reducing the amount of landfill and biological waste from the aforementioned operations is often overshadowed by negative perceptions of the environmental impact of the process in terms of wastewater and odor emissions. Concerns over odor emissions often play a large role in community resistance to site selection for new rendering operations.[2]

Indeed, *untreated* emissions from rendering operations can be detected up to 20 miles away from the source.[3] Modern rendering facilities are equipped with sophisticated mechanisms for controlling odor and particulate emissions. Particulate emissions are controlled by filtering mechanisms called bag houses.[2] Malodorous vapors and contaminated air from the rendering process are controlled by means of several strategies. Common odor control methods include combustion/incineration, chemical oxidation, wet scrubbing, and biological scrubbing. On-site air emissions are often treated with packed bed scrubbers containing chemical oxidants or with masking odors. Cooking vapors are often treated in two stages. First, vapors are cooled and passed through a venturi scrubber. The vapor effluent of the venturi scrubber is then treated with packed bed scrubbers filled with chemical oxidants. Other methods for treating cooking vapors involve the removal of condensable vapors followed by incineration of the non-condensable vapors in the plant boilers.[2,3] Biological scrubbers remove plant odor emissions by passing odorous air through scrubbers containing bacterial biofilms that metabolize the odorous emissions.[2-5]

Despite their sophisticated nature, current odor remediation strategies suffer from some drawbacks. Combustion and incineration strategies are energy intensive processes that result in substantial venting of greenhouse gases. Chemical oxidation requires the use of strong oxidants. Spent chemical and biological packed bed scrubbers must be disposed of properly. Additionally, aqueous effluent from packed bed and venturi scrubbers contribute to the already substantial wastewater flow from rendering operations.[2] Finally, despite these technological advances the process nonetheless still suffers from a substantial odor problem.

Van Langenhove *et. al.* conducted a comprehensive study aimed at identifying the specific volatiles that contribute to malodorous rendering odors that vent from cooking processes. In total, about 110 distinct volatiles were detected and identified, but 26 of that number were confirmed to contribute to the unsavory odors of cooking processes.[6] Chart 1 includes a comprehensive list of the offending odorants as well as a general depiction of their common functional group. The list contains ten short chain aliphatic aldehydes ranging from three to ten carbons in length. Carboxylic acids comprise the second most populous group, containing examples ranging from two to six carbons in length. Sulfur containing functional groups (including thiols, sulfides, disulfides, and trisulfides) comprise the third largest group of offending odorants. Additionally, a single alcohol and a single amine were identified.[6] Most of these volatile organics result from the thermal breakdown of protein and fats during the cooking process.[4] Many of them are highly flammable, corrosive, carcinogenic, and/or toxic to both humans and animals.

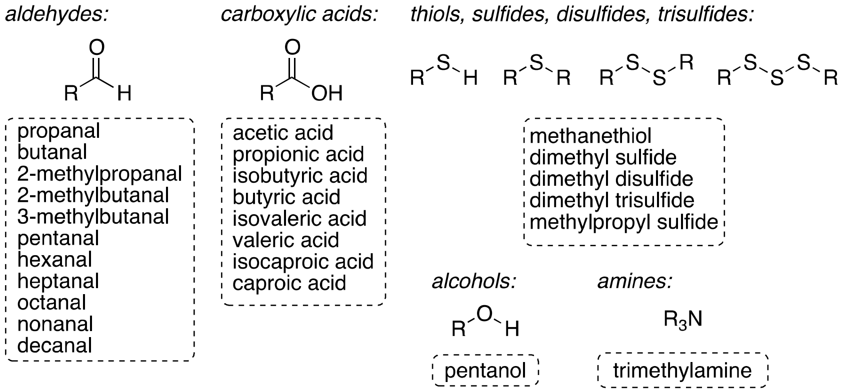


Chart 1. Volatile organics responsible for malodorant cooking emissions organized by functional group.

Other sources of odors in the rendering process include protein degradation products including amino acids and peptides, most notably present during blood storage or emitting from raw material stocks. Wastewater treatment produces several noxious, toxic emissions including hydrogen sulfide and ammonia. Evaporation processes and animal waste product storage also emit hydrogen sulfide and ammonia along with various other amines, aldehydes, carboxylic acids, and thiols. Finally, smokehouse operations emit various aldehydes, acids, and other organic constituents including cresol and acrolein, a potent carcinogen.[4,7]

The large number of offending chemical entities as well as their ill-defined and often variable relative concentrations complicate approaches toward the strategic remediation of rendering contaminant emissions. The situation is further complicated by the fact that the most offending odorants are not necessarily the major constituents of the emissions from rendering processes. For example, the human nose is capable of detecting odorants with thresholds as low as 0.1 parts per billion (v/v).[8]

In summary, the rendering process, while playing a vital role in handling animal by-products form slaughterhouse operations, results in the release of a complex mixture of small molecules that are malodorous. *This on-going project has been devoted to demonstrating the potential of biodegradable functionalized poly(lactic acid) nanoparticles for rendering odor remediation in a series of proof-of-concept studies*.

