Studies on electrospun poly (lactic acid) fibers and low density poly (ethylene) / thermoplastic starch blends as food packaging materials

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NANYANG
TECHNOLOGICAL
UNIVERSITY

**STUDIES ON ELECTROSPUN POLY(LACTIC ACID)
FIBERS AND LOW-DENSITY
POLY(ETHYLENE)/THERMOPLASTIC STARCH
BLENDS AS FOOD PACKAGING MATERIALS**

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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ENGINEERING

A thesis submitted to the Nanyang Technological
University in partial fulfillment of the requirement for
the degree of Doctor of Philosophy

2017

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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Abstract

Polymer based materials have been widely used in food packaging applications due to their flexibility in processing, good sealing properties and transparency. However, most of the polymer-based food packaging materials are poor in their barrier properties which could hinder their effectiveness in maintaining the quality of packed foods. Employing a bio-based functional layer/coating on a polymeric layer as food packaging material could be a potential way to address this challenge.

As compared to traditional cast films, electrospun polylactic acid (PLA) fibers is a potential bio-based candidate to be used as a functional layer/coating because these fibers have high surface to volume ratios and the surface properties can also be altered easily by the addition of nanomaterials during the electrospinning process. However, mechanisms of obtaining electrospun PLA fibers with different surface morphologies (to tune the desired functionality) and the role of these surface characteristics on moisture sorption and microbial growth (moisture and microbes present in food packaging environments) have not been looked at. Hence in this thesis, the first major research focus is dedicated on elucidating the formation mechanisms of varying degree of pores on PLA fibers by changing the electrospinning solution and process parameters. The role of a combination of parameters, such as solvent vapour pressure, solvent miscibility and interaction with water, solubility and relative humidity, in governing pore formation is demonstrated. The results indicated that conventional mechanisms reported earlier such as thermally induced phase separation and vapour induced phase separation were not responsible in generating pores. Instead it was concluded that solvent miscibility and interactions with water caused by high relative humidity environment (moisture, a non-solvent of the polymer) were relatively more important than just solvent volatility. The content of highly volatile solvent in the solution determined the nature of pores (spherical/ elliptical) because this highly saturates the jet-air interface during electrospinning.

Moreover, fibers with and without surface pores and with nanoparticles like Sepiolite (known to absorb moisture easily) and ZnO (known to have antimicrobial activity) are employed in order to understand how the porosity and hydrophobicity of fibers affect moisture sorption and microbial activity. Electrospun fibers were found to be

hydrophobic and moisture sorption results indicated very low amounts of moisture uptake despite the increased surface area of the fibers caused by porosity. But the sorption levels were 3-5 times higher than the cast film counterparts. Microbial test results indicated that hydrophobicity played an important role in initial bacterial adhesion to the substrate. This was demonstrated by using fibers with different levels of hydrophobicity and compared with a hydrophilic surface obtained by spraying of sepiolite particles on the surface of fibers. All fibers except those with ZnO were observed to be bacteriostatic. These fibers do not aid in moisture scavenging but they do not promote bacterial growth. Electrospun fibers cannot be stand-alone packaging materials and they have to be used along with polymeric materials used in food packaging applications.

Low Density Polyethylene (LDPE) is a very abundantly used food packaging material which is obtained from non-renewable resources and it takes decades to be decomposed. Addition of thermoplastic starch (TPS) to LDPE in the weight ratio of 50-50 is a potential way to generate biodegradable material which will reduce the environmental concern. However, the properties of LDPE/TPS blends are not as good as LDPE and this depends on several factors such as plasticization of starch, compatibility between LDPE and plasticized starch, morphology of the blends (phases of TPS in LDPE matrix) and processing conditions. Viscosity of blend components decides the morphology generated in polymer blends and this aspect is not well studied for LDPE/TPS blends. Therefore, the second major research focus of this thesis is to investigate the morphology, mechanical and barrier properties of LDPE/TPS blends when different grades of LDPE that differ in melt flow rate are used to prepare LDPE/TPS blends, with and without organically modified nano clay. The results show that the use of LDPE with higher melt flow rate produces dispersed droplets of TPS in the LDPE matrix while LDPE with low melt flow rate showed no distinct phase morphologies. The presence of clay has shown improvement in the water vapour transmission rates of the blends. In conclusion, the surface properties and morphology of the potential food packaging materials, i.e. electrospun fibers as well as LDPE/TPS blends are greatly affected by the synthesis parameters.

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Abbreviations

FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
MIP	Mercury Intrusion Porosimetry
DSC	Differential Scanning Colorimetry
TGA	Thermogravimetric analysis
DVS	Dynamic Vapor Sorption
VSA	Vapor sorption analyzer
VIPS	Vapor Induced Phase Separation
TIPS	Thermally Induced Phase Separation
LDPE	Low Density Polyethylene
LLDPE	Linear Low-Density Polyethylene
PET	Poly (ethylene terephthalate)
TPS	Thermoplastic Starch
PLA	Poly (lactic acid)
GAB	Guggenheim-Anderson-de Boer
BET	Brunauer–Emmett–Teller
WVTR	Water Vapour Transmission Rate
OTR	Oxygen Transmission Rate

Chapter 1

Introduction

This chapter provides a background to understand the global challenges posed by non-bio-degradable plastics used in food packaging, preventable food wastage, the need for extended shelf life of food products and the role of polymer nanocomposites in active food packaging. Gas/ moisture scavenging/ barrier and anti-microbial coating, identified as the two key controlling strategies in food preservation (by packaging) are discussed in detail. In short, the motivation of this study is to address some of the hurdles in food packaging industry by developing an active food packaging material using nature-friendly materials. This research aims to synthesize Polylactic acid fibers having different surface morphologies by electrospinning method and ascertain the effect of surface characteristics on moisture sorption and microbial growth (moisture and microbes are highly prevalent in food processing/packaging environment). These fibers can find potential use as an insert in the package or a functional layer in multi-layer food packaging. Another aspect dealt is the development of LDPE/ TPS blends which can be used as an alternative to LDPE that is commonly used in single or multi-layer food packaging materials. The last part of this chapter gives an overview of thesis organization.

1.1 Background

Since the pre-historic times of trying to gather and store food grains/hunted meat up to today's desire to buy ready-to-eat foods sold in super markets, man has travelled a long way with regard to finding food for survival. Market globalization has made it possible to transport food across the globe to reach the starving millions in under-developed countries. It is to be noted that modern preservation and packaging technologies are the underlying forces that make the food available even after weeks of harvest or production. Thanks to the development of such advanced technologies and the advent of packed ready-to-eat foods that relieves the problems of cooking and provide ultimate convenience at an affordable cost.

Figure 1.1: Per capita food losses and waste (kg/year), at consumption and pre-consumption stages, in different regions¹.

Although there are constant efforts to increase food production, we must deal with high levels of food wastage, resulting from lack of processing facilities, premature harvesting, poor and inefficient storage practices, unclear information on the food package about the quality of food, in developing countries. On the other hand, large quantities of food displayed and a wide range of products available in super markets, abundance of food (production exceeding demand) and consumer attitudes are the underlying causes for wastage in industrialized countries. Therefore, availability of safe and wholesome foods with high nutritive values to the burgeoning population is a major issue in developing countries. Globally ~1.3 billion tons of food produced every year

(1/3rd of yearly produce) for human consumption is wasted throughout the supply chain (from farm to fork). Figure 1.1 shows the per capita losses and waste in kg/year, at different stages (from production to retailing before reaching consumer and at consumption) in different regions¹. These losses also represent wastage of land, water and energy used in food production, eventually contributing to increased carbon footprint. In Singapore alone, 791,000 tons of food waste was generated in 2016 (14% recycled) and this has increased by ~50% in a decade (542,700 tons in 2005)².

Food spoilage is caused by growth of micro-organisms, ingress of moisture and gases such as oxygen, carbon dioxide and ethylene leading to chemical/ physical reactions and contamination from external environments due to loss of package integrity. In the 1790s, French confectioner Nicholas Appert could extend the shelf life of food by storing food in hermetically sealed jars and heating it in boiling water for a certain time and this was scientifically unknown until Louis Pasteur discovered that heating destroys micro-organisms thus extending the shelf life³. Over the decades, various preservation technologies (physical – chilling, freezing, drying; chemical – adding salt, sugar, exposing to fumigants; external – packaging, to cite a few) have been developed with the aim of preventing undesirable changes in the sensory quality and nutritive wholesomeness of the food, at the same time being economically viable.

Food preservation techniques and packaging go hand in hand although in some cases packaging can be a stand-alone method. Improved packaging materials were developed to provide the basic functions of containment, convenience, communication and protection. Polymers are most widely used now due to their light weight, easy process ability into films, containers, bottles, have excellent strength, resistance to cracking, toughness but are poor barriers to gases and allow light due to transparency. Therefore, the focus was to improve the barrier, and this was done by developing multilayer polymer films with aluminum/ silicon oxide coating and blending different polymers to gain a synergistic effect. Multi-layer flexible films (up to 5 or 7 layers), uniaxial/biaxially oriented polymer films (and blends) were developed to improve the barrier properties as well as their heat sealing capabilities. For example, a five layered metallized biaxially oriented polypropylene (BOPP) film exhibited oxygen transmission rate (OTR) as low as 0.15 cc/m² day bar at 23°C / 75% relative humidity (RH) and water vapor transmission rate (WVTR) of 0.2 g/ m² day at 38°C / 90% RH , along with good

sealing properties⁴. However, reuse and recycling of such films is a serious environmental concern because it is difficult to separate the layers before recycling. Therefore, minimizing the number of layers and achieving the same level of functionality is a major challenge in the food packaging industry.

On the contrary, the basis behind blending different polymers by choosing specific resins and processing conditions is to obtain synergistic properties of both matrices and to obtain a desired morphology that can give the best properties. It has been seen that obtaining a laminar morphology (dispersing small amounts of a good barrier polymer in a highly permeable matrix in such a way that the dispersed phase is in the form of parallel thin laminates) in contrast to fine isotropic dispersions will impede the flow of small molecules and significantly enhance barrier properties. For example, DuPont showed this kind of laminar morphology by blending nylon (copolymer of 6 and 66 - 20 wt. %) and high-density polyethylene (HDPE) ⁵⁻⁶.

Recently, research in food packaging has shifted its focus from conventional passive packaging materials towards advanced high barrier, active and intelligent/smart materials. Polymer nanocomposites can offer innovative results to improve the performance of polymers in food packaging. The presence of nanoparticles can (i) lead to increased thermal stability, high barrier to gases and light, moisture stability; (ii) interact with headspace environment; and (iii) behave like sensors which can indicate the quality of food ⁷⁻¹¹.

Despite the several advantages of polymeric materials, they are regarded as evil in terms of posing environmental concerns as they are ultimately disposed of and people are hardly aware of its role in delivering the product across the globe. 322 million tons of plastics was produced worldwide in 2016, China being the largest producer (27.8%) followed by Europe (18.5%) and South-East Asia (16.7%). In Europe, the packaging segment consumes 39.9% of the plastics produced in Europe (58 million tons in 2015), and 39.9% of the packaging waste generated is recycled (highest recycling among the different segments of plastics waste generated) ¹². Although a certain amount of plastics wastes is recycled, there is need for increased infrastructure to accommodate the high disposal rates of mixed plastics wastes from various segments such as agriculture, automotive, construction, electrical and electronic, packaging and others. The plastic

wastes dumped in Citarum river in Indonesia, the most polluted river in the world, and the Great Pacific Garbage Patch has direct serious effects on marine life/sea birds¹³ (such as reproduction and offspring survival rates) and indirect effects on human beings¹⁴. This calls for an efficient waste management system. Also, to consider the use of 'green' materials that can biodegrade and/or obtained from renewable resources. These facets form the basis of this research on active food packaging using bio-based materials.

1.2 Motivation

Incorporating nanoparticles in polymers is believed to be an excellent option to address some of the issues related to packaging. Mixing polymers with layered montmorillonite (mmt) clay has found to reduce the permeability of gases by reducing the diffusion rate thus increasing shelf life^{15,16}. The clay platelets have a high aspect ratio and are impermeable to gases, thereby forcing the small molecules to take a longer diffusion path in the matrix¹⁷. This is very well accepted as Nielson's tortuosity model, which shows how gas barrier property of polymers is improved on adding clay¹⁸. Electrospun polyamide fibers incorporated with mmt clay deposited on polypropylene films reduced oxygen transmission rate of polypropylene from 963 cc/m² day to 12.5 cc/m² day which has increased the shelf life of potato chips and bread¹⁹. Another issue is the light (UV and visible) transmittance through the package which can cause loss of vitamins and discoloration. In an epoxy matrix, addition of nanoparticles like ZnO showed excellent UV shielding efficiency (~96% transmittance at 320 nm and ~91% transmittance at 370 nm) even at a loading level of 0.07 wt. %²⁰.

A variety of active packaging technologies based on polymer nanocomposites are currently available to suit the needs of different foods. Some of these include packaging materials with anti-microbial, oxygen scavenging, ethylene removing, carbon dioxide emitting/absorbing, and moisture control functionalities^{7, 21}. These are in the form of sachets, inserts or as films sandwiched between polymer films in multi-layer packaging systems. Polymer composites with antimicrobial functions generally contain metal nanoparticles (like silver, gold and zinc), metal oxides (TiO₂, SiO₂, MgO and ZnO) and some natural antimicrobial agents^{7, 22-24}. Broadly, the effectiveness of antimicrobial agents depend on pH, water activity, growth of specific microbes, chemical nature of the antimicrobial agent and temperature²⁵.

Iron powder, ascorbic acid, photo-catalytic metal oxide nanoparticles are typical oxygen scavengers²⁶⁻³⁰. Oxygen scavengers based on iron powder works on the principle of iron oxidation²⁵. Iron powder gets oxidized to $\text{Fe}(\text{OH})_3$ in the presence of oxygen and moisture. TiO_2 act as oxygen scavenger upon UV illumination²⁸, however, as expected, they lose this ability in the absence of UV light.

Despite several developments (a few quoted above, chapter 2 will provide a more detailed review), there is still scope for improvement to achieve similar levels of functionality using reduced number of layers and environmentally friendly materials.

PLA nanocomposites have been widely used as coating material for paperboards (PLA coating in contact with food) in several studies to replace polyethylene coatings and heat sealing and permeability studies have been conducted³¹⁻³². Nevertheless, these serve only as a passive barrier. In order to increase the functionality of this inside coating in food packages and to make them active, electrospinning process could be used to obtain porous fibrous coatings and the morphology can be tuned to suit different functionality. This thesis work addresses some of the key challenges in the development of functional electrospun fibrous PLA coating/layer in multi-layer packaging systems. The functional layer can be the inner layer in multi-layer packaging systems that interacts with the food environment or as inserts in food packages and thus behave as active materials.

Electrospinning is a potential method to produce polymer nanocomposite fibers. Polymeric nano- fibers can also be obtained by several other methods such as drawing, template synthesis, phase separation, self-assembly and electrospinning³³. Electrospinning process has been widely explored in the last few decades to produce micro- and nano- sized fibers with high surface to volume ratios through an electrically charged jet of polymer solution. These fibers find application in tissue engineering/drug delivery, sensors, oil adsorption, catalytic systems, and protective clothing³⁴⁻³⁹. The use of electrospinning process significantly reduces the quantity of polymer consumed as they have a high surface to volume ratio. However, its application in food packaging is not researched extensively.

Another challenge to be addressed in food packaging industry is to develop bio-based materials. However, bio-based materials show poor performance due to their ability to

absorb enormous amounts of moisture. Therefore, these are blended with synthetic polymers to get the synergistic properties of recyclability and performance. This thesis also focuses on the development of LDPE/TPS blends which can potentially replace LDPE, a commonly used packaging material in single or multilayer packaging systems.

Blending thermoplastic starch with LDPE can be an effective solution to reduce the carbon foot-print⁴⁰⁻⁴². Generation of morphology in polymer blends (dispersed/stratified/co-continuous) depends on (i) viscosity (controls degree of dispersion) and elasticity (shaping the phases) ratios of the components; (ii) concentration of the components and (iii) level of interfacial interaction between the components⁴³. This is taken as the base to understand the properties of blends.

1.3 Research Scope and Objectives

Electrospinning process is used to make different kinds of fibers for various applications where high surface area is needed and sorption is critical. The major advantage is the ability to induce pores during spinning which can provide additional adsorption sites. There are several mechanisms reported in literature that explain the formation of pores. These include thermally induced phase separation (TIPS), Vapor induced phase separation (VIPS) and evaporation induced phase separation^{39, 44-47}. Nonetheless there are many questions and it is not easy to conclude with solvent volatility as the only parameter affecting pore formation. It is hypothesized that humidity of the electrospinning chamber and solvent miscibility with (condensed) water in combination with vapor pressure of solvent and solubility parameter differences between solvent(s) and polymer might govern pore formation. Therefore, a thorough study on the mechanisms of pore formation in electrospun fibers is undertaken. Polylactic acid (a widely used bio-based material in food packaging applications) is used with a combination of solvent systems to understand the parameters governing pore formation.

Moisture and microbes are highly prevalent in the food packaging industry. And since the fibers have inter-fiber and intra-fiber porosity, there is a possibility for moisture to be sorbed in the adsorption sites and microbes to attach to the pores and multiply. It is hypothesized that microbes can attach to surface pores that are similar in size to the microbes and start multiplying. The inter-fiber porosity obtained in the electrospun PLA

fibers is of dimensions similar to the size of microbes but the surface pores on fibers are much smaller. Therefore, it is important to understand the effect of morphology and hydrophobicity of fibers on the moisture sorption and microbial activity on the films. Thus, the second part of this study aims to evaluate the performance of fibers when subjected to moisture and microbes.

Blending of thermoplastic starch (TPS) with LDPE reduces the elongation and causes a stiffening effect. Various plasticizers such as glycerol, sorbitol, water, citric acid have been mixed with starch to obtain thermoplastic starch of which glycerol is most widely used^{40, 42, 48-50}. This reduces the hydrogen bonding between the starch molecules and facilitates movement of these molecules. Since thermoplastic starch is very sensitive to moisture it is important to understand moisture sorption and permeation. Several studies have been done to study the effect of plasticizer, content of TPS, use of starch from various regions and effects of processing conditions⁴⁸⁻⁵⁰. However, the effect of viscosity which decides the morphology of the blend is not looked at. It is hypothesized that difference in viscosity of LDPE can produce a difference in the morphology of LDPE/TPS blend. The thermal, mechanical and barrier properties of the blends are studied.

