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MOISTURE TRIGGERED RELEASE OF THYMOL FROM ELECTROSPUN MATS  
TO EXTEND SHELF LIFE OF FRESH TOMATOES

by

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And approved by

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## ABSTRACT OF THE THESIS

### Moisture Triggered Release of Thymol from Electrospun Mats to Extend Shelf Life of Fresh Tomatoes

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Electrostatic fiber formation which also known as electrospinning, a technology to construct fabrics by applying potential differences has gained its popularity in producing nonwoven nano to submicron scale fibers from a wide range of materials (Rutledge & Fridrikh, 2007). The advantages of electrospinning technology in this study are the ambient processing temperature which is suitable to encapsulate volatile compounds and the large surface area produced to make electrospun mats more susceptible to environment conditions (RH, temperature etc.) changes (Vega-Lugo & Lim, 2009). Moisture could interact with electrospinning nanofibers to trigger the release of active compounds from inside the electrospun fibers.

The objective of the research was to study the feasibility of developing the moisture triggered release system of thymol using electrospinning technology to extend the shelf life of fresh tomatoes. The system feasibility was evaluated based on three criteria: first, whether the construction of thymol encapsulated electrospun mats could be successful;

second, whether the moisture triggered release of thymol from the mats could be achieved; and third, could the moisture from fresh tomatoes trigger the release of thymol from the mats and how much could the released thymol extend the shelf life of fresh tomatoes.

Thymol was encapsulated into polyvinyl alcohol (PVA) using electrospinning to produce fiber mats. The retentions of thymol in the fiber mats ranged from 37.9 to 99.2%, depending on the amount of thymol added and electrospinning time. In the first experiment, the rate of release of thymol was found to increase with relative humidity: very low release at 0% RH, somewhat higher at 75% RH, and much higher at 100% RH—this suggests that high RH can be used as a trigger to release thymol in this system. In the second experiment, small pieces of the mat and fresh tomatoes were placed inside plastic containers. The moisture from the fresh tomatoes was able to trigger the release of thymol and extend their shelf life for at least 5 days. Therefore it is feasible to apply this system to real packages.

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## 1. INTRODUCTION

Controlled Release Packaging (CRP) is an emerging technology within food packaging area which can release active compounds (antimicrobials/antioxidants) from packages to foods at controlled rates to enhance food quality and safety (G.G. Buonocoreb, 2003; LaCoste, Schaich, Zumbrunnen, & Yam, 2005). In previous studies, controlled releases of antimicrobials and antioxidants were proven to be more effective than one-time-addition in some applications. Controlled release of nisin was more effective than one-time-addition in inhibiting the growth of *Micrococcus luteus* even at 15% of the amount used in one-time-addition (Balasubramanian, Lee, Chikindas, & Yam, 2011). Targeted controlled release of tocopherol was measured to obtain maximum antioxidant effectiveness (Zhu, Schaich, Chen, Chung, & Yam, 2012). Controlled release packaging is preferred in many cases for its advantages including the protection it provides to slow down or prevent active compounds from degradation and as it can mitigate the sensory influence of active compounds such as essential oils pose on food products by controlled release overtime (Chen, Lee, Zhu, & Yam, 2012; Madene, Jacquot, Scher, & Desobry, 2006; Zhou, Sheen, Pang, Liu, & Yam, 2013). Different polymer processing methods such as film casting (Gemili, Yemenicioğlu, & Altınkaya, 2009), extrusion (LaCoste et al., 2005) and electrospinning (Erika Mascheroni & Giuseppe Di Silvestro, 2013; Schiffman & Schauer, 2008; Sill & von Recum, 2008) were used to construct control release films for various

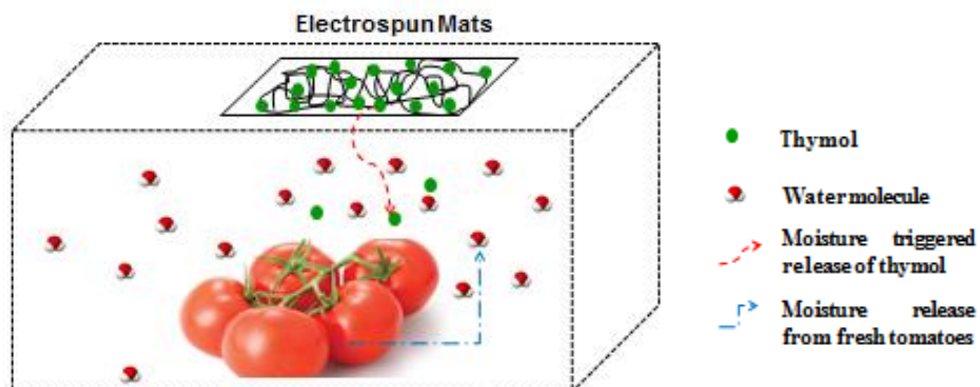
applications.

Electrostatic fiber formation which also known as electrospinning, a technology to construct fabrics by applying potential differences, has gained its popularity in producing nonwoven nano to submicron scale fibers from a wide range of materials (Rutledge & Fridrikh, 2007). Unlike traditional technologies in producing films with limited surface area such as extrusion and film casting, electrospinning produces film with significantly larger surface area (Schiffman & Schauer, 2008). The large surface area and the nano to submicron fiber diameter of electrospun fiber mats might enable them to be more responsive to surrounding environmental changes which suggests the possibility to achieve triggered release of active compounds by controlling environmental conditions include relative humidity (RH) (Vega-Lugo & Lim, 2009). In this study, the moisture triggered system was also achieved by using PVA, a water soluble polymer in electrospinning process (Kenawy, Abdel-Hay, El-Newehy, & Wnek, 2007).

Essential oils including eugenol, cinnamaldehyde, thymol, carvacrol, and their combinations have been studied on their antimicrobial effectiveness against different microbes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008) among which thymol was vastly studied as a single compound or in combination with other essential oils (Nedyalka V. Yanishlieva, 1999; Pei, Zhou, Ji, & Xu, 2009; R.J.W. Lambert, 2001; Xu, Zhou, Ji, Pei, & Xu, 2008). 2-isopropyl-5-methylphenol or thymol, is a natural monoterpene

phenol extracted from the plant of thyme with antimicrobial properties (Lee, Umamo, Shibamoto, & Lee, 2005). Thymol has a pleasant aromatic odor at low doses with limited antimicrobial effectiveness but at high dose thymol could pose undesirable sensory influence on the food products (Burt, 2004; Zhou et al., 2013). As thymol is aromatic and the sensory influence as well as microbial inhibition effectiveness is in proportional to the concentration of thymol in the system (Burt, 2004; Zhou et al., 2013), controlled release of thymol is proposed to minimize the sensory effectiveness while maximize the microbial inhibition over extended time.

Moisture triggered release electrospun mats of thymol were developed in the study to extend the shelf life of fresh tomatoes. The moisture used to trigger the release was from the respiration and moisture losses of fresh tomatoes. The released thymol could inhibit the growth of spoilage microorganisms and thus extend the shelf life of fresh tomatoes (R.J.W. Lambert<sup>1</sup>, 2001; Rosa M. Raybaudi-Massilia & Robert Soliva-Fortuny, 2009). This system was proposed as one of the post-harvesting technologies to reduce the post-harvest losses and maintain the nutritional values of fresh fruits and vegetables (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Kitinoja, Saran, Roy, & Kader, 2011; Mahajan, Caleb, Singh, Watkins, & Geyer, 2014).



**Figure 1. Moisture Triggered Release System overview**

The objective of the research was to study the feasibility of developing the moisture triggered release system of thymol using electrospinning technology to extend the shelf life of fresh tomatoes. The system feasibility was evaluated based on three criteria: first, whether the construction of thymol encapsulated electrospun mats could be successful; second, whether the moisture triggered release of thymol from the mats could be achieved; and third, could the moisture from fresh tomatoes trigger the release of thymol from the mats and how much could the released thymol extend the shelf life of fresh tomatoes.



## **2. LITERATURE REVIEW**

### **2.1 Post-harvesting Losses of Fresh Produce**

The Food and Agricultural Organization of United Nations (FAO) estimated the total food loss or waste was around 32% weight based in 2009 globally (Brian Lipinski, 2013). Fresh fruits and vegetables have the highest waste rate of any food (Bertolini, 2013) due to their intrinsic properties which could cause moisture losses (Dong Sun Lee, 1995; Hung et al., 2011) and microbial and physical spoilages (Ragaert, Devlieghere, & Debevere, 2007). In the meantime, fresh fruits and vegetables are highly recommended in dietary recommendations as they are high in vitamins, minerals and phytochemicals (Slavin & Lloyd, 2012). Due to the high in loss and high in nutritional value of fresh fruits and vegetables, different post-harvesting processing methods were developed to reduce the post-harvest losses in fresh fruits and vegetables (Artés et al., 2009; Kitinoja et al., 2011; Mahajan et al., 2014)

Traditional preservation methods include dehydration, fermentation, thermal processing and freezing have been to preserve food products for a long time (Barrett & Lloyd, 2012). However, in a lot of cases, minimum processing of fresh fruits and vegetables is preferred and is the current trend of the society and industry (Ragaert et al., 2007). Under the circumstance, controlled release packaging is introduced for the controlled release of active compounds to create optimum surrounding environment around the food produce

to inhibit microbial growth and further extend the shelf life of fresh produce.

## **2.2 Controlled Release Packaging**

Traditional food packaging provides functions including containment, protection, convenience and communication to food products (Kit L. Yam, 2005). Nowadays, antimicrobial, antioxidant and shelf life extension packages have been the new developments in food packaging industry among which active packaging is the widely studied one (LaCoste et al., 2005). Active packaging is defined as the package in which compounds been added into or onto the package material or headspace to enhance the package system (Robertson, 2006) or the type of package to extend shelf life, safety and sensory properties by modifying conditions in package (L. Vermeiren\*, 1999). Active packaging enables the package to interact with food system to improve food quality and safety in addition to the traditional functions as different kinds of barriers (LaCoste et al., 2005). Antimicrobial active packaging has been widely studied and showed its effectiveness in quality assurance and shelf life extension on different kind of food products. Bacteriocin nisin was added into cellulose based film and showed 2 log reduction in *Listeria innocua* and 1.5 log reduction in *Staphylococcus aureus* (Amalia G.M. Scannella & Arendt, 2000). Potassium sorbate has been added to starch and derivatives (Marí'a A. Garcí'a, 1998) and whey protein films (M. Ozdemir, 2001) to investigate its antimicrobial properties and release profiles respectively. Other applications for active

packaging include oxygen or carbon scavengers and modified atmospheric packaging using controlled gas permeability packages.

Controlled Release Packaging is a new development within food packaging which can release active compounds (antimicrobial/antioxidant) at different controlled rate to enhance food quality and safety (LaCoste et al., 2005). In previous studies, controlled release of antimicrobial and antioxidant was proven to be more effective than one-time-addition in some applications. Controlled release of nisin was more effective than instant added nisin in inhibiting the growth of *Micrococcus luteus* even at 15% of the amount used in instant addition (Balasubramanian et al., 2011). Targeted controlled release of tocopherol was measured to obtain maximum antioxidant effectiveness (Zhu et al., 2012). Controlled release packaging is preferred in many cases due to its advantages such as the protection it provided to slow down or even prevent active compounds from degradation such as oxidation and the mitigation in sensory influence of active compounds such as essential oil on food products (Chen et al., 2012; Madene et al., 2006).