1.2. Introduction: Poly(Lactic Acid) Polymers and Applications

Polymers of lactic acid (*e.g.* Figure 1) were first discovered in 1932, but their practical applications did not begin to emerge until the early 1970s when they found considerable use as dissolving sutures in surgical applications.[9,10] Fermentation technology developed in the late 1980s resulted in affordable means for the preparation of the requisite monomer for PLA synthesis from D-glucose obtained from corn. Cheaper monomer sources for PLA catalyzed a renaissance of its study, and numerous new applications of the polymer have recently emerged. Current applications include formulations for biodegradable trash/lawncare bags, water bottles, food storage containers, and various fiber products.[11,12] Recent applications in medicine include applications for drug-delivery and controlled drug release. PLA polymers are particularly exciting due to their *biodegradability and toxicological safety*.[9] PLA polymers can be engineered to degrade over a timeline of days, weeks, months, or even years depending on the specific formulation.[9,13] *Their non-toxic nature and tunable degradation properties make them excellent candidates as a medium for the remediation of malodorous rendering emissions.*

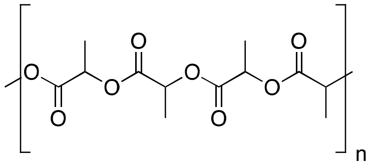


Figure 1. Polylactic acid (PLA)

2.1. *Experimental Design*

With the goal of investigating novel platforms appropriately functionalized to either chemically modify or sequester target malodorous rendering emissions, we designed a novel formulation of amino-functionalized poly(lactic acid) nanoparticles. Figure 2 Panel depicts the target materials in cartoon format along with a photograph of the prepared material. The overall design strategy is based on a non-toxic, biodegradable poly(lactic acid) (PLA) substrate. The PLA core of the materials is then coated with a polyethylene glycol layer, and is then capped with an appropriate functional group via a variable linker region (see Panel B for polymer formulation). The functionalized PLA materials capped with amine (R-NH_2) functional groups are suitable for reaction with a large suite of the most common malodorant VOCs associated with rendering processes (*i.e.* short-chain fatty acids and aldehydes). Specifically, the choice of the amine group was driven by considering the reactivity of the conglomerate of compounds collected in Chart 1 (see Panel C). Considering conventional organic chemical reactivity, nineteen of the twenty-six VOCs associated with offending rendering odors, namely the aldehydes and carboxylic acids, are expected to react with an amino functionality and be sequestered (*i.e.* via chemical transformation). Aldehydes are known to undergo condensation with amines to generate imines (Panel C, eq. 1) via the expulsion of water. Reaction with an amino functionalized PLA nanoparticle would result in the covalent sequestration of the offending odorant onto the surface of the nanomaterial. This interaction would prevent the emission of the compound as well as render it less volatile and therefore less odorous. The high surface-to-volume ratio of nanomaterials and their unique functionalities are expected to significantly reduce the emission of malodorous compounds.

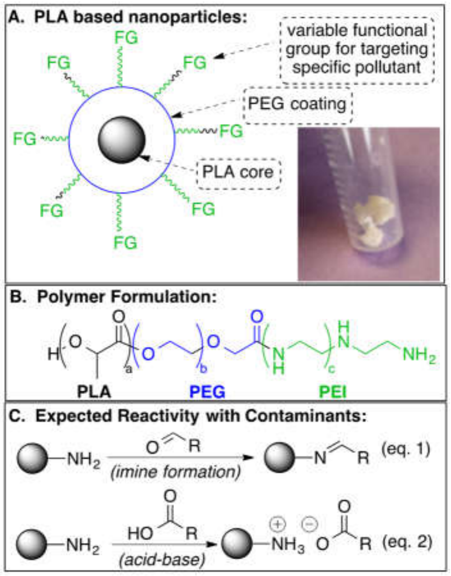
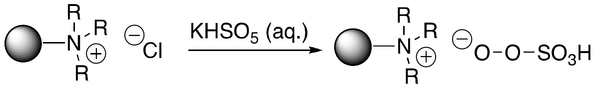


Figure 2. Panel A: Cartoon depiction of poly(lactic acid) (PLA) nanoparticles highlighting design elements. The core substrate is poly(lactic acid) (PLA). The PLA is coated with polyethylene glycol (PEG) to promote water solubility. The variable functional group region can be tuned for compatible reactivity with target pollutants. Panel B: Composition of first-generation PLA-PEG-PEI nanoparticles displaying surface amine functional groups. Panel C: Expected reactivity of amine-capped nanoparticles with aldehyde and carboxylic acid gaseous contaminants.