Based on the above hypothesis and a brief literature review, the key objectives of this thesis work are,

1. To synthesize electrospun Polylactic acid fibers with different surface morphologies and to develop an in-depth understanding of the mechanisms of pore formation during electrospinning process.
2. To study the behavior of PLA fibers on exposure to moisture and microbes in the food packaging environment by evaluating the role of morphology and hydrophobicity of the fibers on moisture sorption and growth of microbes.
3. Develop LDPE / TPS blends in the weight ratio of 50/50 using two different melt flow rate grades of LDPE and evaluate the morphology, thermal, mechanical and barrier properties of the blends.

The objectives 1 and 2 on the synthesis and behavior of electrospun films in a food packaging environment is to utilize its potential high surface areas as compared to the

traditional cast films and PLA is a bio-degradable material. The consumption of polymer in electrospinning process is very minimal and there are developments which allows for mass production of electrospun films⁵¹ but the use of these films in food packaging area is less explored. However, these films can only be used as inserts or an additional layer in food packaging and cannot be a stand-alone packaging material due to its highly porous nature. Therefore, another objective of this thesis (3) is to study the properties of LDPE/TPS blends, an environmentally friendly alternative to replace LDPE which is the most widely used food packaging material that poses serious concerns such as landfills and threat to marine life. Although two different packaging material systems were studied in this thesis, the overall objective of this thesis is to explore newer aspects of environmentally friendly materials and minimize the consumption of synthetic materials.

1.4 Organization of thesis

This thesis comprises seven chapters. The first chapter covers the motivation and introduction to the topic under study. Chapter 2 provides a review on the different kinds of polymer-based packaging materials that have been developed and studied in the last several years along with the theories on sorption and permeation. It elaborates the use of electrospun fibers in different applications and explores the potential to be used in food packaging. It highlights the recent research on bio-based materials with a special focus on thermoplastic starch and its blends with petroleum-based polymers to reduce carbon foot-print. Chapter 3 explains the rationale for material selection, synthesis and characterization techniques used to develop electrospun PLA fibers and LDPE/TPS blends. Chapter 4 elucidates the mechanisms of pore formation in electrospun fibers and a discussion on the different types of surface morphologies obtained. Chapter 5 presents the behavior of electrospun fibers and the role of morphology and hydrophobicity on exposure to moisture and microbes that may be present in food environment. Chapter 6 discusses the effect of viscosity of LDPE on morphology, thermal and barrier properties of LDPE/TPS blends. Chapter 7 comprises the important findings from this study and recommendations for future work.

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Chapter 2

Literature Review

This chapter presents an overview of the deteriorative reactions in food and narrates how packaging can be a useful tool for food preservation and shelf life extension. Different approaches to obtain passive/ active food packaging materials and their properties are furnished. Potential of using electrospun fibers in food packaging applications has been explored and reviewed. The theories associated with sorption in polymers are discussed in detail. Limitations of polymeric materials in terms of environmental concerns and safety aspects are critically reviewed. The importance of biodegradable/ bio-sourced materials has been highlighted and the potential to process these materials via electrospinning and polymer melt blending has been explored to justify the need for this research work.

2.1 Modes of deterioration of food

A good packaging system should keep the food safe and maintain quality, extend shelf life, provide convenience to customer, communicate and address environmental concerns. To design and develop such a system that can maximize retention of flavour, odour, colour, texture, appearance and nutritive value until consumption, it is important to know the modes of deterioration of food ¹. There are many ways by which food can lose its organoleptic properties and become unpalatable. Reactions that affect food quality can be broadly classified into chemical, biochemical, microbial and physical reactions. The rates and magnitude of these reactions can be minimized by choosing the right packaging material.

2.1.1 Chemical/biochemical reactions

Chemical reactions can lead to deterioration in quality of food and impair safety. It can involve different reactants and food substrates under different environments during processing and distribution. Rates of these reactions depends on light, oxygen, temperature and water activity (a_w). Listed below are some of the possible chemical reactions that can lead to food spoilage (also refer figure 2.1).

- i. Enzymatic reactions causing rapid browning in the presence of oxygen (biochemical). It is mostly caused by enzymes such as polyphenolic oxidase which break down polyphenolic compounds into melanin causing dark colours. Examples include oxidation of phenols in cut fruits or the lipid oxidation of raw meat. Red colour of meat is because of the pigment myoglobin which exists in the oxidized form, oxymyoglobin (called fresh meat colour). An excess amount of oxygen present in the package ($>0.05\%$) can lead to oxidation of this pigment forming metmyoglobin and changing the colour to brown. The quality of raw meat can be assessed by the colour, texture and flavour ².
- ii. Non-enzymatic lipid oxidation due to reaction of unsaturated fatty acids with oxygen, causing off-flavours, alteration of colour, loss of vitamins and proteins ³. The lipids become free radicals by losing hydrogen atom under heat and combines with oxygen forming peroxy radicals. These peroxy radicals react with other lipid compounds to form hydroperoxides that can polymerize to produce toxic products

or breakdown to produce volatiles affecting food quality. These products such as aldehydes, esters and peroxides damage proteins and vitamins in food ⁴.

- iii. Non-enzymatic browning, also called Maillard reaction that occurs in dried and concentrated foods due to the interaction between reducing sugars, water and amino acids (in the presence of thermal input) resulting in development of bitter flavours, darkening and loss of proteins ³. Caramelization is also a non-enzymatic reaction which involves dehydration and pyrolysis resulting in oxidised sugars.

Figure 2.1: Chemical interactions in foods - L - lipid pool (Triacyl glycerol, fatty acids, phospholipids) C - carbohydrate pool (polysaccharides, sugars, organic acids) P - protein pool (proteins, peptides, amino acids)⁵

2.1.2 Microbial reactions

Microbial reactions can be both desirable (such as in food fermentations) and undesirable (arise adventitiously and multiply). Changes in food are caused by rapid growth of micro-organisms due to temperature fluctuations during storage/transportation and varying concentrations of moisture and gases inside the package. Bacteria (*Salmonella* species, *E.coli*, *Clostridium botulinum*, *S. aureus*) and fungi (yeasts and moulds) are the microorganisms most commonly found in food ⁶. Both bacterial and fungi follow a similar growth pattern (figure 2.2). At first, they get adapted to the substrate known as lag phase followed by a rapid multiplication (usually exponential) known as the growth phase and a stationary phase where growth and death rate are balanced. Finally, the death phase starts and this maybe possibly due to depletion

of nutrition in the substrate or unfavourable external conditions. At any stage, the micro-organisms need sufficient source of energy, nitrogen, vitamins and nutrients to grow. Botulism poisoning is a highly reported disease caused by the growth of bacterium, *Clostridium botulinum*, which produces toxins that can be fatal ⁷. Any loss in integrity in the package can cause the penetration of microbes from external environment.

Figure 2.2: Microbial growth curve ⁴

2.1.3 Physical reactions

These include sorption of moisture leading to coalescence/ aggregation of food particles and influencing the size, shape and texture (caking of powders, softening of chips, etc.). Physical reactions are quite common in case of poor barrier of the packaging material and/or failure of packaging integrity ⁸.

Figure 2.3: Rate of reaction as a function of water activity ⁹

2.1.4 Factors influencing deterioration reactions

All the above-mentioned reactions are most often catalysed by extrinsic conditions such as temperature, relative humidity, oxygen and light in addition to intrinsic factors such as water activity, pH and redox potential. They are responsible in initiating and accelerating food spoilage. For instance, figure 2.3 shows the effect of water activity on relative reaction rates; figure 2.4 shows how temperature affects the moisture content and water activity. As evident, a small change in water activity can result in large changes in reaction rates. Food stored at constant temperature and relative humidity will eventually reach equilibrium with the environment moisture content and such a steady state is referred to as equilibrium moisture content.

2.2 Theories on Moisture sorption isotherms

Moisture sorption isotherms are obtained by plotting the equilibrium moisture content (mass of water per unit mass of dry matter) against water activity at different temperatures (figure 2.4)⁹. This shows that at constant moisture content, water activity increases with increase in temperature (for foods packed with highly impermeable package). There are various models which predict the water activity of foods and the same applies to sorption of moisture by packaging materials. These are discussed in the following section.

Figure 2.4: Effect of temperature on water activity and moisture content $T_1 < T_2 < T_3$ ⁹

Water activity of a substance is the ratio of the fugacity (escaping tendency) of the water vapour in the system (at equilibrium) to that of pure water at standard temperature and pressure. Relative humidity is the percentage of water activity. BET and GAB are most widely used models.¹⁰

BET isotherm is a very common method to determine the monolayer moisture content, m_0 and surface heat energy constant - C, and these two constants are evaluated from the BET model. It is given by the equation, by measuring m – equilibrium moisture content (g/ 100 g of dry solid) at specific a_w .

$$\frac{a_w}{(1-a_w)m} = \frac{1}{m_0} + \left[\frac{c-1}{m_0 c}\right]a_w \quad (1)$$

$C = e^{(Q/RT)}$ where Q - heat of sorption (cal/mol), R - gas constant and T - temperature.

¹¹⁻¹². $m_0 = 1/(I+S)$ is obtained by plotting $\frac{a_w}{(1-a_w)m}$ and obtaining I = Intercept and S =

Slope. However, BET model has a drawback that it cannot be used in all range of water activity. It is applicable only in the range of 0 – 0.5 water activity. Therefore, GAB (Guggenheim Anderson de Boer) model which has 3 parameters was developed¹³. In 1983, the International symposium on properties of water accepted that this is the best equation for fitting moisture sorption isotherms in the entire water activity range of 0 – 0.95.¹⁴ It is given by,

$$m = m_0 c k a_w / (1 - k a_w)(1 - k a_w + c k a_w) \quad (2)$$

where, m is the equilibrium moisture content at each water activity, m_0 is the monolayer moisture content corresponding to the mono-molecular layer on the surface

of membranes, c - Guggenheim constant, k - corresponds to the properties of the multi-layer moisture with respect to the bulk liquid. The GAB constants are estimated using the quadratic form of equation 2,

$$\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma \quad (3)$$

where, $\alpha = \frac{k}{m_0(1/c-1)}$, $\beta = \frac{1}{m_0}(1 - \frac{2}{c})$ and $\gamma = 1/m_0ck$. The values of α, β, γ were determined by non-linear regression analysis ¹⁵. This equation has been used in this thesis work for modelling the moisture sorption isotherms.

Table 2.1: Various equations to model moisture sorption isotherms

Model	Equation	Application
Oswin (1946)	$M_w = A \left[\frac{a_w}{1 - a_w} \right]^B$	Gives good fit for meat and vegetables, starchy foods.
Smith (1947)	$M_w = A + B \ln(1 - a_w)$	Biopolymers with high molecular weight. Limited to water activity 0.5-0.95 and in some cases above 0.3.
Henderson (1952)	$\ln(\ln[1 - a_w]) = \ln C + b \ln M_w$	Contradictory fit for different foods.
Iglesias-Chirife (1978)	$\ln \left(M_w + (M_w^2 + M_{0.5w})^{\frac{1}{2}} \right) = A a_w + B$	Fruits and high sugar content foods.
Peleg model (1993)	$M_w = A a_w^C + B a_w^D$	Gives good fit but does not estimate m_0 .

Apart from the BET and GAB model, there are several other empirical models for modelling water sorption isotherms such as Smith, Oswin, Henderson, Peleg and Iglesias-Chirife models ¹⁶⁻¹⁸. These are limited to certain range of water activities and are mostly 2-parameter models that are not enough to give sufficient information on the sorption mechanisms, except the Peleg model which is a 4-parameter but still does not give information about the monolayer moisture content. The below table lists the equations for various models and the applicability for different foods.

2.3 Shelf life of foods

Quality of any food is observed to decrease from packaging to consumption due to different mechanisms mentioned in section 2.1. Shelf life of food is the length of time during which the food is safe for consumption and has good quality, starting from the

date of manufacture. Food safety (eat-by/ sell-by dates) is a legal requirement whereas quality is the manufacturer's responsibility to satisfy the needs of the customer. Critical factors affecting shelf life are intrinsic characteristics of the food, type of packaging and environmental conditions during storage and shipping. Identifying and understanding these critical factors helps in estimation/ prediction of shelf life of food.

As a first step in shelf life simulations, it is important to identify the critical factors favouring deterioration of food, define the critical limits of quality indices (may be subjective – measurable by human or objective – measurable by instruments) that indicate the acceptance of food and create methods to quantify them over a period. The next step is to decide the testing conditions to artificially accelerate the factors causing deterioration to quicken the testing time. In general humidity, temperature and light are the factors that needs to be accelerated and the quality indices measured. Figure 2.5 illustrates the shelf life of food (t_{s1} , t_{s2} , t_{s3}) corresponding to different curves that represent the different environmental/packaging conditions. More protective packaging and preserving environment extends the shelf life from t_{s3} to t_{s1} ¹⁹. Although changes in quality indices refer to the nature of food, characteristics of the packaging material may also vary when subjected to adverse environmental conditions. Therefore, characteristics of the packaging material at the time of packaging and across the shelf life of food (especially foods with longer shelf life) influence the quality of food inside.

Figure 2.5: Concept of shelf life defined as time to reach critical limit of quality¹⁹

2.3.1 Environmental conditions and shelf life

In general kinetics of deterioration reactions follow zero, first or multi-order kinetics. Zero order and first order reactions are expressed by the following equations (4) and (5) respectively¹⁹.

$$t_s = ([B]_o - [B]_c)/k \quad (4)$$

$$t_s = \frac{\ln([B]_o/[B]_c)}{k} \quad (5)$$

As mentioned, temperature is one of the most important factors that accelerates aging process and inactivates certain enzymes. Temperature fluctuations can happen in any stage from farm to fork – during packaging, shipping or in warehouses. Arrhenius equation relates temperature and rate of chemical reactions. It is given by

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (6)$$

where E_a - activation energy, R - gas constant (8.314 J/K mol), T - absolute temperature (Kelvin), A - a constant (called frequency factor/ pre-exponential factor) and k - rate constant. A plot of $\ln k$ vs $1/T$ yields a straight line with a slope ($-E_a/R$). Higher values of E_a represent stronger temperature dependence. Shelf life (t_s) and rate constant (k) are related as $t_s \propto 1/k$. Therefore, this implies that shelf life data should be plotted as $\ln t_s$ vs $1/T$. Shelf life tests are done at 3 different temperatures and the values of E_a are obtained from the slope. Alternately, Q_{10} is a term used to express temperature dependence of the reaction.

$$Q_{10} = \frac{k_{T+10}}{k_T} \quad (7)$$

where T is in °C. However, Q_{10} is an approximate estimation of how the reaction rate increases when the temperature is increased by 10 °C. This is based on the assumption that the relationship between rate constant and temperature is linear and that it follows Arrhenius equation. It can be used as a tool to predict shelf life only when the change in temperature is minimal as it cannot be extrapolated beyond measured data.⁴ For instance, in the prediction of shelf life of a food that depends on microbial growth as a quality index, k in the Arrhenius equation represents maximum specific growth rate (μ_{max}). E_a values for microbial growth rate generally range from 50-90 kJ/ mol.²⁰

Another important factor impacting shelf life is the gaseous composition inside (headspace) and outside the food package. Higher levels of oxygen in the headspace can cause detrimental effects on nutritive values of food because oxygen is an active reactant

in many chemical reactions mentioned previously in section 2.1.1. Reducing oxygen levels in the headspace of fresh produce packaging (those that respire even after harvest) will reduce the respiration rate and decrease the production of ethylene thereby delaying ripening. For instance, rate of oxygen consumption was shown to follow mixed order kinetics and was expressed by the equation

$$R_{O_2} = \frac{P_{O_2}}{C_1 + C_2 P_{O_2}} \quad (8)$$

where P_{O_2} is the partial pressure of oxygen and C_1 and C_2 are constants ²¹. The partial pressure of oxygen in headspace is influenced by both consumption in oxidation reaction and by oxygen that permeates through the package. In another case, reaction rate of edible oil was given as,

$$reaction\ rate = k_{O_2} P_{O_2} \quad (9)$$

where P_{O_2} is the partial pressure of oxygen in the headspace and k_{O_2} is the oxidation rate constant of edible oil. Shelf life θ is then predicted using the equation,

$$\theta = \frac{O_2(\max)}{k_{O_2} P_{O_2}} \quad (10)$$

where $O_2(\max)$ is the maximum amount of oxygen allowed to react before the quality of edible oil deteriorates ²². Ingress of moisture can cause many changes and induce microbial growth. In case of potato chips where oxygen partial pressure and equilibrium humidity affect the rate of oxidation, it is represented as

$$Rate = (EXT + \frac{P_1}{RH^{\frac{1}{2}}} + \frac{P_2 EXT}{RH^{\frac{1}{2}}}) (\frac{P_{O_2}}{(P_3 + P_4 + P_{O_2})}) \quad (11)$$

where EXT represents rate of oxidation, RH is the relative humidity and P_1 -4 are constants as mentioned in ²³. The relation between water activity and reaction rates was discussed in section 2.1.4.

2.3.2 Properties of packaging material and shelf life

Gas/ water vapour permeability, light transmittance are very important factors that affect the shelf life of food. Heat seal ability indirectly affects the shelf life as it decides the integrity of the package over time. Oxygen transmission rate (OTR) and Water vapour transmission rate (WVTR) are measured using permeation analysers at a certain temperature, pressure and relative humidity. WVTR, OTR, area and thickness of the

packaging film are the parameters which affect the transport of gas/moisture/flavour/odour molecules into the headspace and out of the package. These values play a very important role in deciding the quality of food packed. As mentioned previously, the oxidation rate of oil (with respect to changes in environmental conditions) is determined using equations mentioned in section 2.3.1. This can be further related to the barrier properties of the packaging material as

$$\text{Permeation rate} = OP \times A \frac{0.21 - P_{O_2}}{d} \quad (12)$$

where OP represents the film's (of thickness, d and surface area A) oxygen permeability²². Kunli et al calculated the shelf life of potato chips packed in PLA- graphene (graphene layer sandwiched between PLA films) composite films (200 g chips in 1 L film package, 100 μm thickness and surface area of 0.1 m^2) as 750 days in contrast to that of 85 days for chips packed in pure PLA when RH outside the package was considered to be 50%.²⁴ The presence of this nanomaterial has increased the shelf life of chips by 8 times. This is because the WVP of pure PLA films was 72 $\text{g mm/m}^2 \text{ day atm}$. whereas that of sandwiched films was 8.9 $\text{g mm/m}^2 \text{ day atm}$.