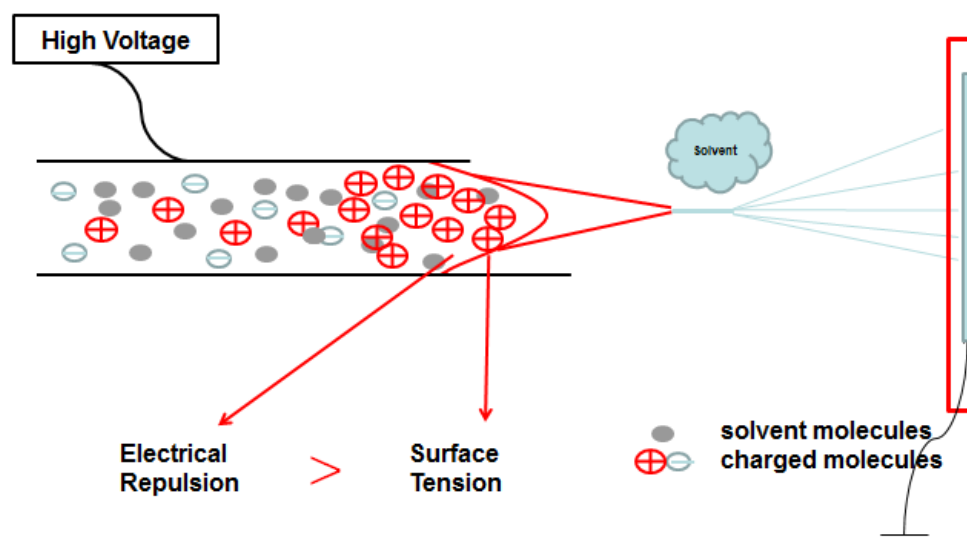
Different polymer construction methods were used to construct control release packaging system for different purposes. Casting films made from EVA, EVOH, LDPE and PP were used for the controlled release of antioxidant tocopherol and quercetin (Chen et al., 2012); cellulose acetate and lysozyme mixture was casted on polypropylene for the controlled

release of lysozyme (Gemili et al., 2009); and smart blending method was proposed to extrude the film for the controlled release of active compounds (LaCoste et al., 2005). In this paper, an emerging technology, electrospinning, is introduced to produce active compounds encapsulated nanofibrous mats for the controlled release of thymol.

### **2.3 Electrospinning**

Electrospinning is a technique to construct nonwoven nanofibers by applying high voltage to form electrically driven jet of polymer solutions (Crette, 2002). The electrospinning process is divided into 3 stages: first, the initiation of jet with the formation and concentration of charged molecules to deform the bead surface; second, cone-jetting with the jetting of fluid from the surface of the deformed bead and third, evaporation of solvent and formation of slender thinning jet (Collins, Federici, Imura, & Catalani, 2012; Rutledge & Fridrikh, 2007). The whole process is initiated when high voltage is applied to create potential difference which causes the induced charges within the polymer solution and separation of positively and negatively charged molecules under electric field. The charged molecules with the same polarity as the polarity of the capillary move towards the surface of the bead and by which increase the charge density of the same charge molecules. With the increase in the charge density on the surface of the bead, there is an increase in the local electrical repulsion within the same charged molecules which counteracts the surface tension force that is holding the bead in its hemispherical shape

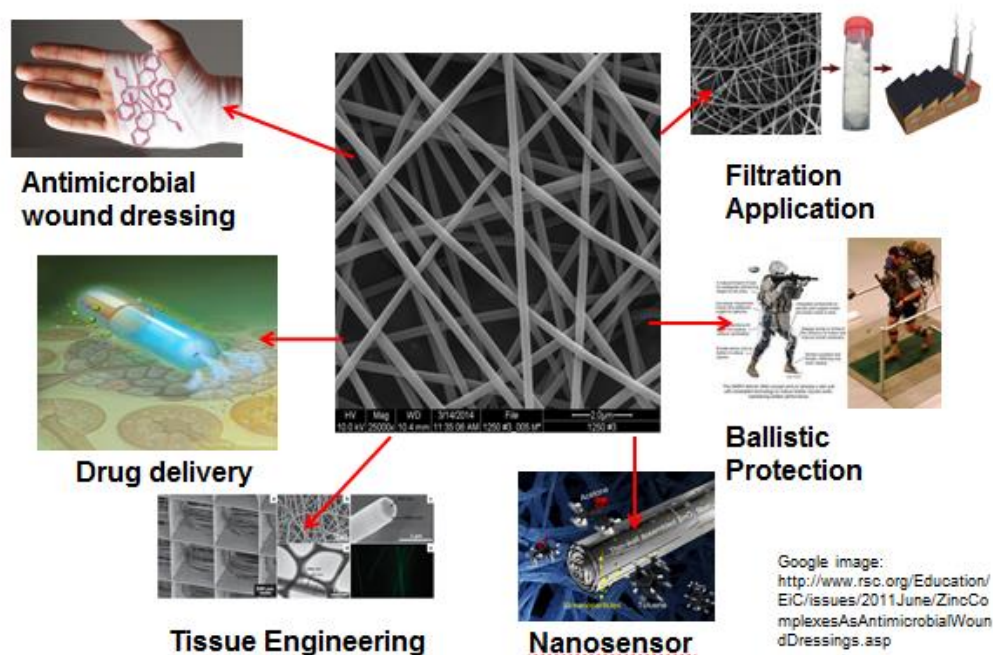
and thus induces the deformation of the bead into cone-like projection. When the electric repulsion continues to increase, at certain point, a fluid jet emerges and solvent is evaporated during the process which results in the collection of non-woven semi/nano scale fibers collected on the collector (Collins et al., 2012). Different polymers could be used in electrospinning process depend on the final application and the active compounds / drug that are added into the system. Processing parameters include applied voltage, flow rate, capillary – collector distance and solution parameters such as polymer concentration, solvent volatility and conductivity are the control measures used to modify the electrospinning conditions for different applications (Sill & von Recum, 2008).



**Figure 2. Electrospinning process illustration**

The application of electrospinning is predominantly in pharmaceutical and medical industry so far. Electrospinning of biocompatible material has been used in the tissue engineering and it has shown great potential in the engineering of tissues including:

vasculature, bone, neural and tendon/ligament (Sill & von Recum, 2008). Electrospinning has been used to encapsulate drug for different release profiles of the drugs under different applications (Jing Zeng & Lixin Yang, 2003). Other applications for electrospinning including antimicrobial wound dressing (Lalani & Liu, 2012; Pim-on Rujitanaroj a, 2008), nanosensor (Ding, Wang, Yu, & Sun, 2009; Zhenyu Li & Ce Wang, 2008) and filtration applications (Gopal et al., 2006). Recently, there has been a development in electrospinning area to develop active compound incorporated electrospun mats to be used in food packaging area for food quality and safety purposes. Controlled release of allyl isothiothyanate was achieved by encapsulating it in soy protein and poly(lactic acid) electrospun nanofibrous mats (Vega-Lugo & Lim, 2009).



**Figure 3. Applications of electrospinning technique**

The advantages of this technology to be applied in this study are the ambient processing temperature which is suitable for the encapsulating of volatile active compounds and the large surface area produced which makes the material more susceptible to environment conditions (RH, temperature etc.) (Vega-Lugo & Lim, 2009). Relative humidity could dissolve the nanofibers to cause the release of active compounds from the electrospun mats. In this research, thymol is encapsulated in poly(vinyl alcohol) nanofibers and its release is triggered and controlled by different RH environment. The effectiveness of the controlled released thymol is studied on fresh tomatoes to extend their shelf life.

### 3. OBJECTIVE

The objective of the research was to study the feasibility of developing the moisture triggered release system of thymol using electrospinning technology to extend the shelf life of fresh tomatoes. Based on the overall objective, three sub-objectives are proposed to test the feasibility of the overall objective:

1. Electrospinning mat construction and system characterization
2. Thymol retention and release kinetics study/modeling
3. Shelf life extension effectiveness study and sensory evaluation

The impact of this study is to propose a new antimicrobial delivery system, active compound encapsulated electrospun nanofibrous mats for the moisture triggered release of active compounds. The innovativeness of the research include: 1. the electrospun nanofibrous system itself for its large surface to volume ratio and susceptibility to surrounding environment and 2. the triggering system from the moisture produced by the respiration of fresh produce which could be a self-generating and self-controlled system. The development of this technology could be a part of the controlled release packaging technologies to release active compounds into food matrix overtime to reduce the post-harvesting losses of fresh produce.



## 4. DESIGN OF EXPERIMENT

### 4.1 Experiment Design Overview

The experiment design is based on the research overall objective and the sub-objectives from the overall objective.

1. For the first sub-objective, electrospinning mat construction and system characterization, it involves the production of PVA electrospun nanofiber mat using fiber forming solutions prepared from different PVA concentrations followed by the addition of surface surfactant compound (Triton X-100) and active compound (thymol). System characterizations include the polymer solution characterization and nanofiber characterization. Polymer solution characterization involves the measurements of pH, conductivity, surface tension and viscosity for the macro understanding of the system. Nanofiber mats characterization includes the fiber diameter distribution analysis and morphology analysis.
2. Thymol retention and release kinetics study/modeling: it includes the loading efficiency study of the electrospun film to evaluate thymol retention after electrospinning and release kinetics study to determine the specific release kinetics of electrospun films under different RH conditions. Modeling of the release is done for a better understanding of the release kinetics and could be used further to estimate the headspace concentrations of thymol in real food system.

3. Shelf life extension effectiveness study and sensory evaluation: real fresh produce application system, fresh tomatoes – thymol encapsulated electrospun mats was proposed to answer three questions: 1. whether the moisture produced by the respiration of fresh tomatoes enough to trigger the release of active compounds and 2. to what extend does the released thymol being able to extend the shelf life of fresh tomatoes and 3. the sensory influences of the exposure to thymol pose on the fresh tomatoes.

## 4.2 Materials and Apparatus

**Materials:** Polymer used in the study was 99%+ hydrolysis PVA with molecular weights (Mw) 89000 - 98000, Triton X-100 (p-tertiary octylphenoxy polyethyl alcohol) was used as surface surfactant, ethanol as solvent and thymol >99.5% the antimicrobial were all purchased from Aldrich Chemical (Milwaukee, WI, USA); deionized water was from Millipore water system; fresh tomatoes were purchased from local market.

**Apparatus:** Nanofiber Electrospinning Instrument, Nabond Technologies Co. Ltd, (Hongkong, China); Guanta 200F Scanning Electron Microscope, FEI (Hillsboro, OR, USA); IKA RCT basic safety control heating plate, IKA (Wilmington, NC, USA); Fisher Scientific Surface Tensiometer 20 (Pittsburgh, PA, USA); UV-1601, UV-Visible Spectrophotometer, SHIMADZU is used for quantitative analysis and CPS-Controller,

SHIMADZU is used for temperature control of the cells; IQ 270G pH meter, IQ Scientific Instruments (Loveland, CO, USA); IQ 270G Conductivity meter, IQ Scientific Instruments (Loveland, CO, USA) TA AR-2000 ETC Rheometer, TA Instruments Rheology Division, (New Castle, DE, USA); 200F Scanning Electron microscope, FEI (Hillsboro, OR, USA);

#### **4.3 Electrospinning mat construction & system characterization**

PVA is generally derived from the hydrolysis or alcoholysis of poly(vinyl acetate), it is available in variety degrees of hydrolysis (DH). DH is one of the major factors influencing the electrospinning spinnability of the material (C.C. DeMerlis, 2003; Chiellini, Corti, D'Antone, & Solaro, 2003). Research showed the poor electrospinning spinnability was observed in DH=99.9% sample due to the high surface tension and strong hydrogen bonding within the polymer (Jong-Chul Park & Kim). It is also showed that for pure DH>99% PVA sample water solution, sporadic electrospaying of droplets were observed which was dissolved in deionized water at 80°C with constant stirring for at least 12 hours (Li Yao & David. G. Simpson, 2003). The reason of the difficulty in handling high DH PVA is due to the homogeneous solution is subjected to aggregation and crystallization of PVA polymers particularly at the surface of the solution (Zhang, Yuan, Wu, Han, & Sheng, 2005) which could cause aggregation on the surface of syringe bead to interfere with the

process. Due to the reasons above, ethanol and Triton X-100 surface surfactants were used for more continuous electrospinning process and higher flow rate. Triton X-100 surfactant was used to lower the surface tension of the polymer solution and to allow the electrospinning of PVA to be carried out at a higher flow rate ( $\sim 2.4$  ml/h). (Xuefen Wang, 2006)

#### **4.3.1 Fiber-forming Solution Preparation**

Various polymer concentration (w/w%), surfactant ratio (w/w%) and active compounds concentrations (% of the polymer used) were added to prepare different polymer solutions.