Similarly, carboxylic acids ought to undergo an acid-base reaction with the amino functionalized PLA nanomaterials (Panel C, eq. 2). The resultant ammonium carboxylate ionic compounds would effectively sequester the odorant VOC onto the surface of the nanoparticle.

Additionally, We designed an oxidant-functionalized PLA nanomaterial to target the five sulfur-containing compounds (see Chart 1) associated with rendering operations. The compounds are particularly noxious, emitting an overwhelming, putrid odor that is detectable even at miniscule concentrations. In order to target these contaminants, we designed PLA nanoparticles and thin films that are end-capped with ammonium peroxysulfates that can serve as the requisite oxidant for odorant destruction.

Briefly, we proposed to prepare engineered PLA nanoparticles and thin films functionalized with quaternary ammonium salts (Scheme 1, **2**). In short, we proposed to cap our base formulation, PLA-PEG-COOH with an appropriate linker that displays a quaternary amine. Employing the method developed by Travis and Borhan,[14] we would then generate the requisite ammonium peroxysulfate functionalized PLA nanoparticles and thin films. Specifically, the active oxidant of commercial Oxone®, potassium peroxysulfate, would be isolated. The PLA nanoparticles and thin films functionalized with quaternary ammonium salts (*e.g.* **2**) would then be treated with an aqueous solution of potassium peroxysulfate in order to displace the counterion (represented in Scheme 1 as Cl^-). The extent of peroxysulfate incorporation onto the PLA nanoparticle or thin film (*e.g.* formation of **3**) could then be determined by iodine titration.[14]



2 **3**

Scheme 1. Strategy for the preparation of ammonium peroxysulfate functionalized PLA nanoparticles poised to react with sulfur containing odorants.

Finally, a logical extension of the approach would be to engineer a hybrid PLA nanoparticle that contains both the amine functional group as well as the ammonium peroxysulfate functional group. This hybrid PLA material would then be adequately equipped to capture aldehyde and carboxylic acid contaminants as well as to oxidize sulfur-containing agents, thus demonstrating the tunability of PLA-based adsorbents. The methods developed for the preparation of the nanomaterials (*vide infra*) are uniquely designed to allow for such fine-tuning of the surface functionalization of the nanoparticles and thin films. Essentially, the nanomaterials can be capped with a statistical mixture of two or more unique functional groups by simply controlling the ratio of the incoming capping groups during the fabrication process. By simultaneously capping the PLA nanoparticle or thin film with a mixture of the appropriate amine and quaternary ammonium salts, a hybrid nanomaterial can be prepared in one operation. Subsequent treatment of the quaternary ammonium sites with potassium peroxysulfate as described above would then produce a hybrid PLA nanoparticle or thin film equipped with both amines and ammonium peroxysulfates (See **4,** Figure 3). Described below is our progress toward achieving the preparation and validation of these three formulations of nanomaterial formulations as a next-generations strategy for odor remediation in the rendering industry.

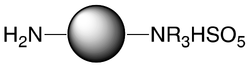


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Figure 3. A hybrid PLA nanoparticle equipped with an amine and an ammonium peroxysulfate.

2.2. Preparation of Amine Functionalized Nanomaterials

As reported previously, our initial efforts on the project were directed toward preparing the amine functionalized nanoparticles that were suitable for sequestration of the aldehyde and carboxylic acid malodorants (nineteen of the twenty-six target malodorants). We end-capped PLA nanoparticles and thin films with low molecular weight polyethyleneimine (LMWPEI). LMWPEI is a dendritic polyamine that presents a number of primary amines (and secondary and tertiary amines) on the surface of the particles and films. The methods described below for the fabrication of the nanoparticles are robust, reliable, and thoroughly optimized.

The amine capped PLA-PEG nanoparticles were generated by dissolving the PEG-PLA-COOH polymer (prepared via ring opening polymerization)[13] in acetonitrile (~ 5 mg/mL). This acetonitrile solution was then added dropwise into water to allow for the polymeric nanoparticles to form over the course of about 1.5 hours. The individual polymer strands self-assemble into nanoparticles by hydrophobic-hydrophobic interactions between the PLA polymer chain such that the hydrophilic PEG outer layer is projected into the aqueous solvent.[13] After the 1.5 h incubation, the nanoparticles were washed by ultracentrifuge filtration to remove residual solvents. The particles were then resuspended in PBS (pH 7.4) and treated sequentially with the EDC and amine end-cap solutions. Specifically, the particles were incubated in a 10X molar excess of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) in phosphate buffered saline (PBS) (pH 7.4) to generate the activated ester. The amine cap (EDA or PEI) was then added as a 10X excess to the activated ester and incubated for six hours in order to load the amine cap via amide formation. The amine-capped PLA-PEG nanoparticles were then washed three times with distilled water using ultracentrifugation and dried using a freeze dryer. In addition to the amine capped nanoparticles, we prepared two control materials for analysis to demonstrate the selectivity of our approach. Specifically, we prepared an ester capped nanoparticle (PLA-PEG-COOMe) and a carboxylic acid capped nanoparticle (PLA-PEG-COOH).

After preparation, the particles were characterized by ^1H nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, and thermogravimetric analysis (TGA). Figure 4 depicts an ^1H NMR overlay of the PLA-PEG-COOH nanoparticles (red line), the PEI capping polymer (blue line), and the desired PLA-PEG-PEI nanoparticle formulation (green line) from 0-6 ppm. The inset spectrum of Figure 4 clearly indicates the appearance of additional NMR signatures in the PEI region of the spectrum of the PLA-PEG-PEI nanoparticles, thus confirming a successful coupling event. Thus the NMR spectrum of the PLA-PEG-PEI formulation is comprised of appropriate signatures from each of the polymer components: ^1H NMR δ (ppm): 5.173 (m, $–C(=O)–C\underline{H}(CH_3)–$), 3.507 (s, $–\underline{CH_2CH_2}–O–$), 2.729 and 2.619 (PEI), 2.505 (m, DMSO-d_6), 1.434 (t, m, $-CH(CH_3)-$).

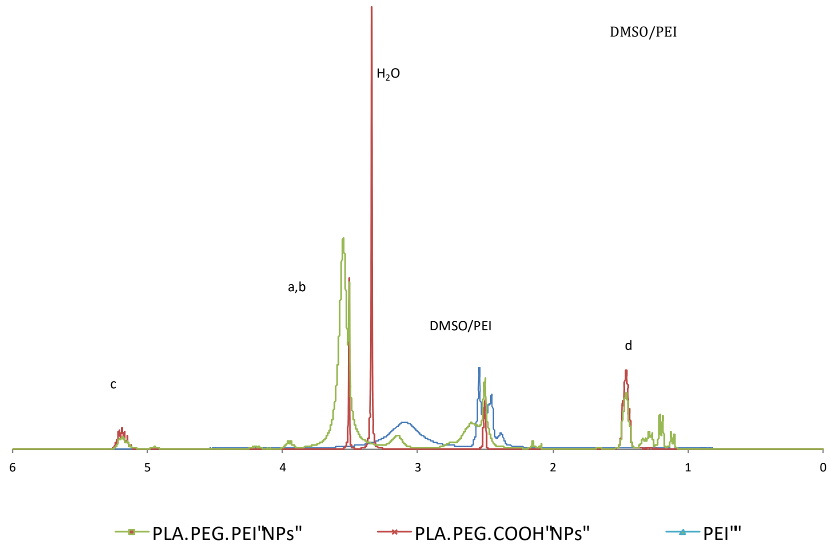

Figure 4. Proton NMR spectrum overlay of PLA-PEG-PEI nanoparticles, PLA-PEG-COOH nanoparticles, and PEI. Inset spectrum indicated diagnostic appearance of PEI peak signatures in the PLA-PEG-PEI NP spectrum confirming a successful coupling.

Infrared spectroscopy of the nanoparticle formulations again revealed diagnostic peaks that confirm the successful decoration of the particles with the amine functionality. Specifically, Figure 5 depicts an overlay of the IR spectra the desired PLA-PEG-PEI nanoparticle (green line)along with the PLA-PEG-COOH (red line) and PLA-PEG-COOMe (purple line) control materials. After amine fuctionalization, a marked increase in absorbance at ~3300 wavenumbers was observed, consistent with the appearance of N-H stretches from the PEI polymer.

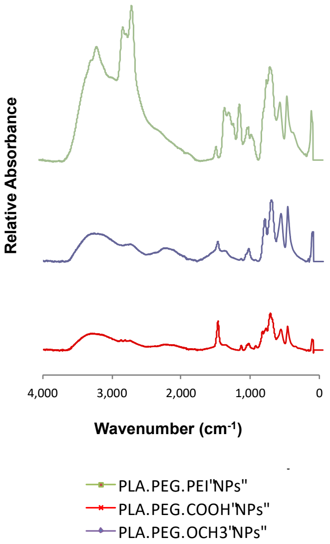

Figure 5. Infrared specta of the three polymer formulations. Note the increase in absorbance at ~3300 wavenumbers, indicative of the presence of PEI amine N-H stretches.

Thermogravimetric analysis of the various nanoparticle formulations was also conducted (Figure 6). The earlier initial thermal degradation of the PLA-PEG-PEI material at approximately 200 ˚C (cf. ~400 ˚C for unfunctionalized materials) further confirms the likely presence of the PEI cap on the desired nanoparticles.

Additionally, the PLA-PEG-PEI nanoparticles were characterized by elemental analysis. This analysis confirmed the presence of nitrogen atoms in the sample, again arising from the successful capping of the material with the amine polymer necessary for contaminant sequestration.

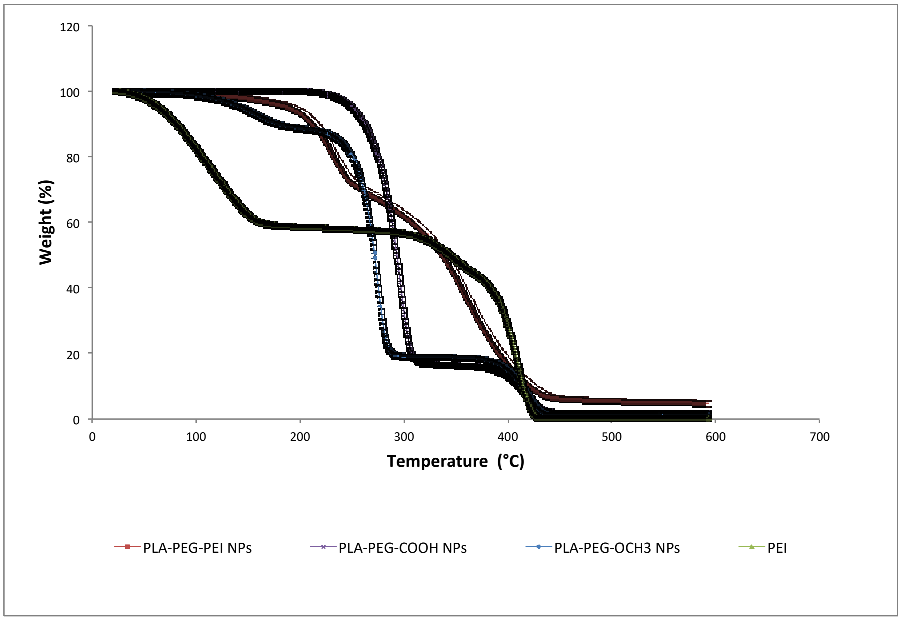

Figure 6. TGA analysis of PLA-PEG-PEI, PLA-PEG-COOH and PLA-PEG-COOMe nanoparticles and PEI starting material.

2.3. Sequestration of aldehyde and carboxylic acid malodorants with PLA-PEG-PEI nanoparticles.

We evaluated the ability of freshly prepared PLA-PEG-PEI nanoparticles (NPs) to capture gaseous samples of target VOCs associated with rendering operations. Briefly, 10 mg of a freshly prepared sample of PLA-PEG-PEI NPs was suspended on a tissue paper barrier above a 1 µL aliquot of analyte in a GC vial (see Fig. 7, panel A). Thus, the NPs were allowed to interact with the vapor portion of the hexanal sample for 30 minutes. Headspace analysis was conducted by gas chromatography (FID detection). Headspace concentration of hexanal was compared between samples treated with PLA-PEG-PEI NPs (Panel A) for 30 minutes and untreated control headspace samples (Panel B). Untreated control samples demonstrated *__no reduction__* in gas-phase hexanal concentration. The bar graph in Fig. 7, Panel C clearly demonstrates the effectiveness of our functional materials: treatment with PLA-PEG-LMWPEI NPs effected a **97% reduction** of the headspace vapor portion of hexanal standards when compared to untreated controls (data collected in sextuplicate). Notably, the reduction in hexanal concentration was only observed with the amine-functionalized NPs. Treatment of hexanal with control NPs end-capped with carboxylic acids (-COOH) or methyl esters (-$COOCH_3$) did not appreciably reduce the concentration of hexanal (Fig. 7, Panel C) underscoring the necessity of a functional group cap with compatible reactivity. These data indicate that our amine-capped NPs likely capture hexanal via covalent reaction (see Fig. 2, Panel C) in lieu of non-specific electrostatic adsorption. Similar experiments with the less volatile eight-carbon aldehyde, octanal, resulted in a **77% percent capture** with our PLA-PEG-PEI NPs. These results clearly indicate that the PLA-PEG-PEI NPs are equipped with appropriate functionality to capture aldehyde VOC pollutants associated with rendering emissions. Similarly, we observed an **86% reduction** of gaseous hexanoic acid (Fig. 7, Panel D), presumably via acid-base reaction as described in Fig. 2, Panel C. From these experiments, it is clear that our first-generation amine-functionalized NPs are capable of sequestering aldehyde and carboxylic acid VOCs associated with rendering emissions.

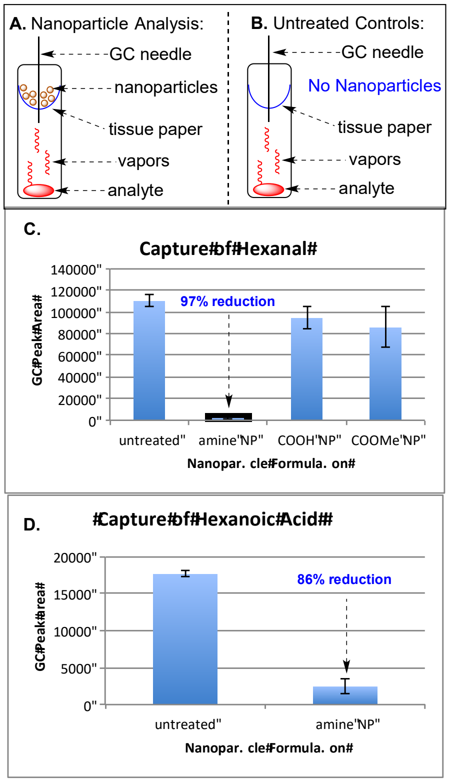

Figure 7. Panel A: Cartoon schematic of headspace analysis experimental design for the capture of VOCs with PLA-PEG-LMWPEI nanoparticles (NPs). Panel B: Cartoon schematic of experimental design for control injections of untreated VOCs. Panel C: Capture of hexanal (97% reduction) with amine functionalized NPs. Panel D: Capture of hexanoic acid (86% reduction) with amine functionalized NPs.

2.4. Functional NPs are Not Fouled by Non-specific Off-Target Binding

Another key to demonstrating target-specific binding of the VOCs was to conduct a "fouling" study where we challenged the NPs with the targeted VOC contaminant along with a non-targeted, incompatible analyte. For this assay, we treated 10 mg PLA-PEG-PEI NPs with a 1:1 mixture of hexanal and 1-nonene. In this experiment, we expected to see preferential binding of the aldehyde, hexanal, via our predicted reactivity. Further, we expected that 1-nonene, containing an incompatible alkene functional group, would fail to react with the amine functionalized NPs and thus would not be sequestered. In the event, co-treatment of our NPs with a 1:1 mixture of hexanal and 1-nonene resulted in the selective sequestration of the targeted hexanal in preference to 1-nonene. Fig. 8., Panel A (next page) clearly indicates the preferential reduction of hexanal and a corresponding increase in the gas-phase presence of 1-nonene after treatment. Fig. 8., Panel B provides a cartoon representation of the analyte system before and after treatment with PLA-PEG-LMWPEI NPs. Prior to NP treatment, a 1:1 mixture of hexanal and 1-nonene are allowed to partially vaporize. After treatment with NPs, most of the hexanal (represented here as red vapors) are absorbed onto the surface of the NPs leaving behind the vapor portion of 1-nonene (represented as purple vapors). Owing to the partial removal of the hexanal vapors from the system, re-equilibration results in a larger vapor concentration of 1-nonene after hexanal capture, accounting for the enhanced 1-nonene signal after NP treatment (see Panel A).

2.5. Functional NPs Exhibit No Acute Toxicity in a Daphnid Model

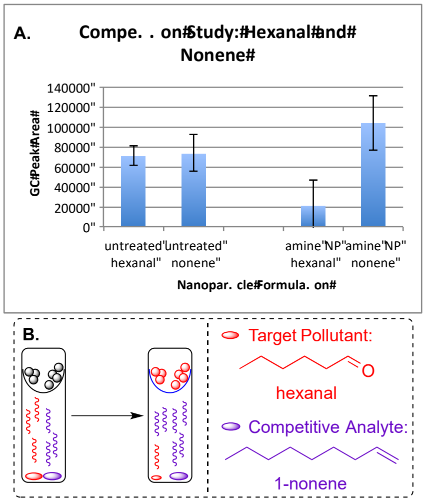

Figure 4. Panel A: Competition study demonstrating the selective removal of the targeted hexanal contaminant in preference to the competitive analyte, 1-nonene. Panel B: Cartoon representation of the competition experiment.

Next we attempted to evaluate the potential toxicity of our NP formulations. As an initial evaluation, Prof. Peter van den Hurk's laboratory (Clemson University Biological Sciences) evaluated our base NP formulation (PEG-PLA-COOH) for acute toxicity in a Daphnid (water flea) aquatic invertebrate model. Briefly, the NPs were tested for toxicity using a standard *Daphnia magna* acute toxicity test, according to US-EPA protocol. The NPs were tested at 7 concentrations ranging from 1 to 5000 ppm, plus appropriate controls without NPs. The test organisms were cultured in standard *D. magna* culture media, and fed a mixture of *Selenastrum capricornutum* and fish food on a daily basis. Daphnid cultures and test beakers were maintained in a climate-controlled room at 20 ˚C and 16/8 h light/dark cycles. On the day of the toxicity test, neonate daphnids (less than 24 h of age) were collected from the culture, and introduced into the test beakers. Each beaker contained 40 mL of media with the NPs dissolved, and 5 neonates per beaker. Each NP concentration was tested in 4 replicate beakers. Neonates were exposed for 48 h and were not fed during the exposure. After the exposure period, test animals were checked for mortality. *The results showed no significant mortality in any of the tested NP concentrations*.

2.6. Conclusions

This result of this funded project have laid valuable ground work toward achieving our ultimate goal of developing a marketable, scalable material for the remediation of rendering malodorants. We have confirmed our ability to synthesize and fully characterize amine-capped nanomaterials. Further, we have demonstrated their ability to capture relevant malodorants of the aldehyde and carboxylic acid functional group classes. Further, we have obtained preliminary data that indicates that our formulations are non-toxic to aquatic organisms. With these discoveries, we are now poised to investigate the development of multi-functional materials that are capable of sequestering a broader range of relevant malodorants. These efforts are currently underway as a part of on-going ACREC and FPRF supported projects.

Impacts and Significance:

The technology that is being developed has the potential to be very beneficial to the rendering industry in terms of providing a next-generation alternative to established methods for odor remediation. The particles described herein might present several uses in an industrial setting. The particles could be employed as a rapid-use odor adsorbent in an emergency or spill situation. Further, the particles could, in principle, be incorporated into existing odor remediation equipment as an added means for odor elimination. The obvious long-term goal would be to develop an appropriate formulation of functionalized PLA nanoparticles that would compete with or ideally supplant existing odor elimination measures. One advantage of such an outcome includes the ability to land-fill the spent scrubbing material without further processing. Indeed, PLA polymers are biodegradable and non-toxic. Additionally, since their means of neutralizing malodorants is based on chemical reactivity instead of thermal degradation, the implementation of this strategy should return significant cost savings in terms of reduced energy consumption and reduced greenhouse gas emissions. Finally, the general strategy of surface decoration of polymer matrices with reactive sites is not limited to PLA polymers. One could envision the development of similar strategies for the preparation of "smart" clothing or other materials that are equipped with an odor elimination mechanism.

Publications:

1. We are currently editing a draft of our first peer-reviewed manuscript from this study. We are targeting a May 1st submission to *ACSNano*.

2. We successfully filed our first provisional patent application to protect this technology under the auspices of the Clemson University Research Foundation, Inc. (CURF):

> "Biodegradable Waste Remediation Method and System" (U.S. Appl. No: 62/110,876); filed on Feb. 2, 2015

Outside funding: The following grant applications were submitted utilizing the data collected in this study:

1. "Biodegradable Functionalized Nanomaterials for the Remediation of Agricultural and Food Process Pollutants", (2013) Sponsor: USDA/NIFA Foundational Program Strengthening Grant, $500,000, Declined.

2. "SusChEM: Functionalized Biodegradable Nanomaterials for the Remediation of Environmental Pollutants", (2013) Sponsor: NSF, $330,000, Declined.

3. "SusChEM: Functionalized Biodegradable Nanomaterials for the Remediation of Gaseous Environmental Pollutants", (2014) Sponsor: NSF, $380,000, Declined.

4. Target Specific Functional Nanomaterials for the Remediation of Environmental Contaminants"; (2014) Keck Foundation Internal Pre-Proposal; $500,000, Declined at University Level.

5. "Biodegradable Functional Nanomaterials for the Remediation of Gaseous Agricultural and Food Process Pollutants"; Sponsor: United States Department of Agriculture – National Institute of Food and Agriculture (USDA-NIFA); $150,000, Declined.

6. Biodegradable Functional Nanomaterials for the Remediation of Environmental Contaminants"; (2015) Sponsor: NSF; $328,647, Under review.

Future Work: What future work do you suggest should be conducted on this project?

In a currently funded ACREC/FPRF project we are working to further vet our materials as effective measures to capture or otherwise destroy target malodorants associated with rendering operations. Further we are actively pursuing the development of multi-functional nanomaterials that can engage a broader range of target contaminants. Newly proposed work (2015 ACREC/FPRF funding cycle) will target the assay of our materials with relevant rendering samples as well as pursue the lab-scale testing of our first generation materials on-site at a member rendering facility. Long term goals include the development of a scale-up procedure that will allow for the large scale preparation of our materials for on-site testing.

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References:

(1) Meeker, D. L.; Hamilton, C. R. An Overview of the Rendering Industry In *Essential Rendering: All About the Animal By-Products Industry*; Meeker, D. L., Ed.; Kirby Lithographic Company, Inc.: Arlington, VA, 2006, p 1-16.

(2) Sindt, G. L. Environmental Issues in the Rendering Industry In *Essential Rendering: All About the Animal By-Products Industry*; Meeker, D. L., Ed.; Kirby Lithographic Company, Inc. : Arlington, VA, 2006, p 245-258.

(3) Bethea, R. M.; Murthy, B. N.; Carey, D. F. ODOR CONTROLS FOR RENDERING PLANTS *Environmental Science & Technology* **1973**, *7*, 504-510.

(4) Shareefdeen, Z.; Herner, B.; Webb, D.; Verhaeghe, L.; Wilson, S. An odor predictive model for rendering applications *Chemical Engineering Journal* **2005**, *113*, 215-220.

(5) Shareefdeen, Z.; Herner, B.; Wilson, S. Biofiltration of nuisance sulfur gaseous odors from a meat rendering plant *Journal of Chemical Technology and Biotechnology* **2002**, *77*, 1296-1299.

(6) Vanlangenhove, H. R.; Vanwassenhove, F. A.; Coppin, J. K.; Vanacker, M. R.; Schamp, N. M. GAS-CHROMATOGRAPHY MASS-SPECTROMETRY IDENTIFICATION OF ORGANIC VOLATILES CONTRIBUTING TO RENDERING ODORS *Environmental Science & Technology* **1982**, *16*, 883-886.

(7) Pearson, A. M.; Dutson, T. R. *Inedible Meat By-Products*; Elsevier Applied Science: London, 1998.

(8) Fazzalari, F. A. *Compilation of Odor and Taste Threshold Data*; ASTM Data Series DS 48A, ASTM: Philadelphia, PA, 1973.

(9) Alexis, F. Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid)-co-(glycolic acid)] *Polymer International* **2005**, *54*, 36-46.

(10) Griffith, L. G. Polymeric biomaterials *Acta Materialia* **2000**, *48*, 263-277.

(11) Drumright, R. E.; Gruber, P. R.; Henton, D. E. Polylactic acid technology *Advanced Materials* **2000**, *12*, 1841-1846.

(12) Lunt, J. Large-scale production, properties and commercial applications of polylactic acid polymers *Polymer Degradation and Stability* **1998**, *59*, 145-152.

(13) Pridgen, E.; Alexis, F.; Farokhzad, O. C.; Langer, R. *Methods in Bioengineering Book Series. Nanoscale Bioengineering and Nanomedicine*; Artech House: Boston, MA, 2009.

(14) Travis, B. R.; Ciaramitaro, B. P.; Borhan, B. Preparation of purified KHSO5 center dot H2O and nBu(4)NHSO(5) from oxone by simple and efficient methods *European Journal of Organic Chemistry* **2002**, 3429-3434.