Most of the thermoplastic polymers (especially those with high amorphous content) are highly transparent. Though it is advantageous for a packaging material to be able to show the colour and texture of food, this is another reason for decrease in the nutritional content of light-sensitive foods such as milk, fruit juices, and dairy products and sometimes causing off-flavours. Therefore, there arises a need for stabilization of polymers against UV/visible light. According to International Dairy Federation, the light transmittance through packages cannot exceed 2% at 400 nm and 8% at 500 nm in order to provide good stability against light oxidation. Mestdagh et al showed the effect of light (both UV and visible in the wavelength range of 200-800 nm) on oxidation of milk by studying the loss of vitamins and formation of peroxides²⁵. They used PET bottles with different light transmitting properties and illumination intensity of 2500 lux for testing the loss of vitamin A along with riboflavin in milk (comparable to the lights found in supermarkets). The presence of oxygen binders could not remove entire oxygen inside the package. UV absorbers offered some protection against light but could not resist the formation of off-flavours during long-term storage. However, PET with an adequate light barrier was found to be the best as its transmittance was very low thus extending the shelf life of milk.

Therefore, a material with excellent barrier (to air/moisture/light), mechanical and heat sealing properties (to maintain integrity) is of great interest in the food industry. Further ahead, it is challenging to develop materials that can maintain this integrity throughout the shelf life of food, keep up the desired headspace environment and thus the extensive research on high barrier materials and active packaging materials based on polymer nanocomposites and multilayer packaging. Since multilayer packaging poses serious environmental concerns for recycling due to difficulties in separating and sorting the layers, reducing the number of layers and incorporating polymer nanocomposites as one of the layers or as single layer packaging materials is a promising approach.

2.4 Polymer nanocomposites in food packaging

Several studies showed the reduction in permeability by adding silicate clays to polymers by reducing diffusion path length ^{26,27}. Incorporation of nanoclays into polymer matrices to prepare nanocomposites is done by (i) in-situ polymerization – monomers are small molecules and therefore they can penetrate the clay layers and yield intercalated morphology, (ii) solution blending in which the polymer and nano particles are dispersed in a suitable solvent followed by evaporation/ extraction of solvent and (iii) melt blending in which the polymer and clay are compounded at temperatures above melting temperature in melt-extruders.

Figure 2.6: Idealized morphology of intercalation and exfoliation of clay in polymer matrix²⁸

Processing conditions used to prepare nanocomposite, organic surfactant used and type of polymer matrix decide the morphology of polymer nanocomposites - phase separated, intercalated and exfoliated. Figure 2.6 represents an ideal morphology indicating

excellent dispersion and distribution of clay in polymer matrix²⁸. Transmission electron microscopy is an imaging technique to provide information on the type of clay dispersion in the polymer matrix and X-ray diffraction is a technique to determine the interlayer spacing which gives an indication of clay dispersion. These techniques are vital in understanding how different morphologies affect the barrier properties.

2.4.1 High barrier packaging

Incorporating nanoparticles in polymers is believed to be an excellent option to address some of the issues related to packaging. Nielson explained the tortuosity model in which polymer barrier is enhanced by adding clays²⁹. Clays are impermeable to gases and the gas molecules are forced to go around these high aspect ratio clay platelets in the polymer matrix thereby increasing the diffusion length³⁰.

Figure 2.7 shows the effect of aspect ratio, loading, orientation and degree of exfoliation of nanoclay layers on barrier properties³¹. The relative permeability, P_s/P_p (P_s and P_p are permeability's of polymer-silicate nanocomposites and pure polymer, respectively) is given by,

$$\frac{P_s}{P_p} = \frac{1-\phi}{1 + \frac{L}{2W}\phi_s\left(\frac{2}{3}\right)\left(S + \frac{1}{2}\right)} \quad (13)$$

where S is the order parameter and $S = 0, -1/2$ and 1 represent random, orthogonal and planar orientations of silicate layers, respectively; L and W represent the length and width of sheets; and ϕ_s is the volume fraction. It was concluded that dispersing longer sheets (>500 nm) increases the tortuosity and reduces the effect of orientation of the platelets on relative permeability. However, a critical factor determining maximum performance is exfoliation of clay in the matrix.

Further, high permeability resistance to O_2 , He, CO_2 , water vapour and many other solvents and gases were also reported with polymer/clay (montmorillonite) nanocomposites although the clay layers were intercalated. For instance, in PET nanocomposites with clay, O_2 permeability is reduced by twice with only 1 wt. % clay³². Polyimide nanocomposites containing 2 wt% mica showed a ten-fold reduction in

Figure 2.7: (a) defines direction of preferred orientation (n) of the silicate sheet normal (p) with respect to the film plane, (b)&(e) - Effect of incomplete exfoliation on relative permeability – relative permeability at different aggregate width and at different sheet length at $\phi_s = 0.05$; (c) relative permeability vs sheet length at several different silicate volume fractions (d) – relative permeability vs sheet orientation in exfoliated nanocomposites at $\phi_s = 0.05$ and $W = 1\text{ nm}$ ³¹.

water vapour permeability as compared to polyimide ³³. Sanchez et al. compared the oxygen permeability of different polymers in the presence of clay nanolayers. They observed that PET nanocomposites showed oxygen permeabilities as low as $0.005\text{ cc}/(\text{m}^2\text{ day atm})$ ³⁴. Besides, many works have indicated that the permeability does not decrease beyond a threshold that exists in polymer nanocomposites containing layered silicates ³⁵⁻³⁶. Similar drop in other physical properties were also reported for polymer nanocomposites ³⁷⁻⁴¹.

2.5 Active food packaging

Food packages are active if they contain an ingredient that can release/absorb substances into or from the food/headspace thereby preserving food quality and providing a reasonable extension of shelf life ⁴²⁻⁴⁸. A variety of active packaging technologies are currently available to suit the needs of different foods. Some of these include packaging materials with anti-microbial, oxygen scavenging, ethylene removing, carbon dioxide emitting/absorbing, and moisture control functions ^{40, 44}.

Figure 2.8: Different types of active food packaging indicating their need in packing different foods.

2.5.1 Antimicrobial packaging

As seen in the previous section 2.1.2, microbes on the surface of food can grow when there are fluctuations in temperature, water activity, pH or in the presence of oxygen and cause food spoilage due to loss of texture or creation of off-flavours/off-odours⁴⁹. The most common foods that are attacked by microbes include fresh produce, milk and dairy products, fresh baked products, meat and fish ⁵⁰. Therefore, control of microbial growth can prove to be effective in improving the storage stability of these packed foods

and help in extension of shelf life. Various kinds of antimicrobial packaging films have been developed based on polymer nanocomposites⁵¹⁻⁶¹ and the readers can refer to extensive reviews^{42, 62-63}. Nano materials have higher surface to volume ratios as compared to their micro-scale counterparts which confers greater efficiency as antimicrobial materials by providing enhanced surface activity and a good barrier⁶¹. Most commonly used antimicrobial materials include metal ions (silver, copper, gold), metal oxides (TiO₂, SiO₂, MgO and ZnO), organically modified nano clays and some natural antimicrobial agents such as nisin, carvacrol and thymol^{40, 43, 47, 64}.

Silver based nanocomposites are most commonly used as antimicrobial films. Elemental silver in the presence of water and oxygen will form Ag⁺ ions according to the reaction,

$$\text{O}_{2(\text{aq})} + 4 \text{H}_3\text{O}^+ + 4 \text{Ag}_{(\text{s})} \rightarrow 4 \text{Ag}^+ + 6 \text{H}_2\text{O}$$

These silver ions bind to electron donors like sulphur, nitrogen or oxygen containing compounds present in the bacteria and form complexes⁶⁵, adhere to cell surfaces, degrade lipopolysaccharides and form pits in the membrane⁶⁶. When the cell membrane is damaged, there is no transfer of nutrients to the cell and hence this stops the metabolism and therefore multiplication of microbes in the food. Silver can also penetrate and damage bacterial DNA⁶⁷. Silver nano- and micro-particles were used in polyamide 6 matrix and their efficiency and long term stability against E coli were compared^{61, 68}. Figure 2.9 clearly illustrates that in nanocomposites, silver ion release rate of $9.5 \times 10^{-4} \text{ mg l}^{-1} \text{ cm}^{-2} \text{ day}^{-1}$ killed all the bacteria (E. coli) within 24 hours. In the case of micro-composites, lower release rates were noted.

Figure 2.9: (a) Silver ion release from PA6-Ag composites vs immersion time; (b) Relative death rate of E.coli within 24 hours vs silver ion release rate. (related to the initial concentration)⁶¹.

Increase in microbial count measured in terms of colony forming unit (CFU) per gram sample can be obtained by colony count method or optical density measurement and this decides the shelf life of food. In most cases 10^6 - 10^7 CFU/g is considered as end of shelf life ⁶⁹.

2.5.2 Oxygen scavengers

Oxygen can directly/indirectly cause deterioration of food by resulting in lipid oxidation and facilitate microbial growth ². Food packages using the Modified Atmosphere Packaging technology were flushed with inert gas such as Nitrogen to remove excess oxygen present inside the package. However, ingress of oxygen through the packaging material can increase the level of headspace oxygen and having an oxygen scavenger can be very effective in preventing food spoilage. Oxygen scavengers were the first type of active materials to be commercialized. In late 1970s, Mitsubishi Gas Chemical Company, Japan, commercialised the oxygen scavenger “Ageless”. Another report showed the effect of Ageless with multilayer packaging materials based on Nylon, ethylene vinyl alcohol and polyethylene and found that headspace oxygen concentration reduced by 99.58% within 24 hours and this extended the shelf life of cat fish to 20 days as compared to 10 days without the active component ⁷⁰. An oxygen absorber reduces the headspace oxygen to less than 0.01% ⁷¹. It must be harmless to human, absorb large amount of oxygen at an appropriate rate, not produce toxic substances/off flavours, and most important, it must be economical. Iron powder, ascorbic acid, photo-catalytic metal oxide nanoparticles are typical oxygen scavengers ⁷²⁻⁷⁶. However, most of the commercial oxygen scavengers were based on iron. It works on the principle of iron oxidation ⁴². Iron powder gets oxidized to $\text{Fe}(\text{OH})_3$ in the presence of oxygen and moisture (see equations). TiO_2 act as oxygen scavenger upon UV illumination ⁷⁴, however, as expected, they lose this ability in the absence of UV light.

Simin Feng and others studied the absorption kinetics of oxygen scavengers based on iron with respect to temperature and humidity. The absorption followed first order kinetics with Arrhenius type behaviour. The absorption rate constant increased with

increasing relative humidity and temperature⁷⁷. The size of the active iron particles also has an effect on the absorption capacity. Mu et al, found that the absorption rate constant of nano-sized and conventional oxygen scavenger was $0.45 \pm 0.044 \text{ h}^{-1}$ and $0.05 \pm 0.006 \text{ h}^{-1}$ respectively (1.4 times that of the conventional). The peroxide values (an important test to determine lipid deterioration by oxidation) of roasted sunflower seeds and walnut was found to only marginally increase in the presence of nano-sized oxygen scavengers thus indicating the effectiveness of using nano-sized particles⁷⁸.

Figure 2.10: (a) Kinetic curves for absorption of scavenger; (b) effect of different oxygen scavengers on peroxide values⁷⁸.

Busolo and Lagaron investigated the use of iron containing kaolinites as oxygen scavengers in PE matrix and found that the absorption capacity was only 4.3 ml of oxygen/g composite, at 24 °C and 100%RH whereas only kaolinite showed 10 times higher (43 ml O₂/g) absorption capacity⁷⁹. This indicates that the efficiency of oxygen absorbers when embedded in polymer matrix needs to be increased. This can be done by using polymers in which diffusion of O₂ is faster so that more oxygen can reach the particles. Another suggestion to improve the absorption capacity of the composite films is by using electrospinning technique to produce polymer nanocomposite films with high surface to volume ratios⁸⁰.

2.5.3 Ethylene scavengers

Ethylene is a natural plant hormone, which causes faster ripening and senescence; so, accommodating the release of ethylene out of the package could extend the shelf life of

fresh produce. Climacteric fruits (fruits having increased respiration rates and characterized by a burst of ethylene as they ripen) such as apples, bananas, apricots, avocados and tomatoes, seem to release more ethylene than other fruits. Low temperature (cold)/ controlled atmosphere (less O₂ and more CO₂) storage of fruits are sometimes employed to reduce respiration rate and thus make the fruits less sensitive to ethylene⁴⁵. Figure 2.11 shows the amount of ethylene produced by some common fruits and vegetables.

Ethylene Production from Different Fresh Produce Types			
Low ($< 1.0 \text{ ml kg}^{-1} \text{ h}^{-1}$)	Moderate ($1\text{--}10 \text{ ml kg}^{-1} \text{ h}^{-1}$)	High ($10\text{--}100 \text{ ml kg}^{-1} \text{ h}^{-1}$)	Very high ($> 100 \text{ ml kg}^{-1} \text{ h}^{-1}$)
Pineapple, artichoke, cauliflower, broccoli, date, orange, rhubarb, spinach, beetroot, green asparagus, celery, lemon, onion	Banana, mango, plum, tomato	Apricot, nectarine, pear, peach	Apple, avocado, cherimoya, passion fruit

Figure 2.11: Amount of Ethylene produced (during ripening) by some common fruits and vegetables⁸¹.

Figure 2.12: Potential adsorbed species on Pd-containing scavenger after exposure to ethylene: (a) π -bonded, (b) di- σ bonded, (c) ethylidyne, (d) ethylidene, (e) vinylidene and (f) vinyl⁸¹.

Traditionally, potassium permanganate (on inert porous substrates such as silica or alumina) is used as an effective ethylene scavenger (by oxidizing it to produce CO₂ and H₂O); but it also removes aroma⁴⁵. Catalysts based on platinum/alumina were also used to oxidize ethylene to carbon dioxide and water, but this occurs only at elevated temperatures ($> 200 \text{ }^{\circ}\text{C}$)⁸². A palladium based zeolite ethylene scavenger was used for effective control of ethylene at low ($5 \text{ }^{\circ}\text{C}$) and room temperature and this helps in extending shelf life of fresh produce such as bananas and avocados. When the palladium promoted zeolites were exposed to ethylene along with one of the gases, nitrogen/air/air-

moisture, it was found that palladium acts more like an adsorbent for ethylene rather than a catalyst and this is interpreted from DRIFT spectra (Diffuse Reflectance Infrared Fourier Transform spectroscopy)⁸¹.

2.5.4 CO₂ emitters and scavengers

Carbon dioxide emitters are generally used to complement oxygen scavengers, particularly with meat products. Their main function is to inhibit the microbial growth by releasing large amounts of CO₂ (as high as 80%) within the headspace⁸³. However excess CO₂ decreases growth of the bacterium, *Clostridium botulinum* (type b), but it can increase production of toxins greatly. This can lead to botulism poisoning. Therefore, careful consideration is required while deciding the amount of CO₂ to be emitted in modified atmosphere packages⁸⁴. Dual action carbon dioxide emitters and oxygen absorbers are commercially available from Mitsubishi Gas Chemical Co., Japan (Ageless® E). These are usually used for storing coffee and are based on ferrous carbonate or a combination of ascorbic acid and sodium bicarbonate⁸⁵.

In contrast, CO₂ absorbers are used to remove carbon dioxide to prevent bursting of the package, mainly for foods like beef and poultry products. These sachets include calcium hydroxide with sodium hydroxide or a mix of calcium oxide with silica gel. The silica gel will absorb water and release it when supersaturated. Calcium oxide uses this water to form calcium hydroxide which scavenges carbon dioxide⁴².

These concepts are incompatible with legislations of several countries apart from being unstable during processing and sometimes inhibition of microbial growth and interaction with food is uncontrollable. These drawbacks provide scope for increased research in this field to develop eco-friendly and food safe materials that comply legally. The above-mentioned materials are mostly available in the form of sachets or as films but the potential to use electrospinning method to produce fibrous mats/coatings is explored in section 2.6.

2.6 Electrospinning

A patent on electrospinning was first filed by Forhmals in 1934⁸⁶. After the 1980s, there is great interest in electrospinning because of the ability to produce micro- and nano-

sized fibers in large scale. It is also a simple and efficient method to prepare fibrous films, non-woven or aligned to suit different applications. A typical laboratory set up consists of a high voltage power supply, pump, syringe, nozzle and a collector. The polymer solution is prepared by dissolving an appropriate polymer matrix in solvent at an optimized concentration and fed into the syringe fitted to a pump that can feed the nozzle at a specific rate. The electric potential is applied between the nozzle tip and the collector that is grounded. As the electric field is applied, the polymer droplet stretches and forms a Taylor cone and when the electrical forces overcome the surface tension of the polymer droplet at the nozzle tip it elongates and thins due to the bending instability and reaches the collector⁸⁷.

Figure 2.13: Parameters affecting electrospinning process

The three main parameters that affect the electrospinning process are polymer solution (molecular weight and molecular weight distribution of polymer, solution concentration, dielectric constant of solvent, viscosity), process parameters (applied voltage, feed rate, distance between nozzle tip and collector) and ambient environment inside the electrospinning chamber (temperature and relative humidity).^{80, 88-89} As the solution concentration increases, larger diameter and bead free fibers are formed. Fiber diameter

also increases with decrease in voltage and distance between nozzle tip and collector, and increase in feed rate. Various types of collectors are used to obtain fibers of different types. These include plate, rotating drums, wire collector and sometimes fibers are collected into a non-solvent of the system used. An increase in temperature in the spinning chamber can reduce the viscosity of the polymer solution and high humidity can cause condensation of moisture on the collected fibers leaving pits/pores on evaporation.

These fibers with varying morphologies have been used in a variety of applications, the advantage being their increased surface to volume ratios and easy process ability using a wide variety of polymer matrices and solvents. In recent years, the potential to use these electrospun fibers in food and agriculture industry are being explored. However, there is concern about the toxicity of organic solvents used to dissolve polymers although they are completely evaporated before end-use. They have been employed for encapsulation of plant extracts and bioactive compounds which makes them thermally and chemically stable. Their morphologies can be tuned for sustained release of these compounds (passive process based on concentration dependant diffusion) compared to their film/sheet counterparts and their high surface area increases its sensitivity to external stimuli such as changes in temperature and humidity. Controlled release of ketoprofen, an anti-inflammatory non-steroidal drug, was studied by encapsulating the drug in electrospun PLA fibers. It was observed that drug release depended on morphology of fibers. Porous fibers released the drug sooner as compared to smooth fibers in which the reaction time to release drug was longer. In case of fibers with wrinkled surface, all the drug molecules on the surface of the fiber was released at a certain time. Efficiency of drug release was diffusion dependant and the diffusion rate increases with increase in temperature ⁹⁰. Another work on PLA, Polylactide co glycolide and Polyethylene vinyl acetate have been used to carry drugs for release at various stages ⁹¹.