The process of preparing active compounds incorporated solutions was:

1. Preparation of polymer solution: Millipore deionized water was weighed and predetermine amount of PVA granules was added slowly for well mixture. PVA granules and DI water were stirred and heated in thermostated oil bath at  $80^{\circ}\text{C}$  for 3 hours on IKA RCT Basic Safety Control Heating Plate (IKA, Wilmington, NC, USA). High temperature should be avoided as it could result in the decomposition of PVA and evaporation of water which causes film formation on top of the polymer solution.
2. The polymer solutions were stirred and cooled down to room temperature.
3. Surfactants were added into the solutions and stirred for 15 minutes. Split the solution into different portions for further addition of thymol.
4. Various percentage of thymol is dissolved in minimum amount of ethanol which were

further added into the solution and stirred for 30 minutes to obtain homogeneous fiber forming solutions.

5. The fiber-forming solutions were added to a 5ml syringe with stainless steel needle for electrospinning.

**Table 1: Experiment Design of Polymer Solution Composition**

<b>PVA</b>	<b>polymer</b>	<b>Ethanol</b>	<b>Triton</b>	<b>X-100</b>	<b>Thymol</b>
<b>concentration</b>		<b>concentration</b>	<b>Ration</b>		<b>Concentration</b>
9%		-	-		-
10%		-	-		-
11%		-	-		-
12%		-	-		-
12%		3%	-		-
12%		4%	-		-
12%		5%	-		-
12%		-	0.1%		-
12%		-	0.1%		5%
12%		-	0.1%		10%
12%		-	0.1%		15%

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12%	-	0.3%	-
12%	-	0.6%	-
12%	-	1%	-
15%	-	-	-

---

#### 4.3.2 Physical properties measurement of the polymer solutions

12% PVA polymer solution, 12% PVA with 4% ethanol addition, 12% PVA with 0.1% Triton X-100, 12% PVA 0.1% Triton X-100 with 5% thymol addition and 12% PVA 0.1% Triton X-100 with 10% thymol addition samples were chosen for the physical properties analysis. PH, conductivity, surface tension and viscosity were measured for the better understanding of the electrospinning system.

**Conductivity:** Conductivity is calibrated using Beckmn Coulter 1000 Microsiemen Conductivity Standard Solution.

**PH:** BDH pH 4, 7, 10 buffer solutions are used for the calibration of the instrument.

**Viscosity:** Viscosity is measured at 25°C with spindle setting 1 and the speed is at 3.

**Surface Tension:** Fisher Scientific Surface Tensiometer 20 was used to measure the surface tension of the solutions. Around 50ml of solution samples were needed for the surface tension analysis. The procedures are below:

1. Cleaned platinum-iridium ring attach to the hood at the end of the lever arm. The arrest

mechanism should be holding the arm at the moment.

2. The torsion arm released and adjusted to a zero reading. Adjust the knob on the right side until the index and its image are exactly in line with the reference mark on the mirror.
3. Sample in glass vessel be placed onto the sample table. Move the sample table until the ring is immersed under the sample liquid. About 1/8 inch immersion is generally considered sufficient. Lower the sample table till the index is in between two lines around the mirror.
4. Slowly turn the knob on the right side clockwise till the ring breaks the surface. The unit of surface tension is dynes/cm.

#### **4.3.3 Electrospinning of Polymer Solution**

Fiber-forming solutions were added in 5 ml plastic syringe with metallic needle and electrospun with a vertical setup (Nanofiber Electrospinning Instrument, Nabond Technologies Co. Ltd, Hongkong, China). The vertical setup composed of a flow rate control pump, a direct current power supply and a stainless steel rotary fiber collector. The rotary fiber collector was attached to the positive electrode and the metallic needle was grounded. Parameters including voltage applied (10kv-20kv) and flow rate of the polymer solutions (0-2.0 ml/h) were tested in the research. The distance between the needle and collector was fixed at 10cm. Thymol incorporate electrospun nanofibers were collected on

aluminum foil affixed on the rotary collector and the process was conducted at room temperature.

**Table 2: Experiment Design for Electrospinning**

<b>Polymer Sample</b>	<b>Voltage (kv)</b>	<b>Flow Rate (ml/h)</b>
<b>9% PVA</b>	10	0.1
<b>10% PVA</b>	10	0.1
<b>11% PVA</b>	10	0.1
<b>12% PVA</b>	10	0.1
<b>15% PVA</b>	10	0.1
<b>12% PVA 3% ethanol</b>	10	0.3
<b>12% PVA 4% ethanol</b>	10	0.3
<b>12% PVA 4% ethanol</b>	10	0.3
<b>12% PVA 0.1% Triton X-100</b>	20	1.5
<b>12% PVA 0.3% Triton X-100</b>	20	1
<b>12% PVA 0.6% Triton X-100</b>	20	1
<b>12% PVA 1% Triton X-100</b>	20	1
<b>12% PVA 0.1% Triton X-100 5%</b>	20	1.5
<b>thymol</b>		
<b>12% PVA 0.1% Triton X-100 10%</b>	20	1.5



---

**thymol**

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**Scanning Electron Microscopy (SEM)**

For SEM analysis, PVA fiber samples were first sputtered with gold (Sputtering Polaron E 5100) for 30s (rate 1nm s<sup>-1</sup>) in argon with 18mA current intensity. Fiber morphologies were examined using Guanta 200F Scanning Electron Microscope, FEI (Hillsboro, OR, USA) at an accelerating voltage of 10kv. The fiber diameter distributions were analyzed from the SEM pictures by taking at least 140 measurements using Ex Docu image processing software.

**4.3.4 Encapsulation confirmation**

The success thymol encapsulation was confirmed using Fourier Transform Infrared Spectroscopy (FTIR) for the distinguish benzene peak in thymol molecules. SEM pictures from control (0% thymol), 5% thymol addition and 10% thymol addition samples were also obtained to confirm the success of thymol encapsulation into the PVA nanofibrous structure.

**4.4 Release Kinetics Study****4.4.1 Loading Efficiency Analysis of Electrospinning Process**

Thymol retention efficiency was defined as the ratio between thymol retained in

electrospun mats and thymol added initially in fiber-forming solutions. Shake-flask extraction method was used for the total extraction of thymol from electrospun mats. In the study, 5 and 10% thymol concentrations fiber-forming solutions were electrospun for 5, 10, 20, and 30 minutes to obtain electrospun fiber mats. The electrospun mats were immediately cut into  $1\text{cm}^2$  square pieces and placed in 30g ethanol in 50ml polypropylene conical tubes. The tubes were then rotary-shaked at 250 rpm at  $26^\circ\text{C}$  in environmental chamber for 48 hours to fully extract thymol in the mats. The filtered ethanol solution samples were measured by UV spectrophotometer (UV-1601, UV-Visible Spectrophotometer, SHIMADZU is used for quantitative analysis and CPS-Controller, SHIMADZU is used for temperature control of the cells in the UV Spectrophotometer) at 274 nm for absorbance. The concentrations of thymol were calculated based on a standard curve of UV absorbances as a function of thymol concentrations. Standard curve samples were prepared by diluting a stock solution containing 10mg thymol /g ethanol (ethyl alcohol). The dilution for standard curve was done with the following concentrations: 10, 1, 0.5, 0.25, 0.125, 0.0625mg thymol /g ethanol. Total extraction was confirmed by reimmerse the samples into ethanol solution after the first analysis is made. The samples were washed for 3 times with ethanol before put into the new system. Sample without active compounds was used to make sure other chemicals do not absorb at the 274nm wavelength. The thymol retention efficiency was expressed in retention percentages as a

function of thymol concentration and electrospinning time.

Thymol retention efficiency(%)

$$= \frac{\text{Thymol extracted from electrospun mats(mg)}}{\text{Thymol added into fiber – forming solution (mg)}} \times 100\%$$

#### 4.4.2 Release Profile Study of Thymol from Electrospun Film

Relative humidities of 0, 75 and 100% were applied in the release profile study of thymol from electrospun mats to understand the influence of RH on the release kinetics of the system.

**Table 3: Experiment Design of Release Profile Study**

Thymol Concentration	Temperature (°C)	Humidity (%)	Time
10%	25	0%	30min
10%	25	0%	1 hour
10%	25	0%	2 hours
10%	25	0%	4 hours
10%	25	0%	6 hours
10%	25	0%	1 day
10%	25	0%	2 days

<b>10%</b>	25	0%	4 days
<b>10%</b>	25	0%	6 days
<b>10%</b>	25	0%	11 days
<b>10%</b>	25	75%	30min
<b>10%</b>	25	75%	1 hour
<b>10%</b>	25	75%	2 hours
<b>10%</b>	25	75%	4 hours
<b>10%</b>	25	75%	6 hours
<b>10%</b>	25	75%	1 day
<b>10%</b>	25	75%	2 days
<b>10%</b>	25	75%	4 days
<b>10%</b>	25	75%	6 days
<b>10%</b>	25	75%	11 days
<b>10%</b>	25	100%	30min
<b>10%</b>	25	100%	1 hour
<b>10%</b>	25	100%	2 hours
<b>10%</b>	25	100%	4 hours
<b>10%</b>	25	100%	6 hours
<b>10%</b>	25	100%	1 day

<b>10%</b>	25	100%	2 days
<b>10%</b>	25	100%	4 days
<b>10%</b>	25	100%	6 days
<b>10%</b>	25	100%	11 days

---

Thymol release profiles were evaluated by enclosing the electrospun mats in sealed 250ml glass jars with 0, 75 and 100% RHs. To prepare the glass jars with specific RH, the jars were purged with dry N<sub>2</sub> gas for 2 minutes to create 0% RH, 5g NaCl and 2.56g DI water were added into the jars to create 75% RH and 7.56g DI water were added to create 100% RH. Moisture meter was used to measure the RH of the bottles to validate the methods of creating the specific RHs to make sure the desirable RH was reached.

After electrospinning, thymol encapsulated electrospun mats were immediately placed in the glass jars and sealed with lids. Direct contact between the salt, water and electrospun fibers were avoided by having extra space on the bottom of the electrospun mats. The mats were placed in the jars for extended periods of 15 minutes, 30 minutes, 1 hour, 2 hours, 6 hours, 1 day, 2 days, 4 days, 6 days and 11 days. The control samples were the mats without exposure to moisture. Ethanol total extraction method introduced in 2.2.4 was applied to extract thymol from electrospun mats exposed to various RHs for different time. The basic theory behind the method was mass balance and the release profiles were analyzed based

on the headspace concentrations of thymol in the sealed jars. The thymol release profiles were expressed as headspace thymol concentration as a function of time.

$$\text{Headspace Concentration } \left(\frac{\text{mg}}{\text{ml}}\right) = \frac{\text{Thymol Extracted (control, mg)} - \text{Thymol Extracted (experiment, mg)}}{\text{Volumn of the Jars}(250\text{ml})}$$

The results obtained were plotted in the form of headspace concentration over time to understand the trend of the release.

### 5.4.3 Modeling of the Release of Thymol from Electrospun Mats

As moisture played major role in the release profile of thymol, the release was unlikely to conform to Fickin's Diffusion. For a better understanding of the release profile and predicting the release of thymol under other RH conditions, empirical model was used to curve fitting the data obtained from the study.

Release kinetics of thymol from electrospun mats under different RHs was modeled using empirical mathematical model. Empirical mathematical model which was based on the pseudo first kinetics which was used to fit the curve of the release profiles, equation below was used for the curve fitting of the system:

$$\frac{C_e - C}{C_e - C_0} = e^{-kt}$$

$C_e$ : equilibrium concentration;  $C_0$ : initial concentration which is 0;  $C$ : concentration at specific time;  $t$ : time;  $k$ : rate constant

#### 4.5 Shelf Life Extension Effectiveness Study

Shelf life extension effectiveness study was conducted on fresh tomatoes in 1L airtight plastic containers. Fresh plum tomatoes were purchased from local market and were without bruising/cuts on the surface, firm, fresh and of similar weight. The tomatoes samples were washed with deionized water, air dried and placed in room temperature for the experiment. Two tomatoes and electrospun mats (control samples had electrospun mats without thymol and experiment samples had electrospun mats with thymol) were put in each of the boxes and three replications were used in each of the samples. Pictures of the tomatoes were taken at 7 days and 12 days to observe the shelf life extension effectiveness of thymol encapsulated electrospun mats on fresh tomatoes.



**Figure 4. Experiment setup for shelf life extension experiment**

In addition to the shelf life extension experiments, a simple sensory evaluation study was

done to understand the influence of thymol on the odor and color of the fresh tomatoes. It is known that essential oils such as thymol could pose strong smell to products which could cause rejections from consumers (Zhou et al., 2013). Hedonic test scaled from 1 to 9 was adopted for the study and the subjects were 12 internal lab mates. Below was the questionnaire for the sensory evaluation:

**Hedonic Testing of Electrospinning Treated Samples**

Participant Information:    Female      Male

Age: \_\_\_\_\_

Nationality: \_\_\_\_\_

Are you familiar with the smell of thymol?

\_\_\_\_\_

Sample Smell: The smell of the treated tomatoes (please choose one below)

Like Extremely

Like Very Much

Like Moderately

Like Slightly

Neither Like nor Dislike

Dislike Slightly

Dislike Moderately

Dislike Very Much

Dislike Extremely

Sample color: The color of the treated tomatoes (please choose one below)

Like Extremely

Like Very Much

Like Moderately

Like Slightly

Neither Like nor Dislike

Dislike Slightly

Dislike Moderately

Dislike Very Much

Dislike Extremely

**Figure 5. Sensory Evaluation Questionnaire Used in the study**

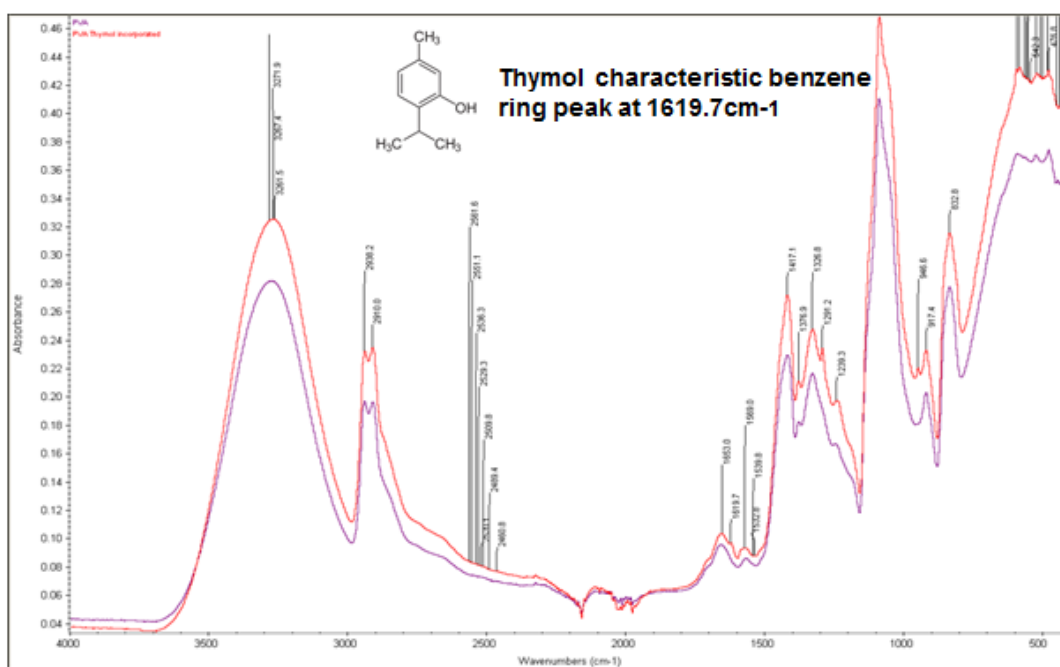


Twenty participants were asked to rate their likeness of the smell and color of the 7-day thymol treated tomatoes samples from dislike extremely to like extremely. They were also asked to write down any comments they had for the samples.

## 4. RESULTS & DISCUSSIONS

### 5.1 Encapsulation of thymol into electrospun mats – FTIR result

From FTIR comparison result of electrospun PVA and thymol incorporated PVA samples, benzene ring peak at the wavelength of  $1619.7\text{ cm}^{-1}$  can be observed. This result confirmed the encapsulation of thymol into PVA electrospun mat was successful.



**Figure 6. FTIR comparison for electrospun PVA (purple) sample and thymol incorporated PVA (red) sample**

### 5.2 Physical Properties of Polymer Solutions

It is known that parameters including charge density (pH), conductivity, surface tension and viscosity influence the electrospinning continuity, morphology and diameter distribution of electrospinning fiber mats. Changes in physical properties of the polymer

solutions could help to understand the observations in the electrospinning processes and SEM pictures. 0.1% Triton X-100 addition, 5 and 10% thymol addition are the samples of importance in this discussion.

**Table 4: Physical Properties of Polymer Solutions of Different Additions**

Sample		pH	Conductivity ( $\mu$ s)	Surface Tension	Viscosity(cp)
<b>0.1%</b>	<b>Triton</b>	6.0	497	33.6	1226
<b>X-100 Addition</b>					
<b>5%</b>	<b>Thymol</b>	6.0	473	44.4	1057
<b>Addition</b>					
<b>10%</b>	<b>Thymol</b>	6.0	468	45.1	1023
<b>Addition</b>					

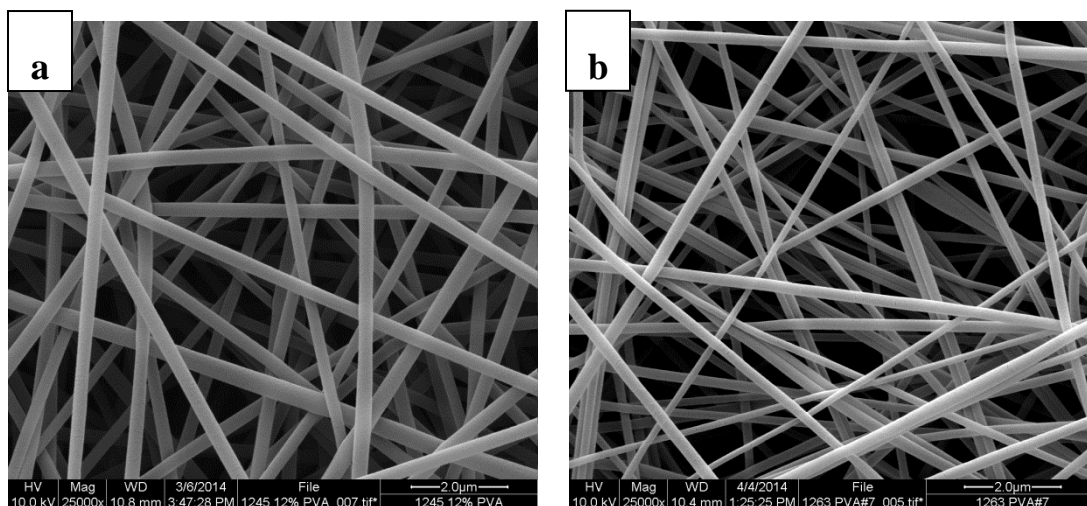
PH is an indication of the charge density of the polymer solutions. The higher the charge density, the stronger the elongation forces could be applied to the electrospinning jets under electrical field and thus, thinner nanofibers (Won Keun Sona, 2005). The pHs of the three samples were identical which indicated its minimum influence on the micro- and macro- characteristics of electrospinning fibers. The similar conductivity of the three samples also showed the unlikelihood of its influence on the morphologies of the samples.

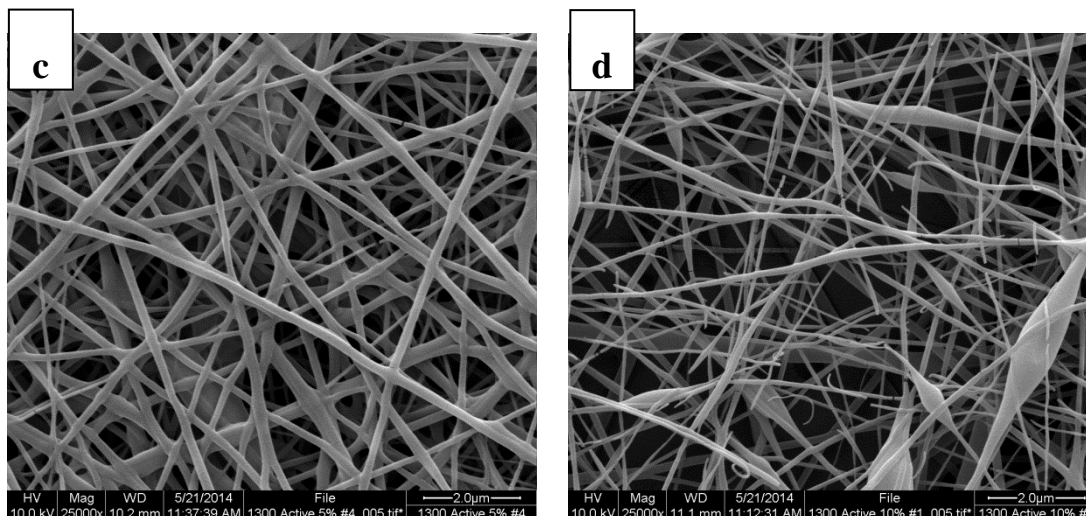
Higher surface tension of 10% thymol addition compared to 5% could be explained by the depletion of surfactant Triton X-100 to reduce the free energy between the

hydrophobic thymol molecules and hydrophilic polymer solution. In electrospinning process, higher surface tension drives the formation of beads which could explain the beads structure in 10% thymol added samples (H. Fong, 1999).

5 and 10% thymol addition samples also had lower viscosity compared to thymol free samples. The reduced viscosity could also attribute to the formation of beads in electrospun nanofibers due to the increasing influence of the solvent on surface tension which favors the formation of spheres – beads (Vega-Lugo & Lim, 2009).

Due to the highest surface tension and lowest viscosity of the 10% thymol addition sample, beads were observed in its nanofibers. Overall, the morphologies of the nanofibers became more disorganized with the addition of thymol.





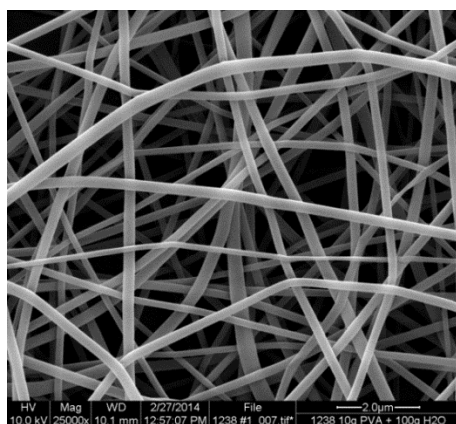
**Figure 7. The SEM pictures (25000x) of electrospinning nanofibers for different polymer solutions (a) 12% PVA Solution; (b) 0.1% Triton X-100 Addition; (c) 5% Thymol Addition; (d) 10% Thymol Addition**

The more and more disorganized fiber structure from 0% thymol addition (control samples) to 10% thymol addition samples also further confirmed the success of thymol encapsulation into the nanofibers.

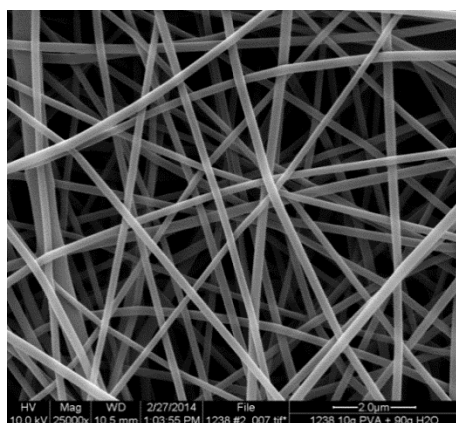
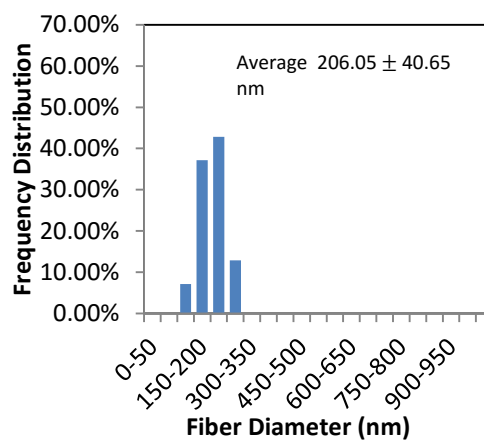
### **5.3 Electrospinning Fiber Morphology and Diameter Distribution**

Electrospun mats fiber morphologies and fiber diameter distributions could influence the moisture triggered thymol release from electrospun mats greatly. In this study, uniform fiber diameter was favored for more controlled release and small fiber diameter was preferred to establish a more RH responsive system. Factors including polymer concentrations were reported to influence the electrospinning nano fiber morphology and

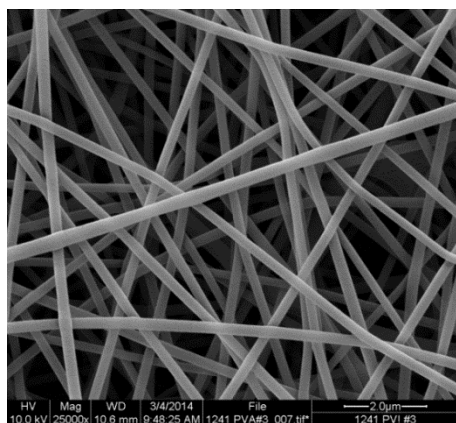
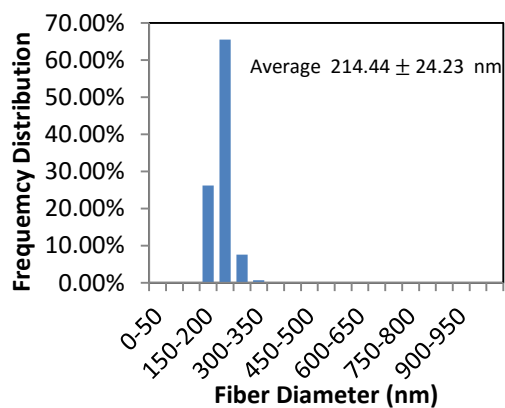
diameter distribution greatly (Jacobs, Anandjiwala, & Maaza, 2010).



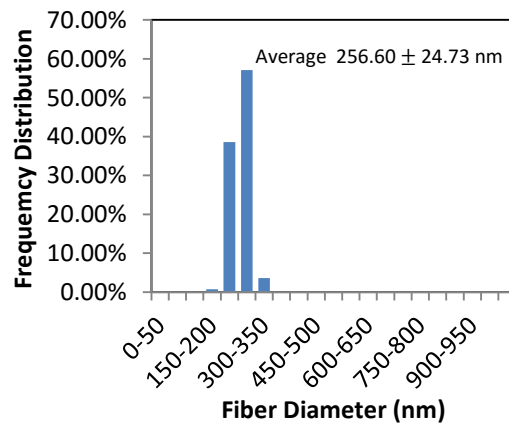
(a)

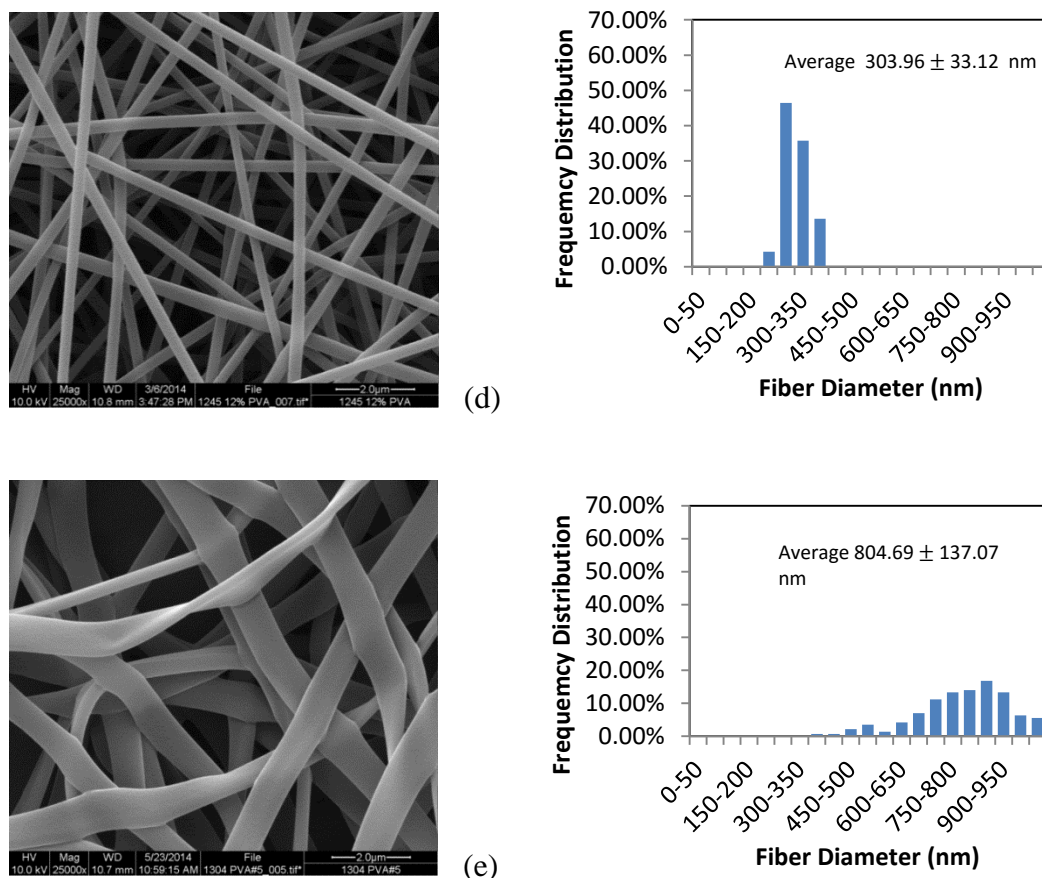


(b)



(c)





**Figure 8 . SEM pictures (25000x) and fiber distribution charts of Poly (Vinyl alcohol)**

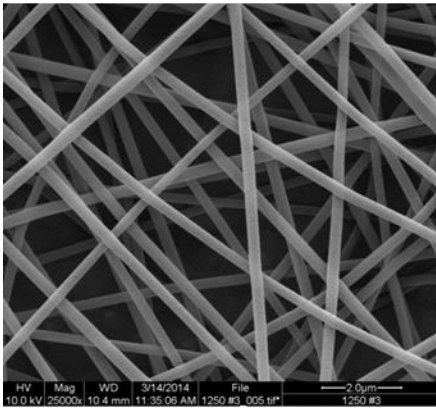
**water solution** (a) 9% wt/wt; (b) 10% wt/wt; (c) 11% wt/wt; (d) 12% wt/wt; (e) 15% wt/wt;

PVA polymer concentrations influenced the fiber diameter distribution and morphology greatly. Fiber diameter distributions showed that the higher the polymer concentration, the larger the fiber diameter, and the more scattered fiber diameter distribution. It was also shown that with the increase in PVA concentration, the fiber morphologies gradually

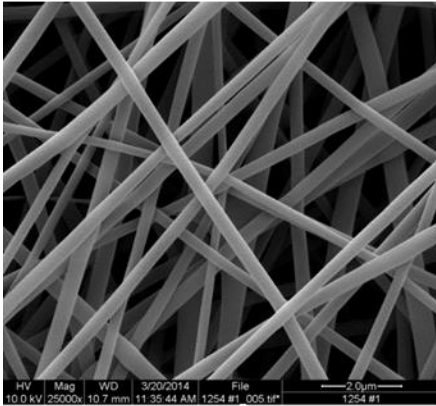
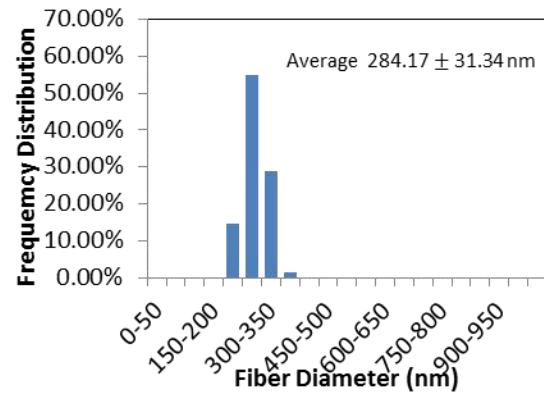
shifted from circular to flat. When the concentration of the PVA reached 15%, the morphology of the fibers flattened. Intrinsic PVA properties could attribute to the morphological changes of the fibers. In PVA water solution, there were intramolecular bindings between hydroxyl groups within PVA and intermolecular hydrogen bonding between PVA molecules and water (Koski, Yim, & Shivkumar, 2004). With the increase in PVA concentrations, there was an increased degree of hydrolysis within the solution which caused an increase in viscosity. The rate of solvent evaporation from the solution was reduced due to the higher viscosity which may further cause the still wet fibers flattened when they hit the collector (Koski et al., 2004). This study helped to determine the best PVA polymer concentration for further research as a balance needed to be reached between fiber uniformity, fiber diameter and PVA concentrations. 12% PVA solution was chosen for its relatively high fiber uniformity and high polymer concentration.

Ethanol and Triton X-100 were also studied to select the more appropriate surfactant for the study. Surfactants' effectiveness to facilitate electrospinning (increase the flow rate), their influence on fiber morphology and fiber diameter distribution were all factors taken into consideration.

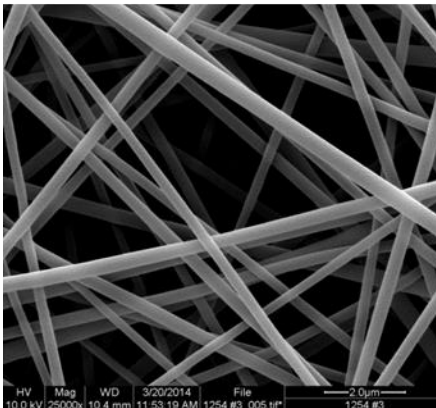
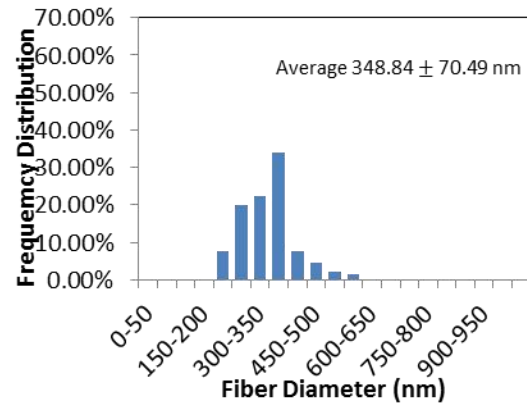




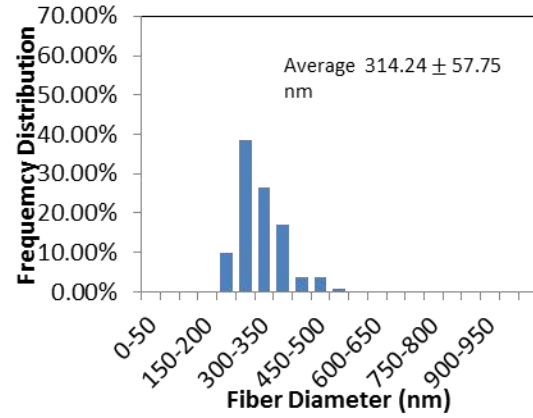
(a)

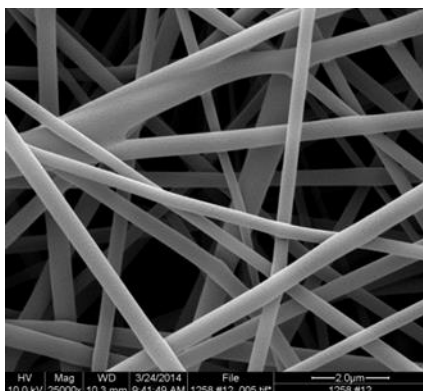


(b)

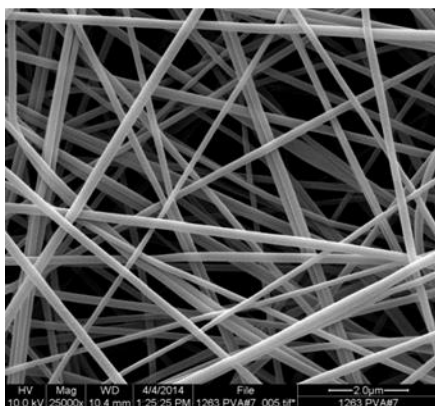
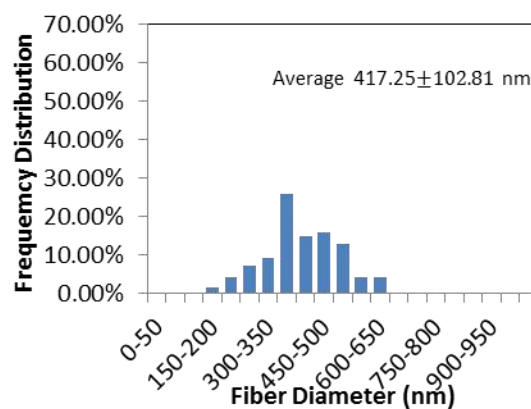


(c)

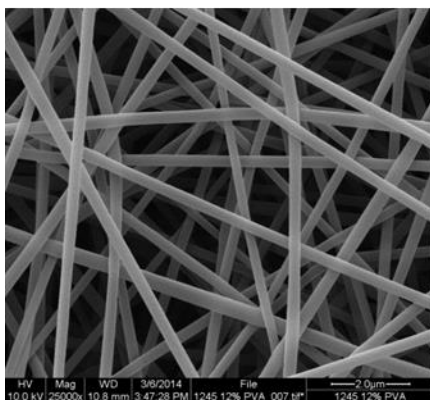
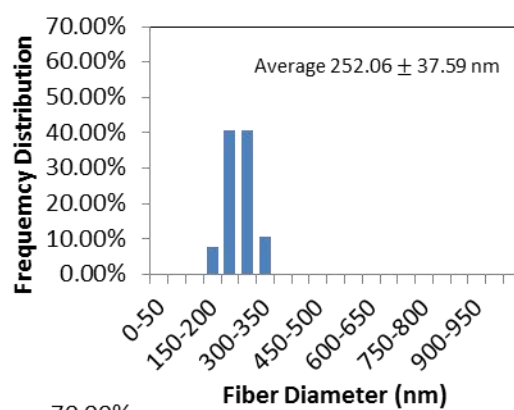




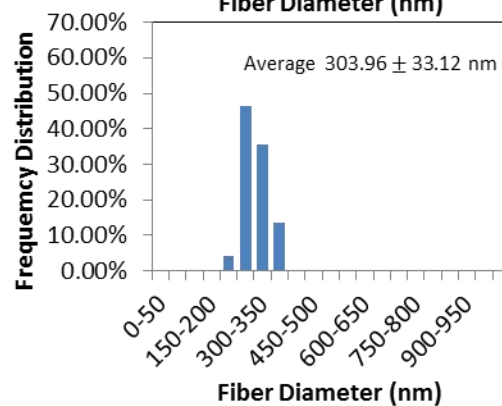
(d)



(e)



(f)



**Figure 9. SEM pictures and fiber diameter distribution charts of surface surfactant**

**addition** (a) 3:97 ethanol: water; (b) 4:96 ethanol: water; (c) 5:95 ethanol: water; (d)

10:90 ethanol: water; (e) 0.1% Triton X-100 addition (f) PVA water solution

From the data above, the addition of ethanol disrupted the fiber morphology and the fiber

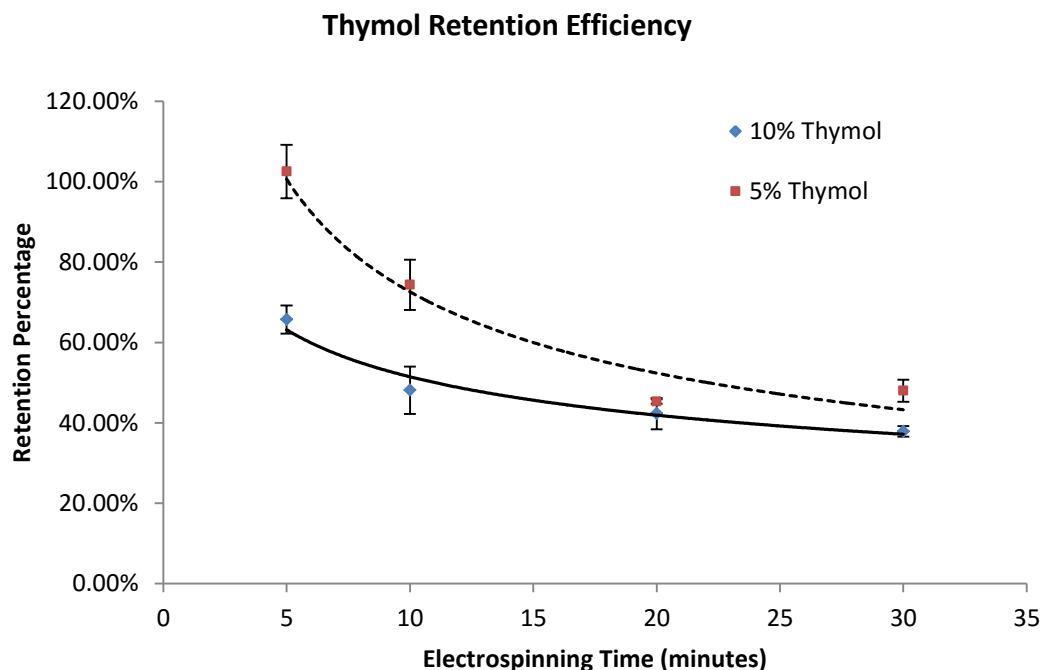
diameter distribution was more scattered. The diameter of the fibers was not largely influenced by the addition of ethanol in 3% ( $284.17 \pm 31.34$  nm), 4% ( $348.84 \pm 70.49$  nm) and 5% ( $314.24 \pm 57.75$  nm) concentrations compared with the control sample ( $303.96 \pm 33.12$  nm). However, with the addition of 10% ethanol, the diameter ( $417.25 \pm 102.81$  nm) was larger and the range of distribution was wider. The effectiveness of ethanol as surfactant was insufficient from the experiment observations and surface tension data.

Triton X-100 was added instead for its higher effectiveness as surfactant. The addition of Triton X-100 reduced the pH of the polymer solution from 6.3 to 6.0 which increased the charge density of the solution which caused the slightly reduced diameter of the nanofibers (from  $303.96 \pm 33.12$  nm to  $252.06 \pm 37.59$  nm). The fiber morphology was not largely influenced by the addition of Triton X-100 into the system. The addition of Triton X-100 increased the flow rate of the fiber forming solution by 15 times from 0.1ml/h to 1.5ml/h which largely increased the amount of thymol encapsulated fiber within time limits.

From the experiment, 0.1% wt/wt Triton X-100 was added as the surface surfactant in the study.

#### **5.4 Loading Efficiency of Electrospinning Process**

Thymol concentrations and electrospinning time were studied to investigate their influences on thymol loadings in the electrospun fiber mats.

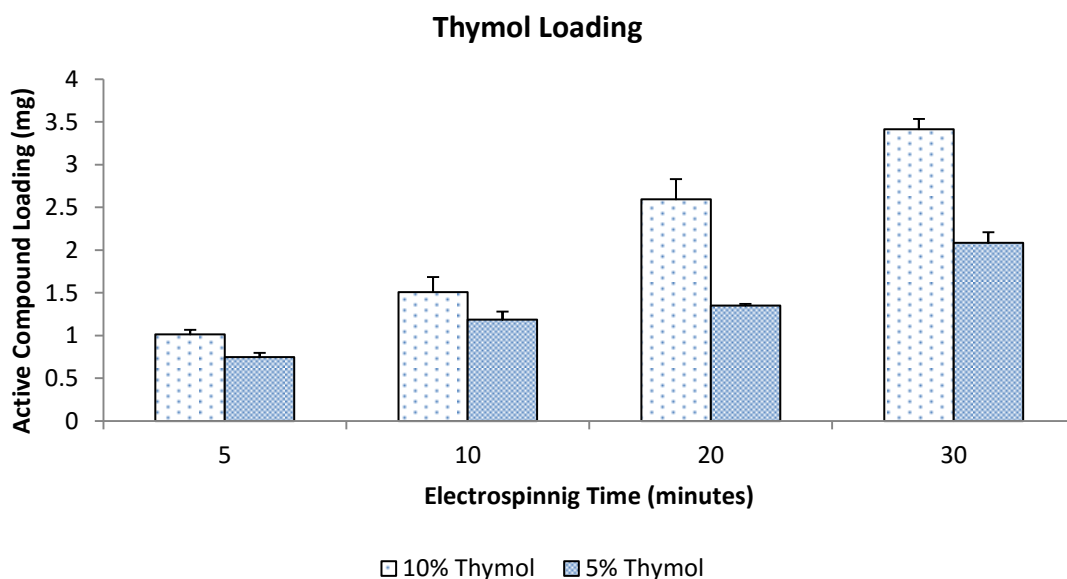


**Figure 10. The retention percentage of electrospun 5% and 10% thymol polymer solutions for 5, 10, 20 and 30 minutes**

The longer the electrospinning time, the lower the retention percentage; and the higher the thymol concentration, the lower the retention percentages were observed from Fig.10. From the result, the retention of thymol during fiber production was largely time dependent ranged from  $37.87 \pm 1.33\%$  to  $65.71 \pm 3.50\%$  retention for 10% thymol electrospun samples from 5 to 30 minutes and  $48.00 \pm 2.73\%$  to  $99.20 \pm 5.36\%$  for 5% thymol concentration samples. The influence of electrospinning time can be explained in terms of

the losses during the process. The combination of large surface area created by nano-fibers and intrinsic volatility thymol were the basis behind the loss.

The influence of thymol concentration on the loading efficiency was more complicated. From Fig.10., it was noticeable that 5% had higher retention percentage compared to 10%. More differences were observed in the beginning stage of electrospinning process (5 and 10 minutes) than later stage (20 and 30 minutes). For 5 minutes samples, 5% thymol addition achieved  $99.2 \pm 5.4\%$  retention while 10% achieved  $65.7 \pm 3.5\%$  retention. It was speculated that for 10% thymol concentration fibers mats there were excess amount of thymol compounds resided on the surface of the electrospun fibers which were exposed to the surrounding environment instead of incorporated into the polymer matrix. Instead of diffusing out of the polymer matrix, the excess amount of thymol directly evaporate into the surrounding environment which caused the higher loss for 10% thymol samples in the beginning stage of process. At later stage, the losses were similar for both concentrations as both of them were majorly caused by the diffusion of thymol compounds from PVA polymer matrix.



**Figure 11. The loading of active compounds by electrospun 5% and 10% thymol addition polymer solutions for 5, 10, 20 and 30 minutes**

The data obtained could also help to select the best thymol concentration and electrospinning time combination in real applications. Despite the lower loading efficiency with electrospinning time, the total thymol loading amount increased over time. This result showed that base on the final application, different approaches needed to be taken to achieve the balance between electrospinning time and thymol concentration.

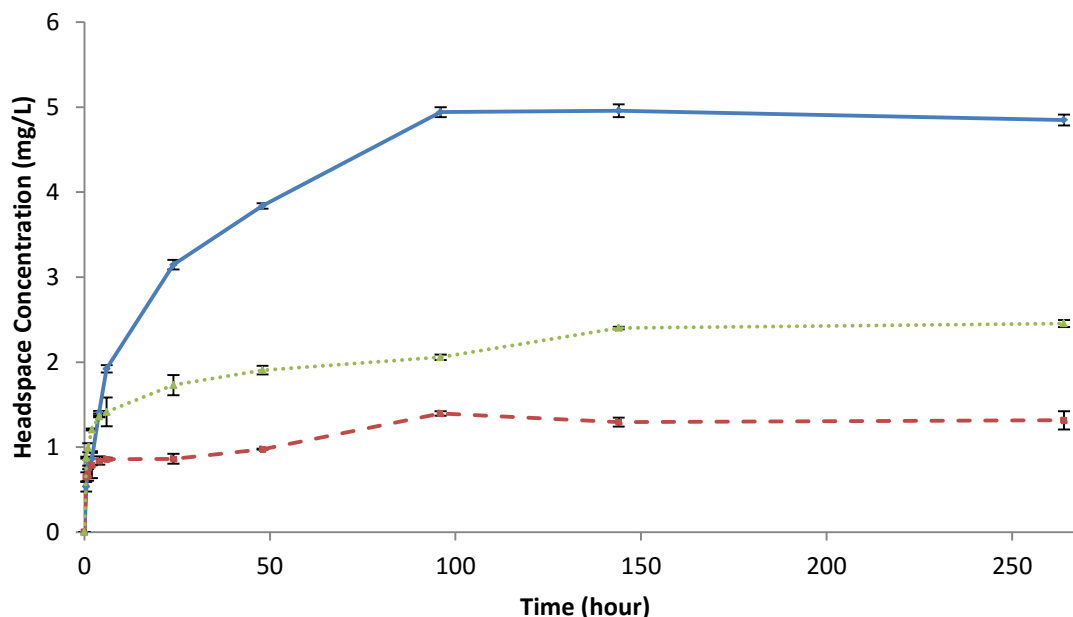
The range of the thymol loading in the study was from 0.74 mg/film (5%, 5min) to 3.9 mg/film (10%, 30min) from calculation which fit the ideal thymol concentration range to achieve microbial inhibition without posing too much sensory influence. This result validated the concept of using electrospinning as the method to construct active

packaging to inhibit the growth of microbes in fresh produce.

The thymol loading efficiency study also laid foundation for the application of thymol incorporated electrospun mats onto food system for antimicrobial effectiveness as an estimation of total amount of thymol in the mats could be made. In addition, if the effective concentration of the active compound on certain microbe was known, the most efficient thymol concentration and electrospinning time combination could be derived from the charts.

#### **5.5 Release profile study of electrospun mats under 0, 75 and 100% RH**

Thymol encapsulated electrospun mats were placed in the jars of 0, 75 and 100% RH to test the feasibility of this moisture triggered release system. The moisture triggered release of thymol from electrospun mats was likely caused by plasticization effect of water and dissolve of PVA fibers.



**Figure 12. The release profile of 10% thymol addition electrospun mats at 100%; 75% and 0% over the period of 11 days**

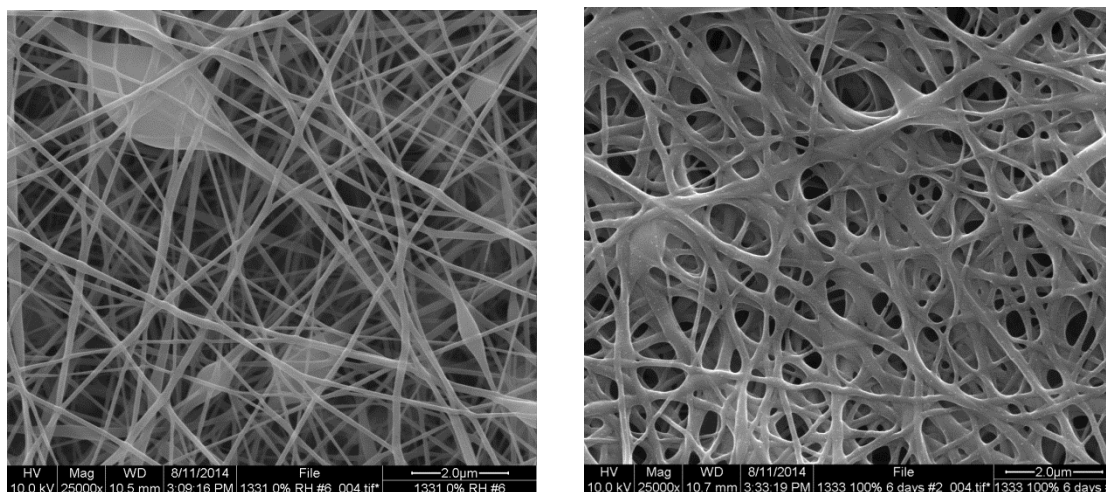
Fig.12. showed the rate of release of thymol was found to increase with relative humidity: very low at 0% RH, somewhat higher at 75% RH, and much higher at 100% RH. The equilibrium concentrations for 0, 75 and 100% RH were 1.3mg/L, 2.4mg/L and 5mg/L respectively. In addition, the release of thymol at 0, 75 and 100% RH were similar in the first 2 hours of the study which indicated the dominant direct evaporation of thymol at the surface of the PVA fibers. At 0% RH, the release of thymol was mostly within the first 2 hours from the direct evaporation of active compounds and negligible release was observed at later stage. At 75 and 100% RH, thymol continued to release for the next hours due to the swelling and dissolve of the PVA fibers. For both 75 and 100% RH samples, it took the



samples around 100 hours to reach equilibrium which showed the slow release of thymol from PVA electrospun fiber mats over time. However, the release thymol in 100% RH was more than double the amount in 75% RH which suggested that high RH can be used as a trigger to release thymol in this system.

The trend of the higher the RH the more active compounds released showed the RH dependent release nature of electrospun nanofiber mats. In addition, for all three RHs, it took the sample around 100 hours (4 days) to reach equilibrium which showed the slow release of antimicrobial thymol over time compared to one time addition.

For a better understanding of the swelling and dissolve of PVA electrospun fibers, SEM pictures were taken for 6 days exposure samples in 0 and 100% RH for comparison.



**Figure 13. The 25000x SEM pictures for 6 days exposure of electrospun mats at the RH of 0% (left) and 100% (right)**

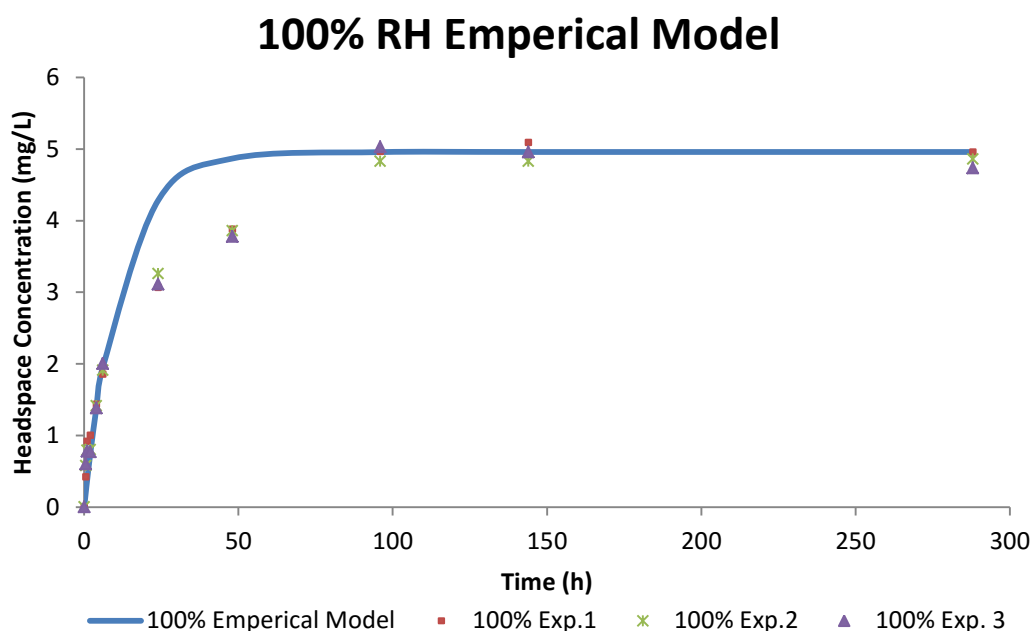
Significant differences in the morphologies of the PVA fibers were observed. The PVA fiber structure swelled and dissolved in 100% RH which could explain the triggered release of thymol from electrospun mats at high RH.

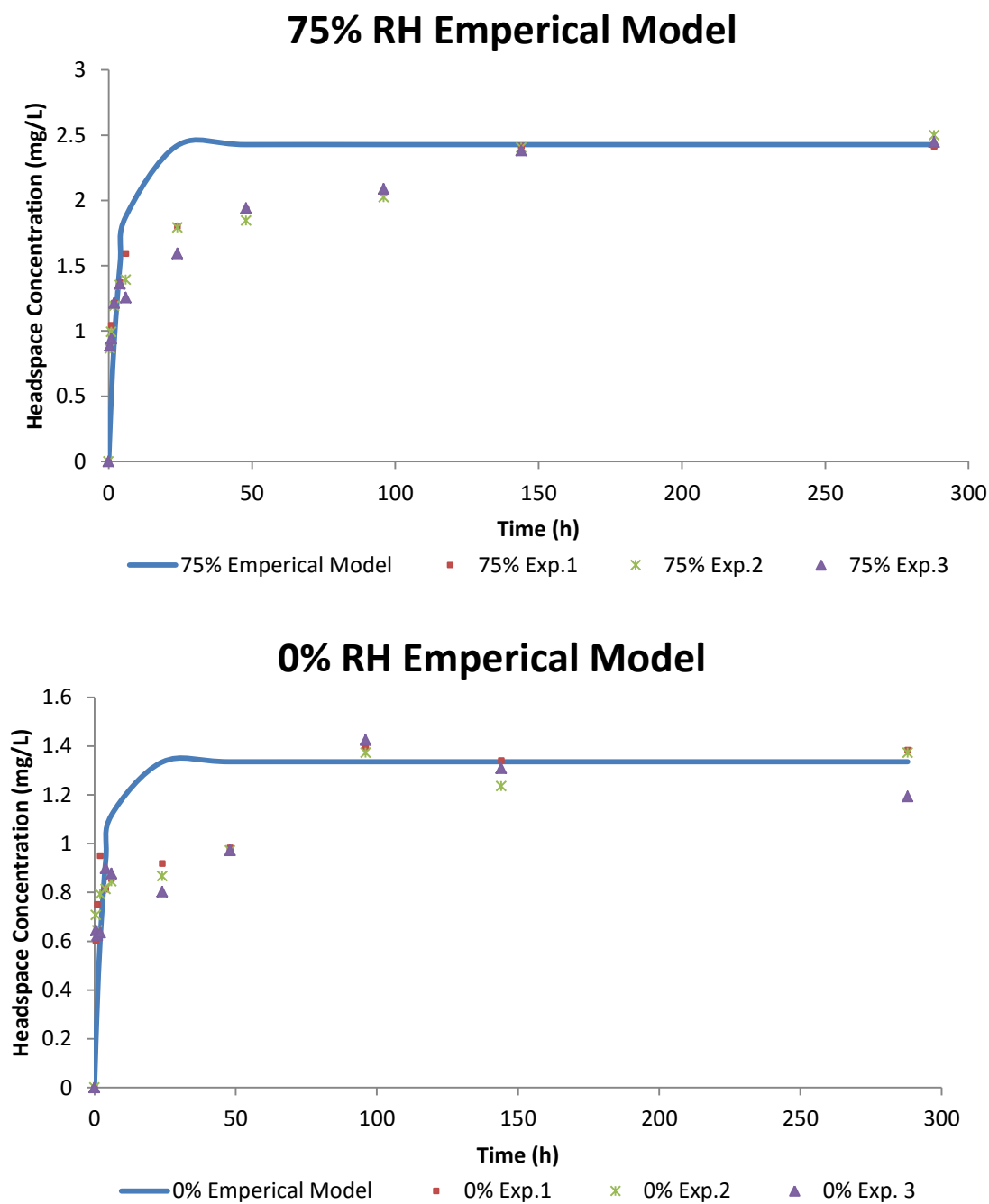
### 5.6 Release Profile Modeling

Empirical mathematical model which is based on the pseudo first kinetics was used to fit the curve of the release profiles, equation below was used for the curve fitting of the system:

$$\frac{C_e - C}{C_e - C_0} = e^{-kt}$$

$C_e$ : equilibrium concentration;  $C_0$ : initial concentration which is 0;  $C$ : concentration at specific time;  $t$ : time;  $k$ : rate constant





**Figure 14. Modeling of the release profile of thymol in RH environment of 0%, 75% and 100%**

Calculating K (rate constant) was one of the major challenges in getting the empirical model for the release profiles. K determines the slope for the release profile which plays major role especially in the beginning of the modeling. The larger the K was, the steeper the model was. From the experiment, there is a small discrepancy between the model and experiment data around hour 50. This discrepancy is also an indication for the two possible release mechanism talked in the thesis before:

1. The fast release from the direct evaporation of active compounds from the surface of the nanofibers due to the saturation of thymol inside the nanofibers.
2. The slow release from the diffusion of active compounds from inside nanofibers and dissolve of the fibers under high RH environment.

The first release mechanism is more prevalent in the beginning stage of the release and the second release mechanism is more in the later stage. The combinations of the two mechanisms made the release fast in the beginning and slow in the latter stage. In addition, as the K values were determined from the data obtained in the beginning of the experiment which explains the discrepancies we obtained in between the experiment data and modeling lines.

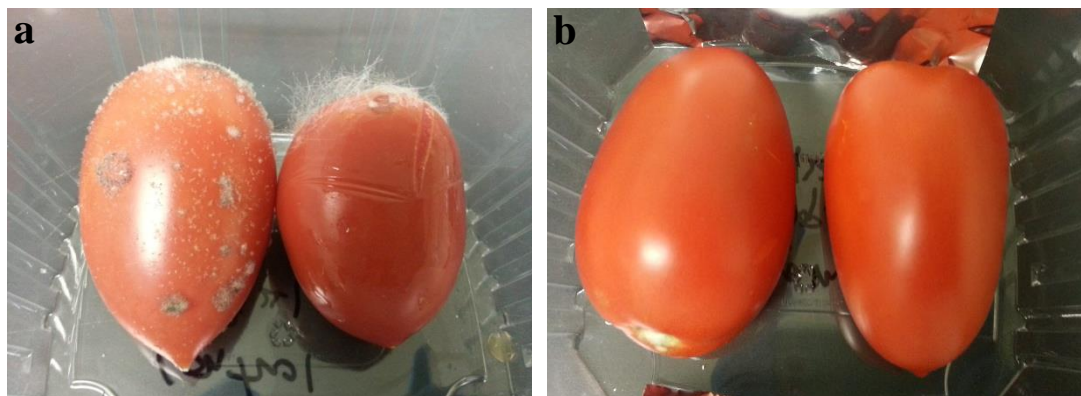
### **5.7 Shelf Life Extension Effectiveness**

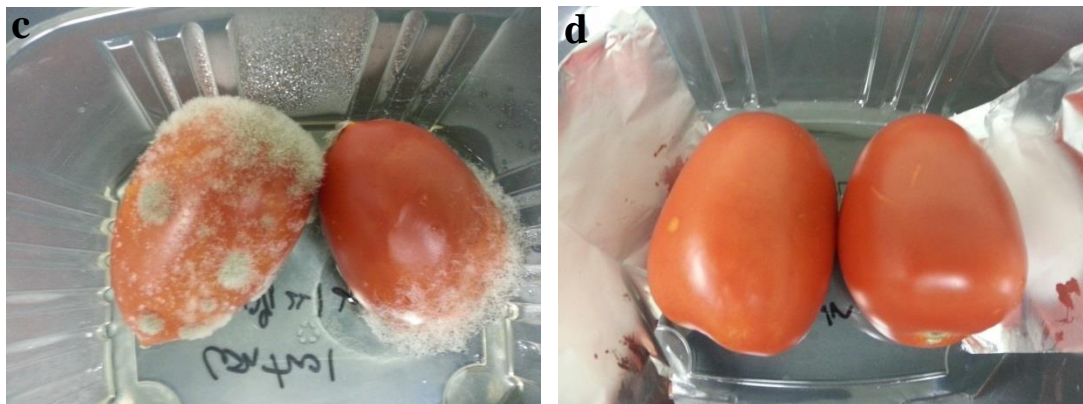
From what was discussed in DOE before, three questions needed to be answered from the experiments: 1. whether the moisture produced by the respiration of fresh tomatoes

enough to trigger the release of active compounds thymol from electrospun nanofibrous mats and 2. to what extent does the released thymol being able to extend the shelf life of fresh tomatoes and 3. the sensory influences of thymol exposure pose on the fresh tomatoes.

For the first question, it is confirmed that the moisture produced from the respiration of fresh tomato was enough to trigger the release of thymol from electrospun mats. The triggered amount was able to inhibit microbial growth on the surface of the fresh produce to extend the shelf life. However, the amount triggered and the release rates needed further investigation.

For the second question, the results below showed the effectiveness of thymol encapsulated electrospun mats in extending the shelf life of fresh tomatoes.





**Figure 15. a and b: 7 days pictures of the control and experiment samples of tomatoes; c and d: 12 days picture of the control and experiment samples**

It is confirmed that the RH conditions created by fresh tomatoes were enough to trigger the release of thymol from electrospun mat and the triggered release could extend the shelf life of fresh tomatoes. From the 7 days samples, the control sample is already moldy with the experiment samples remain fresh from the appearances. When the experiment days extended to 12 days, there were even more distinctive differences between the two samples. From the experiment data, the shelf life of fresh tomatoes could be extended by at least 5 days with the application of thymol encapsulated electrospun nanofibers in the system. This study provided a possible application of this moisture triggered mats on fresh produce to extend their shelf life.

Sensory evaluations were done to evaluate the consumer acceptance of the sensory changes occurred during the 7 days exposure to thymol at the concentrations released from the electrospun mats. The smell and color hedonic tests scaled from 1 to 9 were

used.

**Table 5: Hedonic Test Scores for 7 days Experiment Samples**

Overall Smell	Overall Color	Smell: thymol familiar	Smell: thymol not familiar
<b>6.58</b>	<b>8.17</b>	<b>7.0</b>	<b>6.29</b>

From the results above, the smell of the fresh tomatoes was scaled between “Liked Slightly” to “Like Moderately”. Between the participant who were familiar with the smell of thymol and who were not, there was not significance between their ratings of the experiment samples. For comments from the participants, for the people who were familiar with the aroma of thymol, they could sense thymol on the tomatoes but the smell was not repellent for them and the predominant smell was still tomato. For the participants who were not familiar with the smell of thymol, slight minty, grassy and medicinal smell was detected. In conclusion for the olfactory influence of the thymol encapsulated electrospun mats, the smell was not repellent or even pleasant to all participated subjects. The color of the samples was between “Like Very Much” to “Like Extremely” which showed that for almost all participants, the color of the treated experiment samples looked fresh and normal.

The results further verify the shelf life extension effectiveness of the active compound

thymol released from electrospun mats at the concentration enough to pose antimicrobial effectiveness without causing unacceptable sensory influences on fresh produce.



## 5. CONCLUSION

The results confirmed the feasibility of developing a moisture triggered release system of thymol using electrospinning technology to extend the shelf life of fresh tomatoes. First, research parameters were evaluated to produce thymol encapsulated electrