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Formulation and Characterization of an Antimicrobial Coating Containing Nisin for Large Scale Food Package Converting Processes

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FORMULATION AND CHARACTERIZATION OF AN ANTIMICROBIAL COATING CONTAINING NISIN FOR LARGE SCALE FOOD PACKAGE CONVERTING PROCESSES

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ABSTRACT

This research consisted of formulating an antimicrobial coating containing Nisaplin® intended for large scale production and inhibition of spoilage microorganisms. Secondly, the coating formulated was applied to a flexible film surface using two trials (gravure and flexography) commonly used in large scale food package coating or printing processes. In addition, diffusion and mass transfer theory was applied to discuss the many complications of predicting nisin diffusion or release from a coated material for antimicrobial food packaging applications.

Previous work conducted by predecessors, produced an antimicrobial coating formulation using a 70/30 Methylcellulose/Hydroxypropyl methylcellulose base (MC/HPMC). Some disadvantages of this coating included haze, lack of sealability and percent solids content too low for large-scale gravure and/or flexographic coating application processes (which require 15-50% solids). Due to the characteristics, it was then determined that the coating would need to be re-formulated to maintain these qualities in addition to the ability to be up-scaled to large scale gravure and/or flexographic coating processes and lastly, maintain antimicrobial activity against desired microorganisms.

Multiple materials were tested to determine the antimicrobial coating formulation including four grades of polyvinyl alcohol, plasticizers, emulsifiers and antimicrobials. The first set of testing, differential scanning calorimetry (DSC), was used to determine the melt temperature of the base or matrix for containing this nisin. It is important to

determine the melt temperature of the resin in order to determine the sealability of the final package. DSC testing showed that 88% hydrolyzed, granular polyvinyl alcohol (Mowiol 4-88, Kuraray) resin combined with glycerin (40 phr) resulted in a decreased melt temperature from 189.7°C to 150.9°C and decreased thermal degradation via hydrolysis. These two components were determined to be part of the film forming matrix due to the potential for sealability. Dynamic contact angle testing was also utilized to determine adhesion, critical surface tension to several substrates (LLDPE coex, Bynel®2002; Elvax® 3165, Nucrel® 1202 HC and Surlyn® 1605) and wettability of the coating solution. All substrates were found to have statistically significantly different critical surface tensions from the control LLDPE substrate ($\alpha = 0.05$). All substrates except for corona treated Elvax® and Surlyn® were found to have statistically significantly different dynamic contact angle measurements from the control LLDPE substrate ($\alpha = 0.05$) (p value = 0.1231, Elvax® – corona; p value = 0.5648, Surlyn® - corona). Tape tests were conducted to select the final coating substrate, LLDPE. All of the testing parameters (pH, percent solids, melt temperature) indicated that the formulation was suitable for gravure or flexography coating applications.

Coating trials using the formulated antimicrobial coating showed the potential for implementing a coating containing nisin on large scale production processes. Gravure and flexography trials were conducted on primed and corona treated LLDPE material. Several characteristics of the liquid coating and dried, coated substrate were tested for quality and overall specifications such as pH, percent solids and blocking. Film on lawn testing indicated that treatment films coated using both processes were able to inhibit

Micrococcus luteus compared to control films (Gravure: $P<0.0001$; Flexography: $P<0.0001$). This study showed that the formulated coating had potential to be produced using large scale food package converting processes while maintaining antimicrobial efficacy against a food spoilage indicator bacterium..

Mass transfer of antimicrobial components in antimicrobial packaging systems are governed by numerous variables both extrinsic and intrinsic factors. This study provided literature review and mass transfer theory to predict the diffusion or controlled release of nisin from the produced packaging system to target microorganisms on a food product. Factors such polymer structure, temperature, food product, fat content and polymer swellability and their effects of diffusion and controlled release were discussed. This study showed that antimicrobial packaging systems are complicated multivariable systems that require many assumptions in order to make diffusion prediction mathematically feasible.

The original work conducted by Franklin et al (2004) that this project was based off of was intended for frankfurters. The intended market of the produced antimicrobial film was for ready-to-eat (RTE) foods. These types of foods are those which do not need to be cooked prior to consumption. Due to the rising demand for convenient food products such as RTE foods, this material could be implemented for usage against surface contamination and spoilage microorganisms.

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CHAPTER ONE

INTRODUCTION

In 2012, 14.5% (36.4 million tons) of total municipal solid waste generated in the United States of America was food waste [1]. Food spoilage is one of the major causes of food waste. Approximately 40% of food in the United States goes to waste. This can include wasted food from production, distribution, retail and household environments. Of household foods in the United States, approximately two thirds (66.7%) of products are lost due to spoilage [3].

Active packaging is a growing research area that can reduce food waste via shelf life extension through inhibition of spoilage microorganisms. The demand for active packaging is increasing and part of that is due to the demand for minimally processed food products that can maintain a fresh appearance. According to Food Production Daily, the active packaging sector is expected to grow to 3.5 billion dollars by 2017 in the United States and 17.3 billion dollars worldwide [4]. Additionally, food packaging films and meat packaging products also have projected growth for 2018 and 2019. The demand for meat, poultry and seafood packaging is expected to increase in the United Stated by 3.8% up to $11 billion in 2019 [5]. The research to be introduced is specifically for application in meat type products such as ready to eat (RTE) meats.

Ready-to-eat (RTE) food products are in high demand due to the convenience and a “fresh” product appeal. The category includes food products that require little or no

cooking/preparation prior to consumption, such as deli meats, cheeses and frankfurters [2]. Market growth, specifically in prepared foods such as ready to eat meats, convenience items and various sizes such as individual portions are also expected to exhibit high increases in demand [5].

Ready-to-eat products such as lunch meats or frankfurters are susceptible to post-process contaminants such as the pathogen *Listeria monocytogenes.* The research to be discussed could have potential to be implemented for prevention of listeriosis, which is the infection caused by consuming food products contaminated with *L. monocytogenes.* However, the main focus of the work will be to reduce or slow the growth of spoilage microorganisms to extend the shelf life of food products and reduce food waste. Antimicrobial packaging can be implemented to reduce spoilage. To date it has been difficult to introduce antimicrobial packaging into the market due to cost. The cost inherent from the loss of product due to the growth spoilage microorganisms is a concern for many packaging companies. Antimicrobial packaging is a value added product. If the added cost of the antimicrobial packaging is able to reduce the overall cost of food waste, it would be more readily implemented in the packaging industry.

Nisin is a GRAS approved antimicrobial component contained in the commercially available product Nisaplin® (2.5% concentration). Several studies have shown nisin to be effective in inhibiting gram positive bacteria, showing potential in the food packaging market for the reduction of spoilage microorganisms.

The objective of the first segment of this research is to produce an antimicrobial coating formula containing a 2.5% nisin commercial grade product, Nisaplin® (2.5%) intended for large scale production. The second objective of this study is to take the antimicrobial coating solution formulated and trial the coating on large scale printing or coating equipment. The coated film products will then be analyzed for inhibitory properties and overall quality. Lastly, the theory of mass transfer of nisin will be discussed specifically pertaining to antimicrobial packaging system developed throughout the course of this work.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Food Waste

Total municipal solid waste (MSW) generation in 2012 was 251 million tons. Approximately 36.4 million tons of the MSW was designated as food waste [38]. The Food and Agriculture Organization of the United Nations (FAO) states that approximately 1.3 billion tons of food gets lost or wasted each year. Causes of food waste vary depending on the stage of the life cycle of the product. (i.e. processing, distribution, retail, household, waste) Some examples of causes can include improper storage, physical damage through distribution, insect contamination, spoilage microorganisms, oxidation or even confusion understanding date code [62; 93; 104]. Active packaging is a possible solution to eliminating some of the food wasted due to spoilage microbes. Active packaging utilizes sachets, gases and/or antimicrobials among other components to alter the interior environment of a package in order to maintain desirable food characteristics for an extended period of time.

According to the USDA Economic Research Service, in 2010, the estimated value of meat, poultry, fish and dairy products lost as food waste was upwards of 75.5 billion dollars. The USDA did not differentiate between fresh and ready-to-eat food products in their estimations. At the retail level, 5% of meat, poultry and fish were lost and 11% of dairy products while on the consumer level, 22% of the sold meat and 20% of the sold dairy products were lost as waste [21].

2.1.2 Reduction of Food Waste

There are numerous possibilities for reducing food waste such as educating consumers on proper food storage, changing labels to make handling and instructions of food products more clear and utilizing technology for better preservation methods of food products [104]. Food packaging has the ability to reduce food waste by protecting the food product from physical damage, containing the product in a separate environment inside the package and by providing information for consumers on the labeling [93]. Shelf-life extension through use of antimicrobials, preservatives, barrier materials and more can provide protection against biological and chemical hazards like microorganisms and lipid oxidation.

2.1.3 Food Safety

According to the Center for Disease Control (CDC), approximately 48 million Americans will be affected by a food borne illness, of those people, 128,000 will be hospitalized and approximately 3,000 cases will result in death. A food borne illness is a sickness that can be contracted by eating food or drink that has been contaminated with bacteria, viruses or even parasites [24]. It was also estimated that the cost due to pathogenic foodborne outbreaks totaled approximately $152 billion [39; 119].

There are many opportunities during food processing steps in which a product can become contaminated with a potentially deadly or illness-causing biological hazard. According to the World Health Organization (WHO) in 1995, approximately 25% of the outbreaks in Europe can be traced back to some form of post process contamination [162]. The top 5 factors determined from the survey conducted included insufficient

hygiene, cross contamination, processing or storage in inadequate rooms, contaminated equipment and contamination caused by personnel [112]. In order to reduce incidents involving contamination (biological, physical, chemical) programs such as Hazard Analysis Critical Control Points (HACCP) and Good Manufacturing Practices (GMP) have been implemented.

According to the Food and Drug Administration (FDA), HACCP is defined as "…a management system in which food safety is addressed through the analysis and control of biological, chemical and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product [136]." HACCP was first developed in the 1960's by the Pillsbury Company in order to produce safe food for the NASA space program. The testing precautions produced from this program were then implemented into the consumer food markets in the 1970s, first being used in canning regulations. Since then the HACCP program has grown to become a mandatory food safety program in the United States, as well as in other countries [53].

However, with all of the regulations, sanitation programs and good manufacturing practices in place, the threat of foodborne illness outbreaks still exist. There are particular products and points in processing that can be susceptible to contamination or re-contamination. For example, a packaging material could be dirty or improperly sealed, slicers may not have been cleaned properly or an additional environmental factor could be contaminating food product [112]. Products that are cooked unpackaged, then sliced or further processed and packaged are especially susceptible. Many of these products are called "ready-to-eat".

2.2 Ready-To-Eat Foods (RTE)

Ready-to-eat (RTE) food products are in high demand due to convenience and a "fresh" product appeal. According to the Freedonia Group, a market research group, there is an increased demand for meat and meat products approaching approximately $11 billion in 2019. Ready-to-eat meats are one of the fastest growing sectors driven by the increasing variety of pre-prepared foods being put into the market [131].

RTE foods are products that require little or no cooking/preparation prior to consumption, although some mild heating may be desired for quality preferences. Some examples of RTE foods commonly used in vacuum packaging applications include cheeses, deli meats, frankfurters and smoked meats (such as salmon) with a shelf-life ranging from 60 -90 days [103; 111]. RTE food products are sold with open shelf life dates. Open shelf life dates can be preceded by phrases such as "best if used by date", "sell-by-date" or "better-if-used-by-date" [125]. Open shelf life dates indicate when the product is expected to decrease to an undesirable quality or expected microbial spoilage but does not pinpoint a microbial safety issue [103].

2.3 RTE Food Spoilage

Susceptibility of food products to microbial spoilage vary according to intrinsic and extrinsic properties such as composition of the food product, pH and storage environment. RTE vacuum packaged food products are typically susceptible to microorganisms that can withstand environments with little to no oxygen (facultative or anaerobic microbes) and cold temperatures like that of refrigeration (psychrotrophs). Psychrotrophs can survive and grow within a wide temperature range 0 – 40°C with

optimum growth being around 15-25°C. Examples of spoilage microbes for RTE food products in a vacuum package and refrigerated environment can include *Lactobacillus* spp., *Lueconostoc* spp., *Serratia* spp., *Brochothrix thermosphacta and Enterococcus casseliflavus* [64; 111].

Evidence of spoilage from these bacteria typically shows turbid or cloudy liquid within the package, slime formation, pink and/or green coloration, gas accumulation and off odors [64; 111]. Other undesirable changes in the food products can also include off flavors and textures. For example, some microorganisms are proteolytic using (protein as a nutrient source) which can drastically change the texture of a meat based product or produce a by-product making a food taste "sour" [11]. Some bacteria however do not produce an off-taste or odor. For example, a pathogenic bacterium, *Listeria monocytogenes,* does not produce off odors or off flavors in contaminated food products eaten by unsuspecting consumers.

2.4 *Listeria innocua* and *Micrococcus luteus*

Listeria innocua is a non-pathogenic strain of *Listeria spp.* This strain of bacteria has been used in multiple studies as a non-pathogenic surrogate for *L. monocytogenes* due to the close relation between the two bacteria [13; 8; 65]. *L. innocua* has been found to act similarly when exposed to certain to environmental conditions among other similarities such as inactivation characteristics and genetic stability [8; 105].

Micrococcus luteus is a Gram positive spoilage microorganism. Gram positive microorganisms are those which have a thick cell wall consisting of peptidoglycan (which contains short peptide chains) [111] but lack an outer membrane that would be

found in Gram negative bacteria [14]. *M. luteus* is a heterofermentative lactic acid bacterium that can produce lactic acid, acetic acid, ethanol and carbon dioxide by-products from glucose [30]. This bacterium has also been used in antimicrobial studies testing the antimicrobial efficacy of nisin due to its high sensitivity. It is often used as a reference strain [5; 120].

2.5 Active Packaging

Active packaging is a packaging system that attempts to alter or control the internal environment of a package for the betterment of properties such as shelf-life extension, color and inhibition using one or more specified techniques. Such techniques can enhance the preservation of a food or beverage product in addition to inhibiting pathogenic and spoilage microorganisms [17; 56; 112]. Examples of active packaging technologies include oxygen scavengers, antimicrobials, desiccants for moisture control and ethylene absorbers. For those products sensitive to oxygen, oxygen scavenger sachets are used. These sachets are oxygen permeable pouches typically containing ferrous iron which absorbs the oxygen from the internal environment surrounding the food product [17].

Active packaging is often confused with or combined with the area of intelligent packaging. Intelligent packaging does not adjust the interior environment of a packaging system. Intelligent packaging systems communicate information to consumers or retail associates throughout the distribution chain. Radio frequency identification technology (RFID), spoilage indicators and time-temperature indicators (TTI) are a few examples of intelligent packaging. These technologies are used to track locations, levels of secondary

compounds produced by spoilage microorganisms and to record temperature abuse including duration of said temperature abuse.

2.5.1 Demand for Active Packaging

Active packaging is becoming an increasingly popular area of study due to demands that consumers are putting on the both the food and packaging industries. The "on-the-go" lifestyle requires food products that are convenient, shelf-stable and have the appearance of being minimally processed or fresh [56; 70].Active packaging is necessary for meeting these criteria while also extending shelf-life and preserving the quality of the product [105] According to a 2014 Food Production Daily article, the US demand for active packaging is expected to reach $3.5 billion by 2017 and $17.3 billion globally [124].

Although the demand is high for methods of active packaging, added packaging costs can be unappealing to industry. Active packaging is exceptionally difficult to implement in food packaging due to the low profit margin on food products and the increased expense of active packaging technologies. Many companies will not move forward with a value-added technology such as active packaging if the additional package cost exceeds 1-2 cents per package. In antimicrobial packaging, the most expensive portion is typically the antimicrobial. Due to the added expense it is reasonable to use the lowest amount of antimicrobial needed for inhibitory properties in the packaging in order to maintain economic feasibility. However, the benefit to cost ratio needs to be in favor of implementation of active packaging applications. In some cases the cost of the antimicrobial is too great to meet industry cost standards in the current market. It is

possible for the cost of some antimicrobial products to decrease with technological advances that can lower the production cost, thereby lowering the overall cost for future active packaging projects.

2.6 Antimicrobial Packaging

The consumer demand for a natural, minimally processed product results in the conundrum of decreased shelf life and increased microbial difficulties such as spoilage or pathogenic contamination [4; 23]. However consumers expect the same standards of long shelf life and a safe product with no additional additives. Antimicrobial packaging is a potential solution for extending shelf life, but should merely be used as an extra hurdle to maintain food safety. This type of packaging method does not mean that good manufacturing practices (GMPs) and sanitation standards should be ignored or reduced.

Antimicrobial packaging is the utilization of "food packaging systems that inhibit spoilage and reduce pathogenic microorganisms" [7; 29]. The purpose of antimicrobial packaging is to extend the shelf life of a product while simultaneously maintaining quality and food safety. Shelf-life of products is extended by essentially slowing the lag phase of microbial growth [7; 59] and reducing the overall growth rate of the targeted microorganisms. During the lag phase of microbial growth, the bacterial population does not increase significantly, however the bacteria themselves will grow in size, adapt to their environment and gather nutrients [111].

There are multiple types of antimicrobial packaging technologies which include sachets, pads, films, coatings in addition to other hurdle technologies. Sachets and pads can contain components such as oxygen absorbers, moisture absorbers, ethanol vapor

generators and carbon dioxide generators [4; 127] Sachets and pads are currently on the market in various products in order to reduce lipid oxidation, bacterial and mold growth. For example, ethanol vapor generators prevent mold growth on bakery type items while oxygen absorbers are used to reduce lipid oxidation in products containing higher amounts of fat.

Antimicrobial films can be produced in a matter of three ways: the antimicrobial can be immobilized on the surface or grafted, the antimicrobial can be directly incorporated into the polymer, or it can be coated onto the surface of a film [4].One of the most difficult aspects in producing an antimicrobial packaging material is to determine the antimicrobial agent to be used. In order to produce a viable material, the antimicrobial must be compatible with the packaging material [60; 127; 143] but not so much that the agent is unable to release or maintain efficacy against the bacterial targets. Immobilization is a technique for producing an antimicrobial film that requires that the antimicrobial have the same functional group as the polymer film in order for attachment to occur due to chemical compatibility [4]. This particular technique can be utilized specifically for the treatment of product surfaces because the antimicrobial agent is immobilized onto the surface of the polymer, there is the expectation that it will not migrate into the food product.

The second method of direct incorporation, typically through extrusion, is highly desired by those in industry because of the lack of need for additional processing steps. Not only does extruding the agent directly into the polymer reduce processing steps but there is also potential for the agent to be gradually released from the polymer matrix. This enables the material to have a constant flow of antimicrobial agents to combat target

microorganisms. Immobilized materials do not have this capability because the antimicrobial agent is grafted to the surface of the film. If the agents on the surface were to lose inhibitory properties, then the film would no longer be of use.

Antimicrobial films produced using a coating application utilizes a secondary process in which either a liquid or dried coating is added to a polymer film (or another substrate) through roll coating, spraying, dipping or casting. Some antimicrobial packaging systems are coated with edible films that are intended to dissolve onto the surface of the product and gradually release the antimicrobial agent. These edible films or coatings can be produced from common food additives and natural ingredients such as proteins, polysaccharides, gums and pectin which can be classified as GRAS or safe for human consumptions [23]. For antimicrobial coatings that gradually release the inhibitory agent onto the food product surface, it is assumed as a precautionary method that the coating components will migrate into the food product. Because of this the coatings should also be safe for human consumption under the assumption that they would become indirect food additives. For example, Nisin, an antimicrobial peptide, is GRAS (Generally Recognized as Safe) but limited to a legal limit of 10,000 IU/g concentration in food products.

There are multiple types of antimicrobial compounds. The list of antimicrobials can include: organic acids and their salts, metal ions or nanoparticles, peptides, bacteriocins, enzymes, parabens, plant extracts, fungicides, amines and acid anhydrides [4; 29, 59; 79; 110; 127; 128; 141]. They can be utilized singularly or in combination with others in order to achieve the desired preservative or inhibitory properties. There is no singular antimicrobial that can kill or inhibit all microorganisms [127]. Various

microorganisms can survive in a wide variety of environmental conditions including conditions which may inactivate some antimicrobial agents. For example, some microorganisms can be acid tolerant or resistant to high concentrations of salt. Antimicrobials must be employed that function under these conditions in order to achieve inhibition.

Determining the antimicrobial compound or combination of compounds is one of the many difficulties that can arise when trying to produce antimicrobial packaging or films. In the food and packaging industries, cost is an important factor that can make or break a project. Some antimicrobial compounds can be extremely expensive and therefore less appealing.

Not only is cost a factor but also implementation of an antimicrobial needs to be well thought out. As stated previously, consumers are demanding more natural food products with less processing and additives. Addition of an antimicrobial to a packaging component, if expected to diffuse into the food product, would need to be classified as an additive on the food packaging label [127]. This would "clutter" the label more rather than achieving the "clean label" desired by consumers. Secondly, implementation can be difficult for companies, aside from general consumer acceptance. If the packaging material were to maintain direct contact with the food product, the material would need to be approved for such contact [127].

In addition to cost and consumer acceptance, production or manufacturing antimicrobial materials poses its own difficulties. Single layer and multilayer polymer materials can be produced through many processes which can include extrusion,

lamination, coextrusion, coating, printing and drying. Process conditions can be very harsh on antimicrobial components and can deactivate inhibitory properties partially or entirely leaving the material useless [4; 7; 59]. Antimicrobials can be subjected to high heat, pressure and shear environments deactivating biological agents such as antimicrobial peptides or bacteriocins or those ingredients which have heat sensitivities. Not only is there risk of deactivating antimicrobial activity while manufacturing the packaging material but when subjected to improper storage or distribution conditions.

Components of food products can also deactivate antimicrobial agents or cause a "buffer" disabling the agent's ability to inhibit the desired microorganisms [4; 7; 59; 127]. Deactivation is especially a problem when using biological antimicrobial agents such as bacteriocins or peptides. For example, nisin can become inactivated by increased fat content in food products or simulants. Jung, Bodyfelt and Daeschal (1992) found that nisin antimicrobial activity decreased 33% when added to skim milk and 80% when added to half and half (half milk and half cream) which contained 12.9% fat [77].

One way to implement antimicrobial packaging that can help avoid some of the harsh manufacturing conditions are coating methods. Coating processes will have some shear in the process, but will not exhibit the high pressure and high heat like an extruder barrel would. Coatings can be dried in various ways, typically convection drying for common processes such as gravure and flexography, but residence time in drying tunnels is relatively short compared to other heated production processes.

2.7 Coatings

A solution coating "is a liquid with solids dispersed in the liquid to assist in wetting of the substrate it is applied to [101]." Coatings have been applied to packaging since the early 1900's. In 1906, Kellogg's Corn Flakes had instructed consumers to heat the corn flake products in a pan in the oven in order to restore crispiness [61]. Six years later in 1912, Kellogg's implemented a wax coated carton liner as a moisture barrier which gave them the competitive advantage in the dry cereal market. Since then, coatings have been developed for many different purposes such as abrasion resistance, anti-fog applications, and heat seal coatings for sealability, barrier and antimicrobial applications [61].

There are several ways of coating substrates on a laboratory or smaller scale for product development purposes. Although these types of techniques were not the main focus of this study, many previous studies have been conducted in developing antimicrobial coatings in laboratories using the following techniques: thin layer chromatography, spin coating, Mayer rod drawdowns, casting a specified volume of liquid coating onto glass (or Teflon coated plates) or into vessels such as weigh boats and Petri dishes.

There are also numerous methods for coating substrates with a surface coating on a commercial scale operation. Many of these coating methods differ in the type of metering system. Some examples of coating techniques include gravure, rod, knife, air knife, cast, nip, brush, reverse roll and extrusion coaters [61]. Each of these methods is used for coatings of differing viscosities and different coating weight capabilities. For

example, the air knife technique is commonly used for coatings with a low viscosity. Higher viscosity coatings would require additional rollers in order to work to coating to the desired metered application. Processes such as gravure and flexography require liquid coatings or inks with a relatively low viscosity.

The main focus for the purpose of this study is gravure and flexographic applications which are common printing and/or coating methods commonly used in large scale package converting operations.

2.7.1 Gravure

Gravure (rotogravure) coated materials are produced using an engraved steel cylinder made that is either copper or chromium plated [6].Patterns of cells or wells are laser or diamond engraved into the cylinder and act as pockets to transfer coating to the substrate. These cells are the application method while a doctor blade is used as a metering method to remove excess coating from the cylinder. After the coating is metered by the doctor blade the coating is applied to the substrate which travels between the gravure and impression cylinder. Pressure is applied by the impression cylinder to transfer the coating out of the gravure cylinder cells. A figure of a gravure coating station is shown in Figure 2.1. Gravure coating is a very common method that is used in both printing and coating applications and is used particularly for light weight applications [63]. In particular, gravure is used for longer and more frequent runs because of the durability and expense of the gravure cylinder.

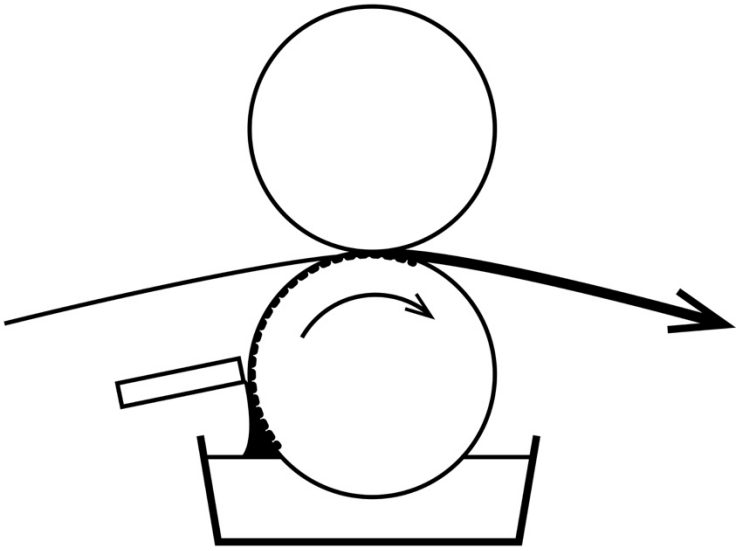

Figure 2.1 Direct gravure coating station. [61]

2.7.2 Flexography

Flexography is common method of printing in the flexible packaging industry. It can be used for a wide variety of substrates such as papers, polymers and foil. It is a comparable process to gravure because it is also used for relatively low viscosity inks or coatings [6]. Flexography uses either rubber rollers or photopolymer printing plates to transfer images or coating patterns from an engraved anilox roll to the printing substrate. These photopolymer plates are produced by exposing UV light plate through a photo negative. The UV exposure crosslinks the photopolymer, making the desired images insoluble during washing and post-cure processing. This results in relief plates in which the image or pattern to be printed is raised rather than engraved cells in gravure cylinders [132]. This coating method also uses evaporation for drying purposes. A disadvantage of flexography is that it is difficult to achieve crisp, high resolution images compared to gravure; however, this was not an issue for this study as no images were printed [6].

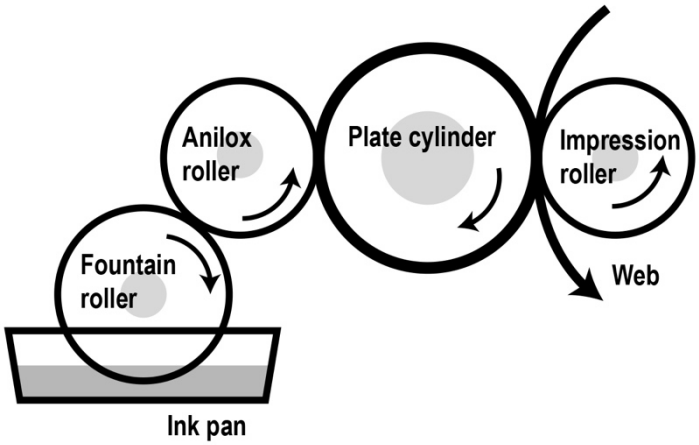

Figure 2.2 Flexographic printing/coating station [145].

2.7.3 Coating, Substrate and Coater Characteristics

Characteristics of coatings such as solids content and viscosity are factors in determining the optimal coating method. Therefore it is important that testing is conducted in order to understand coating qualities and to ensure that the proper equipment is used. Some qualities that were evaluated in the work to be discussed included viscosity, percent solids, pH and coating "class". Additional characteristics to be considered might include shear stability, density and overall composition of the coating including whether the coating is solvent or water based.

The viscosity of a coating solution is the solution's resistance to flow. For example, a solution must have the proper viscosity to be able to be held in the wells of an anilox roller and be properly transferred to a substrate. Low viscosity low yield inks (fluid inks) are commonly used for gravure and flexography processes for ease of roll to roll transfer and for image production. Ink yield is describing the amount of ink that is laid down onto the substrate during the particular printing or coating process. There is a

wide range of other descriptors of inks based on their viscosity and yield such as tacky, stringy, buttery and stiff. Buttery inks are described as low viscosity and high yield which are ideal for screen printing processes [132].

The percent solids of a coating is the amount of solid material left on a substrate after the aqueous (or solvent) portion has been dried, evaporated or removed during the coating process. The percent solids of a coating solution is an important aspect because various printing methods have ranges of percent solids that the methods are able to successfully utilize. Gravure and flexography ink or coating formulations can range anywhere between 20-60% [123]. Because flexography has an additional roll-to-roll transfer during the coating process, inks or coatings used for flexography typically have higher solids content than that used in gravure processes [123].

The pH of a coating can also have an effect on how a coating is run on equipment. pH is the log of the hydrogen ion concentration in relation to water and is measured on a scale of 0-14. A measurement of 0 indicates a highly acidic solution, a measurement of 7 indicates a neutral solution and a measurement of 14 indicates a highly alkaline solution. A low pH coating will require acid resistant doctor blades, ink/coating stations and tubing to prevent rusting and degradation after running an acidic coating on a press. Similar precautions will also be necessary for highly alkaline coatings and inks.

There are multiple classes of coatings that have different requirements. For example, inks are suspensions of a solid pigment within a vehicle (solvent). Suspension coatings require constant mixing during the coating or printing process. As a container of a suspension coating sits waiting to be pumped into the printing press, the solid particles

will naturally settle to the bottom of the container which drastically affects the color being printed due to the lack of pigmentation.

Other considerations that need to be taken into account to determine the proper coating technique include the length of the run, speed range for coating application and drying, percent solids range, appearance of the intended coating (images will require higher quality than coatings) and coat weight range [101; 132]. A thicker coating will give rise to difficulties when trying to dry during a high speed operation. A low percent solids coating will be increasingly difficult to dry if a high coat weight is desired. The ability to dry the liquid solution of the coating off will be greatly affected by drying capacity and the solvents in the composition of the coating. The most common type of drying is an evaporation drying method using warm forced air, that is based on the volatility of solvents and their ability to evaporate fairly rapidly. Both flexography and gravure use this type of drying method.

Lastly the substrate should also be considered when determining a coating method. Some qualities to consider include absorbency, surface tension, tear strength, smoothness, caliper and melt point [101]. Substrates such as paper will absorb excess ink or coating when compared to nonpolar film substrates such as polyethylene or polypropylene and will thus require larger amounts of coating or ink. Paper is also an example of how substrate smoothness is can affect the coating process. A rough surface will need a method of coating that forces the coating to flow rather than a process such as Mayer rod coating that requires the coating to flow out after being added to the substrate. Tear strength and caliper are also important features when determining the process based on the amount of physical abuse that a substrate will undergo during the coating process.

Lastly, surface tension and melting point of the substrate are important factors to consider. These can show the importance of the coating to be able to spread onto the desired surface and preventing melting of the base material during processes such as extrusion coating [101]. Each of these factors should be considered depending on the desired resulting coated material and the intended use of the final material.

2.8 Coating Re-Formulation

The original coating solution formula for this work was based off of Franklin et al (2004) which used a cellulose mixture of methylcellulose and hydroxpropyl methycellulose (70/30 w/w), water-ethanol solvent mixture (50/50 v/v), acetic acid solution (0.02M), Nisaplin® and PEG (polyethylene glycol) 400 [46]. Upon characterization of the formula, it was discovered that the percent solids was 9.5-10%, making the solution unsuitable for a typical gravure or flexographic coating method. There were also desired qualities that were not achievable with this particular formula such as sealability, translucent appearance and slow antimicrobial release. It was due to these characteristics that it was determined that a re-formulation was required prior to pursuing the possibility of up-scaling to a large scale converting process.

The ingredients of the re-formulated coating solution are discussed in detail below. The ingredients are as follows: Nisin (the antimicrobial contained in Nisaplin® (2.5% concentration), polyvinyl alcohol, glycerin, Tween 80®; 0.02 M Acetic acid solution; Water-Ethanol solvent mixture (50/50 v/v).

2.8.1 Nisin

The antimicrobial to be used for the proposed research, Nisin, is a peptide that is 34 amino acids in length. Nisin is an antimicrobial bacteriocin that is produced by different strains of lactic acid bacteria such as *Lactococcus lactis.* A bacteriocin is not an antibiotic. Bacteriocins are classified as antibacterial peptides of which there are several types with differing properties such as mode of action and spectrum of activity against bacteria [2]. It is an antimicrobial peptide that is produced by some bacterial species including those of the lactic acid bacteria [55; 56; 114]. Bacteriocins are naturally produced in the environment by bacteria in order to prevent a higher level of competition with other microbes for nutrients. Nisin is produced during the exponential growth phase of the bacteria and stops once the cell has reached the stationary phase [26; 36; 65; 80; 108].

According to Juncioni di Arauz et al (2009), production of the peptide occurs during fermentation of milk or whey [76]. Species of *Lactococcus lactis* spp. *lactis* which are used to ferment the milk or whey additionally produce Nisin during the exponential growth phase. The broth from the fermentation process is collected, spray dried and milled into a powder [41; 71; 129].

Nisin is effective for inhibiting Gram positive bacteria without the addition of heat treatment or separate additives and has been found to be effective at Nano molar concentrations [85]. It can also inhibit the outgrowth of spores into vegetative cells [34; 108]. Gram negative bacteria are not inhibited alone by nisin but with additional additives or treatments such as chelating agents or a secondary synergistic component [75; 76].

These additives can include that of enzymes such as lysozyme, plant extracts and EDTA. Bacteria that are specifically targeted include *Listeria monocytogenes, Clostridium botulinum, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* and non-pathogenic spoilage microorganisms such as *Micrococcus spp.* and lactic acid bacteria [56; 68].

The molecule is amphiphilic with both a hydrophilic (N-terminus end) and hydrophobic (C-terminus) end [96; 99]. This makes nisin ideal for food matrices, solutions and surface adhesion [108]. It is water soluble except for any residual milk proteins that have been left in the product [71]. Water solubility is one of the properties that make nisin optimal for usage in food products. Other properties include that nisin is heat stable, stable at a low pH, non-toxic, easily digestible, absent of odor and flavor and has a very slight coloration [71; 108].

The heat stability of nisin is important in food production in order for the bacteriocin to maintain its antimicrobial activity while going through high heat food processing steps. If the bacteriocin is inactivated, it will be unable to preserve antimicrobial properties during storage of the food product. Heat stability is also desired in the area of research. Because sterile conditions are required in order to avoid microbial contamination in laboratory testing, the ability to autoclave nisin (121°C) [108] without the loss of antimicrobial activity is optimal. This enables researchers to eliminate one aspect of variability when planning experiments regarding the efficacy of nisin due to loss of activity from heat treatment. However, excessive heat treatment can cause antimicrobial activity to decrease at temperatures above 140°C [69]. If food processing steps were to exceed this approximate temperature, the antimicrobial activity could

decrease. Long-term storage and food component interaction could also produce the same effect [68; 96].

On the other hand, the efficacy of nisin varies with pH. Nisin is stable at a low pH being optimal at a pH of 2 [71] and losing antimicrobial activity as the pH becomes more alkaline. The acid stability is due to nisin being produced by strains of lactic acid bacteria which are naturally acid tolerant microorganisms. In food production, nisin withstands fermentation processes which are naturally acidic. In research applications, for example coatings, an acid solution is added in order to "activate" the nisin by lowering the pH of the coating solution [46]. For example, a nisin mixture (Nisaplin®) was dissolved in a 0.02M acetic acid in water solution to solubilize the nisin and try to optimize the antimicrobial activity prior to adding the solution to the rest of the coating solution to be cast onto glass plates with a thin layer chromatography plater (TLC) [46].

Other properties that make nisin ideal for food additive uses are that it is a non-toxic, absent of odor, flavor, has very slight coloration and is able to be digested easily by those who consume the product. The slight coloration of nisin is a light brown color that results from the use of salts and milk proteins commonly found in nisin mixtures that are commercially available for purchase.

Several modes are proposed that nisin uses to inhibit Gram positive microorganisms has been found to affect the cytoplasmic membrane. Some researchers state that nisin affects the cytoplasmic membrane through the formation of pores and others state that nisin affects the proton motive force. Nisin is a type of bacteriocin which has been found to affect the transport of amino acids by disrupting the proton motive

force and causing the release of the amino acids that had been accumulated within the cell through lysis [84; 108]. On the other hand, the more common consensus is that nisin inhibits microorganisms through inhibiting cell wall synthesis [1; 15; 16; 18; 89]. It is believed that there is pore formation resulting in numerous holes or pores through the peptidoglycan layer of the Gram positive bacteria, causing the cell to lyse and die [90].

The mechanism by which nisin causes pore formation in the peptidoglycan layer is a multi-step process. As stated earlier, nisin has a C-terminus and N-terminus end to its structure. The N-terminus end of the molecule bonds to a lipid II molecule which is a docking molecule in the peptidoglycan layer of the Gram positive bacteria [1]. A single pore is composed of 8 nisin molecules docking to 4 lipid II molecules [15; 16; 18; 89]. The C-terminus ends of the nisin molecules then use the polycyclic structure of nisin to bend the molecule and form a pore in the peptidoglycan layer of the cytoplasmic membrane. This causes the bacteria to lose cellular components resulting in the death of the bacterial cell [84; 144].

Nisin has been approved for use in food products since 1969 and was the first bacteriocin given the status Generally Recognized as Safe in the United States in 1988 [55; 135]. It is GRAS approved by both the FDA (Food and Drug Administration) and WHO (World Health Organization) [84]. Nisin is also approved as a food preservative in over 50 countries. These include China, Brazil and countries within the European Union [85; 115].

The long history of nisin use combined with its' non-toxic natures makes this particular antimicrobial ideal for food additive and packaging applications [94]. Nisin

has been an additive in cheeses and dairy products for many years and there have been no apparent ill effects from the consumption of this product. It is also used in meat products like bologna, some hotdogs and plant-based products [84; 144]. It is approved for usage in over 50 countries [68; 116] and some (but not all) countries have a set limitation for the concentration to be added to food or food packaging. However in the United States, there is a legal limit of 10,000 IU/mL concentration (250 ppm) of Nisin when it is added in food products [55]. The legal limit is set based on the premise that nisin is considered unnatural if it exceeds a concentration that occurs in naturally fermented foods with the proper *Lactococcus* or nisin-producing culture [84].

There are four different types of naturally occurring nisin; Nisin A, Z, Q and U [56; 68; 86; 151]. Both Nisin A and Z are produced from *Lactococcus lactis* while Nisin Z is produced from *Lactococcus uberis* [84]. The two most common types are nisin A and nisin Z. Nisin A is the most commercially available and nisin Z allows for better solubility and diffusion. Because nisin Z has better diffusion properties, larger inhibition zones against target bacteria are observed in comparison to nisin A [1; 114]. Nisin is composed of a 34 amino acid chain with disulfide bonds that assist in the mode of action to be discussed later. Structurally, there is one key difference between Nisin A and Nisin Z. The amino acid at position 27 is histidine in nisin A and asparagine in nisin Z [28; 68]. Nisin Q differs from nisin A by four amino acids [68].

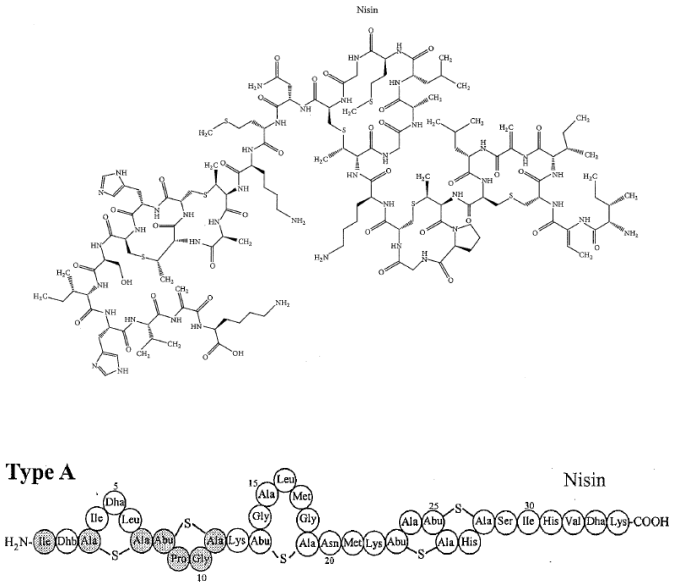

Figure 2.3. Nisin molecular structure [12] and Nisin A amino acid structure [19]

Commercially available nisin products come in a powder form with varying degrees of purity from approximately 99% purity to 2.5% nisin concentrations. Due to the expense of the antimicrobial, use of 2.5% purity is common. One of the most well-known products of this purity is called Nisaplin® from Danisco. It is a product in which the nisin is produced, separated and spray dried before milling into a powder [71]. Numerous laboratory studies have been conducted incorporating Nisaplin® into coatings for polymer substrates, sprays for food products, quantification method studies and more. Because the percent concentration of nisin in Nisaplin® is lower than that of pure, the IU/mL or IU/g is also lower. One gram of pure nisin has a concentration of 4 x 10^6 IU/mL while one gram of Nisaplin® has a concentration of 1 x 10^6 IU/mL [34; 108]. Nisaplin® is composed of 74.4% sodium chloride, 23.8% solids (including nisin and residual milk proteins) along with 1.7% moisture [35; 149]. According to Liu et al, the salts are added as stabilizers to the mixture [88]. Other products produced by other

companies contain similar mixtures but indicated that in addition to the salt and milk proteins, sugars and polysaccharides also stabilize the overall mixture [75].

Numerous studies have been conducted utilizing nisin in coatings for packaging, sprays and dips for animal carcasses before meat fabrication along with tests concerning efficacy alone or in combination with other antimicrobials against target microorganisms. The most common method used to enumerate *Listeria monocytogenes* in the studies was consistently a semi-solid agar well diffusion assay to produce a standard curve of the inhibitory effects of nisin. The standard curve was then compared to that of the tested food product. The standard curve shows known concentrations of nisin and plots the inhibition zone to which nisin could inhibit either a specific strain or cocktail of strains [108]. From this, the authors are able to determine the concentration of nisin that is either still active within the solution that was produced or the amount of nisin that had diffused into the food product. In many cases, a secondary procedure was conducted to enumerate both the bacteria and the antimicrobial concentrations in order to verify findings (i.e. film on lawn, shaker flask and/or ELISA assays) [86].

Utilization of nisin against *Listeria monocytogenes* or other Gram positive bacteria exhibited differences in inhibitory effects based on the strain. It has been determined that different strains of bacteria have less or more resistance against the antimicrobial effects of nisin [96]. Cha et al tested a polyethylene film that has been coated with 3 different solutions consisting of a ratio of methylcellulose/ hydroxypropylmethyl cellulose, polyethylene glycol plasticizer and nisin. In order to achieve an even layer of coating, the film was placed on top of a hot plate in order for the heat to even out any inconsistencies in the coating thickness. The coatings varied in

antimicrobial concentrations with solution concentrations of 100, 500 and 1000 IU/mL. Each of the three coated films was tested against *Micrococcus luteus* and 7 different strains of *Listeria monocytogenes.* Treatments of 100 IU/mL showed no affect against the bacterial strains while concentrations of 500 and 1,000 IU/mL showed a 2-3 log reduction [25].

Another study also showed a 2-4 log reduction when nisin was added to 4 different polysaccharide coatings, swabbed onto roasted turkey slices that had been inoculated with a 5-strain cocktail of *Listeria monocytogenes.* The meat samples were vacuum packed and either frozen or refrigerated. (Frozen samples thawed before tested) The authors determined that the coatings slowed the growth and the treated samples contained 2-4 lower log population than the control which contained a 7 log population [73].

Other studies determined that nisin had the ability to slow the log phase of the bacterial growth or have higher initial reductions in the microorganisms tested. Studies also showed that nisin produced inhibitory effects for a short period of time but the bacteria had the ability to recover and continue to grow after a longer storage time when tested against multiple strains of *Listeria monocytogenes* [74; 95; 100]. Overall, nisin is effective for inhibiting Gram positive bacteria. The studies above tested the antimicrobial at concentrations at least 10 times less than the legal limit at 1,000 IU/mL and showed a slowed log phase and higher initial reductions in the microbial population. In order to obtain a more broad range of antimicrobial activity, nisin needs to be combined with other antimicrobials such as EDTA or organic acids. Utilizing multiple antimicrobials simultaneously will also prevent the likelihood of bacteria building up a resistance to one

antimicrobial. Nisin is GRAS approved and effective but will not achieve high inhibition without the use of additional "hurdles" in food packaging.

2.8.2 Acetic acid solution (0.02 M)

This acid solution is a diluted distilled water and glacial acetic acid solution. Franklin et al (2004) used this solution to dissolve the nisin component prior to mixing the remainder of the coating ingredients together [46]. The low acidity (pH 2) of the solution acidifies the antimicrobial, which has been shown to increase efficacy. Grower, Cooksey and Getty (2004) determined that this acetic acid/nisin solution produced the largest inhibition zones based upon a spot on lawn assay tested against *Listeria monocytogenes* (ATCC 15313) when compared to ascorbic, lactic and hydrochloric acids at the same pH level [55].

2.8.3 Polyvinyl Alcohol (PVOH)

Polyvinyl alcohol (PVOH, PVA or PVAL) is a water soluble, synthetic polymer that is formed through the hydrolysis of polyvinyl acetate (PVAc) utilizing a strong base, such as sodium hydroxide (NaOH), to produce vinyl alcohol monomers and sodium acetate. This hydrolysis reaction is also referred to as a saponification of esters [42; 54; 118]. The structure of PVOH can be seen in Figure 2.4 while the reaction for the formation of PVOH can be viewed in Figure 2.5.

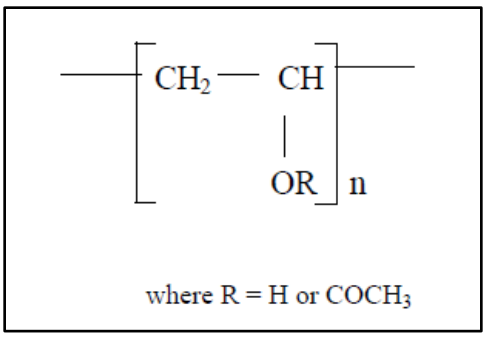

Figure 2.4 Polyvinyl alcohol monomer structure [118].

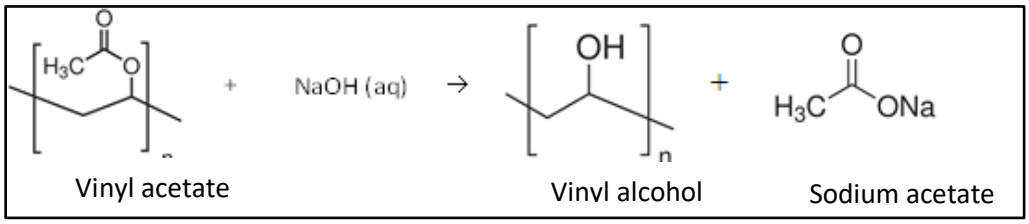

Figure 2.5 Formation reaction of polyvinyl alcohol.

Polyvinyl acetate is formed through a free radical polymerization process which then undergoes the saponification or hydrolysis reaction to form polyvinyl alcohol. Free radical addition polymerization is a process in which free radical or ion formation is initiated using a catalyst or an initiation step, followed by propagation to produce additional ions which link to produce a long polymer chain. The reaction is then terminated via an inhibitor or through consumption of the reactants during the polymerization process [132]. It is possible to produce PVOH through a polymerization

process rather than saponification or hydrolysis of PVAc, however, the desired levels of purity and quantity to be produced are not feasible using this process [42].

There are batch and continuous saponification processes. Batch processes are typically for specialty resins because of the low quantity that is produced in a batch [42; 113]. Continuous processes begin with free radical polymerization for the formation of polyvinyl acetate. The PVAc formed is then hydrolyzed using either a continuous belt or extrusion process. Catalysts for the reactions can include sodium hydroxide, potassium hydroxide, methoxide or ethoxide. Formation and processing of polyvinyl alcohol can be difficult due to an increasing viscosity of the products due to the formation of a gel. The gel is then dried and ground to fine particles which are then sized and packaged accordingly [113].

There are multiple grades of polyvinyl alcohol resins. This variation is due to the degree of hydrolysis of the polymer which causes drastic changes in the characteristics and resulting properties. Degree of hydrolysis refers to the percentage of acetate groups which remain in the resulting PVOH produced from PVAc [54]. There are two general categories of PVOH based upon degree of hydrolysis: partially hydrolyzed or fully hydrolyzed. Partially hydrolyzed resins can range from 80 to 98.5% (1.5 to 20% acetate groups) while fully hydrolyzed resins are higher than 98.5% (1.5% or less acetate groups) [92]. The degree of hydrolysis can have drastic effects on the resulting properties. The table below displays some key property changes:

Table 2. 1 Comparison of properties between fully and partially hydrolyzed polyvinyl alcohol resins.

Partially Hydrolyzed - PH (Lower degree of hydrolysis)	**Fully Hydrolyzed - FH (Higher degree of hydrolysis)**	**Reasons for Difference**
More amphiphilic [92]	More hydrophobic	PH contains more acetate groups containing polar and non-polar components
30-40% crystalline [54; 109]	40-50% crystalline	FH contains more hydroxyl groups enabling more efficient polymer chain stacking
Increased water solubility	Reduced water solubility [54]	PH - more acetate groups reduce inter and intramolecular forces between the hydroxyl groups in the resin molecule therefore making it more water soluble [42; 63]
Lower solvent resistance	Increased solvent resistance [54]	Higher crystallinity of FH resin increases solvent resistance
Lower tensile strength	Increased tensile strength [54]	Higher crystallinity of FH resin increases tensile strength
Lower T_g and T_m	Higher T_g and T_m [72]	Crystalline structure accounts for difference in polymer melt (Melt range 180 - 240°C) [54]
Decreased viscosity [42]	Increased viscosity [42]	Wide range of viscosity of resin in 4% aqueous solution 3.4 – 60 cP
More stable viscosity; Stable in water solution [118; 42]	Gel over time [42]	
Lower surface tension [42]	Higher surface tension	PH - amphiphilic nature
Better adhesion to hydrophobic surfaces [98]	Decreased adhesion to hydrophobic surfaces	FH- Increased hydroxyl groups increased polar nature reducing adhesion to hydrophobic surfaces

Polyvinyl alcohol has been used in many different industry applications due to the variation in properties. PVOH remains stable in water-based solutions and humid conditions. It has also been shown to be chemically resistant, UV stable, exhibit high tensile strength but also maintain good flexibility when utilized in film applications. Other properties such as being tasteless, odorless and a good oxygen barrier can make certain PVOH grades ideal for food and pharmaceutical applications [54; 61]. PVOH is also thermoplastic, giving it the ability to seal when used in a packaging type application. There are limited methods for processing PVOH due to polymer degradation by pyrolysis (also known as the elimination of water) [66]. PVOH begins to degrade at 150°C while the melt temperature range, depending on the degree of hydrolysis, is 180-240°C [54].

Medical, pharmaceutical, food, paper, converting and consumer goods industries have all found applications for polyvinyl alcohol resins. PVOH has been previously used in combination with plasticizers (i.e. glycerol) and bacteriostatic agents to assist in healing for burn victims. It has also been added into dressing and gauze type applications because the material was found to not be harmful when in contact with human skin [109]. Because of this, it has also been proposed that PVOH be used for drug delivery systems [92]. It is currently utilized for tablet coatings because of the materials high oxygen barrier properties to protect oxygen sensitive ingredients or supplements [54].

PVOH has also been used in the food; however, implementation is limited due to the high cost of PVOH [72] and the lower profit margins of food products. Current uses include binding and coating agents within or on the exterior of food products. Different grades have higher moisture barriers which can be used as coatings to prevent moisture loss or gain [54]. Several other applications in various industries include being used as an

adhesive, emulsifier, solvent casting or film forming, a binder for fibers in addition to packaging chemicals in which the pouch is soluble for easy use, even water soluble golf balls and pet waste bags. Some examples of these pouches include laundry detergent pac kets and pesticide pouches which can be dropped directly into a mixing tank [54; 78; 98; 121].

Like any material or ingredient implemented in food products or food packaging, it is subject to regulatory scrutiny. According to a report in 2004 from the Joint Expert Committee on Food Additives (JECFA), a joint committee between the Food and Agriculture Organization of the United Nations and World Health Organization, it is required that there be negligible reactions between the PVOH and the food product under the intended use of the product. When PVOH is used in food products, the intended use is considered to be a neutral pH environment and food products that are stored in either low or room temperature environments [118]. If the application of PVOH has potential to be ingested by a consumer, there are limitations and standards such as no adverse effects from ingesting low concentrations of PVOH and passing through the alimentary canal (contains esophagus, stomach and intestines) unchanged [109].

The intended use of PVOH in the research to be discussed throughout this dissertation is to implement the material as an aqueous coated film for means of carrying and transferring an antimicrobial component to a food product. For this specific application, film for food packaging, there are additional requirements. For example, solvent retention in PVOH films for food packaging are limited to no more 0.5 mg per square inch of material [109]. FAO/WHO JECFA also noted that the PVOH component in an aqueous film coating is not to exceed 2.3 mg/sq. cm [118].

2.8.4 Glycerin

Films cast from a PVOH and water solution can result in relatively stiff and brittle films. Plasticizers are substances known to increase the internal volume between polymer chains producing films that are more flexible and ductile rather than brittle. Plasticizers have also been found to increase both extensibility and workability, increasing the overall toughness of a film [121]. Additional benefits of plasticizers include the ability to reduce processing temperatures by lowering the glass transition temperature (T_g) and melt temperature (T_m) which can reduce the amount of thermal degradation due to less exposure to high temperatures [87; 121]. Reduction of the T_m was a critical aspect concerning this research in order to potentially produce a coated film that could be sealed in packaging applications. Plasticizers have been shown to reduce the melt temperature of polymer crystals through addition of defects into the crystalline structure of the polymer [87].

Glycerin is a thick, clear, colorless, sweet tasting liquid that is produced from hydrolysis of animal and vegetable fats and oils. It has been used for applications in the pharmaceutical industry as a solvent, in the cosmetic industry for products such as hand oils and also in food as a sweetener, emulsifier and humectant. Humectants are substances used to keep foods moist. Glycerin is soluble in water which makes it ideal for combining with a PVOH and water solution to produce a plasticized film or coating [49; 51; 52]. See Figure 2.6 below.

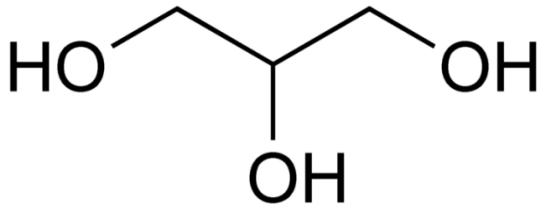

Figure 2.6 Chemical structure of glycerin. [50]

Glycerin (CAS Reg. No. 56-81-5) is a GRAS multiple purpose food substance according to the U.S. FDA under CFR (Code of Federal Regulations) 182.1320. Glycerin is permitted to be used in food for human consumption and food contact materials and is GRAS in accordance with good manufacturing practices [137].

Glycerin can be used to plasticize polyvinyl alcohol resins. According to Lim and Wan (1994) glycerin has the ability to solubilize to the PVOH/water solution in order to decrease the crystalline regions within the polymer [87]. Pyrolysis or elimination of water is the main concern of thermal degradation for PVOH which can be decreased through utilization of glycerin [66; 87]. According to Lim and Wan (1994), the plasticizer will crosslink to PVOH via hydrogen bonding in order to prevent the loss of water associated with thermal degradation [87].

Jang and Lee (2003) found that increasing phr (parts per 100 grams of PVOH) of glycerin resulted in films with lower melt temperatures [72]. If phase separation occurred due to excessive addition of glycerin, the effects of the plasticizer were negated. According to this study, phase separation start to occur for partially hydrolyzed PVOH when glycerin exceeded 40 phr and 65 phr for fully hydrolyzed PVOH [72].

2.8.5 Surfactant -Tween 80

The primary reasons for addition of a surfactant or surface active agent to the antimicrobial formulation were to decrease the overall surface tension of the liquid coating solution, and to aid as an emulsifying component. Surface tension or surface free energy is the "amount of work required to increase the surface by unit area" [132]. Surfactants are defined as "compounds that dramatically lower the surface tension of water and form aggregates like micelles in aqueous media" [134]. Surfactant compounds contain both hydrophilic and hydrophobic ends on the molecule and can be classified as anionic, cationic, amphiphilic and nonionic. These compounds maintain the ability to lower surface tension because adsorption or adherence of the component to both the liquid coating component and the substrate enables the reduction of the surface tension of the liquid, as well as the interfacial surface tension of the substrate [134].

The surface active component chosen for this coating solution was Polyoxyethylene Sorbitan Fatty Acid Ester or Polysorbate (also known by the commercial name Tween®). Tween® 80 was the specific ingredient used for the coating formulation. Tween® is a nonionic surfactant produced through addition of ethylene oxide to sorbitan fatty acid ester (SPAN) resulting in slightly more hydrophilic compounds [134].Tween® surfactants are commonly used as emulsifiers in food products in the United States and nonionic surfactants are "mostly tolerant in aqueous solutions of added salts" [134]. These characteristics were important for this packaging application due to the intention of this material being in direct food contact with potential to migrate into the packaged food product in addition to the Nisaplin® component

containing an additional salt component in the coating solution. See Figure 2.7 for chemical structure.

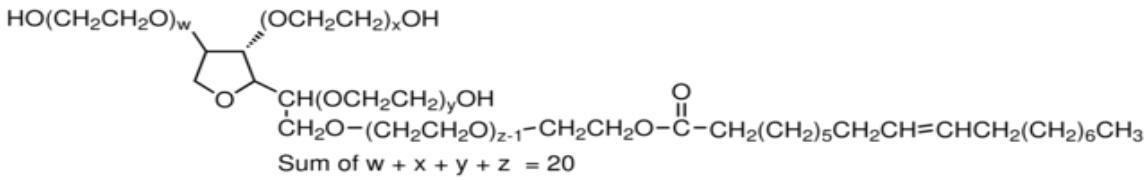

Figure 2.7 Polyoxyethylene sorbitan fatty acid ester (aka Tween® 80) molecular structure [107]

Additional benefits for using Tween® 80 specifically are that it has been shown to increase the effects of nisin in milk. Nisin has been found in several studies to perform in a less effective manner when tested against high or higher fat food products when compared with food simulants such as agar. Although nonionic surfactants have not been found to have antimicrobial effects, [67] they have been found to aid nisin by surrounding the protein and fat components that have potential negative effects on nisin activity. Previous studies have found that an increase in the fat content of milk decreased the overall nisin activity against *L. monocytogenes* strains (Scott A and Jalisco) [10; 77]. Jung, Bodyfelt and Daeschel (1992) found that Tween®80 (0.2%) increased antimicrobial activity when combined with a nisin solution at a concentration of 50 IU/mL. *L. monocytogenes* (Scott A) was reduced from 6.34 log CFU/mL to 2.0 log CFU/mL after a 2 hour exposure to the nisin/Tween® 80 solution. The second *L. monocytogenes* strain tested, Jalisco, was also reduced from 7.60 log CFU/mL population to 1.52 log CFU/mL after a 2 hour exposure to the same solution [77]. Bhatti, Veeramachaneni and Shelef (2004) found that combining 5 μL/mL (0.5%) of Tween®80

with a 125 IU/mL nisin solution resulted in a reduced microbial level below a detectable limit after refrigeration for 15 days. The surfactant on its own did not have an effect on *L. monocytogenes.* In this study the surfactant was described as a means of "displacing" the proteins and fats from the nisin molecules by surrounding or enclosing them. This enabled the nisin molecules to interact with the pathogen cells rather than the protein molecules [10].

According to the US FDA, Tween®80 or Polysorbate 80 is not GRAS approved, however it is used as a food additive and the concentrations are limited for specific food applications. Tween® 80 is approved as an emulsifier or surface active agent under 21 CFR 178.3480 but must also meet the criteria as a direct food additive under 21 CFR 172.840 [138]. However, it cannot be assumed that an ingredient can be used as an indirect additive when approved for specific uses as a direct food additive. According to 21 CFR 174.5 there are several conditions that need to be met to approve an ingredient as an indirect food additive such as "substances generally recognized as safe for their intended use in food packaging". Therefore, the specific use of the concentration used in the coating solution would need to be specifically approved as an indirect food additive in a food packaging application or it could potentially be approved as a surface active agent or emulsifier because that was the intended purpose of the ingredient [138].

2.8.6 Ethanol/Water solvent

The final component of the re-formulated coating solution is the solvent portion. The solvent mixture contained a 50/50 (v/v) mixture of 95% ethanol and distilled water. Both of these ingredients are GRAS approved with the intention of the ethanol evaporating out of the coating upon drying. This mixture was used by Franklin et al

(2004) in order to produce the previous antimicrobial coating [46]. The mixture of ethanol and water enabled a lower surface tension of the overall solution higher surface tensions can cause adhesion difficulties in water-based coatings and is of great importance for processes such as drying. Adhesion theory will be discussed in more depth. Overall, it has been found that increasing the amount of alcohol in an ethanol/water mixture results in a decreased surface tension, which is ideal when trying to coat onto a hydrophobic substrate [46; 55; 81; 94; 100; 140].

2.8.7 Linear low density polyethylene (LLDPE)

Linear low density polyethylene (LLDPE) is a common material used in packaging, known for being low cost while able to maintain strength and toughness. Density of LLDPE can range from 0.91 – 0.94 g/cm^3 [139]. LLDPE made up the sealant layer of a multi-layer coextruded material donated by Sealed Air Corporation for this work. This material is commonly used as a sealant due to its low melt temperature. It is produced using additional polymerization, typically producing a copolymer of ethylene and other monomers such as butene, hexene or octene [35] has the following structure below:

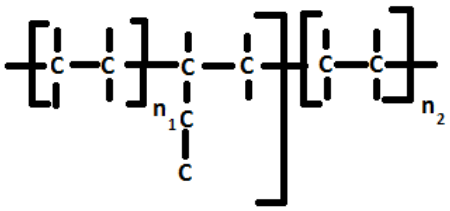

Figure 2.8 Ethylene monomer structure of LLDPE

2.9 Adhesion

Adhesion is an important aspect in packaging and specifically coating technologies in regards to this work. Adhesion can be defined as the joining of two dissimilar materials called adherends or substrates [48; 121]. There are several contributors of adhesion however the two main categories are mechanical and chemical interactions [48; 132]. Additional components can include electrical interactions and interdiffusion of chains. Electrical interactions such as electrostatic attraction are difficult to determine because the attraction between two materials can only be identified after breaking an adhesive bond which can cause an electrical discharge [48; 132]. Interdiffusion of chains primarily occurs when two components are put in close contact with one another and a mechanical pressure is applied. This can occur in heat sealing during which polymer chains from one or both substrates will diffuse into one another based on heat and pressure causing chain mobility over a designated dwell time.

Mechanical adhesion has been found to be more associated with products such as paper that have a rough and fibrous surface. Adhesives or molten polymers are able to interlock with outstanding or protruding fibers in addition to seeping into porous areas of substrates producing a mechanical bond. However, in order to achieve a strong adhesive bond, the materials must be compatible with one another on a chemical level [48].

There are multiple types of chemical interactions that can promote adhesion such as primary bonding including ionic and covalent bonding. Ionic bonds are produced by molecules containing positive or negative charges based on the loss or acceptance of electrons. These charged molecules or ions can then bond to other charged molecules to produce stable electron orbitals. Ionic bonds have energy ranges of 590 – 1050 kJ/mol

producing one of the strongest chemical bonds [132]. Covalent bonding is a chemical bond resulting from the sharing of electrons between molecules and have bond energies of 63-710 kJ/mol [132]. Molecules produced can be a result of polar and non-polar bonding. Covalent bonding is the primary bond type in polymers such as polyethylene.

Chemical interactions can also be produced by secondary bonds, including London dispersion forces, dipole-dipole bonding and hydrogen bonding which could be a component of acid–base chemical bonding reactions [45; 48]. Although the strength of these bond types are not as high energy as the primary bonds, they can still have an effect on adhesive bond strength. London dispersion forces occur in non-polar molecules in which attractive forces are produced by oscillating electron clouds [132]. Dipole bonds are produced between molecules with both positive and negative ends while hydrogen bonding occurs between hydrogen on one molecule and a highly electronegative atom on another. However, without intimate contact between two materials in addition to the chemical bonding interactions mentioned above, adhesion is not likely to be achieved [44]. Aside from the degree of intimate contact, the surface chemistry of both substrates to be in contact affects adhesion [43]. In the case of the research to be discussed, one substrate would be a solid component and the other a liquid coating component.

2.9.1 Surface tension, wettability and contact angle

For coatings and coating technologies, it is generally considered necessary to have surface energies and critical surface tensions that are compatible to facilitate wetting and therefore adhesion. Surface tension is defined as the "amount of work required to increase the surface by unit area" [12]. Surface energy or surface tension refers to the

energy per area in J/m^2 while surface tension is measured in force per length of N/m both being essentially the same [33]. Wetting is a phenomenon that occurs when forces cause a liquid to spread onto a surface [132]. The degree to which a liquid spreads onto a solid surface refers to the wettability of the liquid on that particular substrate surface.

Liquid droplets in a zero-gravity environment would be perfect spheres held together by cohesive forces within the interior of the droplet. The net force within the drop would be zero due to the balance of forces caused by molecules pulling in every direction within the droplet [150]. This is the most efficient way for the liquid droplets to pack molecules together and decrease surface area as much as possible. An environment containing gravity is what causes spherical droplets to distort into the tear drop type shapes among others [132]. When a droplet of coating or some liquid is placed on a substrate, the shape of the droplet on the substrate depends on the amount of work put into the system to break the molecular attraction within the droplet [132]. The droplet could wet out completely, partially wet out or not wet out at all as seen in the figure below. A coating will exhibit wetting when the coating is able to have complete intimate contact with the surface including filling pores and crevices within a substrate [132].

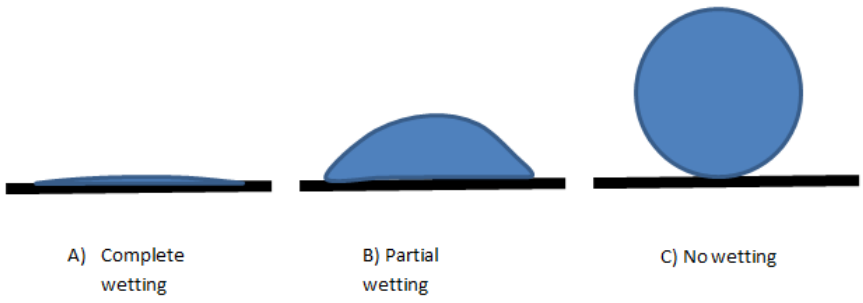

Figure 2.9. Examples of various degrees of wetting for a liquid on a substrate. Adapted from [132].

Numerically, the various degrees of wetting in the figure (2.9) above can be identified using contact angle measurements. Young's equation defined the contact angle of a liquid drop on an ideal surface as "…the mechanical equilibrium of the drop under the action of three interfacial tensions" (Figure 2.10; Equation 1). Out of the three interfacial tensions below shown in Young's equation, only two, $\gamma_{lv}$and γ_{sl} , are able to be measured in addition to contact angle. The variableγ_{lv}, can be measured by a DuNuoy Tensiometer which can provide the surface tension of a liquid using a platinum ring. The amount of force the break the surface tension of the test liquid upon pulling the submerged ring from the fluid is calculated to dynes/cm from a force -displacement curve [148]. The variable γ_{sl} or surface tension of a solid can also be determined by measuring the contact angle on a solid or substrate (using reference liquids with known surface tensions) which will be discussed later.

$$\gamma_{lv} \cos \theta_Y = \gamma_{sv} - \gamma_{sl} \qquad [1]$$

Where: γ_{lv} is the interfacial tension between the liquid component and vapor

γ_{sv} is the interfacial tension between the solid and vapor

$\gamma_{sl}$is the interfacial tension between the solid and liquid components

θ_Y is the contact angle

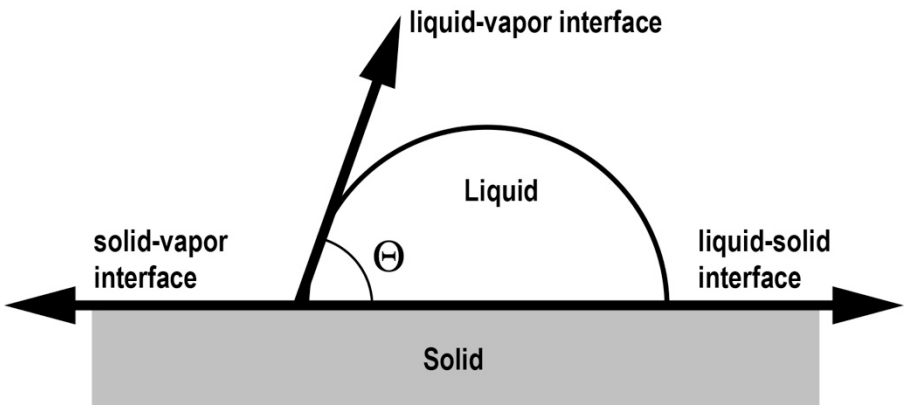

Figure 2.10. Young's equation. [48]

In Figure 2.10, complete wetting occurs when θ is equal to 0 degrees Contact angle measurements less than 90 degrees indicate partial wetting and measurements above 90 indicated non-wetting [48; 132]. There are several ways for measuring contact angle of a liquid on a particular substrate for a known liquid. One category of methods are direct optical methods while the other category contains indirect force methods [150]. Optical methods consist of contact angle goniometers which can consist of measuring static (sessile) droplets or dynamic droplets. For static or sessile drop measurements, a micrometer pipette is used to release a droplet onto a substrate. A back light and protractor eye piece are used to project the droplet silhouette and a measurement is taken [150]. Today goniometers utilize video cameras to record and analyze the droplets via computer programs.

There are multiple indirect force methods for calculating contact angle however the focus will be on the Wilhelmy Balance Method also known as the Wilhelmy Plate Methods. A plate, which can be mounted with or without a polymer sample is lowered into a liquid and lifted out at a constant rate. The weight or force of the liquid on the plate

is recorded using a microbalance for both the advancing and receding portions of the test. The contact angle is calculated from the following formula:

$$\theta = arccos\frac{F}{p*\gamma_{lv}} \qquad [2]$$

Where θ is the contact angle, F is the overall change in force, p is the perimeter or cross section of the sample and γ_{lv} is the surface tension of the liquid. (Retrieved from [83])

There are many factors that can affect contact angle testing results. Because the samples (droplet) sizes are so small in volume, contaminations or impurities can cause inconsistent results. This is also true for plate or film samples being tested using the Wilhemly plate method. It is pertinent to have clean samples free of dirt and debris to avoid skewing results. Aside from contaminations, consistent drop volumes and surface topography can affect direct optical methods and plate speed can also affect the Wilhemly plate method. For both optical and indirect force methodologies, it is important that a single user run all of the testing for consistency in both analysis but also testing procedure and sample preparations [150].

There are now simpler ways for determining the surface tension of a solid rather than conducting contact angle testing on multiple substrates and liquids. Dyne pens are felt tipped pens that contain a liquid mixture of ethoxyethanol and formamide which produce a range of liquid-vapor surface tensions from 30-70 dynes/cm [121]. These pens provide a simple, fast and cheap method for determining the critical surface tension of a solid based upon how the mixture within the pen will wet out onto the surface of the substrate being tested. Critical surface tension of wetting is the surface tension of a solid at which a liquid will wet out completely or produce a contact angle of 0° where cosθ = 1 [132].

The pen solution that wets out after approximately 3 seconds on the surface of the substrate would indicate the critical surface tension of the solid. Dyne pens are commonly used in the coating and printing industries in manufacturing plants due to their simplicity and cost. Some common issues or negatives of dyne pens are that the solutions can become either contaminated or the solution mixture can be altered due to evaporation [126]. It is important that new dyne pens are purchased at least once per year or more depending on the amount of use.

The point at which the contact angle of a liquid reaches zero on a given substrate is called the critical surface tension of the substrate. Dr. William A. Zisman determined the critical surface tension of solids by producing what are today commonly known as Zisman plots in the area of surface chemistry and adhesion. Zisman plots consist of plotting the cosine of a contact angle measurement on the y-axis and the surface tensions of a series of liquids on the x-axis. The point at which the plotted line intercepted $\cos \theta = 1$ was the critical surface tension of the solid [32]. A Zisman plot for a polyethylene film can be seen in Figure 2.11 below:

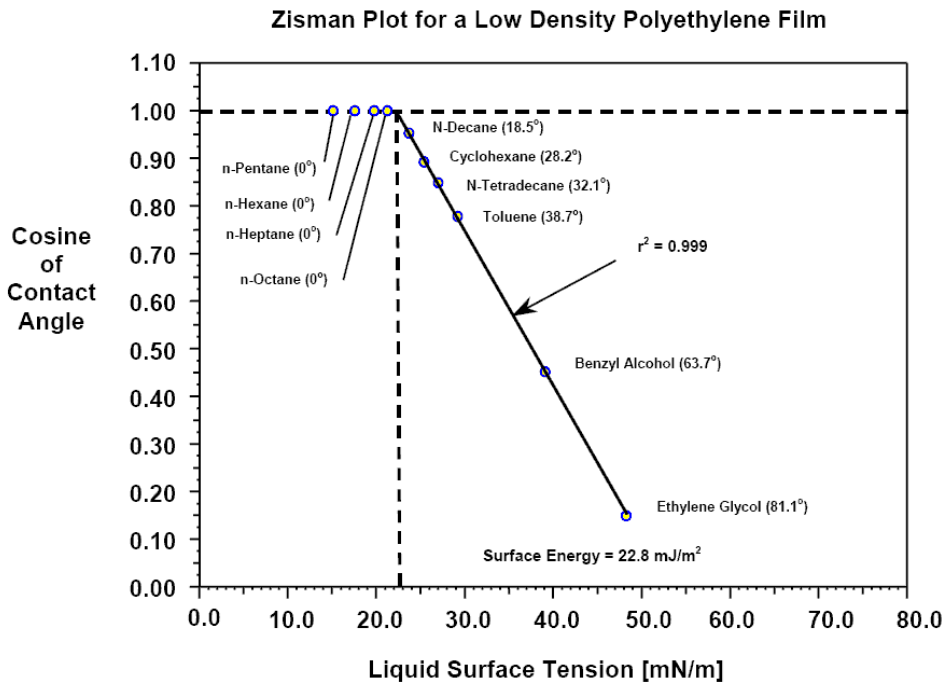

Figure 2.11. Zisman plot for polyethylene film. [117]

The surface tensions of both liquids and solids can be measured in units of dynes/cm or in SI units as mN/m as indicated in the Zisman plot above [132]. The figure above shows that the critical surface tension of the polyethylene tested was approximately 22.8 dynes/cm. This value indicates that the surface tension of a liquid component must be less than 22.8 dynes/cm for some wetting to occur. It has been found that, in order for coating adhesion to be achieved, the surface energy of the liquid coating must be at least 8-10 dynes less than that of the critical surface tension of the substrate being coated [121; 132]. This is critical for wetting to occur, however, as discussed previously, wettability does not ensure adhesion, however it is useful base knowledge.

There are two ways to increase wettability to meet or exceed the demands of the 8-10 dynes surface tension guideline previously mentioned.

One method to decrease the surface tension of the coating solution using solvents. For example, Section 2.8.6 Ethanol/Water Solvent, discussed that in the coating formulation a 50/50 (v/v) solvent mixture was utilized. This was due to the surface tension of water being 72.6 dynes, which can make it difficult for water based inks or coatings to wet out non-polar substrates such as polyethylene. Addition of ethanol solvent to the mixture, 22 dynes/cm, drastically reduces the surface tension of the overall solution. According to Vásquez, Alvarez and Navaza (1995), as the mass percentage of ethanol increased in an ethanol-water mixture, the surface tension decreased. A 50/50 mixture of 100% ethanol and water at 25°C can result in a surface tension of 27-28 dynes/cm [140].

A second set of methods to increase wettability (and potentially adhesion) of a coating onto a substrate is to increase the surface tension of the substrate. Typically in the packaging industry, it is common to both raise the surface tension of a film substrate and decrease the surface tension of a coating solution to facilitate wetting and adhesion. There are many ways that the surface tension of a film substrate can be raised using what are called surface treatments.

2.9.2 Surface treatments

Surface treatments are processes that can "…decrease the amount of work required to increase the surface of a substrate by a unit area" [121]. There are multiple types of surface treatments including flame treating, corona discharge, priming, cold plasma, UV, laser, electron beam, ion beam and metallization [48]. Of these, the most common in packaging are flame treat, corona, and priming. The first two types are physical modifications to the film substrate while priming consists of adding a new, more compatible, chemistry to the film surface.

Each of the physical modifications oxidizes the surface of the material to be treated. This occurs by adding reactive sites such as ions and radicals in excited states. Flame treatment, more commonly used on bottles and molded parts, oxidizes the surfaces of the bottles after they are moved passed a flame or superheated air (1000^0F) [133]. Corona discharge uses electromagnetic fields which ionize the air, bombarding the substrate with electrons and ions in order to oxidize the surface of the film being treated. Priming consists of adding a thin coating or primer that can adhere to both the substrate and the coating or secondary substrate. There are many types of primers of various chemistries to promote the adhesion of multiple types of substrates to one another [133]. The two surface treatments that were used in this coating development research were corona discharge and a polyethylenimine (PEI) primer.

2.9.3 Corona Discharge Treatment

Corona discharge treatment is one of the surface treatments that can achieve increased wetting tensions on film surfaces. As mentioned previously, corona discharge bombards a film surface with ionized air producing oxidized surfaces of films containing ions, radicals and excited molecules via chain scission. The air between two corona treatment electrodes conducts electricity and ionizes the air. Stray electrons impact other electrons in the air making them unstable by putting them into a "higher energy orbit creating an excited molecule" [152]. The excited molecules are unstable which then decompose into radicals and ions [152]. The term corona is used to distinguish the condition of the gas or air between electrodes [152]. Placing a film to be treated between the two electrodes produces a diffuse glow rather than an arc due to interruption of the conductive path. The soft blue glow is what is referred to as corona [152].

Multiple theories have been proposed to suggest the effects of corona discharge treatment on adhesion of polymer film surfaces: addition of polar groups through oxidation, electret formation (electric charge), and increase in surface roughness due to micro pitting, and elimination of weak boundary layers [126]. Oxidation at the film surface has been found to be the primary and most widely accepted effect of corona treatment [40]. Oxidation results in the introduction of polar groups onto the surface of a non-polar material. Some have classified this as production of a layer of low molecule weight oxidized material boundary layer (LMWOM) [146].

Others have described a second significant effect of corona discharge using more topographical methods. Corona can also increase the roughness of a film surface while simultaneously cleaning it by removing dust and debris. The surface morphology described when treating polyolefin such as polypropylene and polyethylene is pitting or "mechanical keying" [152]. Pitting also known as micropittng can increase adhesion and wettability by producing more surface area for intimate contact between substrates.

Corona discharge treatment is applied at varying power densities required to achieve the desired wetting tension. Power density uses the units of watt/(time*surface area). (i.e. watt/(min*ft^2)) Both overtreatment and under treatment can result in insufficient wetting tension after treatment. It has been found that two series of chemical reactions can occur during corona discharge treatments. The first reaction introduces polar groups such as carbonyls, carboxyls and hydroxyl groups through chain scission. If the length of treatment was to be extended or the power density of the treater was too high for the specific material, the carbonyls can convert to ethers, which are nonpolar. This second reaction occurs at a slower rate with increased treatment time and the

production of nonpolar groups can reduce adhesion and wettability [126]. There are additional effects of overtreatment which can result in undesirable wetting and even lack of sealability. Overtreatment can cause what is called fracturing in the surface of the films (reorganization of the polymer chains). This can result in the polar groups produced through corona treatment migrating into the bulk of the polymer making them unavailable at the surface. This can also occur with primers [43].

Overtreatment can also destroy the sealability of polyolefins. Corona discharge treatments can increase the molecule weight of polymers at the treatment surface via cross linking [40; 152]. According to a study conducted by Farley and Meka (1994), any amount of corona treatment has the potential to produce a change in the seal failure of LLDPE from a tear to peel. They found that the cross-linking of the polymer surface reduced chain mobility and reduced chain diffusion at the seal interface. It was also found that cross-linked polymers from corona treatment required higher temperatures to achieve the same seal strength as a non-treated film, if a seal was even achieved. Increasing the temperature or dwell time did not guarantee an achievable seal in cross-linked polymers [40].

If the proper corona discharge treatment were to be achieved on a film, there are additional factors that can cause the decay of the corona treatment over time. Many manufacturing processes include corona treatment in-line with lamination or printing processes to avoid such decay. However, this is not the case for all such manufacturing environments. Corona treatment stability can be affected by time, storage temperatures, relative humidity, migration of film additives, reorganization of polar groups, substrate type and treatment levels [40; 126; 146]. Over time, the electric charge formed on the

surface of the film can degrade. Polar groups can rearrange changing surface morphology, and film additives such as slip additives can migrate to the surface producing a weak boundary layer [126].

Storage conditions can greatly affect the lasting effects of corona treatment. A study conducted found that 1-7% of the corona treatment was lost after 9 days in storage and 23-28% was lost after 37 days. If the storage conditions were at higher temperatures or higher humidity, the corona treatment would have been degraded further [126]. Films that have been temperature abused can result in increased crystallinity. If this were the case, the penetration depth of the corona treatment would be decreased reducing the effect of treatment [146]. High relative humidity levels can also cause the need to increase treatment duration due to interference of hydroxyl molecules in the air [126].

Although corona discharge treatment has been found to be effective in increasing the wettability and adhesion of polymer surfaces, additional surface treatments may be required. As previously stated, wettability does not necessarily produce adhesion. Chemical compatibility is a major factor in two substrates or a substrate and liquid coating to be able to adhere to one another. Primers are a very common method of changing the surface chemistry of a substrate for the adhesion of incompatible substrates. Primers are very thin coatings between layers with typical laydowns of 0.04-0.4 gsm (grams per square meter) or 0.0016 to 0.016 pounds per ream [101].

2.9.4 Polyethylenimine (PEI) Primer

Polyethylenimine primer or PEI is a common primer used in the packaging industry for adhering highly polar and highly non-polar substrates together. PEI is an open chain or aliphatic amine that is also known as a cationic polyelectrolyte, which has

many charged groups. See Figure 2.12 below [47; 82]. PEI is typically diluted in a polar substance such as water prior to coating [27]. This produces additional charged groups on the molecule. Because PEI is a cationic polyelectrolyte, it is attracted to anionic and oxidized surfaces giving it the ability to adhere to both non-polar, corona treated and polar substrates containing ionic components such as sodium chloride [58].

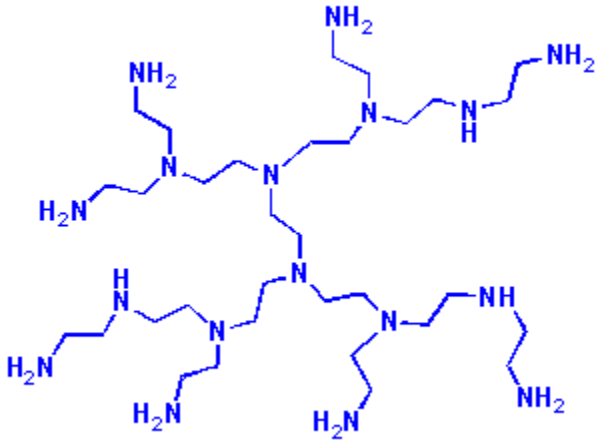

Figure 2.12. Chemical structure of polyethylenimine (PEI) primer. [106]

2.10 Diffusion

Diffusion is "the phenomenon of material transport by atomic motion" [22].

Diffusion can be described by two major categories: Steady state (Fick's First Law) and non-steady state diffusion (Fick's Second Law). Steady state diffusion is a linear diffusion with which the amount diffusing substance moves as a function of time. A longer diffusion time would result a higher quantity of the substance diffused. If the mass transfer or flux remains constant with time the system is undergoing steady state diffusion. Flux is described by the equation below:

$$J = \frac{M}{At} \qquad [3]$$

J = rate of mass transfer or flux (kg/m^2/sec)

M = mass of diffusing substance (kg)

A = cross sectional area of solid (m^2)

t = time (sec)

Fick's First Law (or steady state diffusion) occurs if the flux described above remains constant and is proportional to the concentration gradient. The negative sign in the equation below indicates the direction of diffusion from a high concentration to a low concentration along the concentration gradient [22].

$$J = -D\,\frac{dC}{dx} \qquad [4]$$

D = diffusion coefficient (m^2/sec)

J = mass flux (kg/m^2/sec)

C = mass per volume (kg/m^3)

x = displacement (m)

If the mass flux (J) does not remain constant with time, the system is exhibiting non-steady state diffusion or Fick's Second Law.

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2}\right) \qquad [5]$$

There are many assumptions for Fick's second law:

1. Uniform distribution of diffusing substance at C_0 before diffusion begins
2. Location (x) is zero at the surface and increases moving into the solid
3. Time is zero before diffusion begins

[22]

The diffusion of nisin in antimicrobial coating or film systems has been studied in attempts to produce consistently effective antimicrobial systems. The antimicrobial effectiveness of nisin has been found to be affected by several factors in food systems such as pH, fat content, large particle size of the peptide and non-uniform distribution of nisin in the food product [9; 77; 130]. On the other hand, similar issues have occurred in direct food coatings or antimicrobial packaging materials due to interaction with the food product decreasing efficacy leading to re-growth [46].

For the antimicrobial coating system produced, there are several important aspects regarding diffusion:

1) Diffusion of nisin through the coating material
2) Diffusion through water interface at the food product surface
3) Desorption or release of the antimicrobial onto the surface of the food product
4) Potential migration of nisin into the food product

Diffusion can be affected by many different variables such as temperature, composition of the medium through which the component is diffusing (solid, liquid, gas, crystalline structure of solid), penetrant shape, size, concentration and activation energy. Smaller diffusing molecules will be able to move more freely through a matrix and it has been found that molecules diffuse through amorphous regions of polymer matrices.

Buonocore et al (2004) conducted a study on the controlled release of antimicrobial compounds, including nisin, from a multilayer polyvinyl alcohol (PVOH) structure . The exterior layers of PVOH were cross linked at varying degrees using a cross linking agent, while the interior layer contained non-cross linked PVOH and the antimicrobial components. This study found, using high pressure liquid chromatography (HPLC), that the degree of cross linking affected the time for the system to reach equilibrium. Essentially increasing the cross linking agent resulted in a slow antimicrobial release [20].

Teerakarn et al (2002) found that the diffusion rate of nisin from protein films such as corn zein, increased with increasing temperature conditions [130]. Increasing temperatures leads to higher vibrational motion and low activation energy. This can be shown using the Arrhenius equation below:

$$D = D_0 \exp\left(-\frac{Q_d}{RT}\right) \qquad [6]$$

Q_d = activation energy for diffusion (J/mol) – the amount of energy to produce the diffusive motion of one mole of atoms. Large Q = low diffusion coefficient.

R = gas constant (8.31 J/mol –K)

T = absolute temperature (K)

D_0 = a temperature-independent preexponential (m^2/sec)

If the antimicrobial is unable to reach the food product from the packaging material, then the packaging is essentially useless. Some complications in antimicrobial packaging overall regarding diffusion (Table 2.2) include that the antimicrobial could be so compatible with the packaging material that it can either become trapped in the amorphous regions of the polymer matrix (if producing an extruded antimicrobial film) or diffuses into the material from the coating (if producing an antimicrobial coated film) rather than the food product [60]. This issue becomes more complicated when producing a multi-layer material in which the antimicrobial layer is in between other layers and must diffuse out to produce inhibitory effects on the food product [59].

Diffusion is one of the many challenges to be overcome in antimicrobial packaging which are to be discussed in the following section. According to Teerakarn et al (2002) [130], diffusion of antimicrobial agents applied to food product surfaces are limited due to diffusion into the food bulks [142] which can result in microbial growth and spoilage. Determining the diffusivity of antimicrobial substances is a complex process that needs to be conducted for each food product because of food product/antimicrobial interaction.

2.11 Challenges in Scaling Up Antimicrobial Coatings

There are multitudes of hurdles for scaling up antimicrobial coatings from laboratory concept to a large scale production process. Below in Table 2.2 lists some of these hurdles to be discussed in more detail within this section.

Table 2.2 Summary of challenges for up scaling antimicrobial coated films from small laboratory batch processes.

Summary of Challenges for Scaling Up Antimicrobial Coated Films	
Batch coating formulation	• Physical and chemical properties of coating solution not suited for large scale equipment (i.e. percent solids, pH) • Uncommon ingredients • High cost
Batch production process	• Coating production may not be feasible for large scale production
Batch coating process	• May require process not feasible for large scale production
Regulatory Difficulties	• Exceed legal limit • Toxic for human consumption at any or limited amount • Food contact notification • Food additive status may be required • Determining overall safety • Material not approved for specific use
Antimicrobial efficacy	• Long term storage • Interaction with food product • Large scale processes can deactivate antimicrobial
Diffusion	• Diffuse into food product • Diffuse into material • Encapsulation for slow release • Antimicrobial trapped in polymer matrix
Physical material properties	• Haze • Sealability • Interaction • Coating thickness
Consumer Acceptance	• Additives • Clean label • Antibiotic resistance
Cost	• Determining value-added for material • Ingredient cost • Capital investment cost if use equipment not commonly used in industry

Batch Formulation, Production and Film Coating Processes

The first major sets of hurdles are related to the antimicrobial coating product development process. A batch, for the purpose of this discussion, will be defined as a low volume coating or film coating process that is only suitable for benchtop laboratory work during the product development process. The formulation process can prove to be extremely difficult when attempting to produce a coating using food safe ingredients without adding excessive cost by implementing uncommon or rare ingredients. Many studies have been conducted using newly formulated antimicrobial coating formulas. However, because the coatings were not intended to be scaled up, the physical properties of the coatings were not considered for large scale processes during formulation. Printing or coating processes have specific parameters which coatings or inks need to meet in order to be used on the equipment. Gravure and flexographic processes require a coating to have at least 25-50% solids in order to enable the coating to be transferred to the substrate during the coating process. Viscosity can also have an effect on the coating transfer as well. Too low of a viscosity will result in low to no coat transfer and too high of a viscosity will result in high coat weights and potential for drying issues. The pH of the coating can also have an effect on the coating equipment itself if the coating has acidic or corrosive properties. Some measures can be taken to protect coating equipment from such coatings but at an additional cost.

If the production process of the coating solution is not something that is feasible on a large scale basis, then this can be difficult to later implement in the food packaging industry. This same criterion can be implemented regarding the batch film coating process. Many studies have been conducted on formulated antimicrobial coatings. In

many studies, a thin layer chromatography plate coater (TLC) has been used as the batch film coating process. This piece of equipment contains a "bucket" set at a specific height that draws coating across a single sheet of substrate at a constant rate. Studies have used this piece of equipment to coat directly onto a glass or Teflon coated plate to produce a film or a piece of film that has been secured to the glass plate to use as a strength layer for a coating [46; 100]. This particular method has the ability to control the coating lay down and thickness but not without variability by controlling the coating speed and the height of the gate from the substrate being coated onto. Papers using this method of coating or film formation also required drying for 24 to 48 hours at ambient temperatures. A drying method like this for a batch process would suffice for product development purposes but not for large scale production.

Additional examples of other film coating production methods that have been used as acceptable product development processes include:

- Pouring a specified amount of solution into a Petri dish or other container such as a polystyrene weigh boat
- Pouring solution onto a glass plate or film
- Spin coating
- Heat pressing mixtures with a carver press

Achieving a uniform thickness with these methods is difficult and requires a similar drying period like the TLC method. In order to control for thickness, Cha et al, cast the coating onto a polyethylene film that was placed on a hot plate at 70^{o}C [25; 80; 81; 97; 102]. Spin coating consists of dispensing a solution onto a substrate, then

rotating the substrate at a specified rate until the coating is evenly distributed onto the substrate [57]. Each of these methods is suitable and accepted for product development purposes. This discussion was merely to increase awareness that there is not only variability between methods but that batch process methods cannot necessarily be translated to large scale processes. Therefore batch process methods cannot be expected to produce the same resulting material or antimicrobial efficacy when compared material produced using large scale processes.

Regulatory Difficulties

There are numerous regulatory hurdles regarding antimicrobial coated film packaging. Migration of substances from packaging to the food product is one of the main concerns [31]. The ingredients of the antimicrobial coating discussed earlier in this literature review were investigated to dissolve onto the surface of the food product, releasing the antimicrobial agents for surface contamination and shelf-life extension. For this type of material, a Food Contact Notification would be needed in the United States, but also food additive petition regulations due to the potential to diffuse into the food product. Some ingredients such as Nisin have a pre-approved GRAS certification that also comes with the stipulation of a legal limit. For Nisin the legal limit is 10,000 IU/g. For a 2.5% concentration of Nisin product such as Nisaplin®, this means that there can be no more than 0.01 grams of Nisaplin® per gram of food product.

If a particular ingredient does not have a specified legal limit, measures need to be taken to determine that the coating ingredients are not toxic for human consumption and that all ingredients are approved for the intended use as a component of the antimicrobial

packaging for food products. Determining the overall safety not only includes predicting direct human consumption but also determining that there are no unacceptable changes to the food product composition due to the coating ingredients. For example, no detrimental deteriorative by-products or organoleptic components are produced from food/coating ingredient interactions [31].

Antimicrobial Efficacy

Maintaining antimicrobial efficacy throughout the production process and intended use of the coated packaging film must be achieved in order to have a viable antimicrobial packaging film. Extrusions, laminating and drying processes can all have harsh effects on antimicrobial agents depending on the tolerances of the agents being used. Lysozyme, for example, is inactivated at 80^{0}C to 90^{0}C depending on pH. This is below most processing temperatures in extrusion operations [91]. Not only can the prolonged heat exposure of such processes degrade the antimicrobial agent, the mechanical shear can also deactivate antimicrobials [7; 60; 127; 143].

If the antimicrobial is able to survive the production process, the material needs to be stable during storage prior to or after the film has been filled with a food product. One study showed that films containing the components of basil (linalool and methylchavicol) did not lose inhibitory effects after 1 year of storage at ambient temperatures [128]. Additional studies are needed to determine whether storage over time has an effect on the inhibitory effects of each type of antimicrobial agent contained in films or coated films.

Addition of the food product can complicate the antimicrobial efficacy over the shelf-life of the food product. Many researchers have found that antimicrobial coated

films produce different results when exposed to inoculated food products as opposed to inoculated bacterial media (i.e. TSA (tryptic soy agar), BHI (brain heart infusion broth)) [37]. Foods are complex systems containing organics that can interfere with antimicrobial effectiveness.

Physical Material Properties

The inability to seal a package in general can render a package useless leaving a product susceptible to the hazards of the outside environment. (I.e. oxidation, moisture, pests) This is also the case for antimicrobial films due to their composition. Many edible coating materials are produced from polysaccharides, proteins, lipids and cellulosics which are often non-sealable. For example, methylcellulose/hydroxypropylmethyl cellulose (MC/HPMC) films previously produced by Franklin et al (2004) were unable to be sealed [46]. This may be due to the materials naturally high crystalline structure, or its melt temperature, which is above standard heat sealing conditions. Sealability can also be affected by coating thickness. If a coating requires excessive thickness to remain effective, it will be difficult to seal through.

Many antimicrobial films require direct contact with a food product in order to release the inhibitory agents onto the surface of the food product. Because of this orientation in a package, the antimicrobial layer is also likely to be the sealant layer of the package. Possible solutions to achieve a seal through an antimicrobial film layer would be to either utilize a thermoplastic matrix to contain the antimicrobial or to pattern coat the antimicrobial coating onto a sealant web. Patterned coatings could be achieved through printing processes such as flexography or rotogravure. The coating can be

indexed on the substrate to avoid the seal areas of the web or the pattern can be such that contact areas of the coated sealant are exposed to ensure sealing. Difficulty in this case can arise due to the accuracy of the press. The registration of the coating will need to be accurate as to enable sealant to sealant contact without interference of the non-heat sealable coating. For example, patterns to include can be checkers, circles or stripes, however, channel leaks using stripes are a possibility. If a heat seal is achieved, the seal must also be strong enough to withstand the distribution chain. If the product being packaged is a vacuum packaged RTE product, not only must the packaging material maintain a seal, it must also withstand vacuum conditions.

Additional pertinent material properties that can be affected by the addition of antimicrobial coatings include haze and degradation by interaction with the substrate structure. Haze or clarity of the film can be off putting to consumers who desire to be able to see the food product clearly. Some antimicrobial coatings can appear less translucent than an un-coated substrate due to the crystalline nature of components in the antimicrobial coating (such as salts or cellulose). It is also possible that material quality can suffer if specific components of the antimicrobial (such as plasticizers) were to migrate into the base substrate, causing delamination or deterioration.

Consumer Acceptance

As previously mentioned, haze or lack of clarity in a package can be off-putting to consumers because it hinders a clear view of the product. Consumers have additional concerns beyond aesthetics, such as usage of additives and preservatives in their food products, “clean labels” and concerns about antibiotic resistance. Consumers were found

to have a general concern about the safety of food additives and in a study conducted in Australia, such additives were perceived as a common potential danger [122]. A European study found that consumers did not accept packages that released preservative additives in meat products [3] regardless of the potential benefits. Consumers have been found to exhibit a general fear of the unknown and lack of awareness when asked about antimicrobial packaging technologies such as nanotechnologies, however, consumers were found to prefer active compounds in films rather than sachets [3]. Overall, consumers have the perception that food products with a shorter shelf-life are fresher, therefore active packaging for shelf-life extension interferes with the freshness of the product [31]. Additionally, consumers show a lack of trust in the government and regulatory systems. They have become skeptical about food labelling, particularly relating to food quality, but do trust nutritional labelling that requires scientific testing and evidence [3; 41]. Consumer perception can prove to be one of the most difficult hurdles for scaling up and producing antimicrobial coated films because consumers' lack of insight to the potential benefits of active packaging. Regardless of consumer skepticism and perception, a study conducted by the Flexible Packaging Association found that shelf-life extension is the number 4 concern for consumers regarding food packaging.

Cost

Cost is the last major category of the many hurdles to scaling up and potentially commercializing antimicrobial films. Value added technologies such as antimicrobial packaging technologies should not exceed more than 10% of the package cost [31]. Others have distinguished this cost as no more than 1-2 cents per package. Some

technologies are not yet feasible to be implemented into the packaging industry, although technical progress in the packaging industry has the potential to make these technologies more reasonable in cost [31]. The cost of raw materials and capital investments should be kept to a minimum by implementing common or readily renewable film and coating ingredients into production processes already in place at a manufacturing facility. A study conducted by the Flexible Packaging Association found that consumers are willing to pay for these technologies. It was found that consumers who earn less than $50 k per year would be two times more for a product with an extended shelf life. There is potential for growth within the packaging market for active packaging technologies such as antimicrobial food packaging. The demand for fresh, convenient food products with an extended shelf life is a driving factor, but the technology has overcome many difficulties and hurdles in order to become more common place in the food market.

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CHAPTER THREE

FORMULATION OF AN ANTIMICROBIAL COATING CONTAINING NISAPLIN® INTENDED FOR LARGE SCALE PRODUCTION AND INHIBITION OF SPOILAGE MICROORGANISMS

ABSTRACT

Antimicrobial food packaging could reduce food waste by extending shelf-life in addition to enhancing food safety. Utilization of the antimicrobial peptide Nisaplin®, which is an FDA GRAS approved additive, has the potential to be used in commercial antimicrobial food packaging applications, particularly, ready-to-eat meat products. The objective of this study was to produce a Nisaplin® containing coating formulated for large scale production equipment while maintaining antimicrobial efficacy. Differential scanning calorimetry (DSC) testing was conducted in order to determine a grade of polyvinyl alcohol (PVOH) and compatible plasticizer. Compatible plasticizers were determined based upon the plasticizers' ability to lower the T_m (melt temperature) of the PVOH. Percent solids (%) of liquid coatings and pH testing in additional to general observations were conducted. Dynamic contact angle tests and tape tests were conducted in order to determine whether a secondary base substrate would better suit the formulated coating for increased wettability and adhesion. Film on lawn testing was conducted on dry coated films against *Micrococcus luteus, Listeria innocua* and *Listeria monocytogenes*. Control films did not contain Nisaplin. DSC testing revealed that glycerin lowered the melt temperature of partially hydrolyzed PVOH from 189.7°C

(373.4°F) to 150.9°C (303.7°F), making the coating more suitable for sealing and less brittle. The pH of the antimicrobial coating solution was found to be 5.9. The average percent solids was 20.53 (%). Coated films also achieved inhibition against *M. luteus, L.innocua* and *L. monocytogenes*. Based on the characteristics of the coating and efficacy, it is possible to formulate a commercial grade antimicrobial product containing Nisaplin® that could extend the shelf-life of RTE food products.

INTRODUCTION

In 2012, 14.5% (36.4 million tons) of total municipal solid wastes generated in the United States of America was food waste. [8] Food spoilage is one of the major causes of food waste. Active packaging is a growing research area that can reduce food waste and the demand for active packaging is increasing. According to Food Production Daily [30], the active packaging sector is expected to grow to 3.5 billion dollars by 2017 in the United States and 17.3 billion dollars worldwide. According to the USDA ERS (United States Department of Agriculture Economic Research Service), the cost of food waste totaled approximately $161.6 billion in 2010. [5] Not only could active packaging decrease food waste, but it also has the potential to decrease foodborne illness outbreaks, death and an estimated economic loss of approximately 15.6 billion dollars per year. This estimate was based upon 15 major pathogens included in a study conducted by the USDA. [33] This study showed total cost breakdowns including medical expenses and quality adjusted life expenses based upon any aftermath caused by pathogenic organisms. For example, *Listeria monocytogenes,* a contaminant associated with ready-to-eat foods

exhibited a cost totaling nearly $3 billion out of $15.6 billion for all 15 pathogens in the study.

Ready-to-eat (RTE) food products are in high demand due to the convenience and a "fresh" product appeal. [4] They are food products that require little or no cooking/preparation prior to consumption such as deli meats, cheeses and frankfurters. [14] RTE products are cooked and handled (i.e. cutting, dicing, packaging) after the cooking process which can lead to post process contamination. Because of this, these products are susceptible to pathogenic environment contaminants such as *Listeria monocytogenes* in addition to natural microorganisms that cause spoilage. In order to slow the growth of spoilage microorganisms, products such as preservatives, new packaging methods and additions of antimicrobials have been implemented.

Nisaplin® is a natural antimicrobial peptide that has been utilized in previous antimicrobial coating work for RTE food products. It has been shown to be effective, however, has not been produced in a commercial grade active packaging application. Work previously conducted by predecessors consisted of producing a coating solution with a 70/30 (w/w) base mixture of methylcellulose and hydroxypropyl methylcellulose (MC/HPMC). [Franklin et al 2004; Grower] Several hurdles were discovered when attempting to scale up to a large scale coating application method using the cellulose based formulation. The coated film was unable to be heat sealed due to the highly crystalline structure of the cellulose components. The liquid solution did not contain a high enough percent solids (~9.5%) to meet the properties needed for gravure or flexography coating application methods (15-50%). Lastly, the film was also exhibited a

high degree of haze, which increased over time, potentially due to the precipitation of salts from the Nisaplin® product. Because of these characteristics of the cellulose based formulation, several objectives were determined for a new formulation. The new formulation also needed to exhibit a low enough melt temperature in order to promote sealability and produce a sealable package. It also needed to be translucent or exhibit low to no haze for aesthetics in addition to containing the proper percent solids for implementation onto large scale gravure and flexography coating application processes. The overall objective, however, was to formulate an antimicrobial coating intended for large scale production methods and reduction of a spoilage indicator microorganism.

MATERIALS AND METHODS

Differential Scanning Calorimetry

Carrier Resin Selection

Differential scanning calorimetry (DSC) testing was conducted to characterize the coating base and plasticizers for formulation purposes. DSC can determine the melt temperature of a polymer which is important for determining the sealability of a produced package material. Polyvinyl alcohol (PVOH) resin (10 grams) was heated to 120°C and simultaneously stirred on a stir plate in 30 mL of distilled water for approximately 30-45 minutes until the resin went into solution. PVOH was chosen based upon water solubility qualities for the intention of releasing an antimicrobial compound when in contact with a moist food product. Three different PVOH resins were tested: Mowiol 4-98, Mowiol 4-88 and Mowiol 4-88 GS2 (Kuraray America, Inc., Houston TX, USA) 4-98 was a fully

hydrolyzed (98%) granular resin, 4-88 was a partially hydrolyzed (88%) granular resin and 4-88 GS2 was a partially hydrolyzed (88%) powdered resin. In cases where a plasticizer was utilized, it was added once the resin had gone into solution and had begun to cool. Three plasticizers were tested: Polyethylene glycol 400 (PEG 400), glycerol (Glycerol USP Grade, Thermo Fischer Scientific, Waltham, MA, USA) and glycerin. (Vegetable glycerin, USP Grade, Nature's Oil, Streetsboro, OH, USA) PEG 400 was tested first due to availability. Further literature search showed that both glycerin and glycerol had varying abilities to plasticize PVOH resins based on the degree of hydrolysis. Resin solutions were cooled prior to casting onto a coextruded forming web suitable for thermoforming and vacuum packaging applications donated by Sealed Air Corporation which contained a linear low density polyethylene (LLDPE) sealant web. A size 28 Mayer rod (or wire wound coating rod) was used to achieve an even laydown of the resin solution. Coated films were dried at ambient conditions overnight. LLDPE films were not treated to promote coating adhesion for the intended purpose of removing the coating for DSC testing.

Dried film samples were then prepared for DSC by cutting films with a standard hole punch. Sample weights of 7.1 – 8.9 mg of coating peeled from the substrate were weighed on an analytical balance placed into an aluminum pan and sealed prior to testing. (OHAUS Explorer Analytical Balance, Model #E00640, OHAUS Corporation, Switzerland; Standard Aluminum DSC pans and lids, # T140103 and T131220, TA Instruments, New Castle, DE, USA) A single heating (0°C to 220°C with ramp rate 20°C minute) and cooling cycle program (220°C to 0°C with ramp rate 20°C minute) was run

for each sample (DSC 2920 modulated DSC with a refrigerated cooling system, TA Instruments, New Castle DE, USA). Melt temperature (T_m) of each sample was analyzed along with any anomalies using Thermal Advantage analysis software. (Advantage™ Analysis Software, TA Instruments, New Castle DE, USA)

Figure 3.1. DSC 2920 modulated DSC used for determining polyvinyl alcohol resin grade and plasticizer combination.

Coating Preparation

The coating solution was prepared by heating and simultaneously stirring 10 grams of 4-88 Mowiol PVOH resin in 30 mL of distilled water to 120°C for approximately 30-45 minutes until the resin dissolved into solution. Once the resin had dissolved, 3.2 mL of glycerin (40 parts per 100 grams of PVOH resin) and 185 µL of Tween® 80 (0.25% v/v) (Polysorbate 80, FCC, Spectrum Chemical Manufacturing Group, New Brunswick, NJ, USA) were then added to the cooling resin solution. In a

separate beaker, 1 gram of Nisaplin ® (2.5% - 12,500 IU/mL in solution) (Danisco, Inc. Madison, Wisconsin, USA) was dissolved in 2 mL of 0.02 M acetic acid solution. (Franklin et al 2004) (Glacial acetic acid, Fischer Scientific, Waltham, MA, USA) 30 mL of 95% ethanol was then added, covered and stirred while adding both 0.3 g (0.4% w/v) ascorbic acid (ascorbic acid USP, Avantor Performance Materials, Inc. Center Valley, PA, USA) and 0.22 g (0.3% w/v) potassium sorbate. (Granular potassium sorbate, Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA) Both the resin solution and the ethanol solution were combined upon dissolving all components and cooling the resin solution.

Selected Properties (pH, percent solids and viscosity)

General observations and basic characteristics were recorded during testing and formulation of the coating produced in the previous section. Visual observations of drawdowns (coated with #28 Mayer rod) with the coating such as haze, coloration, evidence of precipitation of solids, delamination or adhesion difficulties were recorded.

pH of the coating solution was tested utilizing a Thermo Fisher-Orion Star A211 pH meter. (Thermo Fisher Scientific, Inc. Waltham, MA, USA). Percent solids of at least 3 batches of antimicrobial coating were tested in triplicate. Approximately 1 gram of liquid coating was weighed into previously dried and weighed aluminum pans. The pans were placed in a 65°C drying oven for 5-7 days. (Lindberg/Blue M Gravity Oven, Model GO1330A, Industrial Laboratory Heaters, Asheville, NC, USA) The pans were re-weighed on an analytical balance and percent solids were calculated.

Viscosity was tested using a Zahn #3 cup. Zahn cups are commonly used in the coating and printing industries as a fast, efficient means to monitor viscosity during a coating or printing process [ASTM D4212-16] The Zahn cup was filled with coating until the cup was overflowing (for a large-scale batch of coating, the cup would be submerged in the liquid to be tested). The cup efflux method involves measuring the time it takes to empty the cup through the hole in the bottom. Higher viscosities take longer to evacuate.

Dynamic Contact Angle, Surface Tension of Liquid Coatings & Critical Surface Tension of Films

Contact angle is a means of quantifying adhesion of a liquid solution to a solid substrate. Dynamic contact angle testing was conducted at a Sealed Air Corporation facility in Duncan, South Carolina in the surface analysis and microscopy laboratory. This set of studies was conducted for several reasons: 1) to determine the wettability of the formulated antimicrobial coating 2) to determine whether the volume of surfactant (Tween 80®) had an effect on adhesion and wettability 3) in an attempt to find a substrate that can eliminate excess surface treatments such as a primer currently utilized on the control film and 4) to determine the overall surface tension of the coatings and critical surface tensions of the film samples.

Dynamic contact angle testing, liquid coating surface tension determination and the critical surface tensions of all substrates tested was conducted using a Dynamic Contact Angle Analyzer (Model DCA-315, Thermo Cahn Instruments, Madison, WI, USA). Prior to each set of testing, the motor was calibrated using the equipment software (Win DCA

32). The balance was also calibrated using the sample holder apparatus followed by a 500 mg calibration weight. All samples were tested in triplicate.

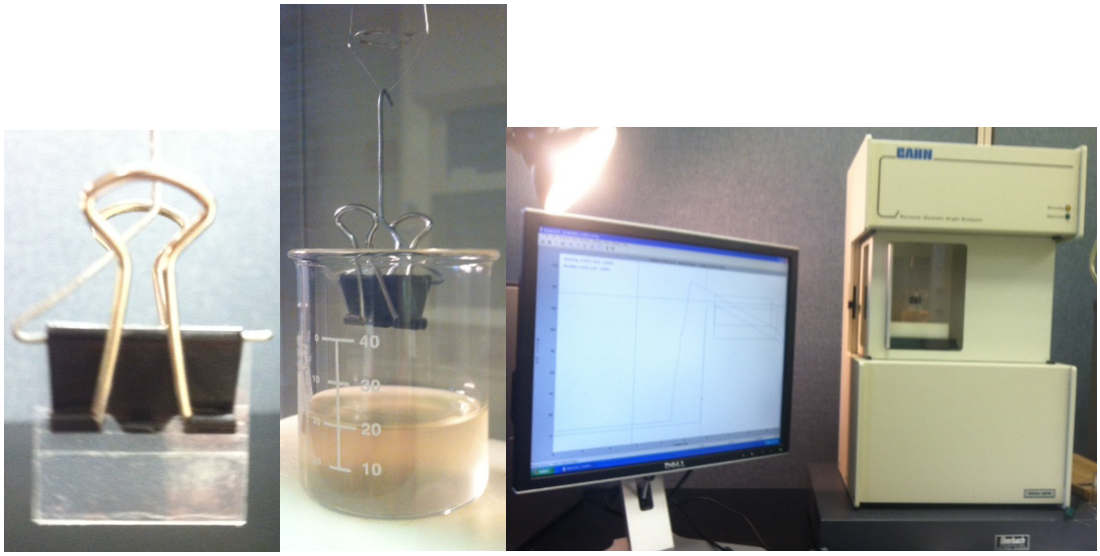

Figure 3.2. Dynamic contact angle (DCA) sample (left); DCA sample set up in apparatus to be tested against coating containing Nisaplin® (center); Model DCA-315 analyzer from Cahn and analysis software (right).

Film Sample Preparation

A common method of surface treatment for flexible packaging is corona discharge treatment. This treatment is needed because most common substrates such as PE and PP are non-polar while coatings and inks tend to be polar. Corona treatment raises the surface energy of a film substrate by cleaning the film surface of debris and dust while simultaneously oxidizing the surface of the film with bombardment of electrons [31]. Film samples for contact angle testing consisted of control LLDPE films treated

with corona discharge handheld treater depicted in Figure 3.3 (Model BD-20 from Electro-Technic Products, Inc., Chicago, IL, USA) followed by and a water soluble primer provided by MICA Corporation. (Houston, TX, USA) LLDPE films were considered the control substrate for this set of testing. Water soluble primer was diluted 1 part primer to 9 parts water and cast onto the LLDPE film with a #3 Mayer rod also depicted in Figure 3.3. The primer was dried at ambient conditions for approximately 4-6 hours.

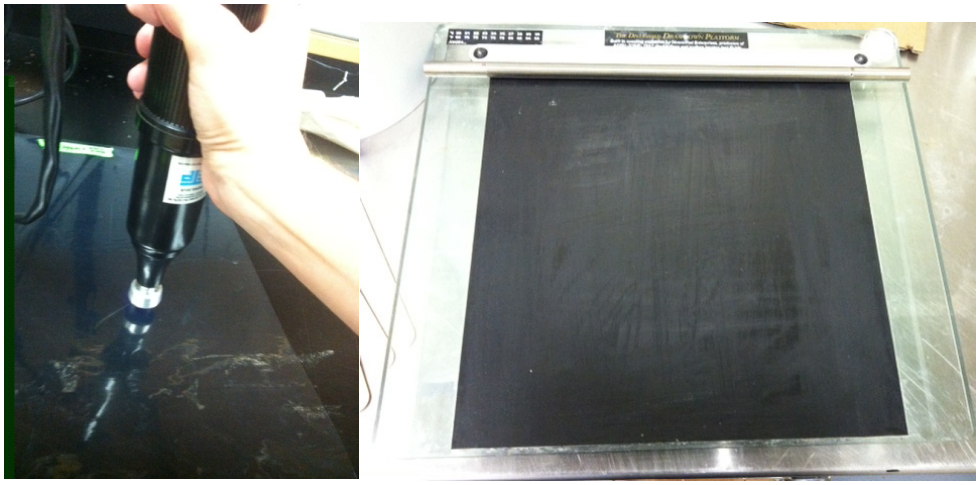

Figure 3.3. Corona discharge handheld treater used for treatment of films (left); drawdown apparatus with a coating rod (right).

Additional substrates (See Table 3.1) were also tested with and without corona treatment totaling 9 substrate types. These additional substrates were tested against the control in order to determine if one of the substrates had contact angle and adhesion properties not significantly different from the control. This would have indicated that there are materials with less surface treatments that have the potential to wet out the

formulated coated. It would be possible to ultimately eliminate additional surface treatments and therefore processing steps in a large scale production setting.

Overall the following films were tested:

- LLDPE - Corona treated and primed – Control
- Bynel® 2002, Elvax® 3165, Nucrel® 1202 HC and Surlyn® 1605 – Corona treated only
- Bynel® 2002, Elvax® 3165, Nucrel® 1202 HC and Surlyn® 1605 - Untreated

Table 3.1. Substrates utilized for dynamic contact angle, surface tension of liquids and critical surface tension of solids testing.

Film Substrate	**Film Description**	**Corona Treatment (Y/N)**	**Primer (Y/N)**
Control (LLDPE)	Linear low density polyethylene	Y	Y
Bynel® 2002	Acid modified ethylene acrylate	N	N
		Y	N
Elvax® 3165	Ethylene vinyl acetate copolymer	N	N
		Y	N
Nucrel® 1202 HC	Ethylene acrylic acid and methacrylic acid copolymer	N	N
		Y	N
Surlyn® 1605	Sodium Ionomer	N	N
		Y	N

Coating Preparations

The coating was prepared in the same manner stated previously, stored in a 50 mL centrifuge tube and wrapped with Parafilm® to prevent solvent evaporation. Coating solutions were also tested with and without the Tween ® 80 (185µL) component while troubleshooting adhesion difficulties.

Dynamic Contact Angle

Sample Preparation for Dynamic Contact Angle and Critical Surface Tension

Double sided tape was mounted onto microscope slide covers. Tweezers were used to remove one side of the tape backing and the adhesive side was pressed onto the desired film sample. A razor blade was used to cut the film from the sheet. After the other tape backing was removed, the film was folded onto the other side attaching it to the microscope slide cover. The razor blade was then used to cut the excess film and tape extending from the outer edge of the microscope slide cover. Prepared samples were then placed in a sample holder apparatus prior to testing. Care was taken to ensure the sample would not enter testing solutions at an angle to avoid skewing results.

Dynamic Contact Angle Testing Procedure

The equipment utilized for this testing was a Dynamic Contact Angle Analyzer. (Model DCA-315, Thermo Cahn Instruments, Madison, WI, USA) Prior to testing, the motor was calibrated using the equipment software (WinDCA32). The balance was then calibrated using the sample holder apparatus and a 500 mg calibration weight. Film

samples were then tested in triplicate. The DCA motor speed was set to run at 40 microns/sec, gravity for the specific location was designated as 979.651 cm/sec^{-1} and the samples were set to dip approximately 4mm into the coating solutions (20mL).The average surface tensions of the coating were input into the experimental settings to ensure accurate readings. Contact angles, cosine of measured contact angles and R^2 values for obtained graphs were recorded.

Surface Tension of Liquid Coatings

Coating Preparations

The coating was prepared in the same manner stated previously, stored in a 50 mL centrifuge tube and wrapped with Parafilm® to prevent solvent evaporation. Coating solutions were also tested with and without the Tween ® 80 (185µL) component while troubleshooting adhesion difficulties.

Sample Preparation

A microscope slide cover was flamed with a handheld torch in order to remove any dust and debris from the surface of the glass. The sample was then placed in a holder with tweezers to ensure that no oils from finger tips would contaminate the surface. Care was taken to make sure the glass slide would enter the testing liquid evenly for accurate readings.

The coating samples (20mL) to be tested were poured into a 50mL beaker prior to testing. A new volume of coating was tested with each measurement and measurements were recorded in triplicate.

Testing Procedure

The tensiometric (also known as Wilhemy Plate) method was utilized. The same equipment was used for this testing method in addition to dynamic contact angle and critical surface tension methods. The motor was set to advance 4 mm into the coating solutions at a rate of 80 μm/sec. The surface tensions of the liquids were recorded once the apparatus had returned to the zero position.

Critical Surface Tension of Films

The critical surface tension of the films was determined by testing the films against two reference liquids with known surface tensions (water and diiodomethane). The critical surface tensions were then calculated based upon the contact angles produced by the reference liquids. This procedure differs from the surface tension procedure because the films were not tested in any coating solutions.

Sample Preparation

The same sample preparations were conducted.

Testing Procedure

The testing procedure to determine the critical surface tension of the substrates in table 3.1 follows the same procedure as the dynamic contact angle testing. However, instead of using the produced PVOH coatings, two liquids with known surface tensions were used as references in order to then calculate the critical surface tensions of the films. Deionized water and diiodomethane were used as the standard tested solutions with surface tensions of 72.6 and 50.8 dynes/cm. Calculated critical surface tensions were recorded based upon the Geometric Mean model.

Tape Test

A tape test is a common practice in the packaging, printing and coating industries to determine to what degree a coating or ink is adhered to a particular substrate. The tape test was conducted according to ASTM F2252 [1]. (Standard Practice for Evaluating Ink or Coating Adhesion to Flexible Packaging Materials Using Tape) Strips of 3M #610 tape (3M, St. Paul, Minnesota, USA) (10 – 2 inch pieces) were placed on dried drawdowns consisting of one of three coated substrates: LLDPE (primed and corona treated), Surlyn® 1605 (corona treated) and Elvax® 3165 (corona treated). Due to the qualitative nature of this type of test, an arbitrary scale was produced in order to provide a ranking system for determining coating adhesion.

Table 3.2. Scale developed for ranking adhesion of antimicrobial coating to LLDPE; Elvax® 3165 and Surlyn® 1605 substrates.

Scale For Adhesion				
1	**2**	**3**	**4**	**5**
100-75% Removed	50-75% removed	25-50% removed	Up to 25% removed	No removal or minimal spots
No adhesion	Minimal	Marginal	Moderate	Excellent

Statistical Methodology

All samples were tested with at least three replicates. Microsoft excel 2010 was used to conduct basic statistical analyses. (Average, standard deviation, coefficient of variation and unpaired t-tests to compare the two formulations) A P value of ≤ 0.05 was considered for statistical significance.

Due to the complexity of analyzing interactions between coating, film and surface treatment types, SAS® Studio (SAS® OnDemand for Academics) was used for factorial analysis of dynamic contact angle and critical surface tension data. Dynamic contact angle factors tested included coating type (Tween® containing formula or No Tween®), film type and corona treatment. The control sample was corona treated and primed LLDPE. (n=54) The factors tested when determining the critical surface tension of the films were film type and corona treatment. (n=27) A P value of ≤ 0.05 was considered for statistical significance.

RESULTS

Differential Scanning Calorimetry (DSC)

DSC testing of a fully hydrolyzed PVOH resin and PEG 400 showed the inability of PEG 400 to increase the intermolecular volume thereby showing minimal effect on the melt temperature or ductility. The results indicated that 98% hydrolyzed resin had an initial melt temperature of 220.0°C which then decreased to 214.6°C with the addition of 40 phr (parts per hundred). Parts per hundred units indicate the mass of plasticizer per one hundred grams of resin.

Two partially hydrolyzed resins were tested with two other plasticizing agents, glycerin and glycerol. The granular PVOH resin had an initial melt temperature of 189.7°C. Both glycerin and glycerol at concentrations of 40 phr decreased the melt temperature of the resin to 158.3°C (glycerol) and 150.9°C (glycerin). The powdered resin of the same grade also exhibited lower melt temperatures after the addition of the chosen plasticizing agents. The initial melt temperature decreased from 193.7°C to 155.1°C (glycerol) and 148.6°C (glycerin). The summary table of these results can be viewed in Table 3.3.

Table 3.3. Melt temperatures of three Polyvinyl alcohol resins (Mowiol 4-98; Mowiol 4-88 and Mowiol 4-88 GS2) with and without one of three plasticizers.

DSC of PVOH resins and various plasticizers				
Resin	**Degree Hydrolyzed (%)**	**Melt Temp (°C) at Concentration (0 phr)**	**Plasticizer**	**Melt Temp (°C) at Concentration (40 phr)**
Mowiol 4-98 (granule)	98	220.0	PEG 400	214.6
Mowiol 4-88 (granule)	88	189.7	Glycerol	158.3
			Glycerin	150.9
Mowiol 4-88 GS2 powder)	88	193.7	Glycerol	155.1
			Glycerin	148.6

Selected Properties (pH, percent solids and viscosity)

The average percent solids of the coating formula containing Nisaplin® was found to be 20.53%. A total of 27 samples were tested. The pH of the antimicrobial coating was found to be 5.853 so the solution was slightly acidic. The viscosity of the coating was approximated to be 175-200 centipoise (cP) using a conversion chart (FTA 1999) from the Zahn cup measurement of 24.47 seconds.

Dynamic Contact Angle, Surface Tension of Liquid Coatings & Critical Surface Tension of Films

Surface tensions of coating formulations (Control; No Tween®) were determined to be on average 31.7 dynes/cm (control) and 31.6 dynes/cm (no Tween®). A summary of dynamic contact angle and critical surface tension measurements can be found in table 3.4.

- All substrates were found to have statistically significantly different critical surface tensions from the control LLDPE substrate. ($\alpha = 0.05$) The average critical surface tension for LLDPE was found to be 44.2 dynes/cm.
- All substrates except for corona treated Elvax® and Surlyn® were found to have statistically significantly different dynamic contact angle measurements from the control LLDPE substrate. ($\alpha = 0.05$) (*P* value = 0.1231, Elvax® – corona; *P* value = 0.5648, Surlyn® - corona) The average dynamic contact angles for LLDPE, Elvax® and Surlyn® were 21.0°, 26.7° and 22.4°.
- Interactive relationships were also analyzed. It was also determined that the control coating yielded significantly different contact angles produced on the same substrate compared to those tested with the coating containing no Tween®. Addition of Tween® yielded lower contact angles than the formula without Tween®.
- Coating type, substrate and corona treatment interactions also had a significant effect on the obtained dynamic contact angle measurements. (*P* value < 0.0001) Corona treatment and substrate interaction also had significant effects on the critical surface tension data obtained. (*p* value < 0.0001)

Table 3.4. Summary Table of dynamic contact angle and critical surface tension results.

Substrate	Film Description	Corona (Y/N)	Average Contact Angle (°)	Average Critical Surface Tension (Dynes/cm)
LLDPE (Control)	Linear low density polyethylene	Y	21.0**	44.2
Bynel® 2002	Acid modified ethylene acrylate	N	57.1	25.9
		Y	37.6	29.0
Elvax® 3165	Ethylene vinyl acetate copolymer	N	41.7	39.1
		Y	24.7**	37.4
Nucrel® 1202 HC	Ethylene acrylic acid and methacrylic acid copolymer	N	47.2	31.1
		Y	30.0	37.4
Surlyn® 1605	Sodium Ionomer	N	40.0	32.1
		Y	22.4**	37.5

** indicates no significant difference ($\alpha = 0.05$)

Tape Test

Tape test samples were ranked on a scale 1-5. The highest degree of adhesion was designated by the number 5, while 1 represented no adhesion. A frequency chart representing results for a total of 30 samples can be viewed in Figure 3.4.

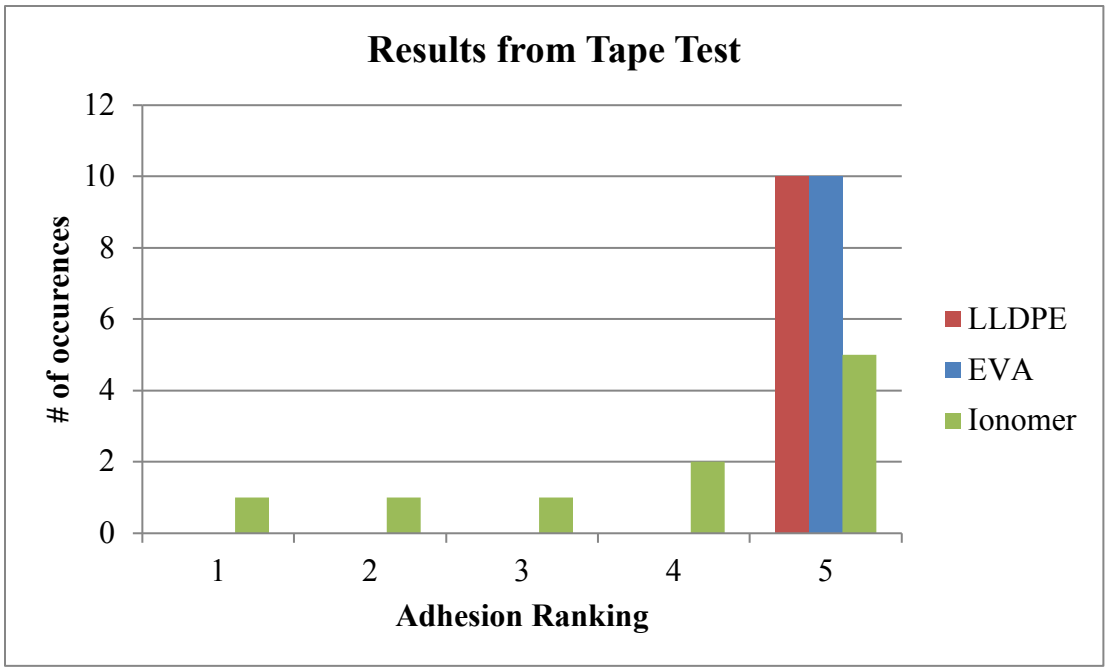

Figure 3.4. Frequency chart indicating coating adhesion rankings results for tape test. (ASTM F2252)

DISCUSSION

Differential Scanning Calorimetry

Drawdowns of water and PVOH resin yielded brittle film formation with little to no adhesion to LLDPE substrate. Differential scanning calorimetry analysis showed that the partially hydrolyzed resin had a lower melt temperature than that of the fully hydrolyzed resin. Partially hydrolyzed PVOH has a higher percentage of acetate side groups on the ethylene backbone of the PVOH resin. Because these side groups are larger in size, there is more interstitial space between polymer chains. The chains are unable to pack together as tightly with acetate groups compared to hydroxyl groups resulting in a less crystalline polymer. The structure of fully and partially hydrolyzed resins has an

overall effect on physical properties. For example, fully hydrolyzed resins demonstrate higher melt temperatures, higher crystallinity, low adhesion to hydrophobic surfaces, lower water solubility and also result in increased tensile strength and oxygen barrier. [16; 27]

DSC testing indicated that the fully hydrolyzed granular PVOH resin had a higher initial melt temperature, 220.0°C compared to the partially hydrolyzed granular and powder resins (189.7°C; 193.7°C). This can be attributed to a higher degree of crystallinity, increased intermolecular forces and lack of acetate side groups within the polymer structure.

Plasticizers have been shown to increase the volume between polymer chains within bulk polymers thereby resulting in a more ductile, flexible and extensible film. Applications of plasticizers can also decrease the melt temperature of the resin thereby providing the ability to use a particular polymer in different processes or provide a wider range of workability. They can increase thermoplastic characteristics and can also decrease the effects of thermal degradation. [22; 27 & 29]

Both glycerin and glycerol have been shown to have plasticizing effects on polyvinyl alcohol polymers. 20% glycerol incorporated into 7.5% PVOH w/w solution yielded a decrease in the melt temperature of the film from 226.0°C to 196.0°C. Glycerin was also used to increase thermoplastic properties of PVOH in 20, 30 and 40% wt. polymer solutions [11 & 12 referenced in 25] Additionally, glycerin was shown to decrease the melt temperature and crystalline regions of both fully and partially hydrolyzed PVOH resins at concentrations of 40 parts per hundred (phr) and 65 phr in

fully and partially hydrolyzed resins. Above 40 phr, phase separation between the polymer solution and glycerin were observed. [22]

Table 3.3 showed that PEG 400 was ineffective as a plasticizer for fully hydrolyzed (FH-PVOH) resin showing a decrease in melt temperature of the FH-PVOH film of only around 6°C. This may be due to the inability for the plasticizer to penetrate the crystalline structure of the FH-PVOH in addition to PEG 400 being incompatible with PVOH. [24] The plasticizer did not dissolve into the resin/water solution and precipitated out as a white cloudy solid.

In this study, glycerol and glycerin plasticizers dissolved into the PVOH/water solution and did not precipitate or bleed out of the polymer upon casting and drying. No visible layer of plasticizer was observed on any dry coated films. PH-PVOH granule films with glycerol showed a 31.4°C decrease in the melt temperature while glycerin showed a 38.7°C decrease. On the other hand, PH-PVOH powder and glycerol films showed a 38.6°C decrease in melt temperature while glycerin showed 45.1°C decrease. Although the powdered resin melt temperature was affected more by the plasticizers than the granular resin, the powdered resin had an initial melt temperature that was higher than the granular resin. It also absorbed water less readily than the granular resin.

An additional peak was found on the DSC thermograms which were determined to be indicative of degradation. PVOH degrades through a process called pyrolysis which is loss of water. [21] It was found that the increasing concentration of plasticizer also decreased the rate of pyrolysis. A figure of an example of this can be found in Appendix A, (Figure A.5). The figure depicts thermograms of powdered PVOH based films

containing 0 and 40 phr of glycerin plasticizer. The pyrolysis peak was visible in the temperature range 60-160°C of the sample not containing any plasticizer. The peak was no visible in the sample containing 40 phr of glycerin. This may be due to the glycerin crosslinking to the PVOH resin via hydrogen bonding in addition to the water binding to the glycerin. Glycerin is a water soluble plasticizer and has characteristics such as a high density in addition to the ability to hydrogen bond essentially trapping the water within the film structure. [22 & 24]

Selected Properties (pH, percent solids and viscosity)

A high percent solids coating will have more versatility for large scale printing or coating methods. Gravure and flexography printing methods are two of the most common methods for printing flexible packaging in the United States. Ideal solids contents for both flexography and gravure processes are in the range of 15-50% but can vary with equipment limitations such as drying abilities. [32] As shown in the results, the formulated antimicrobial coating has sufficient percent solids (20.53%) to be utilized for one or both of these large scale coating processes.

The pH of the treatment coating was determined to be 5.9. This can be attributed to dissolving the Nisaplin® in 0.02 M acetic acid solution. Adjustments to coating equipment can be made for running acidic coatings to prevent corrosion such as switching out easily corroded materials to acid resistant materials in addition to more frequent cleaning after and in between runs.

Viscosity is one of the main factors to consider when formulating a coating especially in regards to application and desired coating weight. It can be defined simply as the resistance to flow. [31] The results indicated that the formulated coating was able to pass through a Zahn #3 cup in an average of 24.47 seconds. Utilizing a conversion chart [13] until further testing, 24.47 seconds in a Zahn #3 cup fell within an approximate viscosity measurement 175-200 cP. Centipoise (cP) is a unit expressing dynamic viscosity which can also be expressed in mPa*s. (milli pascals-seconds) In order to get a means for comparison, water at a temperature of 20°C has a viscosity measurement of 1.009 cP while glycerol has a measurement of approximately 850 cP. [31] Viscosity can have an effect on the type of coating application to be utilized. For example, engraved roller coating applications such as gravure requires a coating viscosity between 100 and 10,000 cP in order to achieve a coating weight between 2-300 g/m^2 (1.2 -184.3 pounds per ream). If the coating contains a viscosity outside of this range, other coating applications, such as knife coating system or a kiss coater method could be used to achieve more desirable coating results. [16] The results indicate that the formulated antimicrobial coating is in the range of viscosity in order to use gravure as the proposed large-scale coating application method.

Dynamic Contact Angle, Surface Tension of Liquid Coating and Critical Surface Tension of Films

Contact angle is a means of quantifying adhesion of a liquid solution to a solid substrate. Droplet angles ranging from 0-90° indicate complete to partial wetting while

90-180° angles are indicative of a non-wetting solution or coating. [31] Ideally, the solution and film substrate should yield a contact angle of 0° to indicate full wettability.

In order to determine if the surfactant (Tween® 80) was having a negative effect on adhesion, contact angle was tested utilizing the original coating preparation and a second coating preparation without Tween® 80. It has been found that surfactant concentrations that are too high can produce a boundary layer of oil between the coating and the film substrate limiting adhesion. [26] Prior to conducting contact angle testing, surface tension of the liquid coatings needed to be determined for the software to determine contact angles in further testing. As indicated previously the average surface tensions of the coatings were determine to be 31.7 dynes/cm (control) and 31.6 dynes/cm (no Tween®). This indicated that the amount of Tween® 80 in the control coating formulation was not a high enough volume to drastically alter the surface tension of the overall liquid solution. However, the volume of Tween® was sufficient to cause differences in the contact angles achieved. Those substrates with the same composition and surface treatments yielded significant differences in the achieved contact angles. The control formulation which contained Tween® 80 resulted in lower contact angle measurements. This is a common effect for the addition of a surfactant material such as Tween® 80.

Additionally, coating solutions containing Tween® 80 remained stable emulsions at ambient conditions in sealed containers for several weeks. The coating formula without Tween®80 exhibited phase separation. (See Figure 3.5) The phase separation appears to be the antimicrobial component, Nisaplin®, due to the brown coloration. The formula

containing Tween® 80 also prevented bubble formation. PVOH has the tendency to foam and Tween® 80 can be used for emulsion, surfactant and foam reduction qualities. Due to the aforementioned effects in addition to achieving a lower contact angle, Tween® 80 will remain in the coating formulation for further research studies.

Figure 3.5. Coating formula stability after 6 weeks. (Left: Control coating formula containing Tween® 80; Right: Treatment formula that does not containg Tween® 80)

Dynamic contact angle and critical surface tension testing were conducted to determine the wettability of the formulated antimicrobial coating on various substrates. A main goal of this study was to find a substrate with properties not significantly different from the control LLDPE. For those substrates that have significantly different properties, only substrates that achieved lower contact angles or higher critical surface tensions than

the control were deemed desirable. This would have indicated a higher degree of wettability and potentially higher degree of coating adhesion. According to the results, there were no substrates that achieved higher critical surface tensions or lower contact angle measurements; therefore focus was on those substrates with performances not statistically significantly different from the control LLDPE.

LLDPE is a common sealant material utilized in the packaging industry. This sealant was coextruded with other materials to produce a material for ready-to-eat meat packaging. In order to achieve any wettability the substrate was corona treated (hand treated). It was later determined that a primer was also needed to achieve a higher degree of wettability. The PVOH coating formulated would readily delaminate from the corona treated LLDPE. A water soluble primer was recommended by MICA Corporation and produced a higher degree of adhesion between LLDPE and the PVOH based coating. This substrate was deemed as the "control" substrate for this study in hopes to eliminate either primer and/or corona treatment processing with a different substrate. Without corona treatment and primer, the critical surface tension of LLDPE was found to be approximately 32 dynes/cm with AccuDyne dyne pens. Corona treatment and primer increased the critical surface tension of the film to approximately 44.2 dynes/cm when tested with dynamic contact angle equipment.

LLDPE was compared to four additional substrate surfaces with and without corona treatments yielding a total of 9 substrates as seen in Table 3.1. The critical surface tension of LLDPE was compared to the other material for adhesion to the PVOH-based antimicrobial coating. As previously stated, all substrates were found to have statistically

significantly different critical surface tensions from the control LLDPE substrate. (α = 0.05)

For "wetting out" to occur between a solid and a liquid, the liquid must be approximately 8-10 dynes/cm lower in surface tension compared to the critical surface tension of the solid component [29 & 31]. LLDPE (primed and treated) resulted in a critical surface tension 12.5 dynes/cm higher than the surface tension of the liquid coating. Because of this, the coating is able to wet out the substrate. However, this indicates that the wettability cannot be based solely upon the critical surface tension of the substrate.

There were two substrates which resulted in dynamic contact angle measurements not statistically different from LLDPE (Average = 21.0°). Corona treated Elvax® (Average 26.7°) and Surlyn® (Average = 22.4°). Because PVOH is produced from polyvinyl acetate, there are remaining vinyl acetate groups on the PVOH after formation which could suggest a chemical compatibility between PVOH and Elvax®. The contact angle however, indicates only partial wetting (0° = completely wets out). This may be due to the polar regions of the Elvax® molecule being buried under the surface of the film leaving the non-polar portions at the surface to make direct contact with the polar coating solution. (Morris, B., personal communication, Jan 21 2015)

According to the dynamic contact angle results, PVOH was also compatible with the Surlyn® substrate. Surlyn® is an ethylene and methacrylic acid copolymer. Ionic polar groups are produced from neutralization of free acid using a strong base such as salts during polymerization [29]. The remainder of the ionomer (Surlyn®) molecule

contains a non-polar ethylene backbone. The polar groups in the Surlyn® structure may have resulted in chemical compatibility with the PVOH based coating. Other highly polar components of the coating could have also aided in the resulting contact angle such as the salt component within Nisaplin® in addition to the surfactant component.

Although it was determined that all substrates had significantly different critical surface tensions compared to LLDPE, both Elvax® and Surlyn® substrates yielded critical surface tensions that were not significantly different from one another in addition to low contact angle measurements. Because of this, it is possible that LLDPE substrate could be replaced with either of the corona treated Elvax® or Surlyn® substrates tested. If this product (antimicrobial coated film) were to reach a large scale operation, the primer could possibly be eliminated from the manufacturing process. The tape test was utilized to investigate this possibility.

Tape Test

The results indicated that the antimicrobial coating yielded the highest degree of adhesion to the LLDPE (primed and corona treated) and EVA (Elvax 3165®) substrates. Each of the ten tape strips resulted either negligible amounts or no coating being removed by the tape. The ionomer (Surlyn® 1605) yielded rankings distinguishing areas of excellent adhesion all the way to no adhesion.

Linear low density polyethylene (LLDPE) is a highly non-polar polyolefin while the main component of the coating is a polar polyvinyl alcohol containing between 12-15% acetate groups among the side groups attached the vinyl backbone. The remainder of the

side groups are hydroxyl or alcohol groups. These two polymers are chemically incompatible therefore surface treatments (corona discharge and primer) of the LLDPE provide a more adequate compatibility for adhesion. Corona discharge is a form of treatment which oxidizes the surface of a film. In this case, solely corona discharge was not enough to promote adhesion of the PVOH coating to LLDPE. The primer used is a polyethylenimine (PEI) resin dispersion, also known as polyaziridine. It is a primer commonly used for adhering polar and non-polar substrates to one another. PEI is an open chain or aliphatic amine. The structure of this resin is shown in Figure 3.6. [15& 23] PEI is known as a cationic polyelectrolyte which has many charged groups. Dissolving the substance in polar solvents such as water can also produce additional charged groups [6]. The charged groups in PEI are primary, secondary and tertiary amines. Because PEI is a cationic polyelectrolyte, it is attracted to anionic and oxidized surfaces [19]. Because of these properties, PEI is able to adhere to both LLDPE which has been oxidized by corona discharge treatment and the PVOH coating which also contains ionic salt components. (Salt is a component of Nisaplin®)

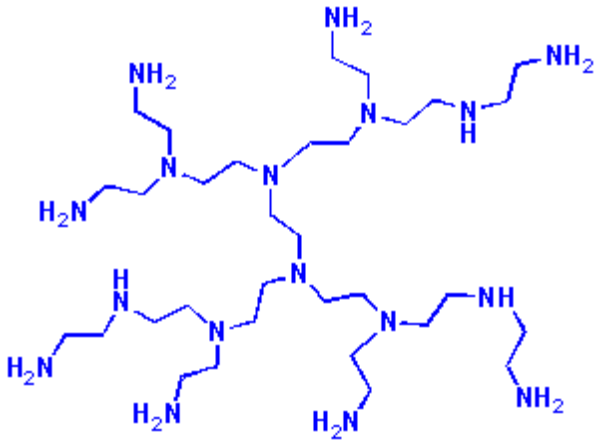

Figure 3.6. Chemical structure of polyethylenimine (PEI) primer. [23]

Corona treated Elvax® 3165 (Ethylene vinyl acetate or EVA) also revealed excellent coating adhesion properties according to the tape test results. Elvax® 3165 is a material composed of a high vinyl acetate (VA) composition, 18%. The general structure of EVA can be viewed in Figure 3.7. The material had a high level of tack and an increase in the VA composition would have essentially turned the substrate into an adhesive rather than a film substrate. The PVOH utilized in the coating, like all PVOH, was produced from the hydrolysis of polyvinyl acetate (PVAc). Because the PVOH in the coating is a partially hydrolyzed grade, (Mowiol 4-88) approximately 12-15% of the side groups on the vinyl back bone are acetate groups as stated previously (Figure 3.7). These acetate groups result in a chemical compatibility with the vinyl acetate groups of the Elvax®3165 material. Therefore no primer was needed for adhesion; however, corona treatment did assist in adhesion properties. Although excellent adhesion was achieved between the Elvax 3165® and PVOH based antimicrobial coating, slight difficulties and a need for corona treatment could have been due to structural considerations as stated earlier. It is possible that polar regions of the Elvax® molecule could have been buried under the surface of the film leaving the non-polar portions at the surface to make direct contact with the polar coating solution. (Morris, B., personal communication, Jan 21 2015)

The final substrate, Surlyn® 1605, was a sodium ionomer. The results indicated that there was little adhesion between the PVOH based antimicrobial coating and the film. Surlyn® is a copolymer of ethylene and methacrylic acid which was then neutralized with sodium hydroxide (NaOH) resulting in ionic sodium attached to what

were once carboxylic acid side groups. The structure can be viewed in Figure 3.7. Although there are polar regions within Surlyn® that have potential for adhesion to PVOH, these polar regions can clump together leaving them unavailable to adhere to additional substrates [28]. The ethylene backbone of Surlyn® also causes poor adhesion due to high hydrophobicity and a low surface tension of 33 dynes/cm [9]. Two studies conducted by España et al [9 & 10] showed that plasma (i.e. corona) treatment resulted in an increased surface roughness of the sodium ionomers tested for those materials with increased treatment times (lower treatment speed) and decreased distances between the treater and the film substrate. It was also concluded that the quantity of oxygen on the surface of the films increased due to oxidation resulting in lower contact angle measurements. Although corona treatment resulted in significantly lower contact angles between the PVOH based coating and Surlyn® 1605 substrate compared to the non-treated Surlyn® 1605, the lack of chemical compatibility was too great to promote adhesion strong enough to survive the tape test.

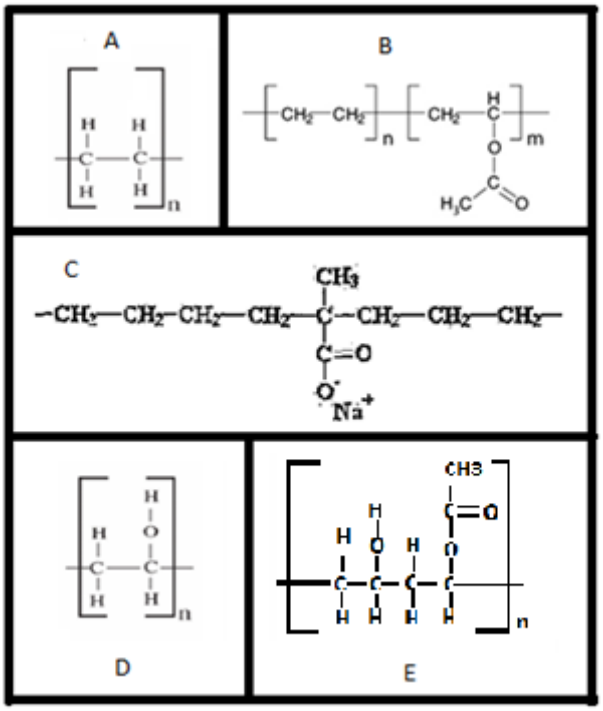

Figure 3.7. Chemical structures of A) LLDPE B) EVA C) Sodium Ionomer D) pure PVOH and E) partially hydrolyzed PVOH. (EVA structure [2]; PVOH structure [17]; Sodium ionomer structure [18])

Coating Formulation Summary Discussion

Polyvinyl alcohol (PVOH) is one of the most common water soluble films and was selected as the polymer base or carrier for the Nisaplin® in the antimicrobial coating. PVOH is a water soluble, thermoplastic resin currently used for food, pharmaceutical and packaging applications such as food additives to reduce moisture loss, tablet coatings and packets for laundry detergent [29]. PVOH has also been found to be UV stable and chemically resistant, hence its ability to contain products such as laundry detergent and pesticide type chemicals [20]. Using a material such as PVOH that is currently used on

large scale equipment in the packaging industry will hopefully allow for utilization without increasing capital cost.

There are two of grades of PVOH resin: partially hydrolyzed and fully hydrolyzed. PVOH is formed through hydrolysis of polyvinyl acetate with a strong base such as NaOH. The reaction that occurs to produce PVOH is also referred to as the saponification of esters. The degree of hydrolysis is a result of the amount of hydroxyl (-OH) groups relative to acetate groups attached to the vinyl backbone. Full hydrolyzed PVOH resin can have 98-100% hydroxyl side groups while partially hydrolyzed PVOH can have 85-89% hydroxyl side groups.

The degree of hydrolysis has varying effects on the physical properties of the resulting polymer. Fully hydrolyzed PVOH resins have higher crystallinity, melt temperature and better barrier when compared to partially hydrolyzed PVOH. On the other hand, partially hydrolyzed PVOH achieves better adhesion to hydrophobic substrates in addition to a lower melt temperature. Degree of hydrolysis can also have effects on properties such as water solubility, viscosity and surface tension. A higher concentration of acetate groups will reduce inter and intramolecular forces within the polymer between hydroxyl groups. This makes partially hydrolyzed PVOH more readily soluble in water. It is because of these qualities that partially hydrolyzed PVOH will exhibit a lower surface tension compared to fully hydrolyzed PVOH. For this application, both increased water solubility and lower surface tension are desired. Thirdly, partially hydrolyzed PVOH will yield a more stable viscosity. Fully hydrolyzed resins will increase viscosity to the point at which the resin solutions will produce a gel [12].

The grade of PVOH was then determined based upon general observations in addition to the results of the differential scanning calorimetry study. Coating drawdowns of PVOH resin and water solution resulted in brittle coatings, which later resulted in delamination from the LLDPE sealant web. Partially hydrolyzed PVOH resin (88%) exhibited a higher degree of adhesion to LLDPE resulting in less delamination or a longer amount of time before delamination occurred. A lower melt temperature for a partially hydrolyzed resin was also found for both granular and powder partially hydrolyzed PVOH, leading to the possibility of sealing a package coated with this resin.

In order to achieve a less brittle coating, plasticizers were tested as shown in the DSC study. Because glycerin exhibited the highest decrease in melt temperature in both partially hydrolyzed resin grades, a carrier resin of partially hydrolyzed resin and glycerin base was determined. The glycerin also appeared to have increased the adhesion of the PVOH resin to an LLDPE substrate. However, delamination of PVOH/glycerin films still indicated either chemical incompatibility or a glycerin-created weak boundary layer. Granular PVOH resin was chosen instead of a powdered PVOH resin for ease of use. Due to the inherent nature of PVOH, the resin absorbs moisture from the air causing clumping. The powdered resin exhibits increased clumping compared to that of the granular resin.

Water-based inks and coatings are particularly challenging to adhere to common non-polar sealing substrates such as LDPE (low density polyethylene) and PP (polypropylene). Water at room temperature (25°C) has a surface tension of 72.6 dynes/cm while PE and PP substrates have critical surface tensions of approximately 30-

34 dynes/cm. In this case, the addition of a solvent, ethanol (95%) was used to lower the surface tension of the overall coating solution. A 25% (v/v) ethanol/water solution at 20°C has an approximate surface tension of 34 dynes/cm while a 60% ethanol/water solution at the same temperature has a surface tension of 27.5 dynes/cm. Not only does the ratio of ethanol to water affect the surface tension, a higher temperature will also decrease the observed surface tension [3 & 7]. The coating produced utilized a 50/50 ratio of an ethanol/water solution at approximately 25°C.

Based upon the lack of adhesion of the antimicrobial coating to the substrate, it was determined that surface treatment of the LLDPE substrate would be necessary. Adhesion can be defined as "…processing by which two initially separate bodies (called adherends or substrates) are held together by intermolecular forces" [29]. Surface treatment will increase the surface energy or reduce the work required to increase the surface of a substrate by a unit area, of the LLDPE substrate [29]. In order for wetting to occur, the surface energy of the liquid coating is required to be at least 8-10 dynes/cm less than that of the critical surface tension of the substrate [29 & 31]. The coating will exhibit wetting when able produce a "homogeneous bond" by filling cracks, crevices and pores of the substrate enabling complete contact with the surface of the substrate [31].

Upon corona treatment of LLDPE, adhesion issues continued for the coating formula therefore it was determined that a primer would be required in order to achieve better wettability and adhesion of the coating. Primers are coatings that are utilized to improve the bonding between two chemically incompatible substrates or a substrate and

an adhesive [29]. Utilizing industry contacts, a water soluble primer, polyethylenimine (PEI), suitable for food contact materials was found.

Dynamic contact angle results indicated that corona treated Elvax® 3165 (24.7°) and corona treated Surlyn® 1605 (22.4°) exhibited contact angles statistically similar to corona treated and primed LLDPE (21°). However, critical surface tension measurements showed that LLDPE yielded the highest surface tension of 44.2 dynes/cm compared to all other substrates tested including those with comparable contact angles. (Elvax® 37.4 dynes/cm & Surlyn® 37.5 dynes/cm) Tween® 80 also had no effect on contact angle measurements observed. As stated earlier, Tween® 80 stabilized the coating formula as an emulsion while also exhibiting foam reduction benefits.

Further investigation of coating adhesion onto LLDPE, Elvax® and Surlyn® substrates was conducted with a simple tape test [1]. The tape test showed that the coating exhibited an excellent degree to both treated LLDPE and corona treated Elvax® 3165. However, no coating was removed from LLDPE while minimal amounts were removed by the tape from Elvax® 3165. Based upon these results the coating formula described in the materials and methods will be coated onto a treated LLDPE substrate.

The coated packaging structure is shown in Figure 3.8. A corona treated sealant web (LLDPE) coated with a primer followed by the antimicrobial coating containing PVOH, glycerin, Nisaplin®, solvents of water and ethanol in addition to the surfactant Tween® 80. Although there was potential to replace LLDPE with corona treated EVA, based upon further investigation through tape tests and observations, it was found to be

more valuable to continue working with a highly common substrate such as LLDPE as opposed to high vinyl acetate content (18%) EVA.

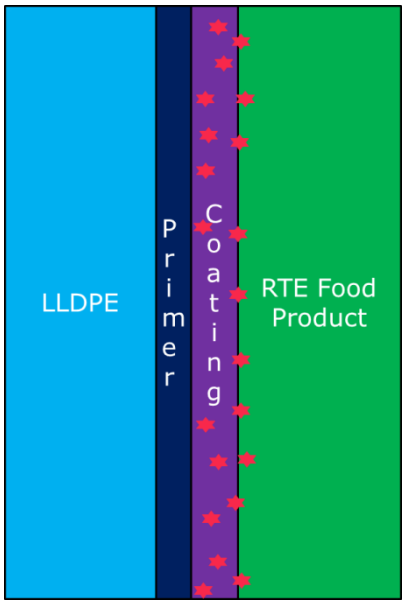

Figure 3.8. Summary of antimicrobial packaging structure.

CONCLUSION

This work demonstrated that there is potential for producing a large scale antimicrobial coating that not only can have the qualities to be run on equipment such as gravure and flexography presses but also has the ability to inhibit spoilage and pathogenic microorganisms. Such a material could be used for extension of shelf-life of RTE food products by reducing food waste and enhancing food safety by inhibition of *Listeria monocytogenes*. The antimicrobial coating formulated will be run on a large-scale gravure coating process in addition to characterizing antimicrobial degradation and efficacy.

Table 3.5. List of abbreviations and trade names for acronyms.

List of Abbreviations and Trade Names	
Bynel®	DuPont ethylene/acid/acrylate terpolymer
DSC	Differential scanning calorimetry
Elvax®; EVA	DuPont ethylene vinyl acetate
FH-PVOH	Fully hydrolyzed polyvinyl alcohol
IU	International units
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
Nisaplin®	2.5% Nisin powdered product
Nucrel®	DuPont ethylene acrylic acid and methacrylic acid copolymer
PEG 400	Polyethylene glycol (molecular weight 400)
PH-PVOH	Partially hydrolyzed polyvinyl alcohol
Phr	Parts per hundred
PP	Polypropylene
PVOH	Polyvinyl alcohol
RTE	Ready-to-Eat
Surlyn®	DuPont sodium ionomer
TLC	Thin layer chromatography
Tween 80®	Polysorbate 80 or Polyoxyethylenesorbitan monooleate

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CHAPTER FOUR

COATING TRIALS OF AN ANTIMICROBIAL COATING CONTAINING NISAPLIN® USING LARGE SCALE GRAVURE AND FLEXOGRAPHIC APPLICATION PROCESSES

ABSTRACT

Numerous antimicrobial films and packaging materials containing nisin have been produced in laboratories and shown to maintain efficacy against targeted microorganisms. However, production of a commercially viable product can hinder materials used due to cost, decrease antimicrobial activity and the proposed packaged system may not be able to transition to a commercial production process. The objective of this study was to produce an antimicrobial coated material using the previously formulated antimicrobial coating containing nisin with large scale gravure and flexography equipment. This study showed that the coating could be run on commercial equipment, however, the overall material quality produced using flexography was superior due to anilox roll availability. The coated material maintained efficacy after production against spoilage indicator microorganism *Micrococcus luteus*. (ATCC 10240)

INTRODUCTION

In recent market studies, it was found that both food packaging films and meat specific packaging products have projected growth for 2018 and 2019. The demand for meat, poultry and seafood packaging is expected to increase in the United States by 3.8%

up to $11 billion in 2019. Growth specifically in prepared foods such as ready to eat meats, convenient items and various sizes such as individual portions are also expected to exhibit high increases in demand [16]. Converting Quarterly also found that the food packaging market is projected to have the fastest growth in film demand [15] increasing from 4.59 billions of pounds in 2013 to 5.11 billions of pounds in 2018 [16].

Nisin is a GRAS approved antimicrobial component contained in the commercially available product Nisaplin® (2.5% concentration). Several studies have shown nisin to be effective in inhibiting gram positive bacteria showing potential in the food packaging market for the reduction of spoilage microorganisms. The cost inherent from the loss of product due to the growth spoilage microorganisms is a concern for many packaging companies. Application of Nisaplin® into or onto a commercially available packaging product for food products could be used to reducing the population of slowing the growth of spoilage microorganisms as a means for shelf life extension. Because Nisaplin® is a higher cost additive, determining an effective yet low cost application process could produce an antimicrobial packaging product that appeals to the industry as a value added product.

Few studies have been conducted on antimicrobial coated materials produced using large scale equipment such as gravure coaters and additional printing methods such as flexography. The main objectives of this study was to produce antimicrobial coated material from the coating formulated in the previous chapter and to characterize the liquid coating and antimicrobial coated films.

MATERIALS AND METHODS: GRAVURE TRIAL

Coating Preparation

Coating solutions, control and treatments, were prepared in 1,750 mL batches due to container and mixing limitations. Multiple batches were produced in order to prepare approximately 2 gallons of each coating type in total. This was to ensure that there would be enough coating to run the coating pump, fill the anilox roll pan and have enough coating to finish the trial runs. Control coating batches did not contain Nisaplin® component but contained all other coating ingredients. The coating ingredients and quantities can be viewed in Table 4.1. The ingredients and proportions are the same as the coating formulation from Chapter 3.

Table 4.1. Coating ingredients and amounts for 1,750 mL batch of coating.

Coating Ingredient	**Amount per 1,750 mL batch**
4 – 88 Mowiol Polyvinyl alcohol granular resin	0.55 lbs
Distilled water	750 mL
USP Pure vegetable glycerin	80 mL
Tween® 80 (aka Polysorbate 80)	4.625 mL
Acetic acid solution (0.02 M)	50 mL
95% Ethanol solution	750 mL
Nisaplin® (*treatment coating only)	25 g

The coating solution was prepared by heating and simultaneously stirring 0.55 pounds of 4-88 Mowiol PVOH resin in 750 mL of distilled water for approximately 1-2 hours until the resin dissolved into solution. The hot plate stirrer was set to 175°C and the water/resin solution was stirred by hand with a wood spoon until later in the preparation

process. Once the resin had dissolved, the solution was removed from the hot plate to allow slight cooling prior to adding 80 mL of glycerin (40 parts per 100 grams of PVOH resin) and 4.625 mL of Tween® 80 (0.25% v/v) (Polysorbate 80, FCC, Spectrum Chemical Manufacturing Group, New Brunswick, NJ, USA). In a separate (1L) beaker, 25 gram of Nisaplin ® (2.5% - 12,500 IU/mL in solution) (Danisco, Inc. Madison, Wisconsin, USA) was dissolved in 50 mL of 0.02 M acetic acid solution [11]. (Glacial acetic acid, Fischer Scientific, Waltham, MA, USA) 750 mL of 95% ethanol was then added. The solution was then mixed using a tissue homogenizer to achieve particle suspension. The ethanol solution was then poured into the resin solution and stirred using a stir bar on the hot plate stirrer for an additional 10-15 minutes. Each batch was poured into either a 2 or 4 liter bottle for storage prior to the trial. Parafilm® and foil was wrapped around the closure to reduce any evaporation of the coating while being stored prior to trials.

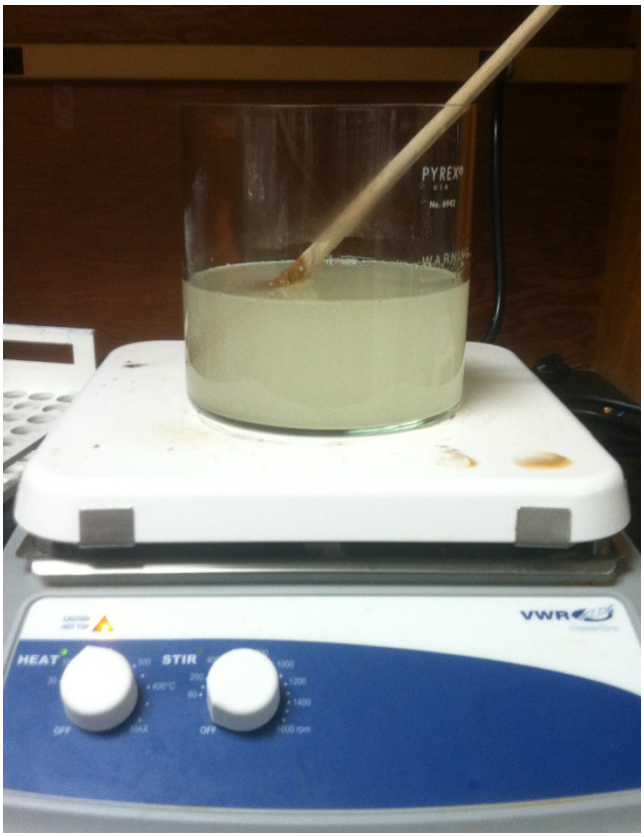

Figure 4.1. Polyvinyl alcohol (PVOH) resin and distilled water solution.

Figure 4.2. Produced control (left) and treatment coatings (right).

Material Surface Treatments and Preparation

The material was a multilayer, 2.5 mil thick, PET (polyethylene terephthalate) coextruded lidding material commonly used for hot dog packaging donated by Sealed Air Corporation. The sealant web of the material consisted of linear low density polyethylene (LLDPE). There was approximately 1400 feet left on the roll after preliminary formulation work. The core containing some specifications of the material can be seen in figure 4.3.

Figure 4.3. Labeled core of donated hot dog packaging material from Sealed Air Corporation.

The web width of the donated roll of material was 17 inches and was slit down to 14.5 inches per the specifications of coating/laminating equipment to be used for the trial. Untreated material, 50 feet, was removed from the slitted roll as a control for future tests.

After the slitting process (slitter seen in Figure 4.4.), material was added to the front and back ends of the web to account for machine equipment set up and adjustments. This leader material was a 48 gauge PET. Approximately 400 feet was added to the front of the roll and 450 feet was added to the back. The roll totaling approximately 2250 feet was then taken to the Sonoco Institute of Packaging Design and Graphics for corona treatment. Preliminary work showed that the handheld corona treater yielded coating adhesion with a water soluble primer at 37 dynes/cm. The initial surface tension of the LLDPE sealant was 32 dynes/cm. Therefore this same level of treatment was the goal level to be achieved at the Sonoco Institute. The corona treater on the OMET VaryFlex 530 was used to treat the material at a line speed of 150 ft/min at 1000 watt*min per m^2.

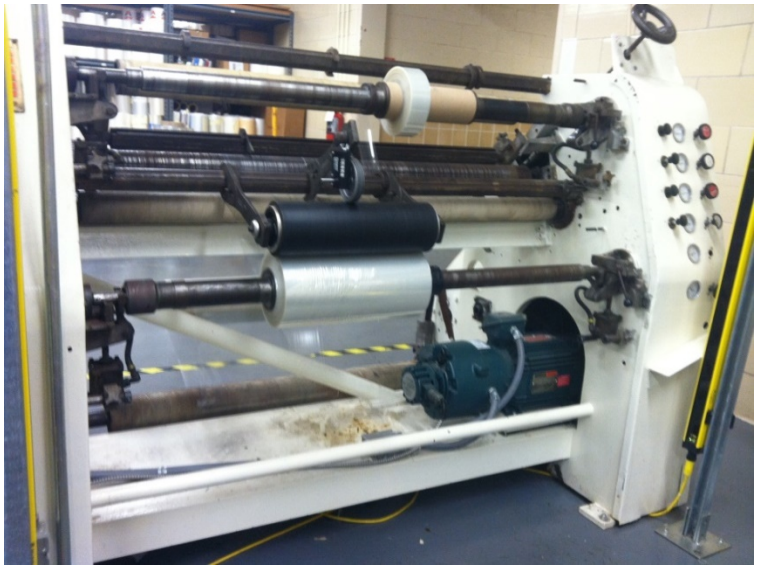

Figure 4.4. Slitting process of coextruded material.

After corona treatment, the material was then primed with a water soluble primer solution donated by MICA Corporation, MICA A-131-X. It is commonly known in the converting industry as PEI or polyethylenimine and is used for adhering non-polar materials to polar materials. PEI solution was diluted 1 part primer (800 mL) to 9 parts (7200 mL) distilled water to produce the priming solution designated by MICA Corporation. The conditions were recorded when priming the corona treated LLDPE coex film as shown in Table 4.2. After priming, the material was stored upright on its side to prevent blocking. The location of the coated side was labeled in addition to indicating the operator side on core for storage (2 days) until coating trials.

Table 4.2. Coater/laminator equipment parameters for addition of primer to LLDPE Coex material.

Priming Conditions of Coater/Laminator in DuPont Lamination Laboratory	
Sample	**Primer**
Primary unwind material	48 ga PET/ 2.5 mil LLDPE Coex/ 48 ga PET
Coat side	In
Tension (1° UW) (psi)	4
Web width (inches)	14.5
Rewind coat side	Out
Tension at rewind (psi)	10
Coater cylinder	200 Quad
Coating	MICA A-131-X Primer (PEI)
Tension - coating station (psi)	13
Dryer 1 temperature (°F)	155
Dryer 2 temperature (°F)	150
Line speed (ft/min)	26
Web break	Off

Coater mode	Tension
Agitate	Auto
Coater draw nip	Close

Percent solids

Percent solids of control and treatment antimicrobial coatings were tested in replicates of ten based on the large volume of coating produced. Sets of measurements were taken once the produced coating had cooled, right before the trial run and after the trial run had ended. This could indicate solvent evaporation during storage or the trial process. Liquid coating was weighed into previously dried and weighed aluminum pans. The pans were placed in a 65°C drying oven for 5-7 days. (Lindberg/Blue M Gravity Oven, Model GO1330A, Industrial Laboratory Heaters, Asheville, NC, USA) The pans were re-weighed on an analytical balance and percent solids were calculated. (n = 60)

pH of coating solutions

pH of the coating solution was tested utilizing a Thermo Fisher-Orion Star A211 pH meter. (Thermo Fisher Scientific, Inc. Waltham, MA, USA).

Coating Trial - Gravure

Control and Nisaplin® containing treatment coating trials were conducted within the same morning. Control coating trial was conducted first in order to avoid contamination should the treatment trial had been conducted first. Percent solids, pH and viscosity measurements were taken just prior to the start of each trial. Trials were run

using the conditions listed in Table 4.3. The solvent-based coater/laminator is depicted in figure 4.5 in addition to the apparatus schematic in Figure 4.6. Masking tape flags were placed in the roll to indicate points of untreated material (for basis weights), coating start points and any mishaps to avoid using the material for testing. The coater was dialed in to the conditions in Table 4.3 using the leader material (PET) and basis weights were taken in line to make sure laydown was being achieved.

Figure 4.5. Solvent-based coater/laminator in DuPont laboratory Clemson University.

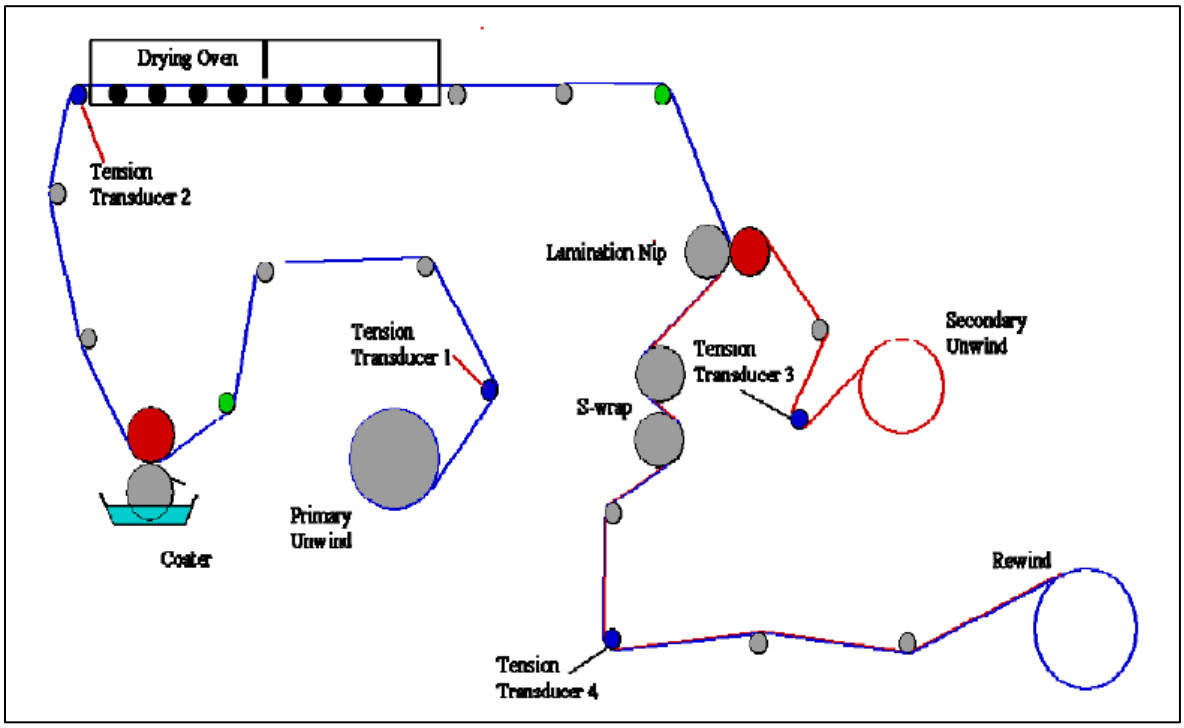

Figure 4.6. Schematic for coater/laminator [14].

A total of 7 rolls (Figure 4.7) were produced from control (3 rolls) and treatment (4 rolls) coating trials. Originally, a 30 day shelf life test was to be conducted in high heat and ambient conditions, however, only Day 0 (ambient) material was tested due to material quality issues to be discussed later. Day 0 material totaled approximately 200-250 feet of coated LLDPE coex material.

Figure 4.7. Rolls of coated material produced during gravure coating trials.

Table 4.3. Coater/laminator equipment parameters for control and antimicrobial coatings to LLDPE Coex material.

Conditions of Coater/Laminator in DuPont Lamination Laboratory for Control and Treatment Antimicrobial Coatings		
Sample	Control	Treatment
Primary unwind material	48 ga PET/ 2.5 mil LLDPE Coex	2.5 mil LLDPE Coex/PET
Coat side	Out	Out
Tension (1° UW) (psi)	1.5	2.0
Web width (inches)	14.5	14.5
Rewind coat side	Out	Out

Tension at rewind (psi)	10	10
Coater cylinder	110 Quad	110 Quad
Coating	Control coating (*no Nisaplin®)	Antimicrobial coating
Tension in coating station (psi)	13	13
Dryer 1 temperature (°F)	155	160
Dryer 2 temperature (°F)	150	155
Line speed (ft/min)	25	25
Web break	Off	Off
Coater mode	Tension	Tension
Agitate	Auto	Auto
Coater draw nip	Close	Close

Viscosity

Viscosity was estimated using a Zahn #3 cup. Zahn cups are commonly used in the coating and printing industries as a fast, efficient means to monitor viscosity over a coating or printing process. The Zahn cup was submerged in each coating solution (control and treatment) and a time was recorded. The time for the stream of liquid coming out of the hole in the bottom of the cup to break was then recorded in seconds. Measurements were collected in triplicate prior to and after trials were completed. A Zahn cup is depicted in Figure 4.8.

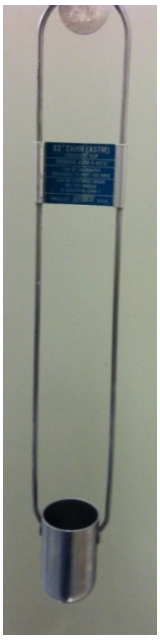

Figure 4.8. Image of a Zahn cup.

Basis Weight

The coating weight or basis weight of the coating on the substrate was determined using ASTM 2217: Standard Practice for Coating/Adhesive Weight Determination [1]. Approximately 25 feet of material was left un-primed in order to peel off control and treatment coatings for basis weight determination.

A 3” x 3” metal template and utility knife was used to cut two samples of equal surface area from each draw down representing a different Mayer rod size and treatment type. Each 3”x 3” inch square of material was weighed on an analytical balance and the weight was recorded. The coating was then peeled off of the substrate and the new mass was recorded. The basis weight of the coating was then calculated in pounds per ream (#/ream). The metal templates and analytical balance can be shown in Figure 4.9.

Locations of samples were also recorded across the web: operator side, center and machine side. (n = 21 per treatment)

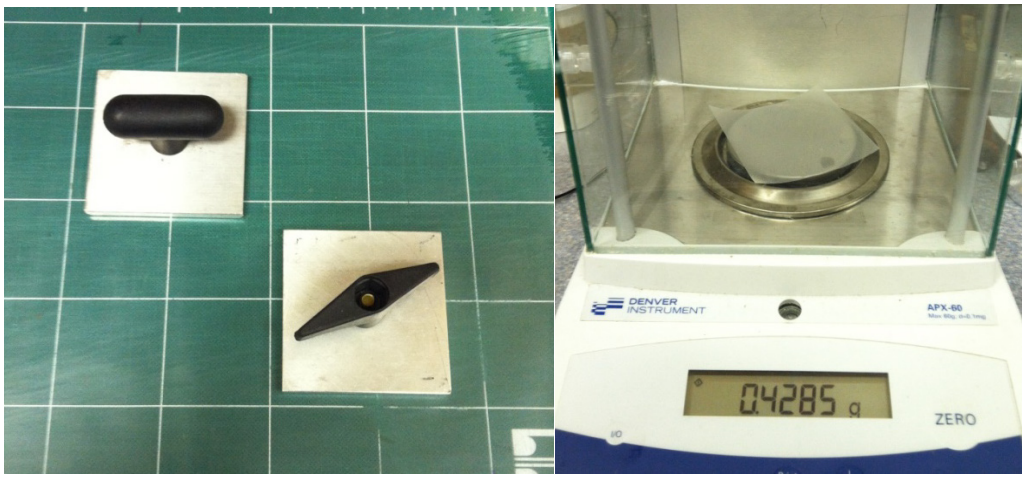

Figure 4.9. Basis weight templates (left) and analytical scale used (right) for basis weight determination.

Haze (ΔE)

ΔE testing was conducted using a Minolta CR-400 chromameter (Konica Minolta, Tokyo, Japan). The colorimeter was calibrated using a white calibration standard and an untreated neat piece of LLDPE coex film. Measurements were recorded in triplicate from each coated or uncoated piece of film using the white calibration standard as a consistent background. (See Figure 4.10) Locations of the measurements (operator, center and machine side of web) were also recorded to note any differences across the web during the coating process. (n=40) ΔE was then calculated using the following formula:

$$\Delta E: \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

Figure 4.10. Haze testing with colorimeter.

Film on lawn

Two bacterial types were propagated from -80°C freezer stocks: *Listeria innocua* (ATCC 33090) and *Micrococcus luteus* (ATCC 10240) . *L. innocua* is a non-pathogenic simulator of *Listeria monocytogenes* and *M. luteus* was tested against as a spoilage indicator organism. Both bacteria were pulled from freezer stocks and streaked onto TSAYE plates (tryptic soy agar with yeast extract) and stored at 37°C and incubated for their respective incubation periods. *L. innocua* incubated for 24-28 hours and *M. luteus* incubated for 48-72 hours. These bacteria were then then transferred to 30 mL of TSBYE (tryptic soy agar broth with yeast extract) and incubated a second time. Both bacteria were propagated twice. The second set of fresh TSBYE was used for the working culture.

Film squares (1/2" or 12.7 mm) were cut from the rolls of film produced during the trial using a ½ inch sample cutter. Control (n = 20) and Treatment (n=20) film

squares were cut for each bacterial type resulting in 80 film pieces total or 40 film on lawn plates containing both control and treatment films.

Film on lawns were conducted by dipping a sterile swab into the working culture and swabbing the entire surface of the agar in the Petri dish. Treatment and control film samples were then faced coating side down onto the inoculated surface and incubated upside down for the correct time for each bacterial type. Zones of inhibition were then measured in both vertical and horizontal directions and averaged. Zones were measured using a digital caliper. Dilution plates were produced to determine the bacterial population of the working culture. The location of each film sample (operator, center and machine side) was also recorded to determine if there were any inconsistencies in the coating process that could effect achieved inhibitory properties. (n=40) A diagram example of a film on lawn is shown below in Figure 4.11.

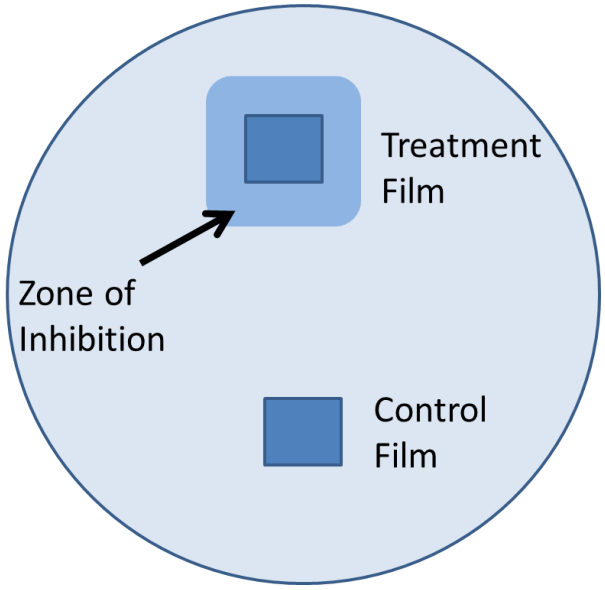

Figure 4.11. Diagram of film on lawn example.

Block testing

Block testing was conducted on both control and treatment rolls produced from the coating trials. (n = 40; 20 per treatment) The blocks depicted in Figure 4.12 were produced at Bishop Branch Machine Work in Pendleton South Carolina according to the specifications in ASTM D3354-15: Standard Test Method for Blocking Load of Plastic Film by the Parallel Plate Method [4]. The blocks in Figure 4.12 were (4 in^2 surface area) of aluminum fitted for the SATEC T10000 Materials Testing System (Instron, Norwood, MA, USA).

Film samples approximately 4.5 in^2 in area and 2 layers in thickness were cut from the roll noting the film sample location: machine or operator side. These samples were left to condition for 40-48 hours as noted in the ASTM standard. A knife was used to separate the edges of the top film from the bottom film. The bottom layer of the film sample was then attached to the lower block using tape. The lower block was then inserted into the Instron and the top block was lowered as close as possible in position to tape the top layer of the film to the top block without causing the two layers to separate. Figure 4.13 shows the sample set up (left) and Instron apparatus (right). Once the film was loaded, the load was balanced and the gauge length was reset (for each sample) in order to calibrate the Instron Bluehill tensile testing software (Norwood, MA, USA) prior to testing. The testing procedure utilized from ASTM D3354-15 followed the constant rate of separation procedure. The blocks were separated at a rate of 0.2 inches per minute (5.1 mm per minute). Max separation was set to 0.75 in (1.9 cm). The max force (gf) for

separation of the film layers was recorded into addition to the thicknesses in triplicate of each film layer.

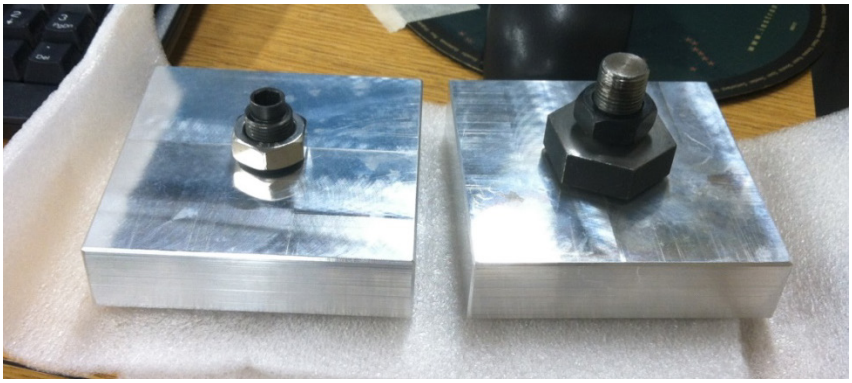

Figure 4.12. Aluminum blocks produced for block testing.

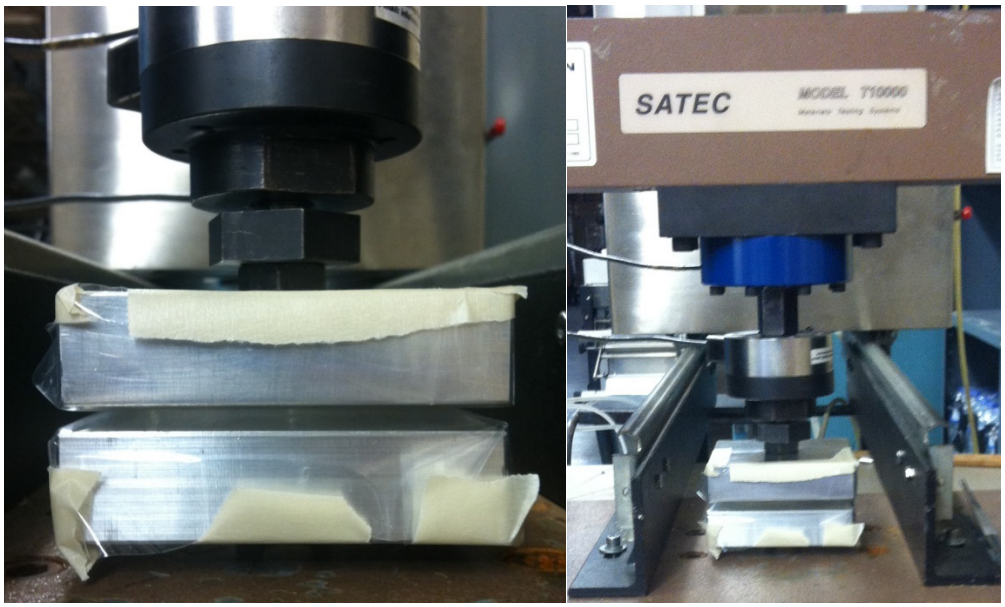

Figure 4.13. Block test in progress (left) and Instron set up (right).

Statistical Methods

All statistical analyses was conducted using SAS® Studio (SAS® OnDemand for Academics) Each of the following data sets were analyzed based on the following list of factors. A *P* value of ≤ 0.05 was considered for statistical significance. All samples were tested with at least 3 replicates.

Factorial analysis was conducted on coating type , time and to determine any significant coating type-time interactions for viscosity, percent solids and pH tests.

Factorial analysis was also conducted on coating type and sample location to determine any significant coating – location interactions for basis weight, haze and blocking tests.

Film on lawn: An exact chisquare test was used to test whether the likelihood of the inhibition zone being larger for the treated sample than the control sample differed by location. Because location was not found to have a significant impact on the likelihood of the inhibition zone being larger for the treated sample than the control sample, a sign test was used to test whether the treated sample was more likely to have a larger inhibition zone than the control sample across all locations.

RESULTS: GRAVURE TRIAL

Coating Film Quality

The produced coated films, as depicted in Figure 4.7 appeared to be in good quality condition. During sample preparations for further testing, it was discovered that the applied coatings were not adhering to the film substrate as predicted. Preliminary

testing utilizing handmade drawdowns indicated that the primer and coating combination would result in sufficient adhesion as to survive a standard ASTM tape test (ASTM F2252) [2]. The films resulting from the gravure trial produced coated films that would lose coating upon unrolling film too quickly by hand.

The material was also unable to be sealed. The dominate mode of failure was either a peelable seal or an adhesive mode of failure. Both of these complications including trouble-shooting are to be further discussed in the discussion section.

Viscosity

The viscosities (n=12) of control and treatment coatings were tested using a Zahn #3 cup. There was a significance difference between the time measurements recorded for control and treatment coating types. (P<0.0001) There was also a significant difference in the viscosities recorded before and after the trial for the treatment coating (P=0.0011), but not for the control coating. (P=0.3053) The average viscosity measurement for the control coating before the trial was 21.53 seconds and 22.06 seconds afterwards. The average viscosity measurement for the treatment coating was 20.10 seconds before the trial and 17.67 seconds afterwards indicating that the coating became thicker during the manufacturing process.

Percent solids

Percent solids measurements recorded from the liquid coating types (n=60) showed that there was no significant difference between measurements taken at varying

times nor were there any coating/time interactions. An overall difference was found between the percent solids of the control and treatment coating types. (P=0.0002) The control coating had an average of 18.73% solids content while the treatment yielded an average of 20.67% solids. This was expected as the treatment coating contained all ingredients from the control coating plus powdered antimicrobial mixture, Nisaplin®.

pH

There was a significant difference in the pH (n=11) values of control and treatment coating solutions. (P<0.0001) The average pH for the control coating was slightly acidic at 6.47 while the treatment coating was slightly more acidic at 5.96.

Basis Weight

Basis weights (n=42) of the coated film material were taken from material that had not been primed for ease of coating removal. There was no significant difference in coating laydown found between coating types (P=0.7041), location of sample (P=0.3681) or coating type/location interactions (P=0.5415). The average control coating weight was found to be 1.50 #/ream (2.44 gsm) and the average treatment coat weight was found to be 1.48 #/ream (2.41 gsm).

Haze (ΔE)

The haze was calculated for 40 measurements taken from control and treatment coating coated film samples. There was found to be no significant different in haze measurements for all variables tested: coating type (P= 0.8675), location (P = 0.0693)

and treatment/location interaction (P=0.1387). The average haze for control coated films was found to be 0.16 and treatments exhibited an average of 0.15.

Block Testing

Block testing results showed that there was no significant difference in the blocking tendencies between coating type (P=0.2210), location (P=0.4802) or coating/location interactions (P=0.9158). The coefficient of variation for this set of testing was well above the 10% standard at 25.78%. The control coated films averaged 290.60 gf while treatment coated films averaged 321.35 gf. (n=41)

Film on Lawn

Two bacterial strains were testing using the film on lawn technique. (n=21 per bacterial strain) No statistics were calculated for results from *L. innocua* samples due to lack of inhibition against a bacterial culture grown to 10^9 CFU/mL.

The working culture of *M. luteu*s was grown to 10^7 CFU/mL. A significant difference was found for control and treatment film samples tested against *M. luteus.* (P<0.0001) An average inhibition zone for treatment samples exceeded the ½" (12.7 mm) film perimeter by 5.78 mm. Images of bacterial film on lawns are displayed in Figure 4.14. Results for all testing previously mentioned can be seen in table 4.4 below.

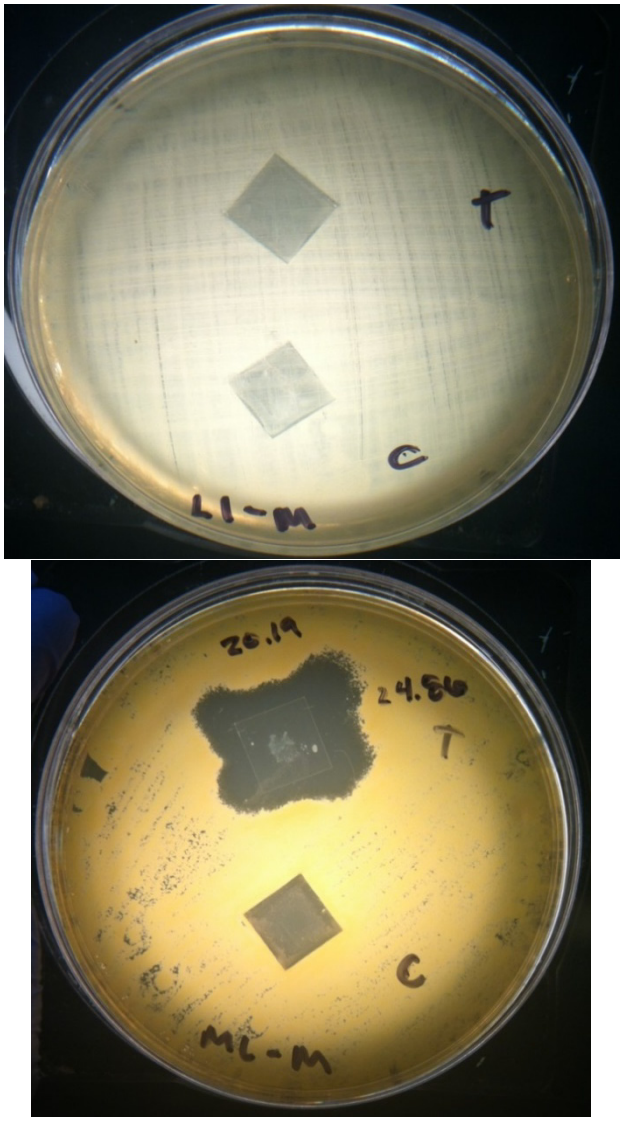

Figure 4.14. Film on lawn images for treatment and control coatings produced during gravure trial tested against *Listeria innocua* ATCC 33090 (left) and *Micrococcus luteus* ATCC 10240 (right).

Table 4.4. Summary of results for coatings and materials produced from gravure trial.

Gravure trial testing summary results for coatings and coated films			
	Control Film	**Antimicrobial Coated Film**	**P values (α = 0.05)**
Solids content (%) *(n = 60)*	18.72±0.69	20.67±2.55	0.0002
Viscosity (sec) *(n = 12)*	BEFORE – 21.53±0.86 AFTER – 22.06±0.41	BEFORE – 20.10±0.72 AFTER – 17.67±0.12	0.0011 (treat*time)
pH *(n = 11)*	6.47±0.03	5.96±0.02	<0.0001
Basis Weight (#/ream) *(n = 42)*	1.50±0.13 (2.44±0.21 gsm)	1.48±0.20 (2.41±0.33 gsm)	0.7041
Block testing (gf) *(n = 42)*	290.60±94.86	321.35±52.89	0.2210
Haze (ΔE) *(n = 40)*	0.16±0.09	0.15±0.06	0.8675
Film on lawn (mm) *M. Luteus (n = 21)*	0±0.0	5.78±2.20	<0.0001

DISCUSSION – GRAVURE TRIAL

Coating Film Quality

Adhesion failure can be defined as "delamination of a coating from its substrate". (Mills 2012) Upon discovery of coating adhesion failure, several measures were taken to troubleshoot the problem. Several possible problems included:

- Excessive corona treatment
- The coating was not fully dry
    - "skinning"
- Poor primer application due to coating not drying

**Note: The adhesion failure could have also accounted for sealability issues

In order to double check the corona treatment, tape was used to remove the peelable coating. Accudyne pens were used to check the surface treatment of the film. The interior surface of the film with the coating removed was approximately 60 dynes/cm. It was possible that the primer, assuming it had been applied properly, still remained on the film after removing the coating yielding the high critical surface tension. This however, would not have been a problem. On the other hand, if there was no primer on the surface, this would lead to other potential issues resulting from excessive corona treatment. There was also the possibility that the coating formulation itself was causing heat sealing and adhesion difficulties. Heavy oil based components such as glycerin, the plasticizer component, or Tween®80, the surfactant component, could have migrated to the surface of the LLDPE sealant producing an oil-like weak boundary layer between the coating and primed substrate.

Seal testing was also conducted with the material mentioned above (coating removed). The sealing range tested was 250-350°F (the original heat seal range specification of the Sealed Air material was 240-356°F). Temperatures at or above 350°F resulted in wrinkles in the PET exterior layer of the material. Pressures of 30 and 40 psi (3/8" seal bar) were also tested in addition to increased dwell times up to 2 seconds. The primary mode of failure for the seals was a peelable seal with predominately adhesive mode of failure.

Some questions had risen from the basic sealing testing such as "is the primer sealable?" if so, it was also possible that the film had been excessively corona treated

which can be found to essentially degrade the sealability of such PE (polyethylene) sealants. (Personal communication with Duncan Darby) Corona discharge treatment increases the surface wetting tension of a surface by bombarding a film surface with ionized air which oxidizes the surface of a film. Excess treatment can result in production of nonpolar ether groups on the film surface [20] or fracturing of the film surface causing a reorganization of the polymer chains making them unavailable at the surface [10]. However, the more likely cause of sealing difficulties was overtreatment causing the LLDPE to crosslink resulting in a higher molecule weight, decreased polymer chain mobility and increased melt temperature of the polymer [9; 20].

A third concern was that the coating may not have been dried thoroughly therefore a layer of wet coating was inhibiting adhesion. This is a film converting defect commonly referred to as “skinning” in which the surface of the coating is dried, but the lower portion of the coating remains wet. This is more common with thicker coatings and associated with user higher drying temperatures in order to compensate for the increased coating laydown. Although the film appeared dry to the touch, gas chromatography methods such as retained solvent would need to be conducted to confirm such a hypothesis. The coating was found to be slightly tacky, after drying during the trial however this was ignored as preliminary drawdowns were also slightly tacky after drying. In order to investigate how well the coating dried, retained solvents on drawdowns were tested courtesy of Printpack, Inc. Analytical Services. Liquid coating samples were sent to Printpack Analytical Services in Villa Rica, Georgia. Drawdowns were produced using a Mayer Rod #16 as indicated from previous work. The samples

were then dried either overnight or in a 160°F oven for approximately 10 seconds. The results were as follows:

Table 4.5. Retained solvent levels of ethanol in antimicrobial coated hand drawdowns.

Sample	Ethanol Level (mg/ream)	Ethanol level (mg/m^2)	Ethanol Level (ppm)
Ambient dry 1	9	0.032	13.2
Ambient dry 2	12	0.043	17.6
Oven dry 1	24	0.086	35.3
Oven dry 2	21	0.075	30.9

*Note: parts per million (ppm) calculated using approximate basis weight value of 1.5 pounds per ream

Sample conversion

Ambient dry 1= 9 gm/ream of ethanol
1 kg = 2.2 pounds (#)
1 ppm = 1 mg/kg

$$1.5\ \frac{\#}{ream} * \frac{1\ kg}{2.2\ \#} = 0.68\ kg\ of\ coating/ream$$

$$\frac{9\frac{mg}{ream}}{0.68\ \frac{kg}{ream}} = 9\frac{mg}{ream} \times \frac{1}{0.68\ \frac{kg}{ream}} = 13.2\ \frac{mg}{kg}\ or\ ppm$$

The results indicate higher retained solvents within the samples that were oven dried at conditions to simulate the gravure trial rather than dried at ambient conditions overnight. This may be due to the ethanol becoming trapped in the coating matrix during the short drying process. Retained solvents are an important aspect in food packaging because they can be indicative of drying issues and high concentrations of retained solvents can result in off odors, flavors or other interactions within packaged food

products. This can cause undesirable food quality and safety issues [5].The particular solvent used in this packaging system, as indicated in table 4.5 is ethanol. Ethanol is commonly used in the package converting industry as a solvent and is also GRAS approved as a food additive on pizza crusts as an antimicrobial prior to baking. (US FDA CFR 21 Section 184.1293) [19]. The detection threshold for ethanol odor is relatively high compared to other solvents. Humans can detect solvents at levels of 1-100 ppm [5] however; these levels can vary depending on the solvent [5; 13].The acceptable level of solvent retained in a packaging system is determined on a case by case basis and can vary by company, product and package type [5]. Although there is no set standard for this packaging system, values presented in Czerny et al (2008) indicated that the values in table 4.5 are below literature values for odor detection for ambient dried samples and in the low end of the detection threshold for the oven dried samples [8]. Threshold ranges were found to be in the 25 – 900 ppm range [8].

Lastly, contact was made with technical representative, Rob Hammond, from MICA Corporation to get a better understanding of the primer that was used during the trial and troubleshoot adhesion difficulties. The discussion produced several conclusions. During the trial, a 200 LPI Quad gravure cylinder was used for the application of the primer. Although this was the smallest cell cylinder available to be used on the gravure coater in the DuPont laboratory, it was pointed out that this particular size cylinder was laying down an excessive amount of primer. The percent solids of the primer solution averaged approximately 0.5%; therefore the 200 LPI cylinder was delivering a high wet weight which was unable to fully dry. Because of this, it is possible that the primer was

re-solubilizing in itself. If any primer was actually laid down and dried onto the LLDPE substrate, it is also possible that the water component of the antimicrobial coating was essentially removing the remainder of the primer. It was recommended that a 400-600 LPI cylinder or a coating laydown of no more than 0.5 #/ream be used for the primer application.

Coating and Film Characterization Discussion

Viscosity and Percent Solids

As expected, the addition of Nisaplin® in the treatment coating produced an increase in the Zahn cup time measurements indicating an increase in viscosity. This resulted in a significant difference between freshly made control and treatment coatings. (P=0.0197) There was a slight increase in the control coating Zahn measurement after the trial however this was not significantly different from the measurements taken before the trial. On the other hand, there was a significant decrease in the Zahn measurements for the treatment coating. The time measurements decreased from 20.10 sec to 17.67 sec after the trial. This could indicate that the gravure cylinder was preferentially picking up solids within the coating due to either attractive forces or that the coating needed more mixing before or slight agitation during the trial. Because the solids within the coating were being removed at a higher rate than the solvents, the resulting viscosity was lower after the trial.

The percent solids of the treatment liquid coating solution was higher (20.67%) when compared to the control (18.72%) as expected with the addition of Nisaplin® to the treatment. (P=0.011) Measurements were taken after coating product, before the trial and

directly after the conclusion of the trial, however, there was no interaction of the percent solids with time. The storage method and short length of time for the trial (1 hour per coating treatment) was not long enough for enough for solvent evaporation to have an effect on the resulting percent solids of either coating type.

The solids content and viscosity of the coatings that were produced are important aspects regarding coating application selection. Coatings with very high solids and thick viscous properties could require multi roll metering systems in order to apply the desired amount of coating to a substrate. Very low solids and low viscosity coatings can be applied using applications that require the coating to spread out over the surface after application such as Mayer rod or air knife coating applications. The percent solids for the treatment (20.67%) falls within the required range of 15-40% [17] solids to be readily used in either flexography or gravure type processes.

pH

The average pH measurements of the control and treatment coatings were both slightly acidic. The treatment coating (pH 5.96) was slightly more acidic than the control (pH 6.47) control coating. Utilization of acidic coatings on production equipment can degrade metal or polymer parts and tubing. It is recommended that thorough cleanings be implemented as a part of the manufacturing processes and to potentially implement corrosion resistant doctor blades, tubes and cylinders to account for the acidity and corrosiveness of the coatings.

Basis weight

There was no significant difference in the coating laydown between the control (1.50 #/ream) and treatment (1.48 #/ream) coatings. This was expected as the gravure cylinder wells hold a specified volume of coating and the Nisaplin® added was no enough to cause a significant change in coating laydown using this process. ($P = 0.7041$) For a direct gravure coating method, the coat weight is determined by the volume of the gravure cylinder wells and coating solids [12]. The same cylinder was used for both coatings and the coating solids were not significantly different enough to cause a significant effect in the coating laydown. The laydown did not significantly vary across the width of the machine. This coating laydown is also within the normal range for coat weight used in industrial applications (up to 4#/ream) (Personal communication with Dr. Duncan Darby)

Block Testing

Some degree of blocking was expected due to tackiness and blocking of drawdowns during preliminary work. The results from the block tested on average exceeded the limit of 200 gf state in the ASTM D3354 standard. There was no significant difference between the average blocking force measurements between the control (290.60 gf) and treatment (321.35 gf). ($P = 0.2210$) This could have been related to the dry ability of laying down 1.5 #/ream coat weight and the drying capacity of the tunnel dryers for this particular gravure system. There was a large degree of variation between the samples tested yielding standard deviations of ±95 grams for the control samples and ±53

grams for the treatment coated samples. This high degree of variation could have been due to the homogeneity of the homogeneity of the coating on the film. This could potentially be investigated in future work using atomic force microscopy or another topographical type microscopy method. It is possible that point of lower blocking could have been due higher concentrations of glycerin or Tween® 80 (oily components) in a particular area versus areas with higher amounts of polyvinyl alcohol.

Haze (ΔE)

According to haze testing standard, ASTM D1003-13 ASTM, haze is defined as "…the scattering of light by a specimen responsible for the reduction in contrast of objects viewed through it" [3]. Without a hazemeter, the measure of the difference between two colors can be calculated using a colorimeter by calculating ΔE from the equation listed in the procedure. The results showed that ΔE calculations for control (ΔE = 0.16±0.09) and treatment (ΔE = 0.15±0.06) were not significantly different. (P = 0.8675)

Values for ΔE of 1.0 or greater are changes in color difference that are perceptible to the human eye [18]. Therefore the results indicated that the differences in the haze between coated and uncoated films for both control and treatment films were imperceptible to the human eye. The amount of nisin added to the treatment coating was not a large enough amount to cause a significant discoloration in the coated film regardless of the liquid coating's brown appearance.

Film on Lawn

No inhibitory properties were achieved against *L. innocua* (ATCC 33090). This was expected based on the minimum inhibitory concentration (MIC) that was determined in a previous study (100 IU/mL) and some materials balance calculations that can be found in appendix B. The activity of Nisaplin® in 1 cm^2 of film was calculation to be approximately 15.8 IU/cm^2 using the formulation for this trial and based on a 1.5#/ream coating weight. The material however, was effective against spoilage microorganism *M. luteus* (ATCC 10240). The minimum inhibitory concentration for *M. luteus* because zones of inhibition had been achieved throughout the studies and preliminary work, however, literature values for MIC have been found to vary due to procedure, media and laboratory. Chandrasekar, Knabel and Anatheswaran (2015) found the MIC of nisin against *M. luteus* (ATCC 10240) to be 0.156 μg/mL or 6.24 IU/mL when using pure nisin [7]. Materials balance calculations shown in Appendix B, estimated that the material contained about 0.006 gram of Nisaplin® for 1 #/ream coat weight and 0.0114 grams of Nisaplin® for a 1/5 #/ream coat weight of the formulation produced. From this, it was estimated that approximately the coating contained 12.97 IU/cm^2. Because thickness measurements were unable to be accurately measured, an estimate of IU/cm^3 cannot be calculated for comparison. (See Thickness section in Appendix B) Regardless, it is assumed that the estimated level of antimicrobial activity contributed to the inhibition of *M. luteus*.

The results of this testing also showed that not only did the treatment samples containing nisin inhibit *M. luteus* but also the control samples. There were no inhibition

zones extending further than the perimeter of the control films, however, there was no growth underneath the control film samples. This could have been due to potential effects from the other ingredients within the coating. Polyvinyl alcohol is a highly swellable polymer. It may be possible that the polymers swellability when put in contact with moisture was desiccating or drying out the bacteria. Other ingredients within the coating such as ethanol and acetic acid solution were expected to evaporate during the process leaving residual or trace amounts in the dry film. Retained solvents testing showed that miniscule amount s of ethanol remained in the film after drying. These amounts were much lower than common materials produced in industry which are not considered antimicrobial films. Glycerin and Tween®80 are both not considered to be inherently antimicrobial. Specifically non-ionic surfactants however have been shown to displace proteins and fats in order to antimicrobials to reach the targeted microorganisms [6].

Potential solutions for second trial

Reduction of the corona treatment would be necessary for the second trial to prevent crosslinking of the sealant web and decreasing sealability. Based upon the troubleshooting conducted, feasible solutions were proposed for a second trial. Clemson University has the capability of engraving copper cylinders for preliminary coating/printing work intended for single time use. One solution proposed was to have a cylinder engraved using the recommended LPI specifications of 400 – 600 LPI and conducting a second trial using the same gravure coater/laminator. A second solution in attempts to ensure a sealable material would be to run the material in three passes to add primer, followed by a heat seal coating and the antimicrobial coating. Cylinder engraving

could be used for this solution in order to product a patterned cylinder if the desired coating process was gravure, however, it would be more economically feasible to use a photopolymer printing plate and flexography process due to the expense of gravure cylinders. This would enable the heat seal coating to only exist around the edges of the packaging material or the material area intended to be sealed. Figure 4.15 show a potential solution for producing heat sealable antimicrobial coated material without coating adhesion problems.

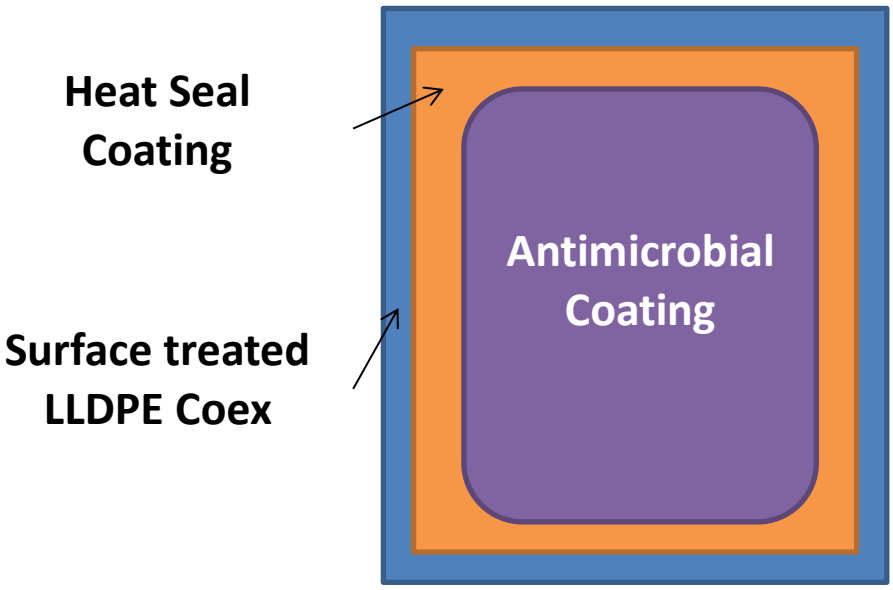

Figure 4.15. Proposed solution using a patterned gravure cylinder or flexography plate.

It was later found that the Sonoco Institute of Packaging Design and Graphics had print cylinders that met the parameters suggested for priming. Using a flexography application would also provide a fast and cheap way to produce a patterned cylinders to coat registered heat seal and antimicrobial coatings shown in figure 4.15 using photopolymer plates.

MATERIALS AND METHODS: FLEXOGRAPHY TRIAL

Coating Preparation

All coating preparations were done using the same procedure as described during the gravure trial however small volumes were produced and the Nisaplin® concentration was increased to account for expected decreased coat weights. Batches of antimicrobial coating (1.5 batches or 2.625 L) were produced using double the amount of Nisaplin® totaling 75 grams for 1.5 batches produced. The same volume was produced of the control coating which did not contain Nisaplin®. Approximately 2 liters of MICA A-131-X or PEI primer was diluted (1:9) with distilled water the morning of the trial.

Coating Trial- Flexography

The same film material donated from Sealed Air Corporation, 2.5 mil LLDPE Coex (H7225B Top non-forming web), was utilized for both the gravure trial and the flexography trial. This material was slit to 14.5" web width and contained approximately 1000 feet of material. A stronger leader material was added to the roll to avoid any wrinkling or breakage that can occur with corona treatment. Approximately 660 feet of 4.5 mil Alox/BoN/CPP (Aluninum oxide coated biaxially oriented Nylon laminated to a crystalline polypropylene) film was added to the front of the roll and 150 feet at the end.

Originally, the concept depicted in 4.15, was to be trialed, however, heat seal coating that was donated for the trial did not arrive in time therefore the trial was to continue without it. The goal of the second trial was to solve the coating adhesion issues discovered after the first trial. The second trial was run using the OMET 530 VaryFlex pictured in figure 4.16 which contained an inline corona treater and 7 coating stations

using a two roll and reversed angle composite doctor blade metering system. (Figure 4.17 & 4.18) The corona treater and last two coating stations were used during the trial. The first coating station was the primer station and the second station was for the control or antimicrobial coatings.

Figure 4.16. OMET 530 Vary Flex Flexography press.

Figure 4.17. Uncoated web at the unwind station (left) moving into the corona treater. (right)

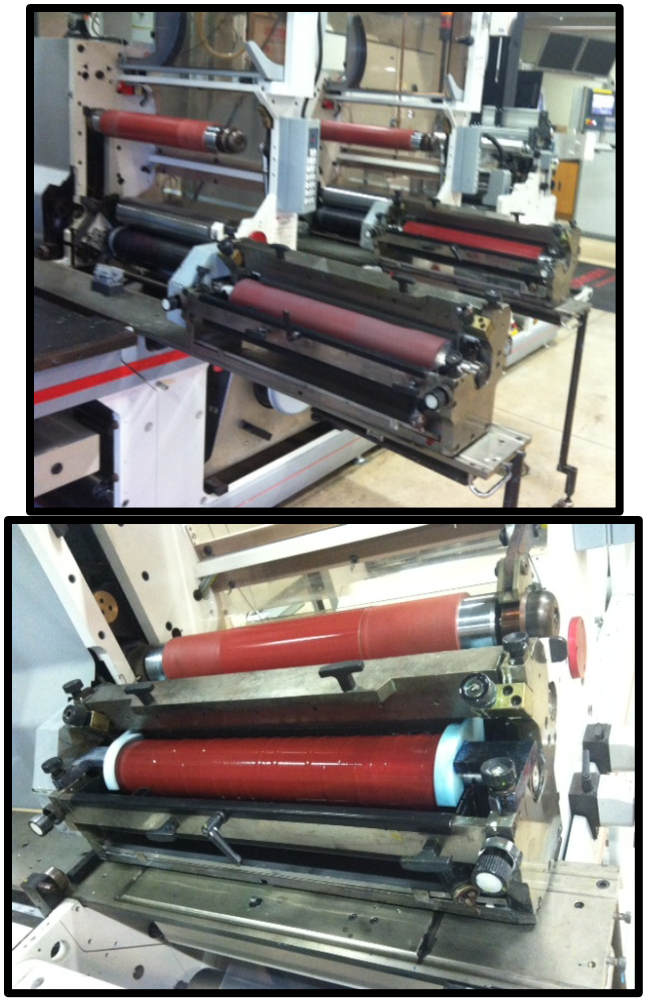

Figure 4.18. Unassembled priming and coating flexography stations (left). Control coating loaded into coating station. (right)

At the start of the trial, the leader material was laced through the press in order to dial in the machine to the desired parameters. Once the unwind had reached the test material, the corona treatment level needed to be determine. The surface tension of the LLDPE coex was approximately 30-32 dynes prior to corona treatment. Several attempts were taken to determine the treatment level using Dyne pens to achieve a treatment level

of 36-37 dynes/cm. The corona treater was then set to 1500 watt*min per m^2 while running at a line speed of 32 feet per min.

The priming and coatings stations contained rubber rollers to flood coat the material. The full web width of the material was not coated in order to avoid extra cleaning for the associates assisting with the trial. The anilox roller used in the priming station was a 5.0 BCM volume (billion cubic microns per square inch), 500 cells per inch cylinder with cells at a 60° angle. The coating station anilox roller was originally a 30 BCM roller. This roll was chosen in order to lay down approximately 1.5#/ream to stay consistent with the coating laydown achieved in the gravure trial. However, the press station hot air dryers were unable to dry off the large volume of solvents even after increasing the dryer temperature from 155 to 175°F. The 30 BCM anilox was then removed and replaced with the next highest volume anilox at 15.2 BCM, 160 CPI to lay down less coating and achieve drying. During the trial, approximately 20 feet of control coated and treatment coated but un-primed material was removed for basis weight testing. Flags were also used to indicate material that was primed and coated for testing. Press parameters can be viewed in Table 4.6.

In total, two rolls of material were produced (control and treatment) during the trial. Approximately 150 of coated material was produced on the control roll and less than 500 feet on the treatment roll. The rolls were stored on end to avoid blocking. (Figure 4.19)

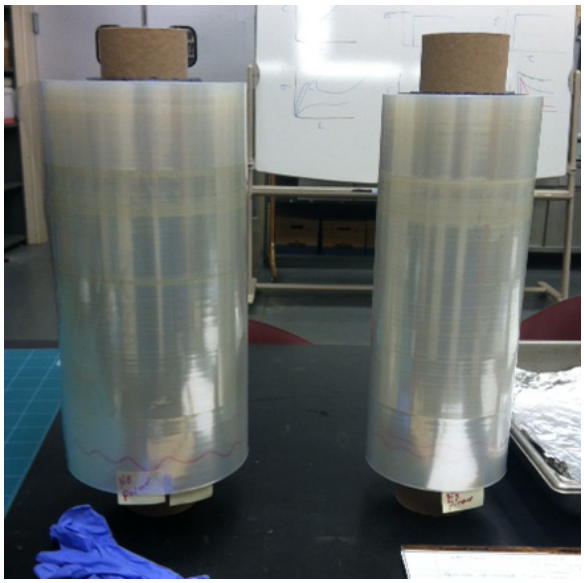

Figure 4.19. Rolls of coated material produced during flexography coating trials.

Statistical Methods

All statistical analyses was conducted using SAS® Studio (SAS® OnDemand for Academics) Each of the following data sets were analyzed based on the following list of factors. A *P* value of ≤ 0.05 was considered for statistical significance. All samples were tested with at least 3 replicates.

Factorial analysis was conducted on coating type , time and to determine any significant coating type-time interactions for viscosity, percent solids and pH tests.

T-tests were conducted to compare treatment and control coated materials for basis weight, haze and block testing samples.

Film on lawn: A sign test was used to test whether the treated sample was more likely to have a larger inhibition zone than the control sample.

Table 4.6. OMET VaryFlex 530 press parameters for control and antimicrobial coatings to LLDPE Coex material.

Conditions of OMET VaryFlex 530 Press in Sonoco Institute of Packaging Design and Graphics for Control and Treatment Antimicrobial Coatings			
Sample	Primer	Control	Treatment
Primary unwind material	4.5 mil Alox/BoN/CPP/2.5 mil LLDPE Coex/ 4.5 mil Alox/BoN/CPP		
Coat side	Out	Out	Out
Tension (1° UW) (daN)	13.8	13.8	13.8
Web width (inches)	14.5	14.5	14.5
Rewind coat side	Out	Out	Out
Tension (rewind) (daN –dekanewton)	15.8	15.8	15.8
Coater anilox	5.0 BCM, 500 CPI, 60°	15.2 BCM, 160 CPI, 60°	15.2 BCM, 160 CPI, 60°
Coating	MICA A-131-X (PEI) primer	Control coating (*no Nisaplin®)	Antimicrobial coating
Station Dryer temperature (°F)	155	175	175
Line speed (ft/min)	32	32	32

** Percent solids, pH, viscosity, basis weight, thickness, haze, blocking and film on lawn testing were conducted using the same procedures described in the gravure trial.

RESULTS: FLEXOGRAPHY TRIAL

Coating Film Quality

The film produced from this trial did not exhibit the adhesion difficulties like that of the material produced during the gravure trial.

Viscosity

The viscosities (n=12) of control and treatment coatings were tested using a Zahn #3 cup. Measurements were taken just prior to and after the trial had been completed. For the control coating, the Zahn measurements average 23.49 sec before the trial and 29.99 sec after. The treatment coating averaged 25.27 sec before and 29.76 sec after. Both showed increases in the viscosity measurements after the trial was completed. ($P<0.0001$) A significant difference was also found between control and treatment measurements before the trial from the fresh prepared coating. ($P=0.0131$)

Percent solids

The percent solids measured were significantly different between the control and treatment coating types. The control coating resulted in an average solids content of 18.72% and the treatment coating was 23.05% solids. ($P <0.0001$) Statistical analysis showed that there was a significant interaction for each coating*time interaction between percent solids measured before and after the coating trials. (P 0.0060) The average solids content increased from 17.81% to 19.63% (control) and 22.54 to 23.57% (treatment).

pH

There was a significant difference in the pH (n=6) values of control and treatment coating solutions. (P<0.0001) The average pH for the control coating was slightly acidic at 6.42 while the treatment coating was slightly more acidic at 5.61.

Basis Weight

Basis weights (n=42) of the coated film material were taken from material that had not been primed for ease of coating removal. There was a significant difference in coating laydown found between coating types (P=0.0001). Location of the sample and location*coating interactions were not tested during this set of data due to lack of significance previously observed testing accuracy of equipment. The average control coating weight was found to be 0.64 #/ream (1.04 gsm) and the average treatment coat weight was found to be 0.74 #/ream (1.20 gsm).

Haze (ΔE)

The haze was calculated for 40 measurements taken from control and treatment coating coated film samples. The average haze for control coated films was found to be 0.18 and treatments exhibited an average of 0.15. No significant difference was found between the haze of each coating treatment. (P=0.2887)

Block Testing

Block testing results showed that there was no significant difference in the blocking tendencies between coating type (P=0.9831). The coefficient of variation for this set of testing was well above the 10% standard at 18.54% (control) and 35.16% (treatment). The control coated films averaged 179.42 gf while treatment coated films averaged 179.08 gf. (n=40)

Film on Lawn

Micrococcus luteus ATCC 10240 was the only bacterial strain tested against this material using the film on lawn technique. (n=19) This material was not tested against *Listeria innocua* ATCC 33090 due to the decreased basis weight and it was later calculated that the MIC of *L. innocua* (100 IU/sq.cm) could not be achieved in 1 sq.cm of coated material.

The working culture of *M. luteu*s was grown to 10^7 CFU/mL. Film samples were tested against films that had been stored for 30 days at ambient conditions. A significant difference was found for control and treatment film samples tested against *M. luteus.* (P<0.0001) An average inhibition zone for treatment samples exceeded the ½" (12.7 mm) film perimeter by 3.60 mm. A summary table of these results can be seen in Table 4.7 below.

Table 4.7. Summary of flexography trial testing results for coatings and coated films.

Flexography trial testing summary results for coatings and coated films			
	Control	**Antimicrobial**	**P values (α = 0.05)**
Solids content (%) *(n = 60)*	AVG 18.72±1.15 Before 17.81 After 19.63	AVG 23.05±0.59 Before 22.54 After 23.57	AVG <0.0001 Time interaction 0.0060
Viscosity (sec) *(n = 12)*	BEFORE – 23.49±1.06 AFTER – 29.99±0.75	BEFORE – 25.27±0.30 AFTER – 29.76±0.36	<0.0001 – B&A 0.0131 - B, C&Trt
pH *(n = 6)*	6.42±0.02	5.61±0.02	<0.0001
Basis Weight (#/ream) *(n = 42)*	0.64±0.07 (1.04±0.11 gsm)	0.74±0.08 (1.20±0.13 gsm)	0.0001
Block testing (g/f) *(n = 40)*	179.42±33.27	179.08±62.96	0.9831
Haze (ΔE) *(n = 40)*	0.18±0.07	0.15±0.07	0.2887
FOL (mm) *M. Luteus (n = 19)*	0±0.0	3.60±1.36	<.0001

DISCUSSION – FLEXOGRAPHY TRIAL

Viscosity

The amount of Nisaplin® in the treatment coating was doubled in order to accommodate for the expected decrease in coating weight application expected using flexography. The additional Nisaplin® produced increased Zahn cup times compared to the values in the gravure trial. Within the flexography trial, the Zahn cup values increased over time. The control coating increased from 23.49 sec to 29.99 and the treatment coated increase from 25.27 to 29.76 sec. The increases in measurements before and after the trial

were found to be significantly different. ($P < 0.0001$) This could have been due to solvent evaporation from the increased agitation of using a two roll metering system while the gravure system transferred coating directly from the gravure cylinder. The flexography system has an addition metering roll because the coating is transferred from an anilox roll to a plate cylinder which transfers the coating to the substrate. The treatment coating also had higher Zahn cup measurements than the control ($P = 0.0131$). This was expected due to the presence of antimicrobial solids in the treatment coating.

Percent solids

The results indicated that there was a significant difference between the average percent solids of the control formulation (18.72±1.15%) and treatment coating formulation (23.05±0.59%). ($P <0.0001$) This was expected due to the addition of the Nisaplin® component to the treatment coating. Both the control and treatment coatings exhibited coating*time interactions (P 0.0060) meaning that the coating type and time the measurement was taken (before and/or after the coating trial) interacted. The control had an average percent solids measurement of 17.80% prior to the trial and increased to 19.63% after the trial. The average percent solids of the treatment also increased from 22.53% to 23.57%. This may be due to solvent evaporation during the coating process. The control coating may have evaporated slightly more than the treatment coating due to the amount of time for equipment set up while the coating was in the coating station.

pH

The pH of the liquid coatings used in the flexography trial was also slightly acidic. The average pH of the treatment coating (pH = 5.61) was significantly lower than that of the control coating (pH = 6.42). (P <0.0001) This was due to an increased volume of acetic acid solution that was added to the coating in order to compensate for the addition of extra Nisaplin® in the solution. Although the coating is only slightly acidic, protective measures should be taken to prevent or decrease corrosion of printing press parts such as corrosion resistance or coated parts.

Basis weight

The coat weight of the material produced was approximately half the desired coat weight. It was estimated that the 30 BCM anilox would produce 1.5#/ream coat weight; however, due to drying difficulties the anilox roll was changed to a 15.2 BCM anilox for the remainder of trial. The material produced by the 15.2 BCM anilox was used as the test material. A significant difference was found between the coating laydown of the control (0.64 #/ream) and treatment (0.74 #/ream) coatings. (P = 0.0001)This may have been due to differences in the critical surface tensions of the control and treatment coatings and how the coatings interacted with the anilox rolls that had a critical surface tension of 21.6 dynes/cm. The control coating may have had more of an affinity for the anilox roll therefore less coating was put onto the substrate. It is also possible that the higher solids content in the treatment coating also increased the laydown of the coating during the process.

Block Testing

The control coated films averaged 179.42 g/f while the treatment coated films averaged 179.08 g/f. There was no significant difference between the average blocking values of the two coated materials. (P = 0.9831) These average values were below the 200 g/f threshold indicated in the ASTM standard that was followed to conduct the set of testing. Although the average values were below 200 g/f, there was a high degree of variation as indicated by the calculated standard deviations. (Control ± 33.27 g/f; treatment ± 62.96 g/f) The calculated coefficient of variation showed 18.54% variation for control coated samples and 35.16% variation for treatment coated samples. These coatings resulted in lower average blocking compared to the gravure coated materials. The lower degree of tackiness may have been due to the decreased coating laydown and potentially increased dryability of the coating.

Haze (ΔE)

As indicated in the results, the average ΔE for both the control (ΔE=0.18±0.07) and treatment films (ΔE=0.15±0.07) were not significantly different. (P = 0.2887) Like the results from the gravure trial, these films also indicated that the coating did not produce a perceptible difference between the coated and uncoated films because ΔE values were less than 1.0 [18].

Film on Lawn

Micrococcus luteus ATCC 10240 was the only bacterial strain tested against this material using the film on lawn technique. (n=19) This material was not tested against

Listeria innocua ATCC 33090 due to the decreased basis weight and it was later calculated that the MIC of *L. innocua* (100 IU/sq.cm) could not be achieved in 1 sq.cm of coated material. (See Appendix B, Minimum Inhibitory Concentration testing)

The working culture of *M. luteu*s was grown to 10^7 CFU/mL. A significant difference was found for control and treatment film samples tested against *M. luteus.* (P<0.0001) An average inhibition zone for treatment samples exceeded the ½" (12.7 mm) film perimeter by 3.60 mm compared to the control which did not achieve inhibition passed the edge of the sample. A summary table of these results can be seen in Table 4.7 above.

CONCLUSION

The coating trials conducted during this study showed that the formulated antimicrobial coating can be implemented on large scale package converting equipment. Like any packaging material converting trial, adjustments were made during the trial and additional coating methods were trialed to produce the material desired. This study also showed that the antimicrobial material maintained efficacy after the production process against spoilage microorganism indicator *Micrococcus luteus*. All of the materials used in the coating formulation can be found in food and packaging industries as additives or films. The substrate and surface treatments were also common methods used in the packaging industry enabling such a package system to be potentially transitioned into a commercial market.

FUTURE RESEARCH OPPORTUNITIES

There are a multitude of research opportunities for this coated material. Atomic force microscopy or other topographical methods would provide insight on the homogeneity of the coating laydown and could possibly explain physical characteristics such as blocking tendencies. Diffusion studies could also be conducted in order to better understand the release mechanism of the antimicrobial and the degree to which the antimicrobial diffuses from the film and onto/into a food product or food simulant. Shelf life testing could be conducted to show whether this material has the potential to extend the shelf life of a product and it could also be tested against multiple types of spoilage microorganisms to determine antimicrobial efficacy. These are just a few examples of the types of studies that can be conducted; however, the possibilities are endless for understanding this particular system and could provide insight to others when producing an antimicrobial coated material.

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CHAPTER FIVE

PREDICTING THE RELEASE AND DIFFUSION OF NISIN FROM A POLYVINYL ALCOHOL MATRIX COATED FILM

ABSTRACT

Antimicrobial packaging systems for food products are complex, multivariable systems that can that be difficult to predict regarding antimicrobial release and diffusion. Factors such as pH, temperature, polymer matrix, food product composition and antimicrobial characteristics can all affect the rate at which antimicrobial can be released from the packaging system. The packaging system proposed is a polyvinyl alcohol (PVOH), Nisaplin® (2.5% nisin) containing coated film. Theoretical diffusion/release mechanisms for this system will be discussed in addition to potential methodology to analyze and predict nisin diffusion from the packaging system.

INTRODUCTION

The increased demand by consumers for fresh, preservative free, natural products has increased the need effective antimicrobial packaging for shelf life extension [8; 12; 27; 31; 36; 39]. The release of antimicrobials from packaging systems can greatly affect packaging effectiveness against targeted microorganisms. Instantaneous antimicrobial release often results in re-growth of the surviving population. On the other hand, gradual controlled release within antimicrobial systems have been found to increase packaging efficacy and reduce overall microbial loads [1; 15; 17; 24; 33]. Highly swellable

polymers such as polyvinyl alcohol (PVOH) have been used in the pharmaceutical industry for controlled drug release [11; 32] and used in antimicrobial packaging studies [5; 6; 35]. The swellability of polymers can affect the diffusion or release rate of antimicrobial through a polymer matrix [5; 34].

Understanding the packaging system can enable a better understanding of the mechanism by which antimicrobial release occurs. Release can occur via diffusion through solid films, swollen or dissolving films and liquid interfaces. An objective of this study is to discuss the potential antimicrobial release mechanisms based upon the proposed antimicrobial system produced throughout this research. The antimicrobial system consists of a film coated with a polyvinyl alcohol coating containing Nisaplin® (2.5% nisin). Additional material components and coating formulation information can be found in Chapter 3 and Appendix A.

There are a number of additional factors that can affect the diffusion and release rate of nisin from a packaging system. Intrinsic characteristics of the packaging system such as the physical and chemical properties the nisin containing matrix, nisin itself and the food product or simulant can greatly affect the overall release and effectiveness of the packaging system. One of the objectives of this study is to discuss some of the major factors and variables presented above that can affect diffusion and/or antimicrobial release.

There are many research opportunities for predicting and understanding antimicrobial release. Several studies have been conducted to determine the diffusion or controlled release rate of nisin into solutions or food simulants [1; 4; 5; 6; 18; 19; 22; 43;

45; 51; 58]. However, this work has not been conducted for a PVOH coated packaging system which has previously shown potential through inhibitory properties against spoilage indicator organism *Micrococcus luteus.* (Chapters 3 and 4) Therefore the final objective of this study was to propose methodology for future diffusion research regarding the PVOH-antimicrobial coated packaging system.

Definition of Diffusion and Desorption

Desorption and/or diffusion are the two most important concepts of mass transfer in antimicrobial packaging. These two concepts describe how the antimicrobial is released from the packaging system and able to target either the desired pathogenic or spoilage microorganism. Desorption is the mode of release which can either be controlled or random. In controlled release, the antimicrobial compound is released at a slowed or gradual rate while random release is typically classified as instantaneous release upon contact with the food surface [6]. On the other hand, diffusion is "the phenomenon of material transport by atomic motion" [7].

There are many challenges and complications when attempting to characterize desorption and/or diffusion within an antimicrobial packaging system. This paper will discuss the theoretical challenges of predicting mass transfer of an antimicrobial nisin from a dissolvable polyvinyl alcohol (PVOH) polymer matrix and propose potential methodology for future research.

Complications Based on the Packaging System and Environment

Antimicrobial packaging systems are complex multivariable systems. Understanding how the packaging system releases the antimicrobial and the assumptions for variables made can drastically affect testing methodology, results and the potential predictive release models. The antimicrobial, nisin, is a 34 amino acid peptide with a molecular weight of 3354 g/mol, is the permeant or diffusing molecule of interest in this system. Most packaging applications refer to the permeation or diffusion of gas components such as carbon dioxide or oxygen through a packaging material. Predicting the mass transfer of a solid molecule such as nisin can produce additional complications in predicting mass transfer, however, it is equally important to understand the packaging system.

Several studies have been conducted using polyvinyl alcohol as a carrier for antimicrobials such as silver nanoparticles and natural spice extracts. Few have used nisin as the antimicrobial agent in a polyvinyl alcohol matrix. Polyvinyl alcohol is a highly swellable and water soluble polymer that is commonly used in food packaging [35]. PVOH was selected as the polymer matrix based upon these qualities in addition to being thermoplastic for the antimicrobial coating depicted in figure 5.1. Although this particular antimicrobial packaging system is intended to dissolve completely at a slowed or gradual rate, this may not necessarily be what occurs in reality. Potential scenarios regarding the state of the polyvinyl alcohol during the dissolution or rate of dissolving into a liquid, (or lack thereof) are to be discussed below. Three general states of PVOH and diffusion through such will be discussed: solid PVOH, PVOH gel and a liquid PVOH

solution. In reality it is possible that one or more of these states of PVOH can exist simultaneously producing a more complicated system. However, this discussion will discuss each of these states in a singular manner for the sake of simplicity.

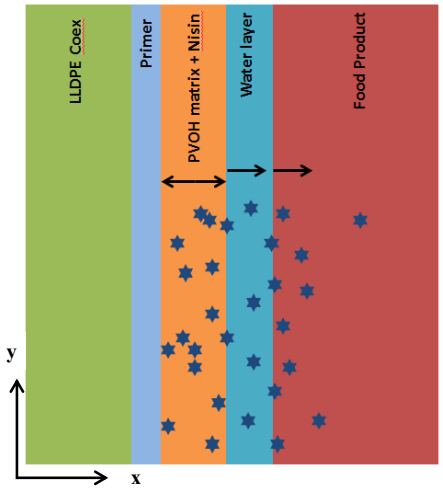

Figure 5.1. Schematic of antimicrobial packaging system with dissolvable PVOH coating containing nisin. **note: this figure is assuming mono-directional diffusion of nisin in x direction toward the food product surface.

Nisin diffusion through solid PVOH matrix

Diffusion of a solid component through another solid component occurs at decreased rates compared to diffusion through gels or liquids [16]. Diffusion of solids through solids has been found to be based upon free volume or diffusion through vacancies within a matrix. The solid diffusing agent (i.e. nisin) can only move within the holes, voids or vacancies. Vacancies can be formed through density fluctuations and/or Brownian movement [2; 55]. Basmadjuan (2004) stated that this type of diffusion has a

strong dependence on temperature. The Arrhenius equation has been discussed through many diffusion studies.

$$D = D_0 exp\left(-\frac{E_a}{RT}\right) \qquad [1]$$

D_0 = constant (m^2/sec)

E_a = Activation energy for diffusion (J/mol)

R = universal gas constant (J/mol*K)

T = temperature (K)

The Arrhenius equation shows that as temperature (T), increase, the fractional component (E_a/RT) decreases leading to an overall increase coefficient of diffusion, D. It has been found that higher E_a value indicated increased interaction between nisin and film matrices due to an increase amount of energy required for diffusion to occur [58]. Increase temperatures can cause the diffusing particle to achieve the energy threshold to move into the opened vacancy or void in a solid matrix. Several studies have found that increased temperatures produced increased desorption of nisin from films structures [22; 58].

For each of the studies cited, various film structures and desorption solutions were utilized for testing. Imran et al (2014) produced hydroxypropyl methylcellulose (HPMC), chitosan, sodium caseinate and polylactic acid (PLA) films which were all individually tested in a desorption solution consisting of 10 mL of a water-ethanol mixture (5:95). All of the film types consist of materials insoluble in organic solvents such as ethanol. However, sodium caseinate can disperse or dissolve slowly in water and HPMC can reach a degree of swelling that can eventually dissolve [14; 47; 48; 49; 52]

Because these film components are found to be insoluble, it could be assumed that desorption was driven by diffusion through the solid film.

Wang et al (2015) produced chitosan and PLA film structures at three ratios, 3:1, 2:1 and 1:1 (CTS: PLA). The films were exposed to distilled water which was shaken using a platform shaker. Nisin desorption was quantified using a UV spectrophotometer. Because neither chitosan nor PLA were soluble in water, diffusion of nisin through the solid film was the driving force of desorption into the desorption solution. Wang et al found that upon contact, a drastic increase in nisin release occurred which could have been due to nisin on or near the surface of the film. Eventually the release rate plateaued upon reaching equilibrium. The component ratios of the film matrix affected the release based on the hydrophobicity of PLA. Increased ratios of the hydrophilic component, chitosan, resulted in decreased diffusion possibly due to nisin having a higher affinity for chitosan. Because PVOH is a hydrophilic component in the proposed antimicrobial packaging, it is possible that nisin could have a higher affinity for PVOH and also exhibit a decreased D, if the PVOH were to remain a solid film. This however can be affected by temperature increase, solvent penetration and polymer swelling and possibly dissolution of the coating [58].

In polymer structures, heating above the T_g, (glass transition temperature), can cause long range segmental motion of polymer chains producing voids for diffusing agents to travel through [22]. For those polymers (e.g. HPMC) with a high degree of swellability when put in contact with solvents such as water, the adsorption of water plasticizes the material. This produces a gel to be discussed in the next section. Solvent

penetration into a film producing a gel can also reduce the T_g of the material increasing diffusion [48].

According to Geankoplis (1978), there are two different types of diffusion within solids which vary depending on whether the diffusing agent or permeant is dissolved within the matrix. For the specific case of this system it is assumed that the nisin molecules are suspended within the polyvinyl alcohol matrix**.** If such were the case, the solid nisin molecules would diffuse through the solid PVOH film structure via vacancies or voids within the film structure through Brownian motion as previously discussed. Figure 5.2 depicts diffusion of nisin through a solid film. Diffusion of a solid through a solid structure occurs at a slower rate than a solid diffusing through a liquid or gel. It is likely that solid diffusion through another solid is so slow that it may not be applicable given this packaging system. Because of this, diffusion through gels and liquids for this packaging system will also be discussed.

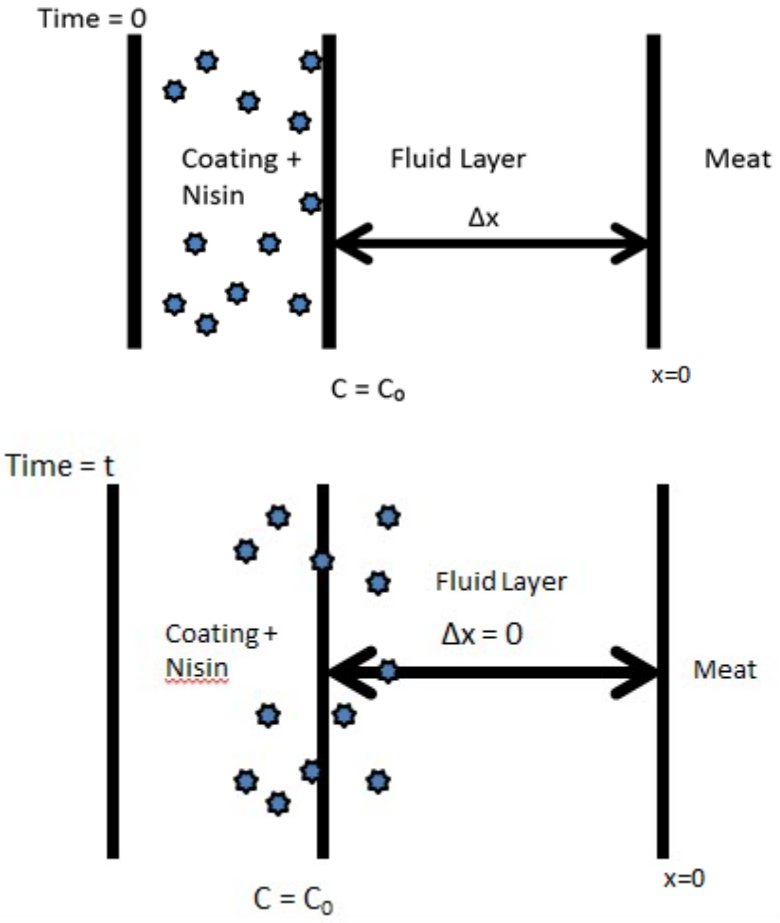

Figure 5.2. Theoretical schematic of nisin molecules diffusing through solid coating matrix.

Nisin diffusion through a gel PVOH

Polyvinyl alcohol is a highly swellable polymer that is commonly used in not only food packaging applications but also in the pharmaceutical industry due to its ability to absorb a large amount of water and swell [6]. When this polymer has absorbed water or some other liquid it can form a gel. A gel is a semisolid porous material in which the open pores within the gel matrix are filled with water or liquid [16]. This is an

assumption of what can occur in the proposed antimicrobial packaging system once the coated film is put in direct contact with a wet food. It is possible that the PVOH can form a gel through which the nisin can diffuse and target the spoilage microorganisms at the food product surface. Buonocore et al (2003) conducted a study to determine the release of nisin from a three layer PVOH structure consisting of cross-linked exterior layers and a non-cross linked interior layer. It was proposed that the release of nisin from such a polymer as PVOH was based on water diffusion into the polymer matrix, relaxation kinetics of the matrix and diffusion of the nisin through the swollen polymer network [5]. Diffusivity of solutes in gels is commonly measured using unsteady or non-steady state methods [16].

Diffusion can be described using two major categories:

Steady state (Fick's First Law) and non-steady state diffusion (Fick's Second Law). Steady state diffusion is a linear diffusion with which the rate of diffusion is constant with time. A longer diffusion time would result a higher quantity of the substance diffused. If the mass transfer or flux remains constant with time the system is undergoing steady state diffusion. Flux is described by the equation below:

$$J = \frac{M}{At} \qquad [2]$$

J = rate of mass transfer or flux ($kg/m^2/sec$)

M = mass of diffusing substance (kg)

A = cross sectional area of solid (m^2)

t = time (sec)

Fick's First Law or steady state diffusion occurs if the flux described above remains constant and is proportional to the concentration gradient (dC/dx). The negative sign in the equation below indicates the direction of diffusion from a high concentration to a low concentration along the concentration gradient. [7]

$$J = -D\,\frac{dC}{dx} \qquad [3]$$

D = diffusion coefficient (m^2/sec)

J = mass flux (kg/m^2/sec)

C = mass per volume (kg/m^3)

x = displacement (m)

Many studies including those which describe diffusivity through a swellable polymer or gel forming system use Fick's Second Law or unsteady state. In unsteady state diffusion, the rate of diffusion varies with time. Fick's Second Law is written as:

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial x^2} \qquad [4]$$

Where the concentration, C, of the diffusing agent varies with time, t, and location, x.

Geankoplis (1978) stated that diffusivity can decrease with an increase in gel weigh percentage. This was also found to be true based on studies conducted by Buonocore et al (2003 & 2004) which found that as the degree of which PVOH was crosslinked (using crosslinking agent glyoxal) increased, the diffusion of nisin from the polymer decreased. Not only was the diffusion of nisin from the polymer decreased but also the amount of water sorbed into the polymer matrix had decreased. This consequently resulted in an increased time for the nisin to reach equilibrium in the test solution [5; 6]. Others have come to this same conclusion in additional studies through

which nisin was diffusing through various gels of increasing agarose concentrations. Ripoche et al (2006) found that diffusion decreased as agarose gel concentrations increased from 3 to 7% and therefore modeled their results using unsteady state theory.

When gels are formed, solvents essentially plasticize the polymer matrix or cause polymer relaxation**.** Buonocore et al (2003) found that nisin release overall depends on penetration and/or diffusion of the food simulating liquid (if using a simulant) into the polymer network, relaxation of the polymer matrix**.** Solvent penetration and swelling can depend on the type of polymer matrix and varying with crosslinking, molecular weight and crystallinity. Both solvent penetration and swelling of the polymer (or water sorption) matrix can affect the degree to which the polymer matrix can relax and the nisin can diffuse through either vacancies or liquid-filled pores within the gel. Buonocore et al (2003) discussed a study by Long and Richman who proposed that once a highly swellable film was placed in direct contact with water, the solvent or water concentration would instantaneously increase drastically and then gradually increase to reach equilibrium within the polymer matrix. The following equation was proposed to indicate that the rate at which the water concentration at the boundary increased was related to the relaxation of the polymer matrix. It is presented as two stages of adsorption [29].

$$\frac{d\propto(t)}{dt} = \left\{\propto_1 * \sqrt{\propto (t)}\right\} * \{1 - \exp\left[-(1-\propto (t)\right]\} \qquad [5]$$

- α (t) = the normalized water volume fraction at the boundaries of the film at time t. –spans from 0-1 and represents the driving force of the macromolecular matrix relaxation phenomenon.

- $\{\propto_1 * \sqrt{\propto (t)}\}$ = early stage of hydration – kinetic constant of the polymer relaxation phenomenon – increasing function of polymer macromolecular mobility
- $\{1 - \exp[-(1-\propto (t)]\}$= later stage of hydration – dat/dt has to decrease as the concentration at the boundary of the film approaches equilibrium – decreasing function of a(t)

Others have also described that nisin release kinetics can be characterized by Fick's second law for a plane sheet with constant boundary conditions, the following assumptions [9; 22]:

1) An initial uniform nisin concentration across the film
2) The nisin concentration in the desorption liquid zero was zero
3) The amount of nisin diffused in the liquid is equal to the amount released from the film
4) Diffusion is not concentration dependent but only affected by temperature changes

Diffusion through a plane sheet with constant boundary conditions is explained in greater detail by Crank (1975). This is under the assumption that no dissolution of any part of the packaging system occurs which can produce changing boundary conditions. Figure 5.3 depicts theoretical diffusion of nisin through a gel produced from a swellable polymer which can result in changing boundary conditions.

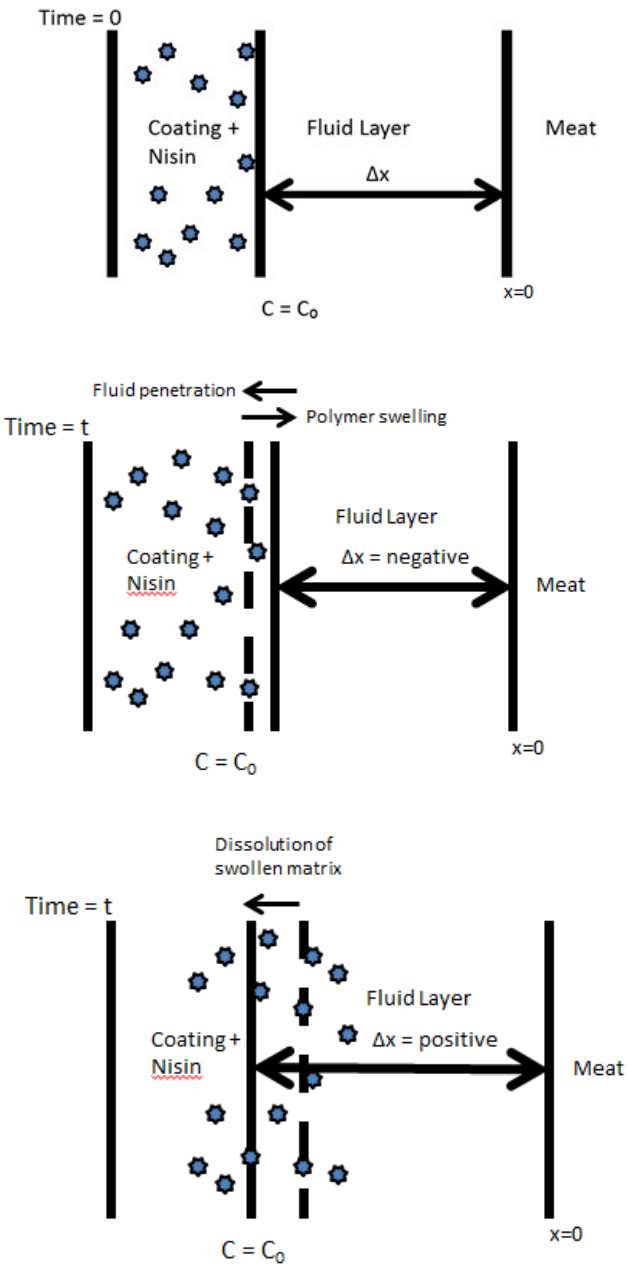

Figure 5.3. Theoretical schematic of fixed nisin molecules within a coating (top) diffusing through gelled coating (middle) matrix which could potentially dissolve (bottom). **Note: The dashed line in image 2 of Figure 5.3 indicates original coating thickness prior to swelling while the dashed line in image 3 depicts the swollen coating thickness prior to dissolution.

It is also important to note that not only is it difficult to characterize molecular diffusion through solid, gel and/or liquid systems contained a multi-component packaging system, but it is also difficult to describe in food products. Diffusion within food products which are also composed of a multitude of ingredients can be complicated simultaneous sorption and transfer of solutes and water components within the food product [45].

Nisin convection through a PVOH liquid solution interface

For this specific packaging system the PVOH matrix is intended to dissolve onto the surface of the food product. This packaging film had been produced for usage with high water content products such as ready to eat meats (deli meats) and frankfurters. The assumption that will be made regarding this dissolvable coated film matrix is that desorption kinetics will follow that of a liquid-liquid mass transfer scenario. The PVOH coating will begin dissolving and present a moving interface indicated in Figure 5.1 enabling the diffusion of released nisin through a liquid layer mixture containing PVOH coating components and the original fluid layer. For such a system, it is possible to assume that the nisin would be held in the polymer matrix until release via dissolution processes.

Regardless of using assumptions to simplify such a system, there are complications. It is possible that the coating may not fully dissolve. This could partially depend on the fluid layer which the coating is intended to dissolve. If there is not enough liquid or the liquid layer becomes saturated with the PVOH prior to completely

dissolving, it could no longer be assumed that this system is a solid-liquid interface mass transfer system. This discussion will present theory for two separate scenarios. The first scenario will focus on the assumption that the release of the coating and diffusion through the water boundary layer to the targeted area of the food product is based on convection rather than diffusion assuming that the coating is able to fully dissolve. It should be noted that it is also possible that the nisin could diffuse through the solid film prior to coming into contact with the fluid layer. The second scenario will be based upon the assumption that the mass transfer of nisin will be based solely upon diffusion rather than convection through the water or fluid boundary layer. It is likely that a combination both diffusion and convection would occur based upon the proposed packaging system.

Figure 5.2 shows a theoretical model of what would occur should the coating containing nisin dissolve. The fluid layer, x, would change with the reduction of the coating layer. The method for mass transfer proposed in Figure 5.2. is mass transfer of nisin through the fluid layer through convection. Siepmann and Peppas (2012) came to a similar conclusion during a study which modeled drug release from an HPMC (hydroxypropyl methylcellulose) containing system which is a highly swellable cellulosic. It was concluded that the characteristics of the HPMC could result in dissolution leading the moving boundary layers as indicated in figure 5.2 based on the PVOH system proposed. It was also stated that the occurrence of dissolution can complicate the solution of Fick's Second Law. (Equation 4)

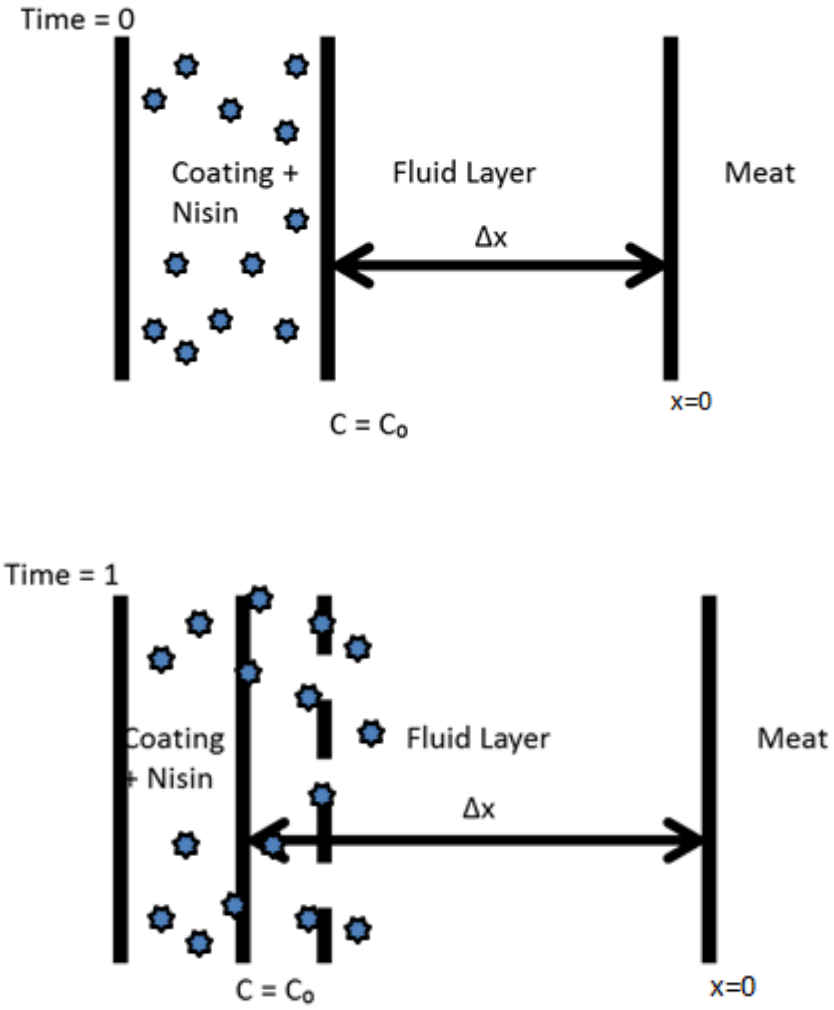

Figure 5.4. PVOH coating dissolution mechanism model with nisin release. (**Note: dashed line indicates original coating thickness prior to dissolution)

Many books and literature characterize the solid-liquid interface by the following equation:

$$-D\frac{\delta C}{\delta x} = h(C_{L.t} - C_{eq}) \qquad [6]$$

D = diffusion coefficient (m^2/sec)

C = mass per volume (kg/m^3)

x = displacement (m)

h = coefficient of transfer by convection

$C_{L,t}$ = concentration of the diffusion substance on the surface of the solid

C_{eq} = concentration of the diffusing substance on the surface required to maintain equilibrium with the concentration of this substance in the liquid at time t.

According the equation above from Vernaud and Rosca (2006), at the packaging-liquid interface, the amount of nisin transferred into the liquid or water layer would be constantly equal to the rate at which the nisin is brought to the surface by diffusion through the packaging material. However, because this material is intended to dissolve into the liquid layer, the assumption can be made that the layer would dissolve faster than diffusion would occur which could alter the left side of the equation to be equal to the rate of the coating loss or the dissolution rate in the same units as diffusion (m^2/sec).

$$-dissolution\ rate\ \frac{\delta C}{\delta x} = h(C_{L.t} - C_{eq}) \qquad [7]$$

This would also require assumptions that the nisin is distributed homogeneously throughout the coating layer and that no antimicrobial activity was lost during the production process and storage. There are multiple factors that can affect dissolution rate which will be discussed in a later section.

On the other hand, if the amount of nisin transferred were based upon the assumption that convection was not significant and that the driving factor was diffusion through the liquid boundary layer, the following equation could be suggested:

$$-dissolution\ rate\ \frac{\delta C}{\delta x} = -D\frac{dC}{dx} \qquad [8]$$

Complications: Variables to be considered

Antimicrobial packaging applications are complex, multivariable systems that are difficult to characterize and predict. This section will provide background information of some of the variables to be considered when characterizing the diffusion and controlled release of an antimicrobial from an antimicrobial packaging system and provide potential methodology given specific variables and assumptions for the packaging system.

Factors effecting diffusivity and controlled release

There are numerous factors that can affect the diffusivity and/or the controlled release of antimicrobials in packaging systems. The following discussion will include:

1. Intrinsic factors: pH, fat content, structure of food and polymer matrices, composition
2. Polymer structure and swellability
3. Temperature
4. Permeant size and Distribution
    a. Factors affecting nisin efficacy
5. Food product
6. Antimicrobial concentration in packaging material and effects of packaging structure
7. Rate of consumption of antimicrobial agent by microorganisms
8. Direction of flux
9. Antimicrobial solubility in packaging system

10. Factors affecting dissolution
11. Volume of liquid assumption
12. Area and thickness of packaging material
13. Convection

Intrinsic factors

There are numerous factors that can affect the degree to which a permeant is released or is able to diffuse through a packaging system. Several studies have researched the effects of factors such as pH, agarose gel percentage, fat content, additive concentrations such as nitrate and nitrite, target microorganisms load among other factors.

A study conducted by Blom et al (1997), tested some of these factors that can specifically affect the diffusion of bacteriocins such as nisin. Agar well diffusion was conducted to test the effects of an indicator bacterial strain load, fat content, agar content, pH and salt concentrations on diffusion of nisin, among several other bacteriocins. It was found that a decreased pH influenced produced increased inhibition zones [4]. However, this may not specifically indicate that the nisin diffused further at a low pH than a higher pH. Guiga et al (2010) also came to a similar conclusion during a study to determine the desorption of nisin from a multilayer structure containing ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) (EC/HPMC/EC) into a solution containing 0.8% w/w NaCl and 28°C tested at two pH levels [19]. (pH = 3.8 or 6.8) Work conducted previously (Appendix A) found that coating solutions adjusted to pH levels 4, 6 and 7

which were then coated to an LLDPE substrate and dried indicated that activity of the nisin increased with decreased pH. The efficacy of nisin has been known to vary with pH but has optimal activity at a pH of 2 which decreases with increasing alkalinity [23].

It is possible that the results of Blom et al (1997) showed the results of antimicrobial activity rather than diffusion as well. It was also stated that the decreased pH (pH 5.5) could have produced decreased ionic forces within the gel reducing the gelling of the agar therefore increasing diffusion. Additional diffusion effects such as increasing the concentration of agar were found to decrease the diffusion rate [4; 45]. In Sebti et al (2004), the rate of diffusion of nisin from a liquid solution into an agar decreased by 50% when the agarose percentage was increased from 3 to 8%. Factors such as fat content, additives, target microorganisms and microbial load are discussed in later sections.

Physical and chemical structure of the polymer & swellability of the polymer

Physical and chemical structural aspects of polymer matrices can greatly affect the degree to which an antimicrobial component can diffuse. As previously discussed, diffusion of a solid component such as nisin through a solid matrix has been found to occur at a slower rate compared to a through a gel or liquid matrix. Other components aside from the physical state of the polymer matrix can affect the diffusion process. The crystalline structure within a polymer can produce a decrease in diffusion rate. Crystals themselves have been found to be either impenetrable or drastically reduce diffusion [55],

in addition to producing a tortuous path for molecules to diffuse through and reducing free volume.

Studies conducted by Buonocore et al (2003 & 2004) used a crosslinking agent to product polyvinyl alcohol films and film structures. The studies found that the release of nisin from the polymer structures decreased as the amount of glyoxal crosslinking agent increased. This was expected as crosslinking produces bonds between polymer chains resulting in less free volume and more tortuous diffusion paths through which the antimicrobial must move.

The swellability of an antimicrobial containing polymer matrix can cause variations in diffusion rates. An increased degree of crosslinking in a polymer matrix, bonding between polymer chains, resulted in a lower swelling ratio in Buonocore et al 2003. The swelling ratio was calculated by immersing film samples (1x1 cm) in 30 mL of distilled water which was removed, blotted with tissue and weighed by a microbalance until equilibrium was reached. The swelling ratio was calculated as:

$$Swelling\ ratio = \frac{g\ Film - g\ dry\ Film}{g\ dry\ Film}$$

Solvent compatibility can affect polymer swelling. Buonocore et al (2003 & 2004) conducted testing on hydrophilic PVOH in water. Due to the chemical compatibility between the solvent and polymer, swelling was able to occur. When a compatible solvent diffuses into an amorphous, glassy, un-crosslinked polymer, the polymer becomes plasticized into a swollen gel layer [34]. Solvent penetration and swelling will fill the free volume of a polymer with the penetration solvent promoting the

diffusion process. Swelling has been shown to increase the mobility of antimicrobial agents in polymers when compared [34].

Factors such as crosslinking and molecular weight have also been found to affect swellability thereby affecting diffusion rate. Studies have found that as the degree of crosslinking increases, swellability or amount of water sorbed by a polymer would decrease. This would result in a decreased diffusion rate [5; 6]. As expected, it was also reported that as crosslinking increased, the time to which the tested PVOH films reached water sorption equilibrium increased [6]. Increased molecular weight polymers result in higher amounts of swelling as opposed to dissolution due to additional disentanglement required prior to dissolving. However, it has also been found that increased molecular weight polymers reduced the rate of diffusion [34]. Numerous intrinsic factors of the food and antimicrobial containing matrices have been shown to affect diffusion. In addition to intrinsic factors, there are also extrinsic factors such as environment conditions, food product and properties of the antimicrobial components utilized.

Temperature

Environmental factors such as temperature can also have an effect on the diffusion of a permeant or the release of a permeant from a material. As mentioned in previous sections, diffusion has been presented as the movement of permeants through free volume within a structure. Energy is required for a permeant to move from one vacancy to another within a polymer structure. This activation energy is required for the permeant to gain enough energy to move through microvoids in polymer structure. It has been

found in several studies of diffusion and controlled release of permeants has increased with increasing temperatures according to Arrhenius Law [18; 22; 27; 45; 51].

[equation 1]

Distribution of the permeant, size of the permeant – factors that affect the efficacy of the permeant

The distribution of the permeant, or antimicrobial for the current research, can also have an effect on diffusion. Larger permeants result in slow diffusion rates. Larger permeants have difficulty moving through the tortuous paths within polymer structures and require larger areas of free volume to accommodate the molecular size. The distribution of the permeant can also have an effect on the antimicrobial release. For the current research, it is assumed that the nisin is dispersed homogenously throughout the coating matrix. Other systems may release differently if the antimicrobial concentration is variable across the coating matrix.

As previously mentioned, the molecular size and distribution can affect the antimicrobial release within a packaging system. However, even if the antimicrobial were released with ideal conditions there are factors that can affect the efficacy of the antimicrobial. Nisin can have increased or decreased antimicrobial activity based upon several factors. The targeted microorganism or microorganisms can be more or less susceptible to the antimicrobial effects of nisin. For example, Gram negative organisms such as *Escherichia coli* are more resistant to nisin due to their cell wall structure compared to Gram positive organisms. The diffusion of the antimicrobial into bulk food

products can also decrease antimicrobial effectiveness because the concentration of nisin may not be high enough to exhibit desired antimicrobial effects within large food volumes. Packaging structure production could decrease antimicrobial activity. For example, high heat, pressurized processes such as film extrusion can denature the antimicrobial protein. Other factors that can affect antimicrobial efficacy within a packaging system include properties of the nisin (heat resistance, activity with pH), chemical or physical changes to the polymer material due to incorporation of the antimicrobial compound, polymer material properties and food composition such as fat content and storage conditions [4; 27; 50; 51; 56; 59].

Food product

Diffusion through foods can be complicated by food product composition, structure, homogeneity, microbial population and other food specific qualities. However, added complications can arise due to simultaneous water and solute sorption and transfer [45]. In addition to food product effects on diffusion, characteristics of food products can also have effects on antimicrobial efficacy. Antimicrobial activity of nisin can be decreased by food qualities such as fat content and pH. The proposed antimicrobial packaging structure was intended for ready-to-eat (RTE) food products such as meats (i.e. frankfurters).

Several studies have found that increased agarose used to simulate diffusion through a gel or solid-type product had produced decreased diffusion [4; 43]. This could be due to the tortuous path produced by the gelling agent as previously discussed.

Ripoche et al (2006) also found that fat content did not have an effect on diffusion. Fat content can be of particular importance in some high fat content products such as frankfurters or any meat product.

In Ripoche et al (2006) vegeteline (or hydrogenated copra oil) was added to three agarose treatments (3, 4 and 7%) to compose 33.33, 66.67 and 100% of the agar. Although it was found that the lipid addition did not affect the diffusion, the activity of the nisin was not tested. It is possible that, although nisin diffusion is occurring, it may have been inactivated by fats.

Other studies have shown that fat content can decrease the antimicrobial activity of nisin when tested against *Listeria monocytogenes* in milk products of varying fat content. A decrease of 33% in antimicrobial activity was seen in nisin added to skim milk and showed an 80% decrease in half and half. (half milk and half cream) which contained 12.9% fat [26].Milk products tested with 2 and 3.5% fat also showed decrease in pathogen reductions [3].

Other properties such as pH have been studied to determine their effect on diffusion. Studies have found that a decreased pH increased diffusion [19; 45] However, meat products such as frankfurters and bologna have a relatively neutral pH 6-7. Once again, the results may not indicate that the nisin had the ability to diffuse or release due to the decreased pH but could have maintained a higher degree of antimicrobial activity due to the favorable lower pH conditions. The study was inferring diffusion though microbial kill. The solubility and stability of nisin have been found to increase with lower pH conditions, while high pH conditions promote instability within the molecule [28].

In addition to pH, other factors that have been found to affect the stability and/or antimicrobial activity of nisin include microorganism type and microbial load, proteolytic degradation, interaction with food components such as fat, amount of nisin, conditions of application or production method (i.e. extrusion, coating) and heat abuse [17; 30]. Studies have found that although nisin is heat stable and autoclavable, temperatures exceeding 140°C can decrease antimicrobial activity [21]. There are a many factors that can affect diffusion and desorption within a true food product based system. However as previously mentioned, it is important to understand how food product characteristics can also affect the antimicrobial activity of the component being utilized.

Concentration of the AM in the package and effects of packaging structure

The concentration of the antimicrobial component within the package must exceed the minimum inhibitory concentration of the targeted microorganisms in order to achieve inhibitory properties. Secondly, the timing of antimicrobial dosage has been shown to effect overall antimicrobial effectiveness. Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial that is required to inhibit bacterial growth. The MIC can vary based upon the type of bacteria, growth phase (lag, log, and stationary phase), and growth medium, growth conditions such as temperature, available oxygen, and available nutrients. MIC data can also vary from laboratory to laboratory based upon the personnel conducting experiments and varying techniques while testing the same bacteria. Consistency among as many variables is important in order to obtain consistent MIC data for targeted microorganisms.

Not only is there a minimum concentration needed to inhibit targeted microorganisms, there is also a legal limit of usage. The concentration of nisin is commonly measured in international units (IU) or activity units (AU) per gram or milliliter depending on whether the food product is solid or liquid. In pure form, nisin has an antimicrobial activity of 40,000,000 IU/g (40×10^6 IU/g). Other products such as Nisaplin® contain 2.5% nisin concentration in a mixture with salts, milk solids and residual moisture [23]. Nisaplin® has an antimicrobial activity of 1,000,000 IU/g (1.0×10^6 IU/g). According to the US FDA, the concentration of nisin is not allowed to exceed 10,000 IU/g of food product [17]. (Nisaplin® = 0.01 grams per gram of food; Pure nisin = 0.0025 grams per gram of food) Calculations of the theoretically available nisin in the current research displayed in Appendix B, show that the current antimicrobial system yields values well below the legal limit per gram of food product.

Additionally, the antimicrobial concentration within the coating solution will not be equal to the antimicrobial concentration within the produced film. The concentration of the antimicrobial within the dried coating or produced film will depend upon the coating weight applied and the initial concentration within the coating liquid or film forming solution. From this information, the theoretically available quantity in international units (IU) can be calculated per square centimeter of the produced film.

The antimicrobial packaging structure can greatly affect the release rate of the antimicrobial. Multi-layer packaging structures and water soluble polymer matrices have been researched in order to slow the rate of release. Slower or more gradual antimicrobial release rates compared to instantaneous antimicrobial doses have been

found have been found to produce longer term inhibitory effects [17]. Although an instantaneous dosage has been found to show initial decreases in the targeted microbial population, studies have found that over time the bacteria will increase in population once again [1; 15; 36].

Balasubramanian et al (2011) came to a similar conclusion when testing the effect of the controlled release of nisin versus instantaneous release against *Micrococcus luteus.* The study concluded that the overall amount of nisin using a controlled release mechanism required to achieve inhibition was 15% of what was required for similar results using instantaneous dosage release. Controlled release required 0.227 μmol which equates to 7.61×10^{-4} grams released in total. The final concentration within 200 mL of TSB (tryptic soy broth) media was 152.95 IU/mL. The instantaneous release experiments showed re-growth occurring after 12 hrs even after the bacteria had been dosed with 7.45×10^{-3} μmol/mL or approximately 1000 IU/mL concentration of nisin [1].

For the current research, the gradual dosage of antimicrobial is intended to be released via dissolution or diffusion through a swollen gel followed by dissolution of the coating. An additional complication for controlled release of antimicrobial using the proposed packaging system is that dissolution is not linear with time. If it was assumed that the antimicrobial was solely released upon coating dissolution, the dosage of antimicrobial released is not linear with time. Mallapragada and Peppas (1996) found that the time required for complete dissolution of a film varied with conditions. This is to be expected as there are numerous factors that can affect polymer dissolution which is to be discussed in a later section. The study conducted [32] found that the timeline for films to

completely dissolved varied from less than a day to several weeks among the polymer films tested. This can greatly affect the release of antimicrobial and the overall effectiveness of an antimicrobial packaging system.

Rate of consumption of agent by microorganisms

The rate of interaction of agents by microorganisms can affect the driving force of diffusion or convection through the liquid layer in the proposed packaging system in the current research. Vernaud and Rosca (2006) discuss the assumptions when considering the antimicrobial consumption rate for microorganisms in food. Throughout this work, consumption will be defined as inactivation as it relates to the mode of action of nisin against targeted microorganisms. For a process that is driven by diffusion of an antimicrobial through a coating or convection at the packaging-food interface, the rate of consumption of the agent can be characterized with the following equation:

$$-\frac{\partial C}{\partial x} = K * C_{f,t}$$

C = mass per volume (kg/m^3)

x = displacement (m)

$C_{f,t}$ = concentration of the diffusing substance in a homogeneous food phase

K = rate constant of the first-order bactericidal reaction (/sec)

For the previously shown equation it was assumed that the diffusion of the antimicrobial was mono-directional and was being brought from the coating to the liquid interface through diffusion. The diffusion rate was assumed to be equal to the rate at

which the antimicrobial reached the target microorganisms on the food surface. Lastly, it was assumed that there was no transfer of antimicrobial on the other surface of the coating. (meaning that the flux was toward the direction of the food product)

Additional variables to consider:

Direction of flux

In order to simplify mathematical calculations, numerous studies modeled diffusion or desorption with the assumption of unidirectional diffusion [43; 45]. It is important to mention that unidirectional diffusion may or may not occur in a realistic system. Diffusion can be driven in any direction within a packaging and food system.

Solubility in Packaging System

The direction of flux for the antimicrobial can be partially affected by the partition coefficient. For example, if the antimicrobial component has a great affinity for the film or other components of the packaging structure, it is possible that the antimicrobial could be driven in the opposite direction of the food product or remains fixed within the polymer matrix. For that matter, it may also affect the nisin becoming available from desorption of nisin in the three diffusion scenarios presented in Figures 5.2, 5.3 and 5.4. The partition coefficient describes the solubility of a component in a polymer media. It is because of the difference in solubility or affinity for one matrix or another that the concentration of the additive or nisin for the proposed system may not be

the same in a liquid coating media compared to the solid film [54]. The partition coefficient is typically written as a ratio:

$$K = \frac{C_{S,\infty}}{C_{P,\infty}} = \text{Food or simulant/package or polymer} \qquad [9]$$

Where K is the partition factor when the system is in equilibrium, $C_{S,\infty}$ is the concentration of the diffusing substance in the food product or simulant at equilibrium and $C_{P,\infty}$ is the concentration of the diffusing substance in polymer or package at equilibrium [22].

The value of the partition coefficient is an important determination that can again determine the affinity of a component such as nisin for either the food or packaging system. For a packaging system that contains a non-polar polyolefin (such as polyethylene or polypropylene sealant) containing an organic diffusing agent tested against an organic solvent or fat, the partition coefficient is <1. With increasing polarity of the food or food simulant the coefficient increases. If water is used as the food simulant the partition coefficient can exceed values of 1000. For extreme conditions or "worst case scenario" values of K =1 or K=1000 can be assumed [38].

According to Imran et al (2014), the partition coefficient can be determined for a food and packaging system using the following equation:

$$K = \frac{M_{S,\infty}/V_S}{M_{F,\infty}/V_F} \qquad [10]$$

Where $M_{S,\infty}$ is defined as the amount (mg) of nisin in the solution or food simulant and $M_{F,\infty}$ is defined as the amount (mg) of nisin in the film. V_s and V_F is the volume of the simulant and volume of the film (cm^3). This can be a useful tool when producing an

antimicrobial system as a means to determine that the system produced will drive the antimicrobial towards the food product rather than remain within the packaging materials.

Factors affecting dissolution

Dissolution is the process by which a substance is dissolved into another substance. In pharmaceutical applications, dissolution of active pharmaceutical ingredients from tablets or pills is widely studied. Dissolution is also an important characteristic in antimicrobial packaging. It is of particular importance for the proposed antimicrobial coated system which is based on the dissolution of a PVOH matrix which releases the antimicrobial nisin to target spoilage microorganisms. The intrinsic dissolution rate (IDR) can be defined as "the dissolution rate of a pure drug substance under the condition of constant surface of the dissolution medium" [41; 61]. There are numerous aspects that can affect the dissolution rate of a substance such as crystallinity, temperature, lamellar thickness, molecular weight, polymer defects and solubility of the polymer within dissolution media.

Mallapragada and Peppas (1996) conducted a study in which the mechanisms of dissolution for polyvinyl alcohol films was analyzed based upon polymer molecular weight, varying crystallization and dissolution conditions, crystal size and distribution in addition to lamellar thickness size. PVOH films with varying molecular weights (M_n = 35,740; M_n = 48,240; M_n = 64,000) were tested. The study found that the amount of time for PVOH films to dissolve varied with crystallinity and dissolution conditions such as the temperature of the dissolution solution. Increased temperatures were found to increase

the dissolution rate of the polymer. It was also found that penetration of the solvent into the films produced a decrease in the crystallinity of the sample. High molecular weight samples showed a more gradual decline in crystallinity compared to lower molecular weight. Mallapragada and Peppas (1996) proposed that this was due to increased difficulty for higher molecular weight polymers to form crystals because of entanglements occurring in long polymer chains. A study presented in Miller-Chou and Koenig (2003) also concluded that dissolution rate decreased with increasing molecular weight but it was also noted that polydispersity also affected dissolution rate. Polydispersity is a measure of molecular weight distributions. The study found that polydisperse samples dissolved two times faster than monodisperse samples of the same molecular weight.

Defects within films have also been shown to increase dissolution. Mallapragada and Peppas (1996) found that crystals containing defects dissolve more readily. A study referenced in Miller Chou and Koenig (2003) stated that imperfections such as cracks in the surface of a film can cause thicker films to dissolve faster due to increased surface area for dissolution media or solvent penetration to contact.

Other factors found to have an effect on dissolution are lamellar thickness and polymer solubility in dissolution medium or solvent. Lamellae are chain folded crystalline regions that radiate outward from the nucleation site of a polymer crystal [7]. Mallapragada and Peppas (1996) found that increased lamellar thickness decreased dissolution rate. Additionally, crystals with greater lamellar thicknesses were more stable.

One of the most important factors influencing dissolution is the solubility of the polymer in the dissolution medium. Chemical compatibility for both solvent and polymer can greatly affect dissolution. ("like dissolves like") [34]. For example, polyvinyl alcohol is chemically compatible with water therefore they are soluble within one another. Gibbs free energy of mixing can also describe the dissolution of an amorphous polymer and can be described by the equation below:

$$\Delta G_m = \Delta H_m - T\Delta S_m \qquad [11]$$

Where ΔG_m = Gibbs free energy change on mixing;

ΔH_m = enthalpy change on mixing

T = absolute temperature

ΔS_m = entropy change on mixing

Gibbs free energy on mixing can be more simply defined as the capacity to do work. Enthalpy change on mixing is the energy available in a system or heat transferred during a constant pressure process. Entropy change on mixing is the unavailability of the thermal energy in a system to convert to work because it is disorder or the system is in the lowest energy state. Therefore ΔG_m, Polymer-solvent miscibility occurs when $\Delta G_m \leq 0$. A negative Gibbs free energy of mixing shows that the mixing is spontaneous. Several models have been proposed to describe the dissolution of amorphous and semi-crystalline polymers which can be found in Miller-Chou and Koenig (2003).

Infinite or finite volume of liquid

The volume of liquid inside of the packaging system could affect the diffusion of the antimicrobial agent. A high volume of liquid would be less likely to become saturated with antimicrobial and more likely to penetrate deeper into the coating and the antimicrobial could diffuse through convection. A low volume of liquid could become saturated quickly, penetrate less into the package coating and the antimicrobial would diffuse through solid films rather than convection through liquid.

Area of the package material and Material thickness

The area of the coated packaging material does not affect diffusion but can affect the overall antimicrobial concentration. Diffusion is typically presented on a per square area basis (For example: cm^2/sec). However, in antimicrobial packaging, the area of the packaging will affect the total concentration of antimicrobial released into the bulk food product. The material thickness on the other hand does affect diffusion. A thicker material will impede mass transfer compared to a thinner material [10].

Convection

The value of the coefficient of convection, h, can affect the overall release of nisin in the packaging system. (See equation 6) A high convection towards infinity would indicate a high degree of constant mixing. An application of a high convection coefficient value could be how release is affected through the distribution chain, while a low convection (natural convection) value could be more indicative of if a package were

sitting on the shelf. Many studies assume infinite coefficient of convection and this assumption leads to the assumption that the concentration of nisin on the surface of the solid ($C_{L,0}$) instantaneously reaches the value at equilibrium (C_{eq}) in the liquid as soon as the release process begins, t=0 [54].

In the current research, a low convection would be assumed meaning that low to no agitation or mixing would occur in the packaging system. Because of this, it can be assumed that a gel layer due to solvent penetration and swelling of the polymer coating matrix will occur before dissolution. A study discussed in Miller-Chou and Koenig (2003) found that dissolution increases with agitation and stirring frequency. An additional study mentioned in Miller-Chou and Koenig (2003) also found that with little to no agitation that a gel layer forms, but decreases with time while high mixing removes layers of polymer without forming a gel.

Proposed Methodology

Discussed below are some suggestions for methodology. These suggestions are based upon the current research of the polyvinyl alcohol coated nisin-containing antimicrobial packaging system. These methods suggested for future work would be utilized to better understand the diffusion and/or controlled release and antimicrobial efficacy of the packaging system.

Methodology for determining the antimicrobial efficacy of the coated film will be presented in addition to discussing the importance of bacterial selection. Secondly, methodology for characterizing the packaging system by determining the dissolution rate

is presented which would assist in determining the rate of mass transfer. This method could be coupled with a protein quantification method (that is not based upon microbial activity) to determine how much nisin is being released from the system. The food simulant for protein quantification method to be used could be either water or a salt-water brine to simulate hotdog exudate.

Assumptions

Diffusion and controlled release mathematics can exponentially increase in complexity without making assumptions to make the math more easily digestible. Several assumptions regarding the packaging system will be made:

1) The direction of flux for the antimicrobial is mono-directional in the direction of the food product or away from the packaging substrate
2) Driving force = rate of consumption by microorganisms
3) Packaging system release of nisin occurs via diffusion and/or convection (dependent on further testing)
    a. Mathematical modeling may require separate models for these two different modes
        i. If the coating dissolves – then there is an assumption of a moving boundary condition. As the coating dissolves its nisin concentration may remain constant but its location in the systems will change.
        ii. If the coating gels – there is no moving boundary as in item i.

1. It is likely that the system both gels and dissolves.
2. One would need to determine if the coating dissolves completely.

4) The nisin is mixed homogeneously throughout the coating.
5) No nisin remains trapped within the coating matrix once the matrix is completely dissolved.

6) The concentration of nisin at the coating-liquid interface is equal to the initial concentration as the release occurs. ($C_{t,0} = C_0$)

Antimicrobial activity

One of the most common methods used for determining antimicrobial efficacy is an agar well diffusion assay with a semi-solid agar overlay [39]. However, because this packaging system is intended as an antimicrobial coated film for direct food contact, a variation of film on lawn is being proposed. Due to the number of replicates and varying antimicrobial concentrations that would be used for this methodology, it is advised that films be produced via drawdowns with Mayer rods correlating to the coat weight that would be produced on a large scale process. The objective of this study:

1) To produce a standard curve with varying concentrations of nisin coated films and corresponding zones of inhibition. An equation can then be produced from this curve to predict effectiveness. (Note: This would only be relevant for a specific coat weight and microorganism type)

2) Compare the inhibition zones for samples and predict concentration based upon standard curve.

This can be compared with mass balance calculations of theoretically available nisin and protein quantification results to be discussed later. This could also show the concentration of nisin released from the film in a scenario with no agitation and the film is in direct contact with microbial growth media. Because this method is dependent on microbial growth, this method will not be used to calculate diffusion.

Bacteria used for testing – sensitivity of the microorganism

Throughout the course of this research the antimicrobial coating produced has been tested against spoilage indicator microorganism *Micrococcus luteus* (ATCC 10240). This work has also shown that the produced packaging system inhibited *M. luteus* through film on lawn studies. It is a Gram positive microorganism that has been used in many nisin studies as a reference strain due to its high sensitivity to nisin [1; 46]. It is proposed that *M. luteus* be used as a control microorganism to ensure that the coating maintains inhibitory properties through further studies, but also additional bacteria should be tested.

Spoilage microorganisms for ready-to-eat type (RTE) products such as hotdogs are typically facultative or anaerobic psychrotrophs. These are microorganisms that thrive in environments with little to no oxygen in addition to surviving and growing within a wide temperature arrange of 0-40°C. Therefore in order to best determine whether the

packaging material can extend the shelf life for such RTE products, it is recommended to test microorganism such as *Lactobacillus* spp., *Lueconostoc* spp., *Serratia* spp., *Brochothrix thermosphacta and Enterococcus casseliflavus* [20; 40]. It is also recommended that the minimum inhibitory concentrations of nisin for each bacteria be determined using the method adapted from Wilson-Stanford et al (2009). This method was used previously to determine the MIC of *M. luteus.*

Dissolution

The intention of the proposed packaging system is to inhibit spoilage microorganisms by dissolving onto the surface of a food product or simulant. In this case, the rate of dissolution can hinder or assist the overall antimicrobial effectiveness of the packaging system. As previously discussed, gradual release of nisin over an extended period of time produced was more effective for inhibition of *M. luteus* compared to a single instantaneous nisin dosage [1] This study would provide information regarding the rate at which the coating would dissolve therefore the rate at which nisin would be released. An assumption for this study would be that the nisin is fixed within the coating until the coating is dissolved within the solvent and released.

For this study, films of consistent surface area (see ASTM F2217) and antimicrobial concentration are to be immersed in pure water or a salt water brine to simulate hotdog exudate. The water or simulant would not be agitated. This system would be intended to imitate a typical packaging system with little to no stirring. (The study could be replicated with high agitation or convection to compare results between

stirring and no stirring.) Samples in triplicate would be removed at each sampling period, lightly blotted to dry. Coating weights would be recorded to show coating loss over time. An equation could be produced from this data to show the dissolution rate.

Buonocore et al (2003) found that PVOH films produced took several days to weeks for complete dissolution to occur and varied with degree of crosslinking. If the proposed study indicated that the coating dissolved "too fast" it would be proposed to determine an optimum degree of crosslinking to achieve the desired dissolution rate. However, this could affect the sealability of the packaging film. On the other hand, if the dissolution were "too slow", it would be proposed to load the coating with a higher concentration of Nisaplin® or potentially pure nisin.

Quantification methods

Many nisin quantification methods have been utilized in previous studies such as agar well diffusion and high pressure liquid chromatography or HPLC. Agar well diffusion can produce variable results that are not comparable between studies, based upon the bacteria used (due to antimicrobial sensitivity), incubation conditions and technician technique among other factors [25; 37]. On the other hand, HPLC methods, although widely accepted can be difficult to interpret over an extended study due to the cleavage of nisin from degradation or conformational changes in the nisin that can occur during the study [42]. Methodology using LC-MS/MS (liquid chromatography – tandem mass spec) or other mass spectrometry methods could provide additional information such as physical structure of the nisin degradation products [41; 60].

The methodology proposed for the continued work has been used in previous studies for nisin quantification [18; 19; 43]. Bicinchoninic Acid (BCA) Protein Assay is a spectrophotometric based method for quantifying proteins. Proteins will reduce Cu (II) to Cu (I) under alkaline conditions which forms a complex with BCA producing a purple color. This can be measured using a spectrophotometer at 562 nm. The concentration of nisin can then be quantified based upon a standard curve [57].

This method could show the amount of nisin available in films coated using large scale application processes or hand drawdowns. Coated film samples of a known square area would be completely dissolved in pure water. Agitation will be required for complete dissolution to occur in a timely manner. The solution would then be measured using the BCA method discussed above. If PVOH or other coating components interfere with the protein quantification methods, filtering processes such as molecular weight filters or microcentrifuge procedures can be utilized. Molecular weight syringe filters vary with size. It would be possible to select a filter that would allow nisin to be filtered from other coating components. Centrifuge procedures could also be utilized to achieve a pure water and nisin solution for protein quantification. Results achieved from this method could be compared with mass balance calculations of theoretically available nisin shown in Appendix B.

Food simulant

The proposed food simulant for diffusion testing should be representative of the type of food for which the packaging will be applied. In the case of this research, the

packaging is intended for hotdogs. This type of product is a fatty food product which can be represented in testing by using a fatty food simulant. Many of the studies presented used water or various desorption solutions agars, however the solutions used were not necessarily food simulants. A hot dog product, according to the FDA would fall under the category of a Food Type III. “Aqueous, acid or nonacid products containing free oil or fat; may contain salt and including water in oil emulsions of low-or high-fat content.” For such product food oil such as corn oil, or mixtures composed or triglycerides or coconut oil were recommended as a food simulants [53]. More recently, the Food Safety Authority of Ireland released a document discussing a transition period of plastics regulation. As of January 1st 2016, food simulants in regulations provided by the European Commission [(EC) No 10/2011] fatty food simulants will consist of 50% Ethanol (v/v) and vegetable oil [13]. However, the packaging system proposed is intended to dissolve onto the surface of a moist food product. The food simulants discussed above may be appropriate for migration testing for food contact notification, but may not be appropriate for diffusion and/or controlled release testing. A water-based simulant such as water or salt-water brine is recommended for diffusion/controlled release methodology.

CONCLUSION

There is much work to be conducted to better characterize and understand antimicrobial release and diffusion in active packaging systems. Coatings utilizing highly swellable and water soluble polymers such as polyvinyl alcohol containing nisin can

produce many scenarios through which nisin can move through solid, liquid and gel and combinations thereof. Each system can be affected by a variety of both intrinsic and extrinsic variables such as pH, temperature, dissolution rate and mechanism in addition to the food product to which the system is applicable. No diffusion or controlled release studies have been conducted on the specific antimicrobial packaging produced throughout this doctoral work. However, these studies would provide insight as to how this system could extend food product shelf life through inhibiting spoilage microorganisms.

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CHAPTER SIX

RESEARCH CONCLUSIONS AND RECOMMENDATIONS

RESEARCH CONCLUSIONS

Research Objective 1: To formulate an antimicrobial coating containing nisin suited for large scale food package converting processes.

This research explored various types of materials, their properties and applications for use as a food contact packaging material. The original antimicrobial coating formulation [in Franklin et al 2004] from which the more recent work had been based upon produced a coating with properties unsuitable for up-scaling to large scale coating processes. Additionally the coated films produced lacked some qualities such as seal ability and transparency. This research study was used to re-structure the previous formulation in order to make it better suited for a transition to large scale equipment in additional to more desirable haze and sealing capabilities.

All ingredients used for the new formulation had all been GRAS (Generally Recognized As Safe) approved or utilized as common additives in the food industry. The carrier for nisin was determined to be polyvinyl alcohol (88%) (PVOH). Polyvinyl alcohol was chosen because it is a water soluble polymer that is commonly used in the food and pharmaceutical industries. A PVOH that is 88% hydrolyzed compared to a higher value contains a higher percentage of acetate groups. These larger side chains

produce properties such as a higher degree of amorphousness which increase solubility in water and decrease T_m or the melt temperature of the resin for increase sealing capabilities. The plasticizing agent was determined to be 100% pure vegetable glycerin based upon differential scanning calorimetry. Glycerin decreased the melt temperature of the PVOH resin to 150.94°C from 189.66°C which was also in the sealing range of the substrate to which the PVOH was to be coated.

Additional ingredients included Tween®80, Nisaplin®, acetic acid solution (0.02 M) and ethanol/water solvent mixture (50/50 v/v). The Nisaplin® (2.5% nisin), acetic acid solution and ethanol/water solvent mixture were all adapted from Franklin et al. Tween®80 is used in the food industry for multiple applications as an emulsifier, surfactant or foam reducer.

Through dynamic contact angle work and tape tests (ASTM F2252) it was determined that the substrate to which the coating would be applied would be a multilayer coextruded material donated by Sealed Air Corporation. This testing was also implemented to determine the necessity for surface treatments such as corona discharge treatment and priming. The sealant layer was LLDPE (linear low density polyethylene). The formulated coating produced the lowest contact angle measurements 21° compared to other substrates except for an EVA (ethyl vinyl acetate) and sodium ionomer. (α=0.05) A tape test was conducted to determine which substrate the coating had the best degree of adherence which was LLDPE.

Basic studies of the coating were also explored during this study to be within the application ranges for large scale processes such as gravure and/or flexography. Antimicrobial efficacy of the coating was also tested through the formulation process against spoilage indicator *Micrococcus luteus* (ATCC 10240) to show that coating ingredients and processing steps did not deactivate the antimicrobial nisin.

Research Objective 2: To conduct coating trials with the formulated antimicrobial coating containing nisin using large scale application coating processes.

This research study explored the ability of the produced coating formulation to be implemented on large scale equipment. Properties of the liquid coating and dry coated films produced were conducted to characterize the materials. Two large scale application methods were used during this study: gravure and flexography. The gravure trial required three passes based on equipment limitations. The substrate was corona treated at the Sonoco Institute of Packaging and Design and priming and coating application was conducted in the DuPont Laboratory at Clemson University.

This study showed that the formulated antimicrobial coating could be implemented on large scale coating equipment however some troubleshooting and adjustments were required. The material produced during the gravure trial exhibited adhesion difficulties. It was concluded that there could have been a combination of factors that affected adhesion such as coating ingredients, priming application and corona treatment. It was concluded that the material had been excessively corona treated and the

primer was not applied with the correct anilox roller. Adjustments were made based upon these findings for the second trial using a flexography press.

Properties of the coating liquid solution that were tested included solids content (%), viscosity (sec) using a Zahn cup and pH. Although the antimicrobial containing coating was slightly acidic (pH =5.96 ± 0.02), corrosive resistant equipment parts can be implemented to reduce acid corrosion. The viscosity and percent solids measurements were also found to be within the range for large scale processes.

Properties of the coated film tested included basis weight (#/ream), block testing (gf), haze (ΔE) and film on lawn. The coat weights or basis weights varied between gravure and flexography processes as expected. The films showed potential for blocking was expected from preliminary testing. The haze of the film was determined to be imperceptible to the human eye. (ΔE < 1) It was also found that the films were effective against *M. luteus.* The gravure coated material produced inhibition zones of 5.78±2.20 mm passed the perimeter of the film sample tested while flexography samples produced zones of 3.60±1.36 mm. The difference was concluded to be due to the gravure samples having a higher basis weight of approximately 1.5 #/ream while flexography films had a coat weight of approximately 0.74 #/ream.

Several studies have been conducted on antimicrobial coatings containing nisin. However few studies have implemented antimicrobial coatings on large scale coating equipment. One of the aims of this study was to produce a material that had the potential to be produced for the food packaging industry. This included implementation of

ingredients, substrates and processes that are common to the packaging industry in order to avoid high ingredient costs and capital costs for equipment purchases. This study showed that it is possible to implement nisin in an antimicrobial coated material using large scale processes without deactivating the antimicrobial from high temperature or high pressure sheer abuse type processing.

Research Objective 3: To apply mass transfer theory for prediction of the release and diffusion of nisin from a polyvinyl alcohol matrix coated film.

The final objective of this research was to review previous studies and apply mass transfer theory to the antimicrobial packaging system that was produced throughout this doctoral work. This work discusses the difficulties of predicting nisin diffusion and release from an antimicrobial coating based upon the film matrix (solid, liquid or gel). The study also discusses some of the many variables that are important to consider when attempting to characterize a system such as partition and convection coefficients, the food simulant to be used, the type of permeant and the polymer matrix containing the permeant. These is additional difficulties in characterizing the diffusion or release in multivariable systems based on Fick's second law of diffusion which are only more complicated by addition of a food product rather than a food simulant.

Several studies have conducted either diffusion and/or controlled release studies of nisin from various film structures into liquids or agar food simulants. Due to the complication of these systems they need to be analyzed on a case by case basis. This

work also presents potential methodology for testing the diffusion or release of nisin from the proposed antimicrobial system.

FUTURE RESEARCH RECOMMENDATIONS

1. The antimicrobial coated film was found to be effective against *Micrococcus luteus.* Additional work could be conducted to determine the sensitivity of other spoilage microorganisms when tested against the films produced.
2. Other properties of the material could be tested such as the seal ability by producing a heat seal curve and determining the thermoforming capabilities. Because this material was originally planned to be applied to thermoformable packaging, testing the thermoforming capabilities and possible nisin deactivation due to heat exposure could be studied.
3. Antimicrobials have been observed to behave differently when tested against a food product compared to microbial growth media. Conducting a challenge study on an actual food product with this packaging film could indicate whether extension of shelf life would be achieved with this material.
4. Diffusion and release studies are recommended to better understand the packaging system and how the nisin is released. It is also important to note that many procedures focus on the diffusion of nisin through detection but exclude whether the nisin maintained antimicrobial efficacy.

APPENDICES

APPENDIX A:

SUPPLEMENTARY FORMULATION TESTING

Nisaplin® is a commercial grade antimicrobial produced by Danisco (a subsidy of DuPont). The material contains a 2.5% concentration of the antimicrobial Nisin. Predecessors at Clemson University have conducted work on producing an antimicrobial coating containing Nisaplin® for reduction of *Listeria monocytogenes* in ready-to-eat food products such as hotdogs and turkey bologna deli meat products. Components from the work of these individuals had resulted in the antimicrobial coating formula as shown in Table A.1.

The following studies in this appendix include work from an original coating formula as described in Franklin et al 2004 (Table A.1). The work was discontinued with this formula due to problems with heat sealing and small batch process thus requiring additional research. The re-formulation process began after determining that the percent solids (9.5%) was too low for sufficient coating transfer to a base film substrate. Typically large scale processes such as gravure and flexography require percent solids ranging from approximately 15-40% [19]. Additionally, it was determined that the tunnel dryer of the gravure coater/laminating line in the DuPont Laboratory in Newman Hall at Clemson University did not have the capacity to dry off a solution containing 90.5% liquid solvents. The formulation produced by Franklin et al (2004) was also composed of cellulosics methylcellulose and hydroxypropyl methylcellulose (70/30 w/w) which are highly crystalline materials that prohibited sealing. This appendix provides preliminary

work using the Franklin et al (2004) formulation in addition to some preliminary studies conducted during the re-formulation process.

Table A.1. Antimicrobial coating formula produced by previous student for continued work.

Franklin et al Antimicrobial Coating Formula	
Ingredient	**Volume**
Nisaplin® (10,000 IU/mL concentration)	2.5 g
0.02 M Acetic acid solution	1.25 mL
Methylcellulose	0.875 g
Hydroxypropyl methylcellulose	0.375 g
Polyethylene glycol 400	25 mL
Ethanol (95%)	0.75 mL
Distilled water	25 mL

*****as prepared in Franklin, Cooksey & Getty, 2004

Materials and Methods

A preliminary study was conducted in order to determine the effects of pH of a liquid antimicrobial coating (which was then cast and dried) on the antimicrobial effectiveness. Films were tested against *Micrococcus luteus (ATCC 10240)* and *Listeria monocytogenes (ATCC 15313). M. luteus* has been used as a spoilage indicator in previous work while *L. monocytogenes* was tested to determine efficacy against a pathogenic microorganisms. The antimicrobial coating was produced utilizing the same formula and process indicated in table A.1 except 0.625g of Nisaplin® was utilized to

adjust the concentration to 2500 IU/mL. [6]. This level of nisin was used because in the study conducted by Franklin et al (2004), 2500 IU/mL was the lowest concentration of nisin that maintained efficacy against a five strain cocktail of *L. monocytogenes* for the 60 day study. Coating solutions were adjusted to desired pH levels (4, 6 and 7) using 0.02 M Acetic acid or 0.02 M NaOH. The coating was then cast onto glass plates using a thin layer chromatography plate coater (CAMAG, Muttenz, Switzerland). The films were peeled from the glass plates and thickness was measured with a Nikon Digimicro MFC-101 micrometer (Nikon Corporation, Excel Technologies, Inc. Enfield, CT, USA). The average film thickness using this casting method was approximately 1.37±0.20 mils. (n=18)

Inhibition testing was performed using a single strain of *Listeria monocytogenes* (ATCC 15313). This strain was grown by taking a single listeria colony from a pre-streaked plate with an inoculating loop and was placed in 20 mL of Brain Heart Infusion (BHI) broth in a sterile Erlenmeyer flask. Microbial work was conducted in a Labconco purifier class II biosafety delta series cabinet. The culture was put in the incubator at 37ºC (Fischer Scientific Isotemp Incubator) and was shaken at a constant rate for 6 hours. Initial population was determined by spread plating dilutions in duplicate onto MOX (modified oxford) media which is selective for *Listeria monocytogenes.* The film on lawn method was then used to test the inhibitory effects of the control and treatment coated films with coating solutions at different pH levels. Film disks were 12 mm in diameter. Film on lawn plates were incubated at 37ºC for 24-48 hours. *Listeria* colonies were counted on the dilution plates using the Leica Quebec darkfield colony counter. Inhibition

zones on the film on lawn plates were measured in millimeters as the clear zones that extended passed the substrate disc using a Cole-Palmer carbon fiber composites digital caliper. (Figure A.1) (n=12)

Results

Figure A.2 showed that the lower the pH of the initial coating solution, the larger the inhibition zone. (n=12) However, it has been shown in the literature that the antimicrobial nisin increasingly activated in a lower pH range and shows reduced activity in alkaline conditions [6; 10]. Nisin is produced during a fermentation process carried out by *Lactococcus lactis spp. lactis.* Lactic acid is a product of the fermentation process, therefore the bacteriocin, nisin, was produced in order to withstand highly acidic environments and eliminate microbes which could be cause for competition [15].

This preliminary study resulted in understanding that the antimicrobial coating should maintain a low pH during the production process in order to achieve inhibitory properties against *Listeria monocytogenes (ATCC 15313).* However, low pH coatings could result in the degradation and wear of highly expensive coating equipment in a large scale operation. Corrosive resistant components would need to be utilized in addition to extra cleaning between coating runs.

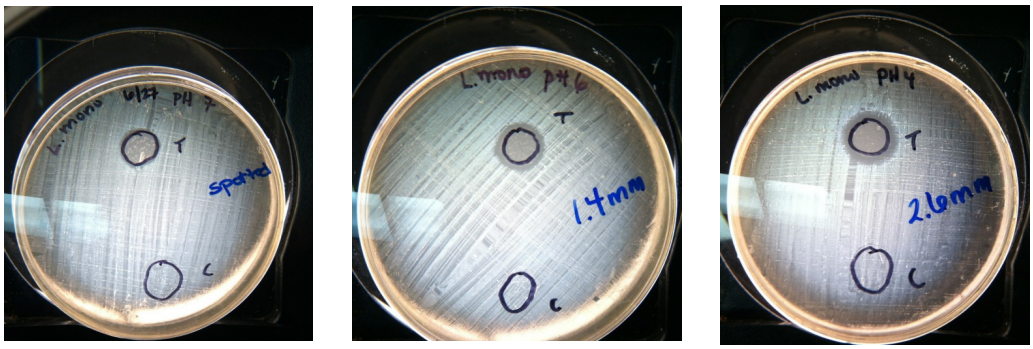

Figure A.1. Film on lawn results of Franklin et al (2004) coating formulation (2500 IU/mL Nisaplin® concentration) tested against *Listeria monocytogenes ATCC 15313* displaying effects of pH on inhibitory properties. (Left: pH 7; Center: pH 6; Right: pH 4)

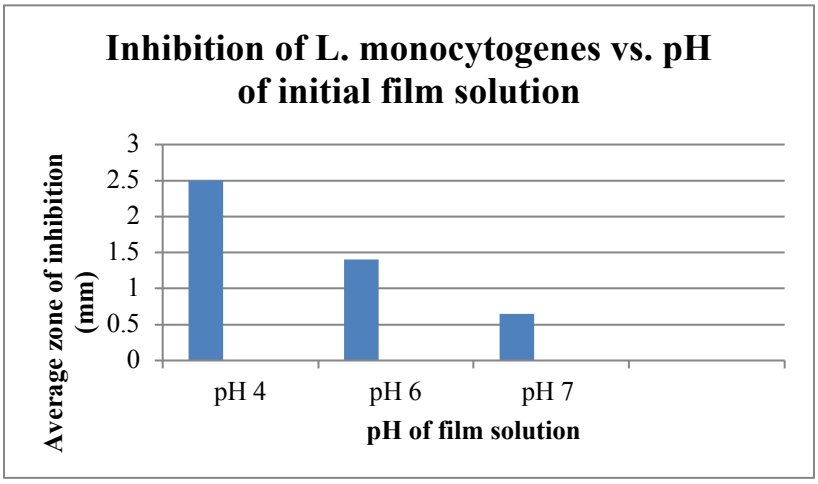

Figure A.2. Average inhibition zones based on pH of antimicrobial coating.

Coating weight determination:

This preliminary study was conducted to determine whether the coating weight of the Franklin et al (2004) antimicrobial coating formula would have an effect on

antimicrobial efficacy. Methyl cellulose coatings contained 12,500 IU/g of Nisaplin® (2.5% Nisin A concentration) as calculated from the formula in Table 1.

$$1 \text{ gram Nisaplin®} = \frac{1{,}000{,}000\ IU/g}{80\ mL\ total\ coating\ liquid\ volume} = 12{,}500 \text{ IU/mL}$$

Sample Preparation:

Coatings were produced same day and coated onto a polyethylene terephthalate or polyester (PET) laminate film containing a linear low density polyethylene (LLDPE) sealant web. Drawdowns were produced by then coating the antimicrobial coating solution onto pieces of film using three different sized Mayer rods. (7, 16 and 28) Films were dried at ambient conditions overnight. Control films were produced by coating LLDPE with a coating solution which did not contain Nisaplin® however, the coating contained all other components in the formulation. Coating weights were determined by following ASTM 2217 [1].

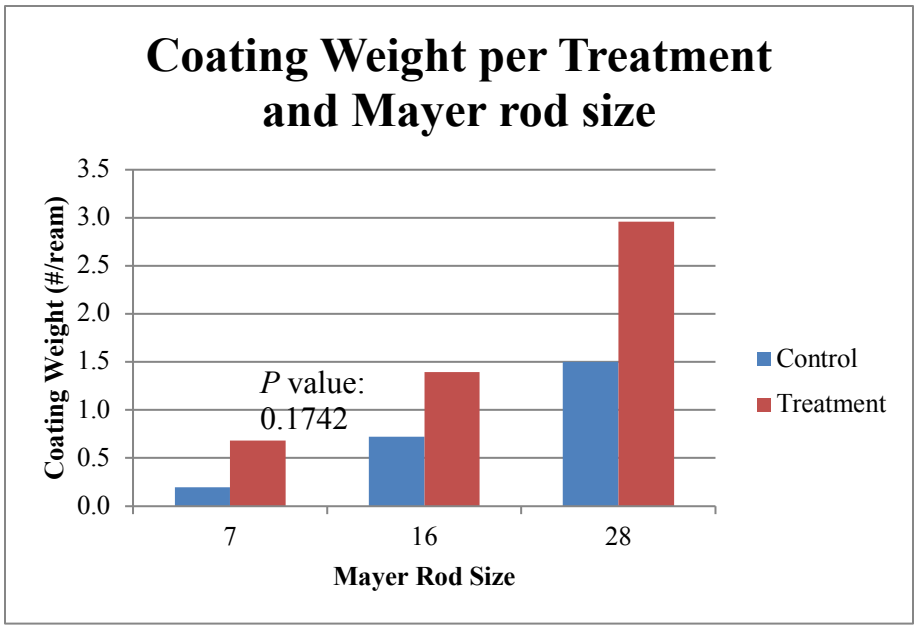

Figure A.3. Average coating weights of films utilizing Mayer rods (size 7, 16, and 28). Control films did not contain Nisaplin®. (α=0.05)

Figure A.3 shows the resulting coating weights. A two-tailed T-test was conducted in excel to determine whether there was a significant difference between the control and treatment coated films. It was determined that there was a statistical difference between control and treatment for Mayer rod sizes 16 and 28, but not for Mayer rod 7 (α=0.05). It was expected that the increase in Mayer rod size would lay down a higher coating weight however, it was uncertain as to whether the Nisaplin® would cause a significant difference in the coating weight of the treated films compared to the control. The coating weight nearly doubled to 3 pounds per ream (size 28 Mayer rod) for the treatment films compared to approximately 1.5 pounds per ream in the control film.

Coating Characterization:

Coating preparation (1.5 times the "recipe" described in Table A.1.):

Acetic acid (1.875 mL of 0.02 M concentration) aqueous solution was added to a 100 mL beaker. Nisaplin® (3.75 g) (Danisco, Inc. Madison, Wisconsin, USA) was then weighed into a weigh boat using an analytical balance (Mettler Toldeo PG 203-S, Mettler Toledo, Columbus OH) and added to the acetic acid solution. This amount of Nisaplin® was chosen to yield the legal limit of 10,000 IU/mL concentration in the finished volume of coating solution. Ultrapure water (37.5 mL) was added to the beaker in addition to 1.3125 grams of methylcellulose and 0.5625 grams of hydroxypropyl methylcellulose (Sigma-Aldrich Corporation LLC, St. Louis MO). The solution was then homogenized for 2 minutes using Vertis Vertishear tissue homogenizer apparatus with a 20 mm shaft. (The Vertis Company, Gardner NY) Ethanol (37.5 mL of 95% concentration) and 1.125 mL of polyethylene glycol 400 (Sigma-Aldrich Corporation LLC, St. Louis, MO) was then added to the solution followed by repeating homogenization. The coating solution containing Nisaplin® was designated as a treatment solution and control solutions were produced in the same manner but lacked the addition of Nisaplin®. A total of 3 treatments and 3 control solutions were produced.

Viscosity

Two methods of testing viscosity were used in order to have both research and commercial methods for determining viscosity. The commercial method used was a Zahn #2 cup which is commonly used in manufacturing plants as a simple and fast on-line test.

The second method was to test viscosity using a Brookfield Viscometer (Brookfield LV-DV-E Viscometer).

The following parameters were kept constant when obtaining measurements using the Brookfield viscometer:

Test time: 2 minutes

Speed of Spindle: 60 RPM

Temperature: ambient temperature (22-25°C)

Spindle type: 02

Beaker size: 100 mL

Volume of solution: ~80mL

The Brookfield spindle was set to spin at 60 RPM and the measurement was taken over a 2 minute period until the torque and viscosity readings stabilized. A range of both the torque and the viscosity in cP or centipoise was recorded for both control and treatment coatings. (n=6)

The Zahn cup testing was conducted by filling the cup with solution till it begins to overflow while plugging the hole in the bottom of the cup manually. The hole is then unplugged and a timer is simultaneously started. The timer was then stopped when the stream of coating exiting the cup breaks or is no longer a continuous stream of coating indicating the cup is nearly empty. The amount of time in seconds for the coating to exit the Zahn cup was recorded. (n=6)

pH

The pH of the coating solution was measured with a pH meter. (Thermoscientific Orion star A214 pH/ISE meter) The pH meter was calibrated prior to sampling with buffer solutions (pH 4, 7 and 10). (n=6)

Percent Solids

The percent solids of coating solutions were tested as this is an important parameter when determining a printing or coating method. Aluminum pans were weighed on a balance and recorded. (Mettler Toledo PG203-S) The coating solution to be tested was mixed to ensure homogeneity prior to weighing approximately 0.5g aluminum pan. The pans were left to dry overnight at ambient conditions. The pans were re-weighed the following day. Percent solids were then calculated as the amount of solid material left in the aluminum pan after the liquid portion of the coating solution had evaporated through drying. Samples were run in triplicate. (n=21)

Coating weight

The coating weight or basis weight of the coating on the substrate was determined using ASTM 2217 [1]. A metal template was used to cut two samples of equal surface area from each draw down representing a different Mayer rod size and treatment type. Each sample was weighed on an analytical balance and the weight was recorded. The coating was then wiped off of the substrate with water and paper towels and the new mass was recorded. The basis weight of the coating was then calculated in pounds per

ream. (#/ream) Mayer rod sizes 6, 16 and 28 were tested. (n=18) Mayer rod size 16 was used for the following drawdowns and drawdowns through the study due to the mid-range coat weight achieved.

Drawdowns

Drawdowns were produced using an apparatus that contained magnetic bar to hold the substrate in place. The mid-range Mayer rod size (16) was utilized for the drawdowns. (The expected coating weight was approximately 1.5 #/ream) The substrate was placed under the magnetized strip (sealant side up), a Mayer rod was placed in front of the magnetic strip and coating was poured in front of the Mayer rod in a length just short of the substrate width. The Mayer rod was then pulled down the length of the substrate at a uniform speed. Each drawdown was dried at ambient conditions overnight.

Haze (ΔE)

Haze (ΔE) testing was conducted using a Minolta La*b* colorimeter (CR-400 Chromameter). The colorimeter was calibrated using a white calibration standard. Three measurements were taken from each coated piece of film. ΔE was then calculated using the following formula: (n=12)

$$\Delta E: \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

Film on lawn:

See previous film on lawn procedure. (n=6 for #16 Mayer rod size)

Statistics

All statistics for this preliminary study were done in Microsoft Excel.

Results

Table A.2. Selected physical coating characteristics of Franklin et al 2004 formulation.

Coating Characteristics – Nathan Franklin (NF) Formulation						
	Zahn #2 (sec)	**pH**	**Percent solids (%)**	**Coat Weight (lb/ream) (#16 Mayer)**	**Haze (ΔE)**	**Inhibition (mm)**
Control	43.1±2.4	4.45±0.1	4.59±0.2	0.72±0.09	< 1.0	0±0.0
Treatment	48.6±9.8	4.38±0.05	9.5±0.1	1.4±0.2	< 1.0	2±0.36

Coating Characterization Discussion:

The results for characterizing the coating formula designated in Table A.1 area shown in Table A.2. Viscosity measurements were conducted using a Zahn #2 cup. The measurements taken using the Brookfield viscometer were not used due to a calibration issue. However, using a conversion chart, the Zahn cup values indicated that the liquid has an approximate viscosity of 100-125 cP [5]. These values indicate that the coating could potentially be used for engraved roller (gravure) or flexography coating/printing processes. There was no statistical difference between the control (no Nisaplin®) and treatment coatings.

Addition of Nisaplin® also had no significant difference on the pH of the produced antimicrobial coating. This coating as stated previously is acidic and may require corrosive resistant coating equipment components in a large scale operation. On

the other hand, the addition of Nisaplin® to the coating increased the solids content from 4.59% to 9.5%. It was later determined that scaling up the coating to a flexography or gravure coating application at the current percent solids level was too low for these applications. Percent solids for flexography and/or gravure applications should be in the range of 15-40% [19].

In addition to low solids content, methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) components prohibited package sealing. These two components are highly crystalline. Preliminary observations showed that the temperatures tested in attempts to seal MC and HPMC coated films melted and deformed the polymer LLDPE substrate. This particular coating also required continuous mixing. The antimicrobial and cellulose components would settle to the bottom of storage containers and beakers indicated that this produced coating was a suspension rather than a stable emulsion.

Haze (ΔE) measurements were less than 1.0 for both control and treatment films indicating that coating did not produce a perceivable color difference when compared to an uncoated film for both coating treatments [20]. Preliminary film on lawn testing indicated that the treatment film had an average zone of inhibition of 2 mm±0.36. The disk itself measured 12 mm totaling 14 mm inhibitory effect overall against *L. monocytogenes.* The control samples showed no zones of inhibition nor any clearing or inhibition under the film sample. This indicated that the Nisaplin® does have inhibitory properties against *L. monocytogenes (ATCC 15313)*. Because of this, in addition to work with Nisaplin®, the new formulation to be developed will contain Nisaplin® as an antimicrobial component.

Re-Formulation Testing

Table A.3. Summary of formulations produced in attempts to yield a coating solution suitable for large scale processing techniques such as gravure coating.

Formulation Trials Summary				
Trial	**Description**	**Average Percent Solids (%)**	**General Observations of Coating**	**Ideas**
Original Formula	• 2.5 g Nisin • 0.875 g Methylcellulose (MC) • 0.375 Hydroxypropyl methylcellulose (HPMC) • 1.25 mL (0.2M) Acetic Acid Solution • 25 mL distilled water • 25 mL 95% Ethanol (EtOH) • 0.75 mL Polyethylene glycol 400 (PEG 400)	9.50	• Too low percent solids for commercial application • exceeds capability of coater/laminator drying in DuPont • unable to be sealed	• Increase solids with additional MC • Pattern coat using flexographic coating method
1	• 10g MC • 1 g ground Lecithin • 1 mL Tween 80 • 100 mL 95% EtOH	12.41	• Essentially paper • Extremely brittle	• Eliminate MC from overall formula
2	• 5 g MC • 5 g PVOH • 70 mL 95% EtOH • 30 mL distilled water • 1 mL Tween 80 • 1 mL PEG 400 • 1 g Lecithin • 2 mL (0.2M) Acetic Acid Solution • 1 g milk solids	13.55	• Does not seal • Looks aesthetically appealing • Brittle • Easily delaminates	

3	• 10 g PVOH (4-98) • 30 mL distilled water • 30 mL 95% EtOH • 1 g ground lecithin • 1 mL Tween 80 • 1 g milk solids • 2 mL (0.2M) Acetic Acid Solution	20.10	• Do not put the Tween 80 in milk solids/acetic • Turns to a solid and makes the solution chunky • Add to PVOH, lecithin and D2O. • Coating is brittle and completely separated from the substrate • Leaves an oily residue on fingers • Does not seal	• According to Literature of Analysis of Coating Failures by George Mills, surfactants can affect cohesion of the coating leaving a weak boundary layer and leaving the coating unable to adhere to the substrate. Remove tween 80 • Decrease lecithin (both surfactants) • Add PEG 400 • Homogenize to make more uniform
4	• 10 g PVOH (4-98) • 30 mL distilled water • 0.5 g ground lecithin • 1 g milk solids • 2 mL (0.2M) Acetic Acid Solution • 30 mL 95% EtOH	19.36	• Clear • Rough to the touch • Adhered better to the substrate in comparison to trial 4but still some delamination • Still did not seal at 400 F - 2.5 sec dwell and 40 psi. • Issue with PVOH? Crystalline structure.	PVOH MSDS states melt at 200 C or 392 F. Will increasing the plasticizer decrease the melt temperature? Run DSC on original resin. Run DSC on coating with increasing amounts of plasticizer? Other options other than PEG 400? Benzyl Benzoate, USP

				used as a food additive and plasticizer. Chemically compatible with PVOH? -
5	• 10 g PVOH (4-88) • 30 mL distilled water • 3.2 mL Glycerin • 185 uL Tween 80 • 30 mL 95% EtOH • 0.3 g ascorbic acid • 1 g milk solids • 2 mL (0.2M) Acetic Acid Solution	21.91	• Delamination issues •Yellow coloration •Haze due to milk solids	• Add potassium sorbate for extra antimicrobial properties • Chemically compatible with PVOH? • Trial with corona treater if finally get seals?• Other options other than PEG 400? • Benzyl Benzoate, USP used as a food additive and plasticizer.
6	• 10 g PVOH (4-88) • 30 mL distilled water • 3.2 mL Glycerin • 185 uL Tween 80 • 30 mL 95% EtOH • 0.3 g ascorbic acid • 0.22 g potassium sorbate • 1 g Nisaplin • 2 mL (0.2M) Acetic Acid Solution	22.82	• Appears homogeneous • Clear - slightly beige • Adhesion issues - Coating delaminates from substrate after several days at ambient conditions or a couple of days in 45 C oven. • Thicker coating yields more	• Corona Treat • Primer • Lessen Potassium salt • Eliminate ascorbic acid • Minimum Inhibitory Concentration Testing

			haze. Potassium salt? • Yellow coloration after storage in oven	
6 (Control -No antimicrobial)	• 10 g PVOH (4-88) • 30 mL distilled water • 3.2 mL Glycerin • 185 uL Tween 80 • 30 mL 95% EtOH • 2 mL (0.2M) Acetic Acid Solution	20.62	• Clear • Adhesion issues	• Corona Treat • Primer
7	• 10 g PVOH (4-88) • 30 mL distilled water • 3.2 mL Glycerin • 185 uL Tween 80 • 30 mL 95% EtOH • 1 g Nisaplin • 2 mL (0.2M) Acetic Acid Solution			

Antimicrobial Determination

Materials and Methods

Coating Preparation

The coating solution was prepared by heating and simultaneously stirring 10 grams of 4-88 Mowiol PVOH resin in 30 mL of distilled water to 120°C for approximately 30-45 minutes until the resin dissolved into solution. Once the resin had dissolved, 3.2 mL of glycerin (40 parts per 100 grams of PVOH resin) and 185 µL of

Tween® 80 (0.25% v/v) (Polysorbate 80, FCC, Spectrum Chemical Manufacturing Group, New Brunswick, NJ, USA) were then added to the cooling resin solution. In a separate beaker, 1 gram of Nisaplin ® (2.5% - 12,500 IU/mL in solution) (Danisco, Inc. Madison, Wisconsin, USA) was dissolved in 2 mL of 0.02 M acetic acid solution [6]. (Glacial acetic acid, Fischer Scientific, Waltham, MA, USA) 30 mL of 95% ethanol was then added, covered and stirred while adding both 0.3 g (0.4% w/v) ascorbic acid (ascorbic acid USP, Avantor Performance Materials, Inc. Center Valley, PA, USA) and 0.22 g (0.3% w/v) potassium sorbate. (Granular potassium sorbate, Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA) Both the resin solution and the ethanol solution were combined upon dissolving all components and cooling the resin solution.

Film Preparation

A multi-layer coextruded film material donated by Sealed Air Corporation was used as the substrate for this coating work. The sealant web of this material consisted of a linear low density polyethylene (LLDPE). The LLDPE underwent corona discharge surface treatment (BD-20 handheld treater) to oxidize the surface of the film to promote adhesion and to clean the surface of debris such as dust. The film was then coated with a water soluble primer donated by MICA Corporation. (MICA A-131-X) The primer, polyethylenimine (PEI), is a common primer that was recommended for adhering a highly polar component such as a PVOH based coating with a non-polar substrate such as LLDPE. The primer was diluted 1 part PEI to 9 parts water and coated to LLDPE using a

#3 Mayer rod. The primer was left to dry at least 4-6 hours at ambient conditions prior to coating with the antimicrobial coating.

Minimum Inhibitory Concentration (MIC)

Listeria innocua (ATCC 33090) was propagated twice and grown overnight at 30°C in TSBYE (Tryptic soy agar with yeast extract). (Difco Tryptic Soy Broth, Becton Dickinson and Company, Sparks, MD, USA; Bacteriological yeast extract, ultra-pure grade, Amresco, Solon, Ohio, USA) The initial population was 10^8 CFU/mL. Semi-solid agar (30 mL) was produced and inoculated with 30uL once the media cooled to 42°C resulting in a 10^8 CFU/mL population for testing. Antimicrobial solutions (10µL) were pipetted into individual wells, and 190 µL of inoculated semi-solid agar were pipetted on top of the solution. Each well was plated in triplicate. The plates were then covered and incubated inverted (after cooling) at 30°C for 24 hrs. Visual observations noting growth or no growth were recorded. This procedure was adapted from Wilson Stanford et al 2009.

The following concentrations of antimicrobial solutions were tested against *L. innocua.* The solutions were produced in distilled water and 0.02 M acetic acid solutions (Table A.4):

Table A.4. Antimicrobial concentrations tested for determining minimum inhibitory concentration of Nisaplin®, potassium sorbate and ascorbic acid against *Listeria innocua* ATCC 33090.

Antimicrobial Concentrations Tested: Minimum Inhibitory Concentration													
Nisaplin® (IU/mL)	500	250	225	200	175	150	125	100	75	50	25	12.5	0
Potassium Sorbate (%)	0.5	0.4	0.35	0.3	0.25	0.2	0.15	0.10	0.05	0			
Ascorbic Acid (%)	0.5	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.05	0			

Film on Lawn:

Spoilage indicator *Micrococcus luteus* (ATCC 10240) and pathogen *Listeria monocytogenes (Scott A;* ATCC 15313*)* were propagated twice prior to being tested. *M. luteus* was incubated for 48 hours at ambient conditions and *L. monocytogenes* was incubated for 24 hours at 37°C. A single colony from each plate was transferred to Erlenmeyer flasks containing 30 mL of TSBYE (Tryptic soy broth with 0.6% yeast extract) which were then incubated at conditions previously stated. TSAYE (Tryptic soy agar with 0.6% yeast extract) (Difco Tryptic Soy Agar, Becton Dickinson and Company, Sparks, MD, USA) plates were spread plated from the stock broths with an 8 log CFU/mL inoculum. Square film samples (15 mm) were placed coating side down onto the inoculated agar. Two types of film samples were tested against *Micrococcus luteus.* Control samples contained no antimicrobials while treated samples contained three. (Nisaplin®, potassium sorbate and ascorbic acid) This was to determine if there was any

inhibitory affects against *M. luteus* in the developed coated film. Inhibition (clear) zones were measured and recorded after incubation.

Four additional types of film samples were tested against *Listeria monocytogenes.* Control films with no antimicrobials and treatments film containing Nisaplin®, Nisaplin® and potassium sorbate or Nisaplin®, potassium sorbate and ascorbic acid were prepared. This was conducted to determine if there was additional, synergistic, antagonistic or no additional affect with addition of potassium sorbate and ascorbic acid to the inhibitory effects of Nisaplin® in the coated materials. Three film samples were tested from each drawdown and three drawdowns were produced from each coating treatment to be tested with each microorganism.

Spot on Lawn:

Listeria monocytogenes ATCC 15313 and non-pathogenic *Escherichia coli* ATCC 9637 were propagated twice and grown overnight in TSBYE. *L. monocytogenes* was grown at 37°C while *E. coli* was grown at 30°C. Both varying concentrations of potassium sorbate in potassium salt and Nisaplin® were produced in PBS (Phosphate buffered solution: pH ~7.35) (Table A.5)

Table A.5. Antimicrobial concentrations of Nisaplin® and potassium sorbate for spot on lawn testing against *Listeria monocytogenes ATCC 15313* and *Escherichia coli ATCC 9637.*

Antimicrobial Concentrations Tested: Spot on Lawn											
Nisaplin ® (IU/mL)	1000	500	250	125	62.5	31.25	15.625	7.1825	0		
Potassium sorbate (%)	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	.04	0.2	0

Populations (10^9 CFU/mL) of *L. monocytogenes* and *E. coli* were spread plated onto petri dishes containing TSAYE. The petri dishes were labeled with antimicrobial solution concentrations in each quadrant. Drops (10 μL) of the corresponding solutions were plated onto the petri dishes. The plates were incubated for 24 hours prior to observations being recorded. Spot on lawns were conducted in triplicate.

Results: Antimicrobial Determination

Minimum Inhibitory Concentration (MIC):

Results showed that neither PS nor AA were able to inhibit *L. innocua* at any of the concentrations tested. Nisaplin® at a concentration of 100 IU/mL inhibited *L. innocua* in all three replicates. Both distilled water and acidified water carrier solutions containing Nisaplin® inhibited at the same concentration (100 IU/mL). (Figure A.4)

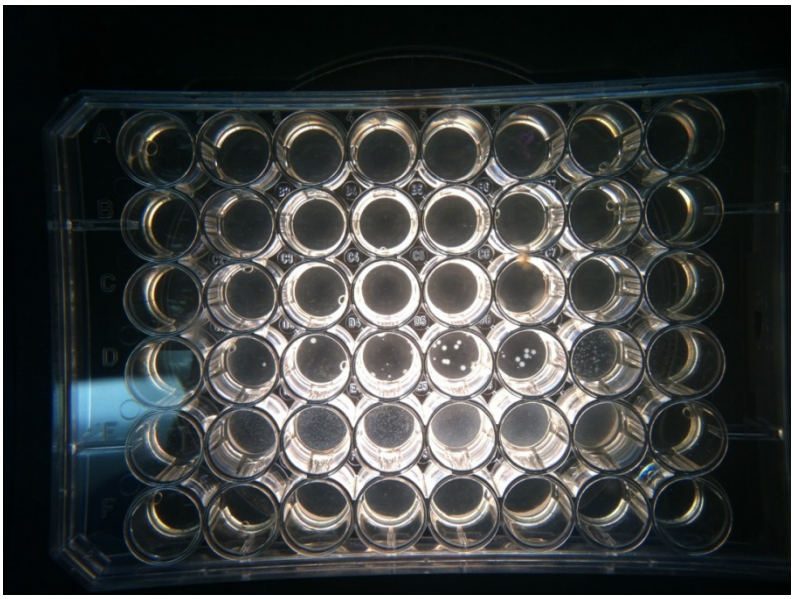

Figure A.4. Minimum inhibitory concentration results of Nisaplin® against *Listeria innocua ATCC 33090.*Clear wells indicated complete inhibition of bacterial strain. High to low concentrations were plated in triplicate from left to right in rows.

Film on Lawn

Control film samples inhibited *Micrococcus luteus*. The treatment film inhibited *M. luteus* resulting in a clearing zone extending passed the outer edge of the film averaging 16.5 mm. Inhibitory effects against *L. monocytogenes* showed no inhibition with the control film and inhibitory effects only in the area where the treatment films were in direct contact with the bacteria (15mm). According to the results, there was a significant difference between the control (no antimicrobial) and treatment (Nisaplin®, potassium sorbate and ascorbic acid) of the films inhibitory effects against *Micrococcus luteus* and *Listeria monocytogenes*. (*P* value < .00001)

In order to determine whether all three antimicrobials were necessary in the coating formulation, coating formulas containing Nisaplin ®; Nisaplin® and potassium sorbate; Nisaplin®, potassium sorbate and ascorbic acid were tested using film on lawn against *L. monocytogenes.* There were no significant differences between inhibitory effects against *Listeria monocytogenes* between coatings containing combinations of Nisaplin ® (12,500 IU/mL), PS (3%) and AA (3%).

Spot on lawn

Results showed that potassium sorbate was unable to inhibit *Listeria monocytogenes* at 2% concentration and below. These results showed that both PS and Nisaplin® were ineffective against non-pathogenic *E. coli* in this test. No clearing zones were visible from the antimicrobial drops plated on the petri dishes at any concentration.

Discussion: Antimicrobial Determination

Minimum Inhibitory Concentration (MIC)

Nisaplin® was shown to inhibit *Listeria innocua* ATCC 30339 at a concentration of 100 IU/mL. There are many variables that can cause changes in the MIC values obtained during testing. The sensitivity of bacterial strains varies resulting in higher or lower MIC values. Neetoo et al (2008) was able to show the sensitivity of varying bacterial strains after having conducted sensitivity testing on 12 strains of *Listeria monocytogenes* prior to selecting the three most resistant microorganisms, to use in a worst case scenario storage study [12]. Nisin A has been found to have an MIC of 6.25

μg/mL or 250 IU/mL against *Micrococcus* luteus, commonly used as a spoilage indicator organism [21]. Nisin MIC values can also vary between pathogenic and non-pathogenic strains, for example, Nisin tested against *Listeria innocua* and *Listeria monocytogenes* Scott A strains exhibited MIC values of 0.002 mM or 268 IU/mL [13] and 156.3 IU/mL. [8] Not only can the microorganisms have an effect on the MIC values obtained during testing but also the testing conditions, media utilized and even the growth phase of the bacteria therefore causing a lack in uniformity of MIC values obtained. Bacteria grown and tested in the stationary phase are hardier which can result in a higher MIC as opposed to the same bacteria grown to a population in the log phase of the growth curve [3].

As stated in the results, neither PS or AA were able to inhibit *L. innocua* at concentrations from 0 – 0.5%.There was no apparent difference in inhibitory effects between distilled water and acidified water carrier solutions. This indicated that the pH of these antimicrobial carrier solutions did not have a significant enough effect to cause differences in the inhibitory effects of the utilized antimicrobial components.

Film on Lawn

The control and treatment film yielded an inhibitory effect against *M. luteus.* Inhibition observed in the control samples could be explained by *M. luteus* being an aerobe therefore the bacteria were unable to survive under the film sample which lacked an oxygenated environment. Treatment film samples yielded zones of inhibition extending from the outer edge of the film indicating the occurrence of diffusion with an average zone of inhibition of 16.5 mm for a 15 mm film sample.

The control film yielded no inhibitory effects against *Listeria monocytogenes* (Scott A) while the treatment film (containing N, PS and AA) showed inhibitory effects. Additional combinations of the antimicrobials were tested in order to determine the need for adding potassium sorbate and ascorbic acid based on a synergistic effect with Nisaplin®. Coating solutions contained combinations (N, NPS, NPSAA) of the following concentrations of antimicrobials: 12,500 IU/mL Nisaplin, 3% Potassium sorbate, 3% Ascorbic acid. The results showed that there was no significant difference between the inhibitory effects of the coating containing just Nisaplin® in comparison to the coatings containing Nisaplin®, potassium sorbate and ascorbic acid. Potassium sorbate in combination with Nisaplin did not increase efficacy. This may be due to potassium sorbate being unable to target Gram positive bacteria or the concentration of the preservative in the coating solution becomes too dilute once it is spread over a large film surface area and dried. The addition of ascorbic acid yielded the same results as stated previously. Although ascorbic acid is not a strong antimicrobial, decreasing the pH of the solution has been shown to increase the inhibitory effects of Nisaplin [7; 17]. To account for the possibility of the concentration of the antimicrobials (PS and AA) not being high enough for a cast film application, 3% solution concentrations were tested using spot on lawn. *Listeria monocytogenes* and nonpathogenic *Escherichia coli* were tested against to determine if the bacterial cell wall composition also had an effect on efficacy.

Spot on Lawn

Because no inhibition was seen from utilization of potassium sorbate or ascorbic acid in against the tested microorganisms, these additives were removed from the coating formula. These ingredients also caused film quality issues including haze from the precipitation of salts and/or yellow discoloration. The yellow discoloration indicated that by the time the films were dried and ready to be tested, the ascorbic acid had already oxidized due to the instability of the molecule and being subjected to drying in an oxygenated environment.

Overall Antimicrobial Determination Discussion

Potassium sorbate is a common preservative and antimicrobial component that is used in the food industry. Concentrations up to 3% of potassium sorbate were tested. Han and Floros (1997) achieved slow growth of yeast using a 1% w/w potassium sorbate [14] concentration. Devlieghere et al 2000 achieved no inhibitory effects using a 5% concentration in an 70 mm EVA/LLDPE film produced, on the other hand, Pranoto et al 2005 was able to achieve reductions against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Listeria monocytogenes* and *Bacillus cereus* using a combination of potassium sorbate and Nisaplin (1020 IU/g of chitosan) incorporated into a chitosan film forming solution.

Ascorbic acid was introduced into this formula because PVOH can be subject to microbial degradation. Antioxidants such as ascorbic acid in combination with organic acids have also been shown to exhibit antimicrobial effects [17]. Tajkarimi and Ibrahim

(2011) showed that 0.4% concentrations of ascorbic acid alone were able to the population of four different strains of *Escherichia coli* 0157:H7 from 8.75 to 5.82 logs. When ascorbic acid was tested in combination with lactic acid, the microbial population was reduced to below the detectable level <10 CFU/mL in carrot juice. (solution pH 4.08) In BHI broth, the same combination of acids yielded a pH of 5.08. It was determined that the inhibitory effects of ascorbic acid were due to a decrease in the pH. Other groups have utilized ascorbic acid in combination with chitosan and lactic acid in a peptone water solution. The causative factor of inhibitory effects was also determined to be due to a decrease in pH as the treatment solution had a pH of 3.2 while the control had a pH of 6.45. The control showed no inhibitory effects [7].

Potassium sorbate and ascorbic acid were originally utilized in the coating formulation in attempt to produce a coating for both Gram negative and Gram positive bacteria. Overall these components were used in an attempt to yield an additive or synergistic effect resulting in overall increased efficacy against specified microorganisms. Although previous work has shown ascorbic acid and potassium sorbate to be effective inhibitors of spoilage and pathogenic microorganisms in addition to yeast and mold prevention, the components did not work well for this system. The components of this system could be acting as a buffer, causing the antimicrobials to lack inhibitory properties.

As stated in the general characteristics, the solution containing all 3 antimicrobials (Nisaplin, PS and AA) exhibited a pH of 4.5-5.0 while the control solution had a pH of 5.5-6.0. The drop in pH may not have been significant enough to cause inhibitory effects

for the specific microorganisms tested. A secondary possibility for the inhibitory effects of ascorbic acid was that because AA is an antioxidant, the ability for AA to absorb oxygen could have potentially starved the pathogenic strain of *E. coli* tested in systems of BHI and carrot juice systems [17]. This causative factor could have potentially removed the oxygen from the immediate environment essentially suffocating the bacteria. In the case of the bacteria tested, *L. innocua* and *L. monocytogenes* are both facultative and do not appear to have been affected. Although, *M. luteus* and the *E. coli* strains tested were aerobes, they also did not appear to be affected by the antioxidant properties of AA nor was the pH of the solutions low enough to cause a bactericidal effect. Lastly, both of these components are susceptible to oxidation and could have been rendered ineffective by the time of testing. Due to the lack of efficacy of potassium sorbate and ascorbic acid discussed previously, the Nisaplin® component was designated as the sole antimicrobial in the coating solution.

PVOH Film and UV Sterilization Preliminary Study

A preliminary study was conducted to determine whether UV (ultraviolet) treatment had an effect on antimicrobial efficacy and also as to whether UV treatment was necessary for conducting this antimicrobial work. It is expected that antimicrobial packaging materials are not treated with ultraviolet light prior to filling with food product.

Materials and Methods

Bacterial propagation

Micrococcus lutes (ATCC 10240) was propagated twice from a -80°C freezer stock. The bacteria was streaked onto a TSA (Tryptic soy agar plate) and incubated for 48 hours at room temperature under a biological hood. The bacteria was then propagated twice in TSBYE (tryptic soy agar with yeast extract) with an orbit shaker.

Coating and Film Preparation

Coated films were produced using the coating formulations 6 and 7 in Table A.3. The coating solution was prepared by heating and simultaneously stirring 10 grams of 4-88 Mowiol PVOH resin in 30 mL of distilled water to 120°C for approximately 30-45 minutes until the resin dissolved into solution. Once the resin had dissolved, 3.2 mL of glycerin (40 parts per 100 grams of PVOH resin) and 185 µL of Tween® 80 (0.25% v/v) (Polysorbate 80, FCC, Spectrum Chemical Manufacturing Group, New Brunswick, NJ, USA) were then added to the cooling resin solution. In a separate beaker, 1 gram of Nisaplin ® (2.5% - 12,500 IU/mL in solution) (Danisco, Inc. Madison, Wisconsin, USA) was dissolved in 2 mL of 0.02 M acetic acid solution. (Franklin et al 2004) (Glacial acetic acid, Fischer Scientific, Waltham, MA, USA) Ethanol (30 mL; 95%) was then added. Both the resin solution and the ethanol solution were combined upon dissolving all components and cooling the resin solution.

The LLDPE substrate used throughout the study was corona treated with a handheld corona treater and primed with PEI primer using a size 3 Mayer rod. The

polyethylenimine (PEI) primer (MICA A-131-X, MICA Corporation, Shelton, CT) was prepared by diluting 1:9 (PEI:water) and mixing. The primer was dried at least 4-6 hours at ambient conditions prior to coating with PVOH solution. Drawdowns of control and treatment coatings were produced using a size 16 Mayer rod and dried overnight at ambient conditions.

Film on Lawn

Micrococcus luteus, 10^8 CFU/mL population, was swabbed onto the surface of TSA plates using a sterile swab. A total of 24 film samples were cut using a 15 mm bore. Half of the control and half treatment samples were UV treated using a Zeta 7400 UV treater for 5 minutes. (Loctite Corporation, Newington, CT) Control and treatment films were placed on a single plate with tweezers. One plate was excluded due to improper sample placement. (n=11)

PVOH Film and UV Results and Discussion

Film samples treated with UV light showed an average inhibition zone of 3.21±1.97 mm while non-UV light treated samples showed an average of 4.27±1.47 mm. A two tailed T-Test conducted using Excel showed that were was no significant difference in the inhibitory properties of UV versus non-UV treated nisin containing film samples. (α=0.05; *P* value = 0.3330) However, there was a significant difference between control (no nisin) and treatment films. (*P* <0.0001) Because of these results, the research

was continued without UV light treatment of films to represent the efficacy of films in a manufacturing environment.

Static Contact Angle and Dyne Pen Preliminary Study

The following preliminary study was conducted in order to determine the wettability and contact angle of an antimicrobial coating from coating formula 6 and 7 (See Table A.3) Both dyne pen tests from ASTM D2578-09 and contact angle testing was conducted. Drawdowns treatment coated films were produced using a 28 Mayer and drawdown apparatus. Treatment films contained Nisaplin®, sorbic acid with potassium salt (aka Potassium sorbate) and ascorbic acid. Films treated with corona discharge treatment were done so using a handheld corona treater (Model BD-20 from Electro-Technic Products, Inc). Films treated with primer were produced by mixing 1 part MICA A-131-X water soluble primer (Mica Corporation) with 9 parts water. Primer was cast onto the film using a Mayer rod (#3) on a drawdown apparatus and left to dry at ambient conditions for 4-6 hours prior to casting the control or treatment coating on top.

The dyne pen test (AccuDyne Dyne Pens) resulted in a surface tension measurement of 32 dynes/cm for both top and bottom web substrates containing LLDPE. (Top web contains an additional additive) Contact angle results were summarized in Table A.6 below.

Table A.6. Contact angle results for Trial 6 coating on coextruded material containing LLDPE sealant web. (U = untreated; CP = Corona and primer)

Static Contact Angle Result Summary				
Sample Treatment	U	U	CP	CP
Web	Top	Bottom	Top	Bottom
Average Contact Angle (°)	64.46±9.52	55.82±7.12	40.76±1.28	40.62±0.88
CV (%)	14.78	12.76	3.13	2.16

Table A.6 shows results for films untreated but coated with the antimicrobial coating and treated films (corona discharge and primer) with the antimicrobial coating. The results indicate that the corona and primed films decreased the contact angle from 64.46° and 55.82° to 40.76° and 40.62° meaning that the primer was compatible with both LLDPE and PVOH. The coefficient of variation also shows that treatment of the films also made the contact angle more consistent decreasing the CV from 14.78% and 12.76% to 3.13% and 2.16%. From this study and previous observations, corona treatment and the water soluble primer will be necessary to continue with the same antimicrobial coating formulation.

Static Contact Angle and Dyne Pen Discussion

Contact angle is a means of quantifying adhesion of a liquid solution to a solid substrate. Droplet angles ranging from 0-90° indicate complete to partial wetting while 90-180° angles are indicative of a non-wetting solution or coating. (Thompson, 1998) Untreated LLDPE yielded average contact angles with partial wetting at 64.46° and

55.82° for top and bottom webs. The coefficients of variation for these samples were 14.78 and 12.76% indicating high variation within the LLDPE substrate surface. The top and bottom untreated web contact angles differed due to additives of a proprietary nature.

In order to achieve a higher degree of wettability, the substrate was corona discharge treated and primed with a water soluble primer from MICA Corporation. After these treatments, the contact angle decreased to 40.76° (top) and 40.62° (bottom) which showed increased wetting but still partial wettability. Although this primer was recommended specifically for adhesion of PVOH to LLDPE, the additional components within the coating may be affecting the degree of wetting. It is also possible that the plasticizer or surfactant concentration could be too high producing a boundary layer of oil between the coating and the film substrate limiting adhesion [11]. Although these components did not appear to be bleeding out of the coating, it is possible that the boundary layer was not visible to the naked eye. On another note, the coefficient of variation dropped to 3.13% (top) and 2.16% (bottom). Therefore, the treatment of the films with corona discharge and a water soluble primer made the surface more consistent by removing dirt and dust, oxidizing the LLDPE film surface and adding a thin, homogenous layer of primer. Ideally, the solution and film substrate should yield a contact angle of 0° to indicate full wettability.

In order to determine if the surfactant (Tween® 80) was having a negative effect on adhesion, contact angle was tested utilizing the original coating preparation and a second coating preparation without Tween® 80. As shown in the results, Tween® 80 did not cause a significant difference between contact angles observed. There was however a

significant difference between contact angles observed on treated LLDPE and untreated ethylene vinyl acetate (7.5% vinyl acetate) substrate. (Elvax® 3120) Because PVOH is product from polyvinyl acetate, there are remaining vinyl acetate groups on the PVOH after formation which could suggest a chemical compatibility between PVOH and EVA. The contact angle however, indicates only partial wetting. This may be due to the polar regions of the EVA molecule being buried under the surface of the film leaving the non-polar portions at the surface to make direct contact with the polar coating solution. (Personal communication with Barry Morris, DuPont) The treated LLDPE yielded lower contact angle measurements indicative of a higher degree of wettability therefore adhesion.

Additionally, coating solutions containing Tween® 80 remained stable emulsions at ambient conditions in sealed containers for several weeks. The coating formula without Tween® 80 exhibited phase separation. The phase separation appears to be the antimicrobial component, Nisaplin®, due to the brown coloration. The formula containing Tween® 80 also prevented bubble formation upon mixing. PVOH has the tendency to foam and Tween® 80 can be used for emulsion, surfactant and foam reduction qualities.

Differential Scanning Calorimetry Thermograms

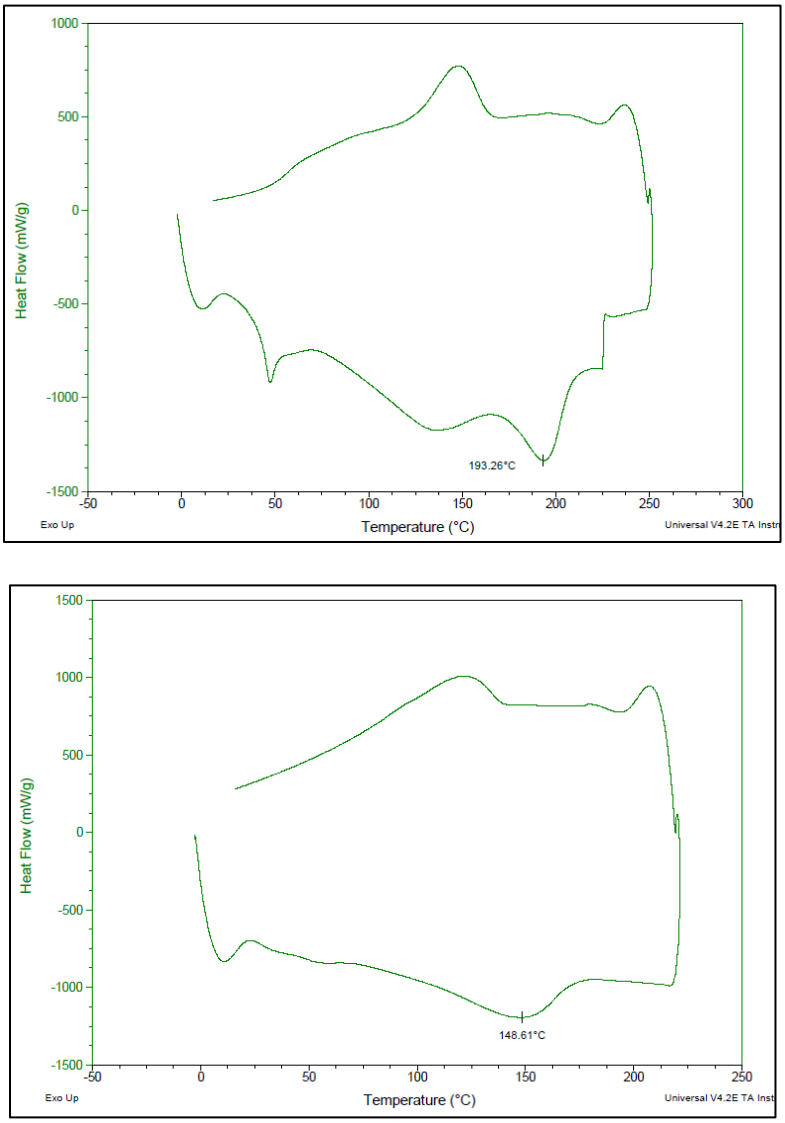

Figure A.5. Thermograms of powdered PVOH (Mowiol 8-88 GS2) containing 0 phr (parts per hundred) glycerin (top) and 40 phr glycerin (bottom). These thermograms display the decrease of the pyrolysis or thermal degradation peak occurring in the temperature range 60-160°C.

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APPENDIX B:

SUPPLEMENTARY COATING TRIAL TESTING AND CALCULATIONS

Materials Balance

This work was originally based off of the done conducted by Franklin et al 2004 in which an antimicrobial coating was produced using Nisaplin® and was used in a challenge study against *Listeria* monocytogenes Scott A on hotdogs. The following calculations were conducted using the coating formulation described in previous chapters containing Nisaplin® in a polyvinyl alcohol matrix in order to estimate the antimicrobial activity in various scenarios. Resulting calculations based upon surface area in contact with hotdog products were conducted assumed an approximated hot dog package surface area of 671 cm^2 based upon measurements of a hotdog package in a local grocery store. Calculations based upon mass assumed a package filled with 16 ounces (1 lb.) of hotdogs. The activity of Nisaplin® per gram of hotdog or per cm^2 of hotdog product was calculated using the conversions and key information below in Table B.1.

Table B.1. Conversion information for Materials Balance calculations.

Key Information and Conversions for Materials Balance Calculations	
1 pound/ream (#.ream)	0.0001627 g/cm^2
1 pound	453.59 grams
1 inch	2.54 centimeters
1 gram of Nisaplin®	1,000,000 (IU/g) International units per gram

**Calculations based on the size and interior surface area of a typical hot dog package*

Table B.2. Measured hotdog dimensions.

Hotdog Dimensions		
	Inches	Centimeters
Length	6	15.24
Width	1	2.54
Depth	1	2.54

Table B.3. Measured hotdog package dimensions and total surface area.

Hotdog Package Dimensions and Area					
	Inches	**Centimeters**	**Package Face area (cm^2)**	**Number of faces**	**Area**
Length	6	15.24	77.42	2	154.84
Width	5	12.7	193.55	2	387.10
Depth	2	5.08	64.52	2	129.04
				Total area of package(cm^2)	670.98 ~671

Table B.4. Results for materials balance calculations for activity of Nisaplin® per gram of hotdog.

Grams of Nisaplin® per Batch of Coating (g)	**Basis weight (#/ream)**	**Basis weight (g/sq. cm)**	**Amount of dry coating per package (g)**	**Amount of Nisaplin® per package (g)**	**Amount of Nisaplin® per gram of hotdog (g)**	**Activity of Nisaplin® per gram of hotdog (IU/g)**
1	1	1.60E-04	1.09E-01	5.98E-03	1.00E-05	13.18
	2	3.30E-04	2.18E-01	1.20E-02	3.00E-05	26.37
	3	4.90E-04	3.28E-01	1.79E-02	4.00E-05	39.55
	4	6.50E-04	4.37E-01	2.39E-02	5.00E-05	52.73
2	1	1.60E-04	1.09E-01	1.14E-02	3.00E-05	25.07
	2	3.30E-04	2.18E-01	2.27E-02	5.00E-05	50.14

	3	4.90E-04	3.28E-01	3.41E-02	8.00E-05	75.21
	4	6.50E-04	4.37E-01	4.55E-02	1.00E-04	100.28
3	1	1.60E-04	1.09E-01	1.64E-02	4.00E-05	36.10
	2	3.30E-04	2.18E-01	3.28E-02	7.00E-05	72.20
	3	4.90E-04	3.28E-01	4.91E-02	1.10E-04	108.30
	4	6.50E-04	4.37E-01	6.55E-02	1.40E-04	144.40
4	1	1.60E-04	1.09E-01	2.10E-02	5.00E-05	46.28
	2	3.30E-04	2.18E-01	4.20E-02	9.00E-05	92.57
	3	4.90E-04	3.28E-01	6.30E-02	1.40E-04	138.85
	4	6.50E-04	4.37E-01	8.40E-02	1.90E-04	185.13

Table B.5. Results for materials balance calculations for activity of Nisaplin® per square centimeter of hotdog.

Grams of Nisaplin® per Batch of Coating (g)	**Basis weight (#/ream)**	**Basis weight (g/sq. cm)**	**Amount of dry coating per package (g)**	**Amount of Nisaplin®/ 1 pkg (g)**	**Amount of Nisaplin® /sq. cm of hotdog surface area**	**Activity of Nisaplin®/ sq. cm of hotdog area (IU)**
1	1	1.63E-04	1.09E-01	6.00E-03	1.05E-05	10.54
	2	3.25E-04	2.18E-01	1.20E-02	2.11E-05	21.07
	3	4.88E-04	3.28E-01	1.79E-02	3.16E-05	31.61
	4	6.51E-04	4.37E-01	2.39E-02	4.21E-05	42.15
2	1	1.63E-04	1.09E-01	1.14E-02	2.00E-05	20.04
	2	3.25E-04	2.18E-01	2.27E-02	4.01E-05	40.07
	3	4.88E-04	3.28E-01	3.41E-02	6.01E-05	60.11
	4	6.51E-04	4.37E-01	4.55E-02	8.02E-05	80.15
3	1	1.63E-04	1.09E-01	1.64E-02	2.89E-05	28.85
	2	3.25E-04	2.18E-01	3.27E-02	5.77E-05	57.71
	3	4.88E-04	3.28E-01	4.91E-02	8.66E-05	86.56

	4	6.51E-04	4.37E-01	6.55E-02	1.15E-04	115.42
4	1	1.63E-04	1.09E-01	2.10E-02	3.70E-05	36.99
	2	3.25E-04	2.18E-01	4.20E-02	7.40E-05	73.98
	3	4.88E-04	3.28E-01	6.30E-02	1.11E-04	110.98
	4	6.51E-04	4.37E-01	8.40E-02	1.48E-04	147.97

The calculations shown in tables B.4 and B.5 show that the theoretically available Nisaplin® per square centimeter or per gram of hotdog product are well below the legal limit of 10,000 IU/g. Therefore if a specific target microorganism required a higher concentration of antimicrobial in order to be killed, then it is possible to add more Nisaplin® to the coating solution without reaching or exceeding the legal limit concentration.

Thickness – Digital Micrometer

Thickness measurements were taken using a Nikon Digimicro MFC-101 micrometer (Nikon Corporation, Excel Technologies, Inc. Enfield, CT, USA) on neat and coated (control and treatment) films. (n = 150) Locations of the measurements (operator, center and machine side of web) were also recorded to note any differences across the web during the coating process.

Gravure Thickness Results

Control, treatment and neat films were tested for thickness. (n=150) There was a significant difference in the film thickness found based on the film type. ($P<0.0001$) There was no significant difference between thicknesses measured based on location ($P =$

0.4657) or film/location interaction (P = 0.0554). Neat (uncoated) films had an average thickness of 2.53 mils. Control coated films were 2.68 mils on average and treatment coated films averaged 2.59 mils. These values were determined to not be precise enough to determine an accurate coating thickness measurement. No measurements were taken using the digital micrometer on the material produced during the flexography trial. Both materials produced gravure and flexography trials (control and treatment) were sent to the Clemson Light Imaging Facility located in the Life Sciences building on campus to determine a more precise coating thickness in microns.

Thickness – Clemson Light Imaging Facility (CLIF)

The following procedure was developed by Rhonda Reigers Powell from CLIF.

"Ten small samples of 1-2 cm long by less than 1 cm wide were removed from the larger samples at random using a razor blade, and in some cases, samples were trimmed further with scissors. At least 3 separate pieces of the larger samples were used to generate representative samples. If the sample was coated, a paint marker was used to indicate the top side (coated side) of the sample.

A ball of play-doh was used to mount the samples, so that each piece could be imaged in cross-section to determine base layer plus coating thickness.

The sample was placed on the stage of an Olympus LEXT OLS4000 3D confocal laser measuring microscope. All samples were first identified using a 5X objective and were then imaged using a 20X objective (numerical aperture 0.60) with 2X zoom. The top and bottom limits of the sample were set in the software, and images were collected using a 405 nm laser. The Olympus LEXT collects multiple Z-planes and merges them into a single image. The LEXT boasts resolution capabilities of at least

120 nm in the XY plane and 10 nm in the Z plane, and is calibrated by Olympus annually.

Measurements of the width of the cross section (representing thickness of the original sample) were collected using the Olympus LEXT software package. For each image, 3 measurements were taken on each piece. These regions roughly correlated to a measurement on the left, center, and right regions of the image. In each case, a screenshot was collected to demonstrate the region where the measurement was taken. Measurements were exported to an Excel Spreadsheet.

During imaging of the control sample (no coating), it was observed that the thickness of the samples cut from different pieces varied widely in thickness. Small, but likely acceptable, variations were observed in samples cut from the same larger piece.

Wide variations were also observed in the coated sample. This wide variation resulted in no net difference observed as a group in the thickness of the coated samples as compared to the control samples, and therefore, no measurements related to film thickness could be collected.

In the future, if all coated samples are produced from the exact same base piece, it is possible that this technique could be used to collect information about film thickness. This may be unrealistic, though, due to the manufacturing process. The ideal situation would be to image a piece that is half uncoated/half coated and measure the height of the interface. This, too, seems difficult given the manufacturing process."

Rhonda Reigers Powell

Clemson Light Imaging Facility
College of Agriculture, Forestry, and Life Sciences
Clemson University
8 January 2016

Results

The uncoated control film averaged 99.0±24.7μm and the flexography coated Nisaplin® containing film had an average thickness of 87.0±15.35 μm. (n = 60)

Discussion

Based upon the results, it appeared that the coated material was on average thinner than the uncoated material. There was a large variation in the thickness measurements found for both the uncoated control and the coated treatment. Added complexity arose due the lack of coloration in the film. Previous attempts were made to just measure the coating thickness; however, the coating was also clear and indistinguishable from the film. Recommendations for future thickness testing would be to add a slight coloration to the liquid coating such as a water soluble food coloring. Figure B.1 below shows images of film cross-sections.

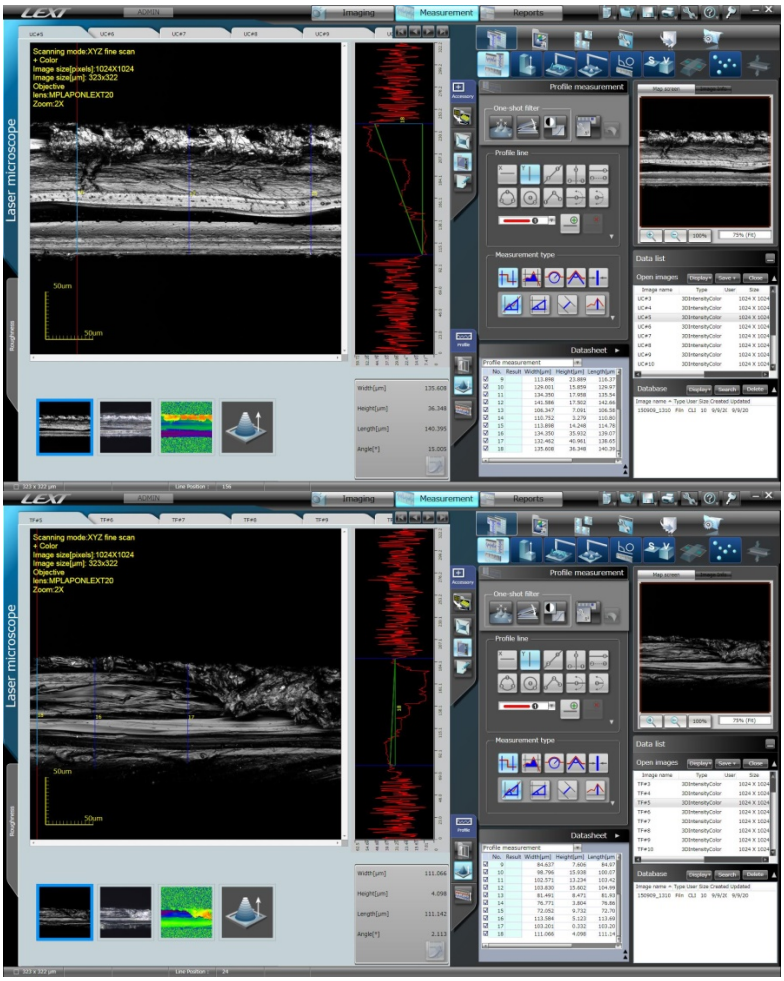

Figure B.1. Images of cross-sections for uncoated film (top) and flexography antimicrobial coated (bottom) film for thickness measurements.

Pounds per Gallon of coating for estimating Coat weight

In order to determine the specifications for the anilox roll to be used in trial #2 which could achieve the same coat weight (~1.50 #/ream) as achieved in the gravure trial, an online industry calculator was used after determining the weight per gallon of coating.

(Table B.6) The calculator was found in the link below from Pamarco Global Graphics, an equipment supplier for the printing and converting industries:

http://www.pamarco.com/resources/calculators/coat-weight-calculator/ [3]

Based upon the percent solids of the treatment coating, pounds per gallon and intended coat weight, it was estimated that of the choices of anilox rolls at the Sonoco Institute, the 30 BCM anilox roll would be best suited to produce the desired coating weight.

Conversions:

1 pound = 453.6 grams

1 gallon = 3785.41 mL

1 batch of coating ~ 1750 mL

2.16 batches of coating = 1 gallon

Table B.6. Calculation of pounds per gallon of coating for online coat weight calculator.

Pounds per gallon calculation of coating formulation			
Ingredient	Volume or Mass Used per gallon	Density (g/cm^3)	Mass in pounds
PVOH	1.188 lb		1.188
Water	1620 mL	1.0	3.571
Ethanol (95%)	1620 mL	0.807	2.882
Glycerin	172.8 mL	1.26	0.48
Tween® 80	10 mL	1.03	0.023
Acetic acid solution (0.02 M)	108 mL	~1.0	0.238
Nisaplin®	0.119 lb Gravure (0.238 lb) Flexo		0.119 - Gravure 0.238 - Flexo
		Pounds per gallon	Gravure: 8.50 Flexography: 8.62

Cost Analysis

Cost is one of the challenges for implementing antimicrobial into the food packaging market. Therefore cost analysis was conducted for the antimicrobial coating material produced. It is important to note that these calculations are based upon the measured hotdog package area of 671 cm^2. It is also likely that the overall coating cost presented will be lower due to the higher cost of lab grade, smaller volume materials. For larger operations, bulk items are produced. This cost analysis excludes converting and overall machine costs.

Table B.7. Coating cost calculation for 1#/ream coating to cover 671cm^2 area of hotdog package.

Ingredient	**Unit Cost ($)**	**Unit Volume**	**Amount used per package**	**Amount of packages produced per unit volume**	**Cost per package ($)**
Distilled water	3	1 gallon (3785.41 mL)	0.05 mL	1.32E-05	0.00003960
95% Ethanol	28.5	4000 mL	0.047 mL	1.18E-05	0.00033500
Tween 80®	87.08	4000 mL	.000287 mL	7.18E-08	0.00000625
Glycerin	13.49	32 oz (907.184 mL)	0.005 mL	5.51E-06	0.00007430
Nisaplin®	80	1000 g	0.00155 g	1.55E-06	0.00012400
Acetic Acid solution	99.11	4000 mL	.00036 mL	9.00E-08	0.00000892
Polyvinyl alcohol	12	1000 g	0.0155 g	1.55E-05	0.00018600
			**1 #/ream	Cost per package ($)	0.00077407

In 2014, approximately 1 billion hotdog packages were sold in retail stores in the United States totaling $2.5 billion in sales [2]. If this coating was used solely for the hotdog market, the cost per package shown in Table B.7 would result in an overall increase value added cost shown in Table B.8.

Table B.8. Cost of coating based on 2014 hotdog consumption in U.S.

Cost of antimicrobial coating for hotdog market		
Basis Weight (#/ream)	**Cost of coating per package ($)**	**Cost of coating per billion packaging ($)**
1	0.000774	774,000
2	0.001548	1,548,000
3	0.002322	2,322,000
4	0.003096	3,096,000

These calculations show that the coating cost could be relatively inexpensive enough to be implemented into the packaging market provided that the package extends the shelf life of the product. This coating has yet to be testing against a real food system and is recommended for future research.

REFERENCES

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