Below are several studies on using electrospun fibers in food packaging materials, however these have not been materialised commercially. PLA is gaining popularity in food packaging industry as a bio-based and environment friendly material ⁹²⁻⁹⁸. Polylactic acid fibers were employed to carry silver nanoparticles as anti-bacterial agents and the release of silver ions was studied and related it to the anti-bacterial

activity⁹⁹. In a work by Vega-Lugo et al., allyl isothiocyanate was encapsulated as an antimicrobial agent in PLA and soy protein-polyethylene oxide electrospun fibers by direct addition and by complexation of allyl isothiocyanate with β cyclodextrin (to prevent evaporation of allyl isothiocyanate) and then electrospinning it. It was found that the release of anti-microbial agent depended on humidity, increasing with increase in humidity¹⁰⁰. Another work shows the antimicrobial effect of triclosan/cyclodextrin complex encapsulated in PLA fibers. It was observed that these fibers shows excellent anti-bacterial activity and this was due to the increased surface area of fibers along with the efficiency of triclosan complex with cyclodextrin to kill microbes¹⁰¹.

A natural antimicrobial/anti-fungal agent Eugenol was incorporated in electrospun PVA fibers by Kayaci et al. In this study, it was found that the thermal stability of Eugenol was increased as it was encapsulated within the fibers and the electrospinning process does not involve high temperatures. However, complexation with cyclodextrin to reduce volatility didn't show significant differences¹⁰². A few studies also show the encapsulation of natural phenolic compounds such as gallic acid and raspberry extracts in electrospun polymer fibers¹⁰³⁻¹⁰⁴. Neo et al. evaluated the potential of using gallic acid, a natural phenolic compound with anti-oxidant properties, as anti-microbial agent in zein electrospun fibers. These fiber mats were thermally stable even after storing for 60 days at 21.5°C. Quick release of gallic acid and efficient inhibition of the growth gram-positive and gram-negative bacteria was attributed to the increased surface area of electrospun mats, thus exhibiting the potential to be used as edible coatings on dry foods or as part of multi-layer packaging films¹⁰⁵. Mascheroni et al. encapsulated aroma compound (perillaldehyde) in pullulan and β - cyclodextrin and found that the release of aroma compounds happened only above a relative humidity of 90% (water activity dependant)¹⁰⁶.

From the above cited literature, it is inferred that morphology of the fibers and response of electrospun fibers to external conditions such as humidity/ temperature play a very important role in controlling functionality. Therefore, it is important to study the fundamental mechanisms in obtaining fibers with different morphologies. It has been reported that porosity in fibers can be induced by preparing solutions using highly volatile solvents which phase separate into solvent rich and polymer rich regions during electrospinning. Thermally induced phase separation (TIPS), Vapour induced phase

separation (VIPS) and Evaporation induced phase separation are a few mechanisms reported.¹⁰⁷⁻¹¹¹. This is not comprehensive as it only considers vapour pressure of solvent, leaving all other parameters of the polymer solution and ambient environment. Therefore, the focus of this thesis work is to elucidate the mechanisms of obtaining fibers with different surface morphologies taking into account all the properties of the solvent such as vapour pressure, dielectric constant and solubility parameter. Another focus is to understand the sorption of moisture and gases (from ambient) by these electrospun fibers. Also, there are few studies which relate the surface characteristics (morphology and hydrophobicity) of fibers on the control of microbial growth without adding any anti-microbial agent.

Electrospun fibers cannot be stand-alone packaging materials but can be used as a layer in multilayer packaging materials or as inserts in food packages. So, another aspect that this thesis deals with is the use of PE-TPS blends as the packaging material. TPS is blended with different synthetic polymers to reduce environmental concerns caused by landfills of mixed polymer wastes. The next section gives a brief introduction to polymer blends and PE-TPS blends in specific.

2.7 Polymer blends

A polymer blend is a mixture of two or more polymers/ copolymers with or without compatibilizers to obtain the benefit of all components. Polymer blends provide the best properties of each polymer, reduce cost, improve functionality for the desired end use. Polymer blends may be homogenous or heterogenous. In case of homogenous blends, the final properties of the blend are an average of both components because each component loses some of its innate properties. However, in some cases, the strength of one polymer camouflages the weakness of the other component. In heterogenous blends, synergism is hard to predict and depends on the morphology of blend obtained. This morphology can be either dispersed or co-continuous and this is decided based on the blend composition, nature of blend components and the viscosity ratios of the blend components at the processing temperature. At low concentration of one component, a dispersed phase is observed where the dispersed phase can be in the form of spherical drops, cylinders or as laminates. Polymer miscibility in homogenous blends requires a negative free energy of mixing,

$$\Delta G = \Delta H - T\Delta S \quad (14)$$

Entropy gain is negative while mixing two polymers, therefore free energy is negative when the mixing is exothermic, which depends on the interaction between components. Miscibility is reliant on the temperature because solubility/interaction parameters of the two polymers is characterized by the processing temperature.

There are three types of polymer blends, (i) Completely miscible blends in which homogeneity exists at a nanometre scale if not at a molecular scale. The free energy of mixing is negative and such blends show only one glass transition temperature (T_g) and this is in between that of its components and closely correlated with the composition; (ii) Partially miscible blends in which one component is partially dissolved in the other showing good interfacial adhesion. These can be called compatible blends and they show a fine phase morphology; (iii) completely immiscible blends in which the components show a coarse morphology and they require a compatibilizer to improve the interfacial adhesion of the components ¹¹².

Increased need to reduce environmental concerns calls for the use of biodegradable polymers. However, these polymers have limitations on mechanical strength and barrier properties due to its ability to absorb enormous amounts of moisture. Therefore, they are blended together with petrochemical based polymers or other bio-based materials in different ratios to get the desired properties. Starch is one such bio-based polymer that is most commonly blended with polyolefins such as Polyethylene, Polypropylene and with bio-based polyesters such as PLA.

Starch is a most abundant and cheap carbohydrate polymer containing linear amylose and branched amylopectin units. There is strong interaction between these units via hydrogen bonding. In order to reduce these interactions, facilitate movement of molecules and prevent retrogradation, various methods have been employed to chemically or physically modify starch to make it easier to blend with polymers. One common method is to add plasticizers such as glycerol, sorbitol, water, citric acid have been mixed with starch to obtain thermoplastic starch of which glycerol is most widely used ¹¹³⁻¹¹⁷. Starch is modified chemically by addition of esters such as nitrates, sulphates, phosphates, alkenyl succinates and fatty acids ¹¹⁸⁻¹¹⁹. In this process, some of

the hydroxyl groups are replaced by ester groups and the starch becomes hydrophobic and this improves thermal stability and mechanical properties. These starch modification processes, although have several advantages, limit the ratio of starch that can be added to polymers. Therefore, preparation of thermoplastic starch is preferred in this study.

2.7.1 Polyethylene-Thermoplastic starch (PE-TPS) blends

Polyethylene is the most widely used synthetic polymer in food packaging industry due to its excellent strength, puncture resistance, transparency, resistance to chemicals, low permeability to moisture and easy process ability into containers and films by injection moulding and cast/blown film processing methods respectively. It has also been widely used in agricultural applications and in construction industry. But these are difficult to be recycled and it takes years or decades before it can decompose. Therefore, to reduce the consumption of polyethylene, PE-TPS blends have been highly researched in industry and academia.

Figure 2.14: Simple scheme showing (a) addition of maleic anhydride to PE and (b) reaction of maleated PE with starch.¹²⁰

Blending polyethylene with TPS needs a compatibilizer that can improve the miscibility of blends. Polyethylene is non-polar hydrophobic, and starch is polar hydrophilic in nature. Maleic anhydride grafted PE (PE-g-MA) is most widely used compatibilizer among others such as ethylene acrylic acid (EAA) and ethylene vinyl alcohol (EVA). EVA is again highly hydrophilic and therefore limits its use as compatibilizer. EAA is effective but needs to be added in large quantities and this means that biodegradability is reduced. PE-g-MA is very effective in blending starch and polyethylene.¹²¹⁻¹²² It is easily synthesised and less toxic. Figure 2.14 shows the reaction mechanism of PE-g-MA with polyethylene and starch¹²⁰.

There are several studies which show the use of TPS up to 50% by weight of polyethylene. It is found that different processing conditions and ratios lead to different morphologies and this affects the mechanical and barrier properties of the blends.¹²³⁻¹²⁷ This thesis work studies the influence of morphology of PE-TPS blends using two different film forming grades of PE.

2.8 Summary

To summarise, there are several developments in the field of food packaging using various processes and methods. The potential to use electrospun fibers in food packaging is less explored. Some of the potential areas that can be explored further are researched in this work and they include the following. The mechanisms involved in obtaining different surface morphologies of fibers (chapter 4) and the effect of this on the sorption and microbial growth (chapter 5) will be evaluated. The properties of PE-TPS blends will be studied and finally they are combined to see the effect on water vapour transmission rate (chapter 6).

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Chapter 3

Materials and Methods

This chapter gives the rationale behind materials used in this research work. The preparation of electrospun PLA fibres, melt extrusion of thermoplastic starch (TPS) and LDPE/TPS blends are described. Characterization techniques employed to study the morphology, thermal, mechanical, chemical, barrier properties of blends and tests to estimate porosity and sorption performance of fibres are explained. Protocols to test the antimicrobial activity of the electrospun fibres are also detailed.

3.1 Materials selection and preparation

As discussed in chapters 1 and 2, there is an increasing demand for development of green bio-based materials to be used in food packaging applications that reach their end as soon as the food is consumed. It is also observed that the polymeric flexible films/cartons/rigid containers currently used in packing food consist of multiple layers to obtain the desirable barrier and functionality ¹. However, on disposal, separating the layers, sorting and recycling is a very expensive process and such facility is only scanty. Therefore, minimizing the number of layers and optimizing the performance is a big challenge. This thesis work attempts to develop electrospun fibres having different surface morphologies using PLA as matrix. For it to find potential use as a functional layer in food packaging it is important to study the mechanisms of obtaining such fibres and to study how these surface features respond to moisture and microbes present in food environment.

Another part of this work to develop LDPE/TPS blend will be an environmentally friendly alternative to replace LDPE, a widely used material in food packaging applications as a single or multi-layer system.

3.2 Fibrous PLA membrane

PLA is aliphatic thermoplastic polyester produced by ring opening polymerisation of lactic acid monomer which is obtained from bacterial fermentation of carbohydrates or by chemical synthesis. It has reasonably good mechanical strength and can be easily processed. However, its thermal resistance and gas/moisture barrier is lower as compared to its fossil fuel based counterparts such as PET (that is widely used in packaging) ²⁻³. In addition, PLA falls under the category of “Generally Recognised as Safe” after it passed several migration tests based on the guidelines issued by Food and Drug Administration (FDA). Only limited migration of potential migrants such as lactic acid, lactide and lactyollactic acid was observed and there is no significant risk as the migrants are expected to be converted to lactic acid which is safe ⁴. Also, PLA is easily soluble in organic solvents which facilitate the ability to be processed via electrospinning method to obtain fibrous films and coatings. Therefore, this polymer is chosen as matrix for the inner electrospun layer. A high molecular weight PLA, ‘PLA Polymer 2003D’, with a specific gravity of 1240 kg/ m³ and glass transition temperature

of 57°C was purchased from Nature works® LLC, UK in the form of pellets. Table 3.1 shows a list of solvents used to prepare PLA solutions for electrospinning. Analytical grade solvents were used as received.

Table 3.1: List of solvents used in the study

Solvent	Supplier
Dichloromethane (DCM)	Merck, Singapore
N, N – Dimethylformamide (DMF)	Tedia, US
Methanol	Fisher Scientific, UK
1,4 – Dioxane	Merck, Singapore
1,2 – Dichloroethane (DCE)	Sigma-Aldrich, USA

Sepiolite particles used along with PLA to produce electrospun membranes was obtained from Tolsa S.A, Spain. Its chemical formula is $\text{Si}_{12}\text{Mg}_8\text{O}_{30}(\text{OH})_4(\text{OH}_2)_4 \cdot 8\text{H}_2\text{O}$ (hydrated magnesium silica). Sepiolite particles are needle shaped, 1-2 μm long and 0.01 μm wide. It has the highest surface area ($\sim 300 \text{ m}^2/\text{g}$) and the highest density of silanol groups among different clays and acts as a good adsorbent. Zinc oxide (ZnO) of 99+% purity and average particle size of 20 nm was obtained from Nanoamor, USA.

3.2.1 Preparation of fibrous PLA membranes by electrospinning

Electrospinning is a technique to produce ultra-thin fibres from polymer solutions by applying a high voltage usually in the range of 1-30 kV. As the voltage is gradually increased, the pendant drop of polymer solution at the nozzle tip experiences electrostatic repulsion between surface charges and the coulombic force exerted by the electric field. These forces distort the droplet into a cone (Taylor cone) and once the threshold electric field is crossed, the electrostatic forces can overcome the surface tension of the polymer solution thus ejecting a liquid jet that stretches and whips to form thin fibers.⁵ There are many excellent reviews on electrospinning discussing about the underlying physics, interaction of surface tension of solvents and electrical forces, solution properties, etc.⁶⁻¹¹. But to obtain fibres with consistent diameters in electrospinning, several variables must be considered and controlled. They include

polymer molecular weight and concentration, vapor pressure and dielectric constant of solvent, applied voltage, feed rate, nozzle to collector distance. In addition to these, external conditions such as humidity of the spinning environment might as well play a role.

Figure 3.1: Top-down Electrospinning set up⁵.

A laboratory scale electrospinning unit, Nanospinner NE 300 (Innovenso Ltd., Turkey) with a bottom-up setup was used. The advantage of using a bottom-up set up is that there is no problem of polymer solution dripping (as the solution is pumped against gravity) to the collector plate which may result in inhomogeneity in the obtained fibres. Humidity inside the electrospinning unit was varied by using Olee UV air dehumidifier (OL-608) and monitored using a digital humidity indicator. Various solvent systems and humidity levels (ranging from 25–90%) were employed. 8 wt% PLA solutions were prepared by dissolving the required amount of PLA pellets in the solvent systems (refer table 3.2 below) while continuously stirring (using a magnetic stirrer) for 24 hours at room temperature. The solutions were spun at a feed rate of 0.9 ml per hour and a voltage of 15–19 kV, while maintaining the distance between nozzle and collector at 120 mm. The fibers were collected on an aluminium foil attached to the plate collector. The membranes were dried for about 6 hours at room temperature followed by vacuum drying at 45°C for 12 hours to remove traces of solvent.

Table 3.2: Electrospinning of PLA (8 wt. %) with different solvent systems (in volume %).

Electrospinning solution combinations		Designation
Single solvent system	PLA + DCM	A1
Binary and ternary solvent system	PLA + (70%DCM + 30%DMF)	A2
	PLA + (40%DCM + 40%DCE + 20%DMF)	A3
	PLA + (90%DCM + 10%DMF)	A4
	PLA + (90%DCM + 10%Methanol)	A5
	PLA + (70%DCM + 30% 1,4-Dioxane)	A6
	PLA + (40%DCM + 40%DCE + 20%DMF) + 5 wt% sepiolite	A3S
	PLA + (40%DCM + 40%DCE + 20%DMF) + 5 wt% ZnO	A3ZnO

3.2.2 Characterization studies

Scanning electron microscopy is a tool used to obtain images of micro- and nano-sized features. It uses high energy electron beam (generated by a field emission or thermionic electron gun and guided by electromagnets) to scan the sample surface systematically and this is detected by electromagnets thus generating signals that give information about the surface features. As the electrons interact with the sample surface, secondary electrons may be emitted from the atoms near the sample surface providing a very sensitive, detailed and high-resolution image of the surface morphology, or back scattered electrons may be emitted from atoms at a greater depth in the sample thus losing their resolution. The former known as secondary electron imaging (SEI) is used in this study to observe the surface morphology of electrospun fibers.

Morphology of electrospun fibers was observed using a field emission scanning electron microscope (FESEM) - JEOL 6340 F. Fibers were sputter coated with platinum before examination at an accelerating voltage of 5-10 kV. From SEM micrographs, average fiber diameter was measured by considering around 200 fibers using Image J software.

Figure 3.2: Scanning electron microscopy¹².

Transmission electron microscopy (TEM) is another method to obtain images of nanoparticles and to observe the dispersion in polymer matrix. A high energy electron beam is focussed on a very thin section (<100 nm) of the sample and as the electrons interact with the sample a contrasting image is captured and magnified by the CCD camera used as the detector in TEM. The polymer and particles have an atomic mass difference and the contrast is differentiated based on this difference. In this case, a few electrospun fibers are quickly deposited on coated copper grid by placing it on the collector and quickly moving the collector to get a fine layer of fibers so that it is thin enough to be observed in the TEM. The contrast between polymer fiber and

nanoparticles can be easily detected. Carl Zeiss TEM Libra 120 is used in this study at 120 kV.

Mercury intrusion porosimeter is a technique used to measure porosity and pore size distribution of various porous materials. Mercury is a non-wetting liquid and cannot naturally penetrate the pores by capillary action. Therefore, it must be forced by applying external pressure. This equilibrated pressure and pore size are related inversely, meaning that only slight pressure is needed for mercury to be forced into large pores.¹³ Under applied pressure mercury leaves the penetrometer (electrical capacitance dilatometer) stem and enters the sample. The stem is a capillary that is coated with a metal plating which along with the mercury inside acts as a coaxial capacitor. At each change in pressure, the volume of mercury entering the pores in the sample (has a sensitivity of under 0.1 μL) is measured by the decrease in the capacitance (meaning decrease in mercury in the stem).¹³ This method can be used to determine pore sizes in the range from 0.003-1100 μm .

Figure 3.3: Mercury in equilibrium with and entering an opening under increasing forces¹⁴

Porosity and total pore area were measured using mercury intrusion porosimeter, Micromeritics AutoPore IV 9520. The amount of material needed for porosimetry test, $m \pm 10\%$, is calculated using the formula,

$$m = 0.206 \rho_B [(100/\%P)-1] \quad (1)$$

where, ρ_B = skeletal density of material in g/cc (without pores), $\%P$ = approximate open porosity as volume %, and m = approximate mass of material in g.

The fibers were dried overnight in vacuum oven at 45 °C before analysis to ensure the sample is completely dry. The sample was placed in the cup of a powder penetrometer (stem volume 0.412 ml) and tested first at low pressure ($6.8927 \times 10^2 - 6.8927 \times 10^4$ Pa) and then at high pressures ($6.8927 \times 10^2 - 6.8927 \times 10^7$ Pa) at a mercury filling pressure of 3.5824×10^3 Pa and equilibration time of 10 seconds. The pore radius and pressure are related by the Washburn equation (assuming that pores are cylindrical),

$$r = -2\gamma\cos\theta/p \quad (2)$$

where, θ = contact angle of mercury with the sample (130°), γ = surface tension of mercury (0.485 N/m), r = pore radius, and p = applied pressure (Pa). The volume of pores at the corresponding sizes are measured based on the volume of intrusion of mercury into the sample and thus the total pore area can be determined.

Surface wetting was studied by measuring the contact angle of the membranes using Dataphysics OCA 20 contact angle instrument. This is an optical contact angle measuring and contour analysis system in which the set-up captures an image of the liquid droplet on the surface of the sample (sessile drop). Based on the grey scale evaluations of the image, a baseline (line of contact between liquid droplet and solid sample) is detected and tangents are applied at the intersection of the droplet arc and the baseline to determine the left and right contact angle. A water droplet of 6 μ l was dropped at a medium speed and the static contact angle was measured. All reported values are an average of 5 readings measured.

Figure 3.4: Schematic of a set-up for sessile drop method

Moisture sorption isotherms are measured gravimetrically using a high precision magnetic force balance and a chilled mirror dew point sensor under two modes – Dynamic dewpoint isotherm (DDI) that gives dynamic isotherms and the Dynamic vapour sorption (DVS) that gives static or equilibrium isotherms. In DDI mode, the humidity is not controlled, rather the water content and water activity are measured as the sample is allowed to dry (flowing air from the desiccant tubes) and wet (flowing air from the water reservoir). In the DVS mode, the water activity is increased/decreased incrementally and the water uptake/loss by the sample is measured gravimetrically. Thus, DVS mode is used in this study as the amount of water uptake by the electrospun fibers is of interest.

Moisture sorption isotherms are obtained using a vapour sorption analyser (Aqualab VSA) in the Dynamic vapour sorption (DVS) mode. The water activity (a_w) was increased from 0.1 – 0.95 in steps of 0.1 at a temperature of 25°C and the moisture sorbed is recorded on dry basis (g water/g solid) when equilibrium is reached for each a_w . Guggenheim–Anderson–de Boer (GAB) model was used to fit the adsorption data of the membranes.

$$m = m_0 c k a_w / (1 - k a_w) (1 - k a_w + c k a_w) \quad (3)$$

where, m is the equilibrium moisture content at each water activity, m_0 is the monolayer moisture content corresponding to the mono-molecular layer on the surface of membranes, c is the Guggenheim constant, k is a factor corresponding to the properties of the multilayer molecules with respect to the bulk liquid. The GAB constants are estimated using the quadratic form of equation 3,

$$\frac{a_w}{m} = \alpha a_w^2 + \beta a_w + \gamma \quad (4)$$

where, $\alpha = \frac{k}{m_0(1/c - 1)}$, $\beta = \frac{1}{m_0} (1 - \frac{2}{c})$ and $\gamma = 1/m_0 c k$. The values of α, β, γ were determined by non-linear regression analysis¹⁵.

The goodness of fit of the GAB model with the experimental data was determined using the equation for root mean squares,

$$\%RMS = \frac{\sqrt{\sum_i^n \left[\frac{m_i - m_{pi}}{m_i} \right]^2}}{n} \times 100 \quad (5)$$

where, m_i is the experimental moisture content, m_{pi} is the predicted moisture content and n is the number of experimental points ¹⁶.

Microbial growth on fibrous membranes was tested by colony count method as described here. This method is similar to the method used by Shankar et. al.¹⁷ *Listeria monocytogenes* and *Escherichia coli* were chosen as representatives of gram-positive and gram-negative bacteria respectively. These are the most prevalent food borne pathogenic bacteria. *L. monocytogenes* was inoculated in 20 mL Brain Heart Infusion (BHI) and *E. coli* in Tryptic Soy Broth (TSB) broth (BHI and TSB were obtained from BD). Subsequently it was incubated for 16 h at 37 °C. The culture was centrifuged at 5000 rpm for 10 min. The cell pellet was then suspended in 100 mL of the respective sterile broth followed by 10 times dilution with sterile water. 50 mL of diluted broth (10^6 - 10^7 CFU/ mL) was taken into 100 mL conical flask containing a fibrous membrane sample (5 cm x 5 cm). This was incubated at 37°C for 12 h under mild shaking. This diluted broth without any sample was the control. Bacterial cell viability was calculated by colony count on the plates at a 3 hours interval. In order to test the attachment of bacterial colonies on the surface of fibrous membranes, the following method was used. An aliquot of the diluted broth of *E. coli*, as prepared above was spread on the fibrous membranes and the membranes were incubated at 37°C. After 9 hours, the membranes were washed to remove the non-attached bacteria and dried. This was then sputter coated with platinum and observed in FESEM for presence of attached bacterial colonies. All films were tested in triplicate.

3.3 LDPE/TPS blends

3.3.1 Preparation of Thermoplastic starch

Starch is a natural, inexpensive and renewable polymer with amylose (linear polysaccharide) and amylopectin (branched polysaccharide) units. Corn starch used in this thesis work was obtained from Roquette Riddhi Siddhi Pvt Ltd., Gokak, India. The hydrogen bonding between the starch molecules is very high and therefore in order to lessen the order and crystallinity and allow free movement of starch molecules, to be

blended with LDPE, plasticizers are added. In this work glycerol (obtained from Aik Moh Paints and chemicals Pvt. Ltd.), Glycerol monostearate (GMS) and Urea (obtained from Sigma Aldrich) are used to prepare thermoplastic starch (TPS). Starch is dried in vacuum oven at 80°C for 6 hours. Preparation of TPS is done in 2 steps. First, appropriate amounts of starch, glycerol, urea and GMS are manually mixed using a cake mixer for about 20 minutes until the clumps are removed to get a uniformly mixed powder. This is left overnight in a desiccator and this allows the starch molecules to react with glycerol.

Second step is melt mixing of this starch using a co-rotating twin screw extruder (Leistritz Micro twin screw extruder) at 80 rpm. Twin-screw extrusion is the most widely used method for melt mixing polymer with fillers and additives or to blend polymers as it can be used in commercial scale. The intermeshing screws provide good dispersion and the output is high in this method. The temperature profile used from feeding zone to die (6 zones) is 70°C, 120 °C, 130 °C, 135 °C, 140 °C, 140 °C. Extruded strands were air cooled and granulated using pelletizer and stored in desiccator.

Table 3.3: Ingredients for TPS 1

Material	Weight (g)
Starch	100
Glycerol	37
Glycerol Monostearate	2
Urea	0.75

Table 3.4: Ingredients for TPS 2

Material	Weight (g)
Starch	100
Glycerol	35
Glycerol Monostearate	1
Urea	1

Figure 3.5: Schematic of a twin-screw extruder.

3.3.2 Preparation of LDPE/TPS blends

LDPE of two different melt flow indices (MFI) were purchased from The Polyolefin Company, Singapore. MFI of LDPE 210 is 2 g/10 minutes and that of LDPE 410 is 4 g/10 minutes. LDPE 210 was blended with TPS 1 and LDPE 410 was blended with TPS 2 in a 50/50 weight ratio. Compatibilizer used is Polyethylene grafted maleic anhydride (PE-g MA). This was purchased from Sigma Aldrich. CLOISITE 15 (C15) is purchased from BYK. It is layered montmorillonite organically modified with bis-(hydrogenated tallow alkyl) dimethyl salt. The clay is dried over night at 105°C prior mixing. Appropriate quantities of all ingredients were measured and melt mixed using the twin screw extruder at 100 rpm. The temperature profile used from feeding zone to die (6 zones) is 120°C, 150 °C, 165 °C, 170 °C, 180 °C, 175 °C. Extruded strands were air cooled and granulated using pelletizer and stored in desiccator.

Table 3.5: Ingredients of different films prepared

Designation	Ingredients
210	LDPE 210
210 C	LDPE 210 + C15 3 wt%
210 TPS	LDPE 210 / TPS 1- 50/50 wt%
210 TPS C	LDPE 210 / TPS 1- 50/50 wt% + C15 3wt%

410	LDPE 410
410 C	LDPE 410 + C15 3 wt%
410 TPS	LDPE 410 / TPS 2 - 50/50 wt%
410 TPS C	LDPE 410 / TPS 2 - 50/50 wt% + C15 3wt%

The blended pellets were then made into films using a single screw blown film extruder (Dr. Collin GmbH Teach line BL50) at 50 rpm and a temperature of 160°C at 3-4 bar pressure and 240 rpm roller speed. Blown film extrusion is the most significant polymer processing method to produce flexible packaging films and bags. Molten polymer exits a circular die vertically to form a free bubble which is later guided by rollers and wound onto bobbins. The processing variables are screw speed, roller speed, internal pressure of bubble¹⁸. thickness of films obtained was 80-100 μm . The films are always stored in desiccators as starch is prone to absorb moisture.

3.3.3 Characterization studies

The morphology of films is observed on etched samples using FESEM (JEOL 6340 F) at an accelerating voltage of 5-10 kV. Etched films were sputter coated with platinum before examination. Etching is done by immersing the films in deionised water, heated to 60°C for a few minutes followed by ultra-sonication at 60°C for 6 hours. The films are then removed and washed with deionised water and dried completely with tissue paper and then in oven at 60°C for 2 hours.

Thermal properties of polymer are studied using Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA). DSC works on the principle of recording the changes in the heat flow differences to sample and reference as the temperature is increased/ decreased at a specific rate. From the heat flow vs temperature curve obtained, glass transition temperature (T_g), melting temperature, crystallization temperature, enthalpy of fusion can be obtained. Thermogravimetric analysis is a very simple method to determine the composition of the compound based on the loss in weight as each ingredient decomposes at different temperature on heating at a specific rate in air/ N_2 atmosphere. From the weight loss vs temperature and the derivative of weight loss vs temperature, the onset degradation temperature and peak degradation temperature can

be calculated. In this study, the thermal properties of the films were studied differential scanning calorimetry (DSC: Q 10, TA Instruments) at a heating rate of 10°C /min from 30 °C to 250 °C, isothermal for 2 minutes and cooling from 250°C to 30°C in nitrogen atmosphere. The onset and peak degradation temperatures are studied using thermogravimetric analysis (TGA: Q500 TA Instruments) at a heating rate of 20 °C/ min from room temperature to 800 °C under N₂ atmosphere.

Mechanical properties of the films were measured using universal testing machines (UTM, Instron 5567 Instruments) using a 500 N load cell. Ultimate tensile strength (MPa) and elongation at break (mm) were recorded. The extension rate was kept at 50 mm/min and the load was applied till the sample breaks. The dimensions of the sample used were 15 cm × 2 cm. 5 samples were studied for each type of film. Bluehill software was used for the calculations.

Fourier Transform Infrared Spectroscopy (FTIR) is a technique to identify the functional groups present in the sample. The molecules in the sample will absorb certain frequencies of IR radiation and the rest is re-radiated. The interference of this re-radiated radiation with unperturbed radiation of the instrument produces an interferogram. Fourier transform is applied to this interferogram to get absorbance or transmission spectra of intensity vs wavenumber. The peaks are unique for each molecule identified and is its chemical fingerprint. The advantage of using Attenuated Total Reflectance (ATR) method is the short path length to the sample. This was used to analyse the surface chemical bonds and ATR method was used to scan the sample in transmission mode for 32 scans from wavenumber 4000-600 cm⁻¹.

Water vapour transmission is measured using 15 cc Payne cups. 3 cm diameter films are used for testing and the exposed area is 10 cm². The cups are filled with 5 ml of water and sealed tightly with the films. The initial weight of the cups (with film and water) is measured. The cups are placed in the humidity chamber at a temperature of 38 °C and a relative humidity (RH) of 90% with a water vapor partial pressure of 0.05826 atm. The transmission of water through the films is measured as the loss in weight of the cups at specific time intervals. Then weight loss vs time is plotted and the slope gives the transmission in g/day. Then the water vapour permeability (WVP) is calculated using the formula,

$$WVP = \frac{(\text{transmission} \times \text{thickness of film})}{(\text{area} \times \text{water vapour partial pressure})} \quad (6)$$

The WVP has a unit of g.mm/m².day.atm. All measurements are made in triplicate.

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Chapter 4

Mechanisms of Pore Formation in Electrospun PLA Fibers*

This chapter elucidates the mechanisms of formation of varying degrees of surface pores/pits on polylactic acid (PLA) fibers during electrospinning. The role of a combination of different parameters in governing pore formation was demonstrated. They include solvent vapor pressure (p_v), solvent miscibility/ interaction with water, solubility parameter, and relative humidity (RH) within the spinning unit. The results indicated that traditional mechanisms like thermally induced phase separation (TIPS) and vapor induced phase separation (VIPS) were not responsible in the generation of surface porosity/pits. Instead, higher RH (water vapor, a non-solvent of the polymer), and its miscibility/interaction with solvent(s) were concluded to be relatively more important than the simple presence of a high p_v solvent or a combination of high p_v and low p_v solvent system. Further, content of high p_v solvent in solution determined the spherical or elliptical nature of pores/pits by affecting the level of saturation of nearby region of the interface between jet and air during the electrospinning process.

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4.1 Introduction

Recently, electrospinning technique, which enables production of fibres with high surface-to-volume ratios, has seen application in different fields where sorption is a critical parameter. Some of these fields include tissue engineering/drug delivery, sensors, oil adsorption, catalytic systems, and protective clothing. There are many excellent reviews on electrospinning discussing the underlying physics, interaction of surface tension of solvents and electrical forces, solution properties, etc.¹⁻⁵. As food packaging materials, use of electrospun fibers is less explored. They show an advantage in terms of maintaining integrity of encapsulated plant extracts/ bio-active compounds thus protecting from chemical/thermal degradation and in controlled release of active ingredients. Active packaging materials based on electrospun fibers have higher sensitivity to changes in the surrounding environment⁶⁻⁷. This can be achieved for different applications by tuning the surface morphologies of the fibers, the type of polymer matrix and active ingredients used.

For many biological and chemical applications involving adsorption and desorption of various organic/inorganic species on a solid, surface morphology of the latter plays an important role. To this extent, significant experimental and theoretical efforts were diverted towards understanding the effects of surface features like roughness, pores and functionalization on sorption/desorption⁸⁻¹². Rechendorff et al.¹² showed that fibrinogen protein adsorption on evaporated tantalum films was influenced by nano-scale surface roughness. The uptake increased by almost 70% when the root-mean squared roughness increased from 2.0 to 32.9 nm. For many filtration applications and oil spill clean-up processes, the approach of surface functionalization is commonly used to allow for superior selectivity of hydrophobic hydrocarbons¹³. In addition, for faster adsorption kinetics, porous surfaces are developed with remarkable enhancements in surface area, which provide additional binding or adsorption sites¹⁴. The sorption mechanisms, in general, include pore-filling, hydrogen bonding interactions and hydrophobic interactions. In some cases, π - π bonding interaction and exchange of carboxyl/hydroxyl functional groups were also ascribed as sorption mechanisms¹⁵.

Another major advantage of electrospinning is the ability to induce porosity on the fibers in situ during spinning, which provide additional sites for adsorption. But there are many

differences (and questions) on the proposed mechanisms of pore formation in the literature. Some of the commonly reported mechanisms are TIPS, VIPS, and evaporation induced phase separation (due to difference in vapor pressure of solvents)¹⁶⁻²⁰.

TIPS is based on the phenomenon that the solvent quality usually decreases when the temperature is decreased. Conventionally, after demixing is induced, the solvent is removed by extraction, evaporation or freeze-drying.¹⁶ In electrospinning, fibers could be directly spun into a cryogenic liquid, and as a result of sudden drop in temperature, TIPS occurs between the solvent-rich and solvent-poor regions. Subsequently, after the solvent is evaporated in a controlled manner, pores form throughout the fiber (not just on the surface). However, this method generally yields bigger pores and thicker broken fibers due to immediate freezing, which affects fiber whipping and elongation.¹⁸ VIPS is based on precipitation by absorption/penetration of a non-solvent like water from the vapor phase into fibre jet.¹⁹ Bognitzki et al.²¹ even showed that porous fibres could be obtained by selective dissolution of electrospun polymer blends. They verified this by electrospinning polylactic acid (PLA)/poly(vinylpyrrolidone) (PVP) with dichloromethane (DCM) as solvent. Subsequently, porosity was obtained by either annealing the fibres to remove PLA or using water as a solvent to remove PVP.

Further, some have reported that porous fibres could be obtained during electrospinning by using a single (highly volatile) solvent through thermodynamically driven events.^{18, 21} In here, it was believed that the solvent-rich regions were transformed into pores. Due to fast evaporation of the solvent, phase boundaries were crossed resulting in rapid phase separation and structure formation. However, it was noted that these phase morphologies exist only on the surface of fibres, which is debatable. In another investigation, the importance of solvent volatility in inducing pores on fibres rather than phase separation was emphasized.¹⁹ This was established by using tetrahydrofuran, THF ($p_v \sim 21.6$ kPa at 25 °C and boiling point, $T_b \sim 66$ °C), dimethylformamide, DMF ($p_v \sim 2.7$ kPa at 25 °C and $T_b \sim 153$ °C) and their combination (THF/DMF - 75/25% and 50/50%) as solvents for PS. With 100% THF, high density of pores was observed on PS fibres and they disappeared as the volatility of the mixed solvent system decreased (that is, with increase in DMF content).

Nonetheless, there are many questions on pore formation during electrospinning and it is not so direct to conclude based on only one particular factor like solvent volatility. In fact, in many studies, humidity (at which the fibres are spun) and solvent miscibility with (condensed) water are not considered (or mentioned). We believe that these in combination with vapor pressure of solvent and solubility parameter differences between solvent(s) and polymer might govern pore formation. This forms the fundamental basis of this study, that is, to elucidate the mechanisms of pore formation on fibres during electrospinning. For this purpose, PLA is chosen as the matrix and various solvents with different properties are chosen strategically (see Tables 4.1 and 4.2).

Table 4.1: Electrospinning of PLA (8 wt. %) with different solvent systems (in volume %) and the end result on porosity after electrospinning at ~75% RH.

Electrospinning solution combinations			Porosity at ~75% RH
Single solvent system	PLA + DCM	A1	Pores
Binary and ternary solvent systems	PLA + (70%DCM + 30%DMF)	A2	No pores
	PLA + (40% DCM + 40% DCE + 20% DMF)	A3	Pores
	PLA + (90% DCM + 10% DMF)	A4	Pores
	PLA + (90% DCM + 10% Methanol)	A5	No pores
	PLA + (70% DCM + 30% 1, 4 – Dioxane)	A6	Pores

PLA solutions were dissolved in different solvent systems in the weight ratio of 8%. This was optimized by trial and error with different ratios from 6-10% to get uniform diameter fibers and to prevent beads (observed at low concentrations of PLA due to low viscosity) and flattened fibers (observed at high concentration of PLA due to high viscosity). Figure 4.1 shows the size distribution of fiber diameters for two systems, A2 and A3.

Table 4.2: Vapor pressure (kPa)²², dielectric constant²² and Hansen solubility parameters²³ (MPa)^{1/2} of solvents used.

Solvent	Vapor pressure	Dielectric constant	Hansen solubility parameter (MPa) ^{1/2}			
	(kPa)		δ_d	δ_p	δ_h	δ_t
Dichloromethane	58.1	8.93	18	12.3	7.2	22.9
N, N-Dimethylformamide	0.49	36.71	17.4	13.7	11.3	24.8
Methanol	16.937	32.66	15.1	12.3	22.3	29.7
1,4-Dioxane	4.95	2.209	19	1.8	7.4	20.5
1,2 -Dichloroethane	11.11	10.37	19	7.4	4.09	20.8

Figure 4.1: Fiber size distribution of A2 and A3 systems obtained by image analysis using imageJ software

4.2 Results and discussion

4.2.1 Influence of solvent properties on pore formation

4.2.1.1 Single solvent system

Before discussing the electrospinning results of PLA with different combinations of solvents, it is important to identify if the mixed solvents form azeotropes or heteroazeotropes, which then demands the calculation and understanding of mixed solvent properties (like boiling point, vapor pressure, etc.). However, this is not the case here in the ratios added (see Table 4.2).²⁴ But to have a quick comparison across the systems, simple Raoult's law was used to calculate the total vapor pressure of each solvent combination. Obviously, the single solvent system of DCM has the highest p_v of ~58.1 kPa, whereas 40% DCM + 40% DCE (1,2 -dichloroethane) + 20% DMF mixture yielded the lowest ~30.3 kPa, and the rest of the systems are in-between ~43-52 kPa. But these differences could not be translated into porosity/pits on the electrospun fibers.

Figure 4.2: SEM micrographs of PLA fibers spun at 75% RH with 100% by volume of DCM (a) top view; and (b) cross-section.

An SEM micrograph of PLA fibers spun at a RH of ~75% using a single solvent system (PLA/DCM) is shown in Figure 4.2 that reveals high density of porosity. Although the temperature in the spinning unit is constant, high p_v of DCM results in rapid solvent evaporation followed by evaporative cooling of the charged jet as it travels to the collector. This could result in TIPS. Obviously, evaporative cooling will be more effective on the surface of the fibers compared to the bulk and might as well explain the

fact that these fibers only shows surface porosity and not throughout the cross-section (see Figure 4.2b). Apart from condensation, moisture in the ambient could also act as a non-solvent by absorption and penetrating into polymer solution during electrospinning process, as explained earlier, and result in VIPS. For this mechanism, the difference in the p_v of solvent (used in the electrospinning process) and non-solvent (from the ambient) is important as it determines which one will saturate the nearby region of the interface between jet and air. In the present case of DCM ($p_v \sim 58.1$ kPa) and water ($p_v \sim 2.3$ kPa), with reasonable confidence it is assumed that DCM will saturate the nearby region of the jet-air interface. This suggests that VIPS may not be the mechanism in this case. Another important parameter is the diffusion coefficient of the non-solvent in the selected polymer. This will govern the penetration efficiency and in turn determines whether pores are formed only on the surface or throughout the cross-section of the fiber.

Scheme 4.1: Mechanism of pore formation in a single solvent system (A1).

Nonetheless, the spherical nature of the surface pores suggests that TIPS and VIPS are not the operating mechanisms (where elliptical or elongated pores along the fiber axis are more common). This means that the porosity is induced after fibers are deposited on the collector as a result of condensation of moisture in the air (as water is a non-solvent for PLA). Subsequently, these water droplets leave their imprint in the form of pores or pits on the surface of fibers (see scheme 4.1). This is similar to the concept of ‘breath figures’, which was originally introduced by Aitken²⁵ to explain the formation of water

droplets on clean glass surfaces when exhaled breath condenses on those surfaces. Later, this was extended to describe condensation on different types of surfaces; for example, see ²⁶⁻²⁷. Even analytical solutions that describe the evolution in time were proposed²⁸. In many cases, breath figures were also used to characterize the degree of contamination on a (homogeneous) surface²⁹. If the surface is contaminated, the condensation was expected to be strong leading to film formation²⁷. Needless to say, that %RH plays a critical role in this process.

4.2.1.2 Binary and ternary solvent systems

As discussed before in earlier studies, higher p_v solvent systems (either single or a combination) and/or higher RH levels should induce porosity. Also, porosity should decrease (and gradually disappear) as the volatility of the mixed solvent system decreases. However, this is not the case here. A2 (containing 70% of high p_v solvent, DCM) showed no pores or pits on the fibers, Figure 4.3a. In contrast, A3 (containing 60% of low p_v solvents, DCE and DMF), showed surface pores, Figure 4.3b. These differences clearly illustrate that p_v /solvent volatility is not the sole reason in inducing porous morphology on the electrospun fibers.

To explain this, it is important to understand other properties of these solvents like their miscibility and interactions with water. In A2, the solvent system was DCM/DMF in the ratio 70 to 30 by volume. Since the p_v of DCM is very high compared to DMF, it is expected to evaporate relatively quickly during the electrospinning process leaving behind DMF. Therefore, at high RH levels, after the moisture in the air condenses onto the fiber surface, the H-bond interaction of DMF with water, $C-H\cdots O$, plays an important role in hindering the deposition of condensate as separate droplets. Instead it is presumed that the condensate is coalesced and spread across the fiber surface. Subsequently, after the evaporation of water, a smooth surface morphology is generated without any pores or pits. This in fact is supported by the result that if the DMF content is reduced to 10% by volume, pit/pores were observed, however at a reduced density compared to system A1 (Figure 4.3c).

Figure 4.3: SEM micrographs of PLA fibers spun at 75% RH with different solvent combinations: (a) 70/30 DCM/DMF; (b) 40/40/20 DCM/DCE/DMF; and (c) 90/10 DCM/DMF

To further elucidate on the effect of solvent miscibility/interaction with water, methanol and 1, 4-dioxane are chosen in combination with DCM. Both methanol and dioxane are miscible with water but their hydrogen bonding component and p_v s are different (Table 4.2). Although methanol's p_v is much higher than that of DMF, methanol has H-bonding component twice as that of DMF, thus enabling even a lower amount of methanol to bond with moisture/droplets. This explains why there are no pores on the surface of fibers even with 90/10 DCM/methanol (Figure 4.4a, A5); while 90/10 DCM/DMF showed reduced density of surface pits (Figure 4.3c, A4) as explained before. Also, from the SEM micrograph of A5, it is evident that the fibers are flat and more like ribbons than tubular. This indicates the slower evaporation of solvent in combination with water, thus collapsing the fibers.³⁰

On the contrary, even with 30% dioxane (A6), the morphology is similar to 90/10 DCM/DMF with surface pores/pits. But 70/30 DCM/DMF didn't yield porous

morphology, Figure 4.3a. This is despite dioxane having a similar p_v as DMF. This again reiterates the effect of solvent interaction with water.

Figure 4.4: SEM micrographs of PLA fibers spun at ~75% RH with different solvent combinations: (a) 90/10 DCM/methanol; and (b) 70/30 DCM/dioxane.

Scheme 4.2: Mechanism of pore formation in a ternary solvent system (A3).

From the above results, it is evident that at high RH levels, in the presence of low p_v solvents, their interaction with water plays a dominant role in inducing surface pores/pits. This further explains why there are pores on the fibers of A3. The solvents in A3 have huge differences in p_v s and their miscibility/interaction with water is different. DCM ($p_v \sim 58.1$ kPa) and DMF (~ 0.49 kPa) were discussed earlier, whereas DCE with a p_v of ~ 11.1 kPa is immiscible with water. Also, the density of pores at these high RH conditions is determined by the magnitude of these interactions as well as the amount of low p_v solvent. But interestingly, many of the pores are elongated along the fiber axis,

giving an average aspect ratio of ~ 2.3 . As the amount of high p_v DCM is only 40% by volume, expectedly, it cannot saturate the nearby region of the jet-air interface. This provides a chance for the water vapor in the ambient to be attracted to the positive charges distributed on the surface of fiber and condense as small droplets, thus undergoing stretching along with the fiber. This process is shown in Scheme 4.2.

4.2.2 Effect of humidity on pore formation

RH is another important factor that could influence the pore formation on the surface of fibers.³¹ Previously, it was noted that when PS with a molecular weight of 190,000 g/mol was electrospun with THF as solvent, average surface pore size increased from ~ 85 nm to 135 nm as the RH increased from 30% to 70%.³² The pore size further increased to 350 nm at 70% RH when a high molecular weight (560,900 g/mol) PS was used. Change in viscosity was thought to be a possible reason for this. In contrast, Lu and Xia¹⁷ showed that when PS (molecular weight of 300,000 g/mol) was electrospun with DMF, a hierarchical structure was formed (each fiber contained bundles of entangled fibrils) only in the RH range of 40-70%. But when the solvent was changed to THF (higher p_v of 21.6 kPa compared to DMF's 0.49 kPa), surface pores were formed at RH levels of 22%, 42%, and 62% and no pores were generated at a low RH of 2%. Nonetheless, in the present work, to understand the effect of RH, A1 and A2 systems were chosen because of their surface morphological differences and electrospinning was carried out at RH levels ranging from 25% to 75%. As expected, at relatively low RH levels ($\sim 25\%$), both A1 and A2 did not show any porous surface morphology (and therefore, SEM micrographs are not shown here). However, A1 showed pores at 35% RH and above; and the level of porosity increased with RH. An example of this behaviour is shown in Figures 4.5a (RH $\sim 35\%$), 4.5b (RH $\sim 45\%$) and 4.2a (RH $\sim 75\%$). Also, in A1, the fibers are fairly uniform in diameter at low RH due to lesser dissipation of charges during spinning. This is quite reasonable because DCM has a low dielectric constant and a high p_v , which can cause production of inconsistent fibers if charges are dissipated easily. A2 did not show pores at all levels of humidity pointing to the effect of miscibility/interaction of DMF with water. Examples of this behaviour are shown in Figures 4.5c and 4.5d.

Figure 4.5: SEM micrographs of A1 (a and b) and A2 (c and d) systems electrospun at different relative humidity levels (a, c – 35% and b, d – 45%).

4.2.3 Total pore area on the fibers

As discussed earlier, the objective of making porous fibers is to influence adsorption kinetics by enhancing surface area, and thereby providing additional binding or adsorption sites. To understand how much extra surface area was obtained by inducing porosity on the surface of fibers (and accompanied surface morphological changes like roughness), mercury intrusion porosimeter was utilized. It is well known that thermodynamically, gas/vapor condensation-evaporation and mercury intrusion-extrusion into and out of pores are similar processes. But the only difference is that they happen in opposite directions.

Figure 4.6: Pore size diameter versus intrusion volume and cumulative pore area for A2 and A3.

A2 and A3 systems were considered for porosity measurements because both these systems showed similar fiber diameters, $1.00 \pm 0.20 \mu\text{m}$ and $1.08 \pm 0.27 \mu\text{m}$, respectively. By having this commonality and minimizing the differences in density of deposition, differences in inter-fiber porosity could be controlled. This enables an easy comparison of the effect of pores present on the surface. In A2 and A3, the area corresponding to inter-fiber porosity is $8.1 \text{ m}^2/\text{g}$ and $8.2 \text{ m}^2/\text{g}$, respectively (based on Figure 4.6). This value is comparable to that of previous work by Yang et al.³³ in which they reported the area of inter-fiber porosity to be $\sim 8 \text{ m}^2/\text{g}$ for PLA fibers of similar size. By relating the average pore size as observed in SEM images with the porosimeter curve, it is clear that the peak in A3 (which is absent in A2) corresponds to the pores present on the surface. The calculated total pore area was $\sim 31.2 \text{ m}^2/\text{g}$, corresponding to an average pore size of $\sim 63.0 \text{ nm}$. The total area ($39.3 \text{ m}^2/\text{g}$) is also comparable to that of $37.1 \text{ m}^2/\text{g}$ for porous polyacrylonitrile fibers obtained by Nayani et al.³⁴ However, in that study, the area corresponds to core-shell fibers having a hollow core and porous sheath. The porosity was obtained by non-solvent induced phase separation mechanism by directly spinning

the fibers into a bath of non-solvent. In another recent study, Touny et al.³⁵ synthesized porous PLA fibers using reactive electrospinning where water was liberated as a by-product during the formation of monetite (which was formed in situ by a reaction between calcium hydroxide and orthophosphoric acid). As a result of this water-induced pore formation mechanism, as expected, pores are elongated and present throughout the fiber. But even here, the surface area of porous fibers was found to be 26.5 m²/g at a monetite content of 28 wt. %.

In summary, this study underlines the importance of understanding various intrinsic and extrinsic parameters on pore formation during electrospinning. The obtained pore size, shape and density are independent of the fiber diameter. This was also confirmed by changing the collector distance. However, further work is required to quantify the extent of (a) moisture condensation as a result of evaporative cooling when different solvents are used; and (b) hydrogen bonding and its relation to spreading of water droplets on the fiber surface.

Nonetheless, as different applications require different levels of porosity, carefully choosing the solvent system and controlling the ambient conditions could help meet the requirements in a single step. This simplistic approach in enhancing the adsorption behaviour will provide a comprehensive platform for a broad variety of applications including active food packaging, oil adsorption, moisture and odour management in fabrics.

4.3 Conclusions

PLA in single, binary and ternary solvent systems with different properties were studied to understand the mechanisms of pore formation. The approaches discussed provided a simple way to fabricate fibers with different levels of porosity (surface features).

- 1) The presence of spherical pores on fibers suggested that pores were formed by condensation of moisture after fiber deposition (due to evaporative cooling of fiber surface) similar to the concept of breath figures. This suggested the importance of solvent vapor pressure, relative humidity and saturation of fiber jet-air interface by a high vapor pressure solvent in inducing (spherical) pores.

- 2) In the presence of low vapor pressure solvents and at high RH levels, solvent miscibility/interaction with water played a dominant role in inducing pores. This was demonstrated by considering binary and ternary solvent systems in which one of the solvents was miscible with water and had different hydrogen bonding parameter.
- 3) RH played a determining role in pore formation mechanisms. But at relatively low humidity levels of less than ~30%, no pores were observed on the fibers in all systems.
- 4) The total pore area of fibers (A_3) was $\sim 39.3 \text{ m}^2/\text{g}$, as measured by mercury porosimeter.

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Chapter 5

Surface Characteristics, Sorption and Microbial growth on Electrospun PLA fibers

This chapter focuses on the effect of surface characteristics (morphology, hydrophobicity) of electrospun PLA fibers on the growth of microbes and moisture sorption. Surface morphology, surface wetting properties, moisture sorption of PLA fibers with various surface morphologies and with incorporation of nanoparticles such as sepiolite and zinc oxide are discussed. The growth of bacteria (against gram-positive and gram-negative bacteria) on these fibers with and without moisture scavenger (sepiolite) and antimicrobial agent (ZnO) is evaluated.

5.1 Introduction

The need for antibacterial surfaces in the food industry is increasing and the fact that food processing environment is prone to humidity and temperature changes and that bacterial growth and formation of biofilms may be a threat was identified and discussed by Zottala et al. ¹. Electrospinning is one successful technique among several other techniques (such as nanolithography, photolithography, e-beam lithography which may be expensive or simpler methods such as surface roughening, polymer demixing, self-assembly in which it is difficult to get desired features and shape) used to produce nano- or micro-size patterns/features on the surface of substrates/films used for biological purposes and food processing equipment/ packaging ². Creating such surface features is a promising alternative to the use of antibacterial chemical agents which can sometimes promote resistance to antibiotics and release cytotoxins ³⁻⁴. The features influence the surface wetting properties which affects the way microbes respond to these structures but there is limited insight on the response mechanisms and prospect of such featured surfaces to behave as microbe repellent surfaces ².

When microbes come in contact with any surface, they first try to attach themselves and form a biofilm (a biologically active colony of mixed or mono species of microbes in contact with the surface). The process of biofilm formation happens in 4 stages – at first, gravitational and hydrodynamic forces, Brownian and flagella induced motion causes bacteria to encounter a surface at a favorable temperature and pH followed by the second stage, adhesion. Adhesion can be reversible or irreversible and this depends on the physico-chemical interactions between bacteria and the surface which is dependent on the material property of the surface. The surface features and its chemical nature are found to influence this stage ⁵. In the third stage, bacteria proliferate and synthesize a biofilm ⁶. The fourth stage involves maturation of biofilm and specific metabolic activity and functioning ⁷. The bacteria interact among themselves through organelles and pass biochemical signals leading to a possible lysis of the cell matrix which can cause detachment of the biofilm or release of free bacteria into the immediate surrounding. The properties of the material can influence cohesion of mature biofilms ⁸.

E. coli was found to significantly adhere to the patterned PDMS surfaces having 2-100 μm features at a spacing of 5-20 μm . This is very large as compared to the dimension of bacteria. This shows that micro-topographies promote bacterial adhesion⁹. A study by Whitehead et al. also reiterated that size of surface features plays a role in retention of bacterial colonies (bacteria of suitable size). Surface pits of varying diameters (0.2, 0.5, 1 and 2 μm) were produced on stainless steel surfaces and studied for bacterial retention. They showed that there was more retention in bigger pits¹⁰. Hsu et al. found that bacterial colonies proliferated on the surface between pits but not inside pits¹¹.

In recent studies, it has been hypothesized that if surface features are smaller than the size of bacteria, then there is limited accessibility to attachment meaning reduced bacterial growth and prevention of biofilm formation¹²⁻¹³. PDMS surfaces with submicron features (2.7 μm height, 3 μm diameter and 440 nm trenches) were used to study the adhesion of *Escherichia coli*. It was observed that the adhesion of bacteria was reduced for 2 hours initially but found to be drastically increasing after 24 hours. This was attributed to the increase in surface wetting of the surface (receding contact angles were found to decrease from $\sim 90^\circ$ to $\sim 10^\circ$) which favours bacterial flagellar movements (flagella can access even small crevices)¹³. Xu and Siedleckis observed that pillared nanofeatures of 400 and 500 nm on Polyurethane urea films significantly decreased the adhesion of *Staphylococcus aureus* (~ 600 nm)¹². Wang et al observed that contact angle and surface wetting has lesser impact on bacterial adhesion than dimension of surface features which is contradictory to the previous reported literature¹³⁻¹⁴. They studied using PET substrates and surface features with dimensions comparable to bacterial cell dimensions. It was found that sharp edges promoted bacterial adhesion than curved edges and attributed it to the tendency of bacteria to find spots that enable them to have higher surface contact¹⁴. These studies reveal that surface topographies have an effect on bacterial adhesion to the surface but still there are questions on the effect of size, shape and hydrophilicity/hydrophobicity of these structured surfaces.

As already discussed in chapter 2, there are several works that exhibit the potential of electrospinning process in the food industry although they have not been of commercial importance. These include encapsulation of bioactive compounds and enzymes. The main objectives of these studies are (i) for making functional foods (ii) protecting the

food from oxidation, moisture and light (iii) slow and sustained release of active ingredients such as anti-oxidants and anti-microbial agents (iv) masking unpleasant taste and odor. The above functionalities are discussed in view of the effectiveness of the active ingredients and the rate of their release based on the size of fibers in which they are encapsulated.¹⁵⁻¹⁶ In case of situations where the active ingredients have not started to release or are completely released (due to sudden environmental changes as the high surface area fibers are very sensitive even to small changes) or they are not able to be released due to complete encapsulation, the effect of these fibers on providing the same integrity on exposure to humidity and microbes that may be present in the food environment, has not been well studied. In such a scenario, the morphology and surface characteristics of fibers play a role and therefore it is important to gain understanding on the effect of these parameters on exposure to the food processing/shipping environment (humidity and microbes mainly).

Here we focus on the adhesion, of two most commonly found bacteria in food industry, gram-positive (*Listeria monocytogenes*) and gram-negative bacteria (*Escherichia coli*) on PLA based electrospun fibers with different surface morphologies and characteristics and estimate the growth of colonies. If surfaces of interest can repel bacterial adhesion, the process of biofilm may be nipped off at an early stage. This together with the evaluation of moisture sorption by electrospun fibers and a measure of the hydrophilicity/hydrophobicity will give an insight on the use of these fibrous films as a functional layer in food packaging without using any chemical agents.

5.2 Results and Discussion

5.2.1 Surface characteristics of Electrospun PLA fibers

Fibers with different surface morphologies are obtained by electrospinning PLA dissolved in different combination of solvents as listed in table 5.1. PLA with smooth morphology (A2) was obtained using a solvent combination of 70/30 DCM/DMF (volume %) whereas A3 (40/40/20 DCM/DCE/DMF) showed surface pores. The factors responsible for and the mechanisms of obtaining smooth and porous morphology were clearly elucidated in chapter 4. The viscosities were similar and therefore similar size fibers, $1.00 \pm 0.20 \mu\text{m}$ for A2 and $1.08 \pm 0.27 \mu\text{m}$ for A3, were obtained. The pore area

of fibers increased with the presence of surface pits observed on A3 fibers. This led to an increased surface area of $39.3 \text{ m}^2/\text{g}$ ($\sim 8.2 \text{ m}^2/\text{g}$ accounts to inter fiber porosity) for A3 as compared to $8.1 \text{ m}^2/\text{g}$ for A2 as measured by mercury intrusion porosimetry. These values are comparable to previous work reported by Yang et al¹⁷ and Nayani et al¹⁸. The average size of pores on A3 fibers was $\sim 63 \text{ nm}$.

Surface wetting depends on the surface roughness of the fibers. According to Wenzel model, increase in surface roughness increases water contact angle. This does not consider the surface porosity.¹⁹ Cassie model suggests that grooves created by rough surfaces trap air and this increases the contact angle.²⁰ As compared to the cast films of PLA, A2 and A3 fibers showed a higher water contact angle making it more hydrophobic (table 5.2). The interfiber porosity causes air to be trapped, thus increasing the contact angles. This observation is similar to that reported in other works.²¹⁻²³

Table 5.1: Electrospinning of PLA (8 wt. %) with different solvent systems (in volume %)

Solvent combination	Designation
PLA + (70%DCM + 30%DMF)	A2
PLA + (40%DCM + 40%DCE + 20%DMF)	A3
PLA + (40%DCM + 40%DCE + 20%DMF) + 5 wt% sepiolite	A3S
PLA + (40%DCM + 40%DCE + 20%DMF) + 5 wt% ZnO	A3ZnO

Figure 5.1: SEM micrographs of PLA fibers spun at 75% RH with different solvent combinations: (a) 70/30 DCM/DMF; (b) 40/40/20 DCM/DCE/DMF

Table 5.2: Water Contact angle of PLA cast films and electrospun fibers

Contact angle	PLA C	A2	A3
Average (°)	73	118.2	124.4
Standard deviation	2.1	3.3	3.2

In general PLA is hygroscopic as the -COO groups in PLA can react with water and undergo hydrolysis leading to reduced molecular weight. But this is possible only on long term exposure of PLA to water. Nevertheless, the electrospun fibers were found to be hydrophobic. The increase in water contact angles of electrospun fibers can also be attributed to the fact that electrospinning process conditions influence polymer chain rearrangement. Solvent-polymer interaction, interaction with electrical forces and polarity of polymer may cause certain groups in the polymer chain to move to the surface. This was shown by Cui et al. in a study where they characterised the surface groups on cast films and electrospun fibers of Poly (D,L-lactide) using x-ray photoelectron spectroscopy. They observed that methyl groups (low binding energy groups) were found to be increased on the surface of electrospun fibers thus showing a contact angle of $132.2 \pm 1.5^\circ$ as opposed to the contact angle of $76.4 \pm 1.3^\circ$ for cast films. ²⁴

Fibers with sepiolite and ZnO particles were prepared as per the systems mentioned in table 5.1. Ternary solvent system was used to dissolve PLA and nanoparticles (5wt% wrt PLA). The fibers obtained had lesser diameters as compared to the neat PLA fibers (A3). There were no pores on the surface although some roughness was observed (figure 5.2). This reduction in fiber size can be attributed to the reduction in viscosity of the composite solution. A similar behaviour was observed by Beatrice et al., in polyamide 6 fibers containing montmorillonite clay. ²⁵ Reducing the fibers diameters leads to more contact points thus increasing the surface roughness thereby increasing the contact angles. The contact angles of A3 ZnO and A3 S (table 5.3) were higher than that of the neat polymer fibers (A3).

TEM images show the presence of particles in the fibers (Figure 5.3 a and b). It is seen that the sepiolite particles slightly aggregate but individual particles of ZnO can be seen

although they aggregate together. This could be the insufficient dispersion of the particles in the solvent system used.

Figure 5.2: SEM micrograph of PLA fibers spun at 75% RH with ternary solvent system and nanoparticles (a) A3 ZnO; (b) A3 S

Table 5.3: Water Contact angle of nanocomposite fibers.

Contact angle	A3ZnO	A3S
Average (°)	133.7	136
Standard deviation	5.0	3.0

Figure 5.3: TEM image of A3 ZnO (a) and A3 S (b)

PLA fibers were electrospun using ternary solvent system and sepiolite particles were sprayed on the surface of the fibers using a spray coater. Sepiolite particles were dissolved in deionised water in the weight ratio of 1:100 and stirred for 3 hours at a speed of 1000 rpm using a mechanical stirrer. This enables the sepiolite particles to be uniformly dispersed in water and therefore allows uniform spraying. Figure 5.4 shows the individual particles of sepiolite sprayed on electrospun PLA fibers. In this case, the water contact angle is $\sim 34^\circ \pm 2.9$. This is because of the high water absorption capacity of sepiolite particles. This system was used to generate a hydrophilic surface as compared to other systems discussed above which are hydrophobic.

Figure 5.4: SEM micrograph of Sepiolite particles sprayed on electrospun PLA fibers

5.2.2 Moisture sorption isotherm

Moisture sorption isotherms are determined, for PLA cast film and electrospun fibers without surface pores (A2), with surface pores (A3) and with sepiolite particles (A3 S), using a gravimetric vapour sorption analyser that measures the increase in mass of the sample (equilibrium moisture content) at each level of water activity (relative humidity is the percentage of water activity). The isotherms were fitted using the GAB model and the constants were obtained using non-linear regression analysis. % RMS (root mean square) was calculated using equations mentioned in chapter 3, section 3.2.2. %RMS gives an indication of how well the model fits with the experimental data obtained and values less than 5% is considered a good fit.²⁶ Here we see that (figure 5.5 and table 5.4) the best fit was observed for A2 fibers whereas cast film showed a poor fit. This could be due to the very low values of moisture sorption by PLA cast film (experimental value

of ~ 0.6). But such low values of moisture sorbed by cast film is comparable to values reported in literature.²⁷⁻²⁹ Carcinoss and others showed that moisture sorption on cast PLA films ranged from 0.6-1.1 for amorphous and crystalline PLA films but the difference was not significant. They also showed that as the PLA degrades, the moisture sorption levels were found to decrease and with more hydrophilic end groups, the sorption levels were higher. They could not conclude why crystallinity didn't affect the sorption levels to a great extent. Dunghan et al., pointed out that molecular weight of PLA plays an important role in affecting the sorption by PLA films because at lower molecular weights, there are more hydrophilic end groups and therefore higher sorption.

Here the focus is on whether the surface features of electrospun fibers (inter-fiber porosity and surface pits) can aid moisture sorption. A3 fibers showed the maximum equilibrium moisture content among the 4 samples. It would be expected that A3 with surface pores and higher total pore area will show increased monolayer moisture sorption but this was not observed. A2 showed the highest monolayer moisture sorption but the overall sorption was less than that of A3. This increase in overall sorption observed in A3 can be attributed to the surface pores and higher total pore area. However it should be noted that the contact angles of A3 is higher than that of A2, which gives a probable explanation for the low values of monolayer moisture content in A3. This explains the hypothesis that hydrophobicity may repel the monolayer moisture or allow it to move into the inter-fiber pores through capillary action. A3 S also showed considerable sorption (higher than A2) that can be attributed to the slightly rough surface and decreased fibers diameters. These features should increase sorption but again it is to be noted that the water contact angle of A3 S is $\sim 136^\circ$ which is higher than that of $\sim 118^\circ$ for A2.

The reason for adding sepiolite to the fibers is that it would assist sorption, because sepiolite has the highest number of silanol groups among the different clays. However as seen from the SEM and TEM micrographs, the sepiolite particles are in aggregates and mostly embedded in the polymer fibers. As a rule of thumb, individual particles of sepiolite can absorb more moisture than aggregates. Therefore A3 S also did not show increased moisture sorption. The other GAB constants k and c are dependent on temperature, c increases with increase in temperature and k decreases with increase in temperature. k represents the interaction between the multilayer water molecules with

reference to the bulk liquid. The values of k are less than 1 and c between 1-20.³⁰ The values of k for all the 4 samples fall within the range except for c values. However the values of c are found to be higher as reported in another work but the reason is not discussed.³¹

Figure 5.5: Moisture sorption isotherms of PLA C, A2, A3, A3S at 298 K

Table 5.4: GAB constants calculated by non-linear regression analysis

GAB constants	K	C	M_0 (g/100 g)	% RMS
PLA C	1.08	11.12	0.04	12.42
A2	0.46	13.49	2.27	2.23
A3	0.93	40.28	1.33	6.42
A3 S	0.75	470.66	1.42	2.25

From the moisture sorption experiments and the contact angle data we find that hydrophobicity of fibers does not allow for increased sorption in PLA electrospun fibers despite the different surface features. These fibers can be readily used in food packaging industry without any limitation of increased moisture sorption that may be expected to be caused by the surface features.

5.2.3 Microbial adhesion and growth

From the water contact angle measurements it can be seen that these fibers are hydrophobic. As discussed in section 5.1, it is still unclear whether hydrophobic surfaces repel bacterial attachment and this also depends on the size of surface features and chemical nature of the polymer. It can be seen that microbial retention is higher in pits that are of the same size as the bacterial cells.¹⁰ The electrospun fibers used in this study show inter-fiber porosity in the range of $\sim 1 \mu\text{m}$ (as seen from the mercury intrusion porosimetry data shown in Chapter 4, figure 4.6). This is similar to the size of bacteria used for this study - Gram-negative bacteria *E. coli* and gram positive bacteria *L. monocytogenes*.

In the study to check bacterial attachment to surfaces, the films were immersed in bacterial suspensions and incubated at 37°C for 9 hours. It is shaken at a speed of 150 rpm and this causes some shearing forces. Three samples were taken for this study with different levels of hydrophobicity - A2, A3 ZnO and A3 sprayed with sepiolite particles. From figure 5.6 it can be seen that bacterial colonies are found only on the sepiolite particles sprayed over the electrospun fibers (5.6f). This sample is very hydrophilic and therefore tends to retain bacteria. Due to shearing forces caused by incubator shaking, the bacteria are repelled by the hydrophobic surfaces. This is similar to the results reported by Xu and others.³² In this study, they used smooth, micron and submicron size patterned polyurethane urea having hydrophilic and hydrophobic surfaces. Under shear they observed a reduction rate (66-91%) of *Staphylococcus epidermidis* on patterned surfaces as compared to smooth hydrophobic surfaces. In case of hydrophilic surfaces, only an increase in shear rate could cause decrease adhesion. Under static conditions they observed a reduction on submicron size features but bacterial adhesion to micron sized features still exists in both hydrophilic and hydrophobic surfaces. Similar results were obtained by Wang et al., on PET surfaces¹⁴. Therefore in our current

study, although there are features which are similar to the size of bacteria, there was no adhesion because of the hydrophobicity and decreased interaction with moisture as seen from moisture sorption isotherms.

Figure 5.6: SEM micrographs showing microbial attachment before and after incubation in *E. coli* at 37 C for 6 hours – A2, A3 ZnO and Sepiolite sprayed A3 before incubation (a,c,e) and after incubation (b,d,f with zoom in to show microbial colonies)

Another test was performed on these fibers to evaluate the effect of these fibers on the growth of microbial colonies. It can be seen from figure 5.7 that the fibers cannot be

bactericidal. The growth of colonies were similar to that of the control sample. Whereas, ZnO showed reduction in the colony count of E coli and L monocytogenes. It is well known that ZnO can act as antimicrobial agent. The proposed mechanisms are that the nano size ZnO particles cause abrasive damage to the cell membrane and therefore causing intracellular contents to leak thus causing cell death. Also ZnO in aqueous suspensions can produce reactive oxygen species which can cause damage to bacterial cells³³⁻³⁴.

Figure 5.7: Microbial growth on electrospun fibers - gram positive and gram-negative bacteria.

5.3 Conclusion

Effect of surface characteristics of electrospun PLA fibers on moisture sorption and microbial growth are studied. It can be seen that (i) electrospinning process increases the hydrophobicity of PLA and this reduces the moisture sorption despite the porous morphology, (ii) There is no adhesion of bacterial cells on hydrophobic surfaces. The fibers remain bacteriostatic. The hypothesis that bacterial cells adhere to surface features of the same size as them does not apply here because the inter-fiber porosity and the size of E coli are similar. One would expect an increased adhesion and increased chance of biofilm formation but this was not observed. Therefore, where porous fibers are needed in food industry to increase the sensitivity as sensors or for sustained release of active agents, the concern of bacterial cell adhesion in these pores and their proliferation can be eliminated. These can find potential use as functional layers/inserts in packages along with conventional food packaging materials like LDPE but cannot be used a packaging

material by itself. Therefore the next chapter focuses on the development of LDPE/TPS blends to replace LDPE the most widely used food packaging material.

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Chapter 6

Effect of Viscosity of LDPE on Morphology and Barrier of LDPE-TPS Blends

This chapter focuses on evaluating the effect of viscosity of LDPE on the properties of LDPE/TPS blends. LDPE of 2 different viscosities (MFI – 2g/10 minutes – LDPE 210 and MFI - 4 g/10 minutes – LDPE 410) are used to prepare LDPE/TPS blends in the weight ratio of 50/50. Blow molding process is employed to make the films and the properties are studied. It can be seen from the morphology that LDPE with different viscosities produced blends with different surface morphologies. The thermal, mechanical and barrier properties are studied for the prepared blends.

6.1 Introduction

Highly increasing demand for more environmentally friendly materials to replace the synthetic polymers and reduce serious environmental concerns such as landfills and threat to human and marine life calls for the need for research and development of bio-based materials. Approximately 40-50% of plastics produced is used in packaging industry and LDPE is a very widely used material due to its excellent mechanical properties, transparency, resistance to chemicals, low permeability to moisture and easy process ability by cast/blown film processing methods. It has also been widely used in agricultural applications and in construction industry. But these are difficult to be recycled and it takes years or decades before it can decompose.

Starch is one of the most abundant polysaccharides having linear amylose and branched amylopectin units which strongly interact via hydrogen bonding preventing the flexibility of molecules to move. Therefore, in order to prevent retrogradation and facilitate easy movement of amylose and amylopectin, starch is either chemically or physically modified to enable it to be blended with synthetic polymers. Therefore, blending TPS with LDPE was attempted and it has been shown that TPS will aid the degradation of LDPE and reduce consumption of synthetic polymer ¹. But these blends are highly immiscible and therefore show poor mechanical and barrier properties because of poor interfacial adhesion. Therefore, compatibilizers such as ethylene acrylic acid (EAA), ethylene vinyl alcohol (EVA) and Polyethylene grafter maleic anhydride (PE-g-MA) have been used to improve the miscibility between non-polar hydrophobic LDPE and polar hydrophilic TPS ²⁻³. This increase in miscibility will lead to an enhancement of mechanical and barrier properties of the blends.

Chemical modification of starch involves addition of compounds such as nitrates, sulphates, phosphates, alkenyl succinates and fatty acids ⁴⁻⁵. This process replaces some of the hydroxyl groups by ester groups thus making starch hydrophobic and this improves its thermal stability and mechanical properties. This chemical modification method, although have several advantages, limits the quantity of starch that can be added to petrochemical based polymers. Another route to make starch blend with polymers is via plasticization using glycerol, sorbitol, water or citric acid as plasticizers ⁶⁻¹⁰. This produces thermoplastic starch which can be easily melt extruded along with synthetic

polymers. Therefore, preparation of thermoplastic starch is preferred in this study using glycerol as the plasticizer.

Generation of morphology in polymer blends (co-continuous/dispersed/stratified) depends on concentration of blend components, level of interaction between components and the viscosity and elasticity ratios of the blends¹¹. Viscosity ratio controls the degree of dispersion whereas the elasticity ratio controls the shape of the two phases. There are several works to study the morphology of obtained blends by adding different quantities of TPS, using different plasticizers under different processing conditions¹²⁻¹⁴. Most recently, Mortazavi and others showed that there is formation of elastic networks in compatibilized blends and that TPS and compatibilizer drastically increase the elasticity and viscosity of blends¹³. Sabetzadeh et al. also showed that TPS effectively increased the elasticity and viscosity of blends¹⁵. This highly depends on the plasticization of starch. The above-mentioned studies have evaluated the effect of plasticizer, content of TPS, use of starch from different origin and effect of processing conditions, but the effect of viscosity of LDPE on the generation of morphology, mechanical and barrier properties of LDPE-TPS blends has not been looked at. In this study two LDPE grades that differ in their melt flow properties are used to study its effects on blend properties.

Table 6.1: Designation and composition of various films prepared.

Designation	Ingredients
210	LDPE 210
210 C	LDPE 210 + C15 3 wt%
210 TPS	LDPE 210 / TPS 1- 50/50 wt%
210 TPS C	LDPE 210 / TPS 1- 50/50 wt% + C15 3wt%
410	LDPE 410
410 C	LDPE 410 + C15 3 wt%
410 TPS	LDPE 410 / TPS 2 - 50/50 wt%
410 TPS C	LDPE 410 / TPS 2 - 50/50 wt% + C15 3wt%

In the present study two different TPS are prepared, TPS-1 and TPS-2. 20-40 % glycerol was used in several studies reported earlier ⁶. Based on this, several trials were made using 25- 27 % Glycerol, 1-2 % GMS and 0.5-1% Urea with starch. Depending on the ease of process ability, 27 % was used to prepare TPS-1 along with 2 % GMS and 0.75% Urea. Glycerol monostearate is a processing aid to help in melt extrusion of TPS and LDPE-TPS blends. Urea has amide groups that can prevent retrogradation of starch but amide containing substances can be toxic, therefore used in very small amounts. This TPS-1 was blended with LDPE 210 in the weight ratio of 50/50 and the final melt extrudates were observed to be slightly sticky and the morphology generated (further discussed in section 6.2) did not show distinct dispersed phases of TPS. Therefore, percentage of glycerol and GMS were reduced to 26% and 1% respectively and urea was slightly increased by 0.25%. This is designated as TPS-2 and is melt extruded with LDPE 410 that has better flow properties (higher MFI) than LDPE 210. Distinct phase morphology was obtained and the interaction between TPS and LDPE could be observed. PE-g-MA content in all blended samples was 5 wt%.

6.2 Results and Discussion

6.2.1 Morphology of LDPE-TPS blends

Two different grades of polyethylene were used to prepare blends by melt extrusion in the weight ratio of 50/50. The pellets were blown into films using blown film extrusion process. SEM micrographs were obtained by etching the blown films in deionised water to remove the starch. From figure 6.1, it can be seen that 410TPS and 410 TPS C show distinct phases of starch where as there is no formation of phases observed in 210 TPS and 210 TPS C. This can be attributed to the viscosity of LDPE, processing conditions and the plasticization of starch. Lower melt flow rate of LDPE 210 decreases the ease of processing and morphology generation because this depends on the viscosity and elasticity of the blend components. Increasing the melt flow rate of LDPE by two-fold and decreasing the plasticizer and processing aid in 410TPS blends shows a distinct phase and a dispersed morphology with spherical to oval shaped droplets of TPS in LDPE matrix. This kind of morphology is hypothesized to decrease the transport of small molecules (gas/molecules) as the phases of starch are not connected to each other.

If the TPS droplets are linked, there is a possibility for small molecules to diffuse through the thickness of the film via capillary action.

Figure 6.1: SEM micrographs of LDPE-TPS blends in the weight ratio of 50/50 without clay (a-210 TPS, b-410 TPS) and with clay (c-210 TPS C, d-410 TPS C).

6.2.2 Chemical composition and Thermal properties

FTIR spectrum of all the obtained films are shown in Figure 6.2. The broad peak at $\sim 3450\text{ cm}^{-1}$ indicates the O-H stretching indicative of glycerol and starch. The peaks between $2800\text{--}2900\text{ cm}^{-1}$ represent -CH_2 stretching of alkanes which is characteristic of LDPE. Medium intensity peaks are observed at $\sim 1450\text{ cm}^{-1}$ and they represent the -CH_2 deformations in LDPE. The peak at $\sim 1050\text{ cm}^{-1}$ indicate the O-H bending of TPS. These spectra are comparable to that shown by Mina et al.¹⁶ The small peaks around $1050\text{--}1150\text{ cm}^{-1}$ also represent the stretching of Si-O-Si in Cloisite 15. This peak is combined with the O-H bending peaks of starch in the blends but can be clearly seen in 210 C and 410 C samples. A small peak observed at $\sim 1690\text{ cm}^{-1}$ indicates the reaction between anhydride group in maleic anhydride and -OH group in starch to form ester. This shows

the effect of compatibilizer on forming chemical bonds between LDPE and TPS. A similar phenomenon was reported by Oromiehie et al.¹⁷

Figure 6.2: FTIR spectrum for LDPE 210 based films (a) and LDPE 410 based films(b).

Figure 6.3: TGA curves of LDPE 210 based films (a) and LDPE 410 based films (b).

Figure 6.4: DSC curves of LDPE 210 based films (a) and LDPE 410 based films (b).

Thermogravimetric analysis in N_2 atmosphere shows the decomposition profile of LDPE-TPS blends (figure 6.3). It is observed that TPS starts to decompose around $\sim 320^\circ\text{C}$. Glycerol is lost at about 290°C . This shows its stability at processing conditions. The onset degradation temperature of 410 C and 210 C are higher than that of the respective grades of neat LDPE— 465°C for 410 C as compared to 413°C for 410 and 441°C for 210 C as compared to 418°C for 210. This is enhanced further by the addition of PE-g-MA. A similar inference was made by Xie et al.¹⁸ and Hwang et al.¹⁹. 210TPS and 210 TPS C have similar onset degradation temperatures of 468°C . However, 410 TPS C has higher onset degradation of 441°C as compared to 410 TPS which starts to degrade at 431°C . Differential scanning calorimetry is used to measure the melting and crystallization temperature of the blends. It was observed that all samples showed crystallization temperature of 96.5°C and melting temperature of $108\text{--}109^\circ\text{C}$ and there is no significant difference.

6.2.3 Mechanical properties of blends

The composites of LDPE – 410 C and 210 C - with 3 wt. % clay shows a reduction in tensile strength and elongation at break in comparison with neat LDPE. Reduction in mechanical strength of the blends is directly dependent on the quantity of TPS component. The ultimate tensile strength of the blends in the LDPE/TPS weight ratio of 50/50 is reduced by ~50% in both 210 and 410 grades of LDPE. Adding brittle TPS to a tough LDPE matrix causes embrittlement of the polymer blend. However, the elongation at break values were significantly higher compared to those reported in literature. A recent study by Nguyen et al showed a 50% reduction in elongation at break of 250 μ m thick films of LLDPE-TPS blends in the weight ratio of 50%. However the yield stress of the blend reduced to a lesser extent ²⁰.

Figure 6.5: Mechanical properties of LDPE 210 based films and LDPE 410 based films - Ultimate tensile strength (UTS in MPa) and Elongation at break (in mm).

A work by Oromiehie et al., showed that elongation at break for LDPE/TPS blends in weight ratio of 50/50 was only 5.42% and tensile strength was 8.5 MPa. Glycerol at 30wt% of starch and LDPE with 2g/10 minutes melt flow rate was used ¹⁷. In the current

study, 410 TPS and 210 TPS showed an elongation of 90 and 100 % and a tensile strength of 7.53 MPa and 6.99 MPa respectively. This improvement in elongation can be attributed to the improved miscibility of the blends, good plasticization of TPS and the morphology generated. GMS and Urea helps in plasticization of starch to obtain TPS and prevents retrogradation. Also, PE-g-MA aids in compatibilizing the TPS and LDPE domains. Therefore, this is a determining improvement. Glycerol changes starch from discrete particles to TPS, a plasticized material that can be blended with LDPE. The viscosity of LDPE and the presence of glycerol determine the dispersion of TPS in the LDPE matrix and therefore the morphology of the blend. This affects the mechanical properties. Several studies show that the morphology of the blends obtained and the degree of dispersion (depends on the viscosity) influence the mechanical properties of the blend²⁰⁻²¹. In a study reported, 29% and 30% glycerol were used to plasticize starch and mixed with LDPE in the ratio of 49 wt%. This shows an elongation at break of ~230%.⁹ However in this study, within the 2 grades 410 and 210, there was not much difference in the elongation at break values as the elongation is largely governed by the amount of TPS in the blend. Addition of clay decreased the tensile strength and elongation at break of the blends which is characteristic of nanocomposites.

6.2.4 Barrier properties

TPS is very hydrophilic and tends to absorb lot of moisture. Water vapour transmission rate (WVTR) was measured using Payne cups at 38 °C and 90% humidity by gravimetric method as explained in chapter 3. WVTR increased from 0.79±0.01 for 210 to 96.14±7.70 g.mm/m² day atm. for 210 TPS and from 5.58±1.59 for 410 to 90.22±12.9 g.mm/m² day atm. for 410 TPS. From the results obtained, the morphology alone does not seem to play a role in increase of WVTR because WVTR also depends on the polarity of the TPS domains and its hydrophilic nature. If the TPS domains are linked, then water molecules can easily pass through the hydrophilic portions by capillary action and diffuse through the matrix. However, in case of 410 TPS the starch was dispersed in a discrete way and not linked as seen in SEM micrographs. On addition of clay the WVTR has decreased by ~34% for 210 TPS C and ~14.5% for 410 TPS C as compared to 210 TPS and 410 TPS. Clay layers impedes the flow of water molecules creating a longer path for the water molecule to diffuse through the thickness of the film and this can be explained from the well-known tortuosity model. These values are close to that

of the WVTR of PLA films which is 72 g.mm/m² day atm. measured under the same conditions. But the elongation at break values of PLA (~200%) is much higher than the LDPE-TPS blends.²⁰

Figure 6.6: Water vapour transmission rates of LDPE 210 based films (a) and LDPE 410 based films (b).

6.3 Conclusion

LDPE-TPS blends with different melt flow rate LDPE was prepared in the weight ratio of 50-50 and blown into films. The discrete droplet morphology was observed in films that had high melt flow rate LDPE (LDPE 410). 210-TPS did not show such a clear phase morphology. It is observed that the viscosity of LDPE played a role in generating different morphology but the difference in morphology did not have a significant difference in the mechanical properties of the blended films. However, the water vapour transmission rate showed some difference. Addition of clay further improved the barrier to water vapour.

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Chapter 7

Conclusions and Future Recommendations

This chapter concludes the work done in this thesis on electrospun PLA fibers and development of LDPE/TPS blends and provides a summary of new findings based on the obtained results. The underlying mechanisms in obtaining different kinds of films was studied and the effect of morphology on moisture sorption and microbial growth were evaluated. LDPE/TPS blends with different viscosity grades of LDPE was prepared and tested for thermal, mechanical and barrier properties. Future recommendations include continuing work which can be improved that can lead the materials studied in this thesis to commercial utilization in food packaging industry.

7.1 Conclusions

This work involves some fundamental studies in evaluating the potential to use electrospun PLA fibers as functional films in food packaging applications and the development of LDPE/TPS blends that can be used to replace LDPE. The idea behind these studies is to gain further understanding in the use of bio-based materials in food packaging to reduce environmental concerns. Use of electrospinning process reduces polymer consumption due to their low density and use of TPS reduces consumption of synthetic polyethylene, a polymer that is used abundantly in the food packaging industry.

This thesis explored the underlying mechanisms involved in electrospinning process to obtain fibers with different surface morphologies. It signifies the importance of gaining knowledge on the various parameters (both intrinsic and extrinsic) that influence pore formation during electrospinning. The pore size, shape and density were independent of fiber diameters which were confirmed by changing the distance between collector and nozzle.

The mechanisms reported in previous works considered only the solvent vapour pressure and proposed mechanisms such as VIPS, TIPS and evaporation induced phase separation. However, there are many other properties of the solvent such as solvent miscibility with water, solubility parameter and dielectric constant that were overlooked. The approach we used here to understand the mechanisms was changing the solvent systems used to dissolve PLA. Single, binary and ternary solvent systems were used. Another approach was by changing the relative humidity of the ambient environment.

The key findings include (i) spherical pores suggested that moisture condenses after fiber deposition due to cooling caused by evaporation of solvent and this is similar to the concept of breath figures. This showed the importance of relative humidity, level of saturation of high vapour pressure solvent in the jet-air interface and role of solvent vapour pressure in inducing pores. (ii) At higher relative humidity and in the presence of low volatile solvents, miscibility and interaction of solvent with water in the ambient played an important part in inducing pores. This was demonstrated by the use of binary and ternary solvents in which one solvent was miscible with water but had different

levels of hydrogen bonding parameters. (iii) At low humidity below ~30% no pores were formed in any of the systems employed. Therefore, humidity does play an influential role.

This thesis also explored the effect of these surface characteristics on the moisture sorption and growth of microbes (moisture and microbes are highly prevalent in the food processing/packaging environment). This was demonstrated using fibers with and without surface pores and with nanoparticles like Sepiolite (that can absorb moisture) and ZnO (that can behave as anti-microbial agent) and compared to their cast film counterparts. The total pore area of fibers with surface pores (A3) was ~39.3 m²/g, and that without pores was ~8.1 m²/g as measured by mercury porosimeter. The fiber diameters of neat PLA fibers were about 0.75-1.25 μm whereas with nanoparticles, the fiber diameters reduced to 400-500 nm. The electrospinning process led to formation of hydrophobic surfaces due to their surface roughness. The fibers showed very little moisture sorption despite the increase in total pore area and this can be attributed to the hydrophobicity of the fibers. The presence of Sepiolite also did not increase the sorption as the particles were embedded within the polymer matrix. The fibers show about 3-5 times increase in moisture sorption as compared to cast films.

Also, to consider that the size of inter-fiber pores ranged around 1 μm that is similar to the size of bacteria considered for this study. It is reported in literature that microbes can adhere to surface pits that have dimensions similar to their size so that the bacteria can have maximum contact surface. However, the microbial test results show that hydrophobic fiber surfaces did not show any adhesion of bacterial colonies. To further confirm the argument, Sepiolite particles were sprayed on hydrophobic fibers and the contact angles were greatly reduced because Sepiolite is highly hydrophilic. There was occurrence of bacterial colonies on the surface of hydrophilic sepiolite particles. All the fibers except those with ZnO nanoparticles were bacteriostatic and did not promote growth. From the results obtained, we can see that these fibers are not efficient enough to be used as moisture scavengers but certainly they are not sufficient enough to promote microbial activity.

This is a simple and cost-effective approach to obtain fibers of different levels of porosity that may be needed for a variety of applications such as food packaging, odour management in fabrics, moisture management in fabrics and oil adsorption.

Another important hurdle in food packaging industry is the use of green materials which can reduce environmental concern such as land-fills. The last part of the thesis addressed the preparation and properties of blending LDPE and TPS. Several studies reported the effect of plasticizers and compatibilizers on the effect of the thermal and mechanical properties of the blends. This thesis explores the use of LDPE with different melt flow rates and its effect on the morphology generated and evaluated the thermal, mechanical and barrier properties. LDPE/TPS blends with different melt flow rate LDPE was prepared in the weight ratio of 50-50 and blown into films. The discrete droplet morphology was observed in films that had high melt flow rate LDPE (LDPE 410). 210-TPS did not show such a clear phase morphology. It is observed that the viscosity of LDPE played a role in generating different morphology but the difference in morphology did not have a significant difference in the mechanical properties of the blended films. However, the water vapour transmission rate showed slight difference. Addition of clay improved the barrier to water vapour in the blends.

7.2 Future recommendations

Fundamental studies on the mechanisms of obtaining different surface morphology electrospun fibers and the effect of such a morphology on moisture sorption and microbial activity was performed. However, there are still some continuing questions that can lead to a deeper insight on the potential use of these fibers to gain commercial importance in the active food packaging industry.

- i. Further work is needed to quantify the extent of condensation of moisture during the evaporative cooling of solvents during electrospinning process. This can help in understanding the interaction of solvent with the condensed moisture by hydrogen bonding. This can be related to the spreading of droplets on surface of fibers.
- ii. Further study on the hydrodynamic forces and shearing forces on the adhesion of bacterial cells to electrospun fibers and the integrity of fibers under such forces.

- iii. As a next step, active ingredients can be incorporated in the fibers and tested for performance in physical food packages under conditioned environments to check for shelf-life extension and retention of quality of food packed.

The study on LDPE/TPS blends can be further explored to understand the thermodynamics and kinetics of moisture sorption under the conditions of practical end-use. This will help address the issue of increased moisture uptake by starch and help in suggesting suitable approaches to reduce the moisture uptake and improve barrier to small molecules. There are several studies that show the modification of starch to make it hydrophobic. But use of these chemical modification methods will reduce the degradability of starch. Another interesting study could be to integrate the electrospun fibers and the LDPE-TPS films to study the reduction in moisture sorption as these electrospun fibers are found to be hydrophobic and show low levels of moisture sorption while the surface features can add to functionality.

List of Presentations and Publication

Poster presentation

“Effect of Humidity and solvent parameters on porosity of electrospun fibers”

SIMTech, Annual poster exhibition, 16-Aug-2013

Journal

L. Natarajan, J. New, A. Dasari, S. Yu, and M. A. Manan, "Surface morphology of electrospun PLA fibers: mechanisms of pore formation," *RSC Advances*, vol. 4, pp. 44082-44088, 2014. DOI: 10.1039/C4RA06215A

Oral presentation

“Polymer nanocomposites in active food packaging: effect of surface characteristics of electrospun fibers on microbial growth” L. Natarajan, A. Dasari in ISPAC 2016, Singapore (International Symposium on Polymer Analysis and Characterization) (15-June-2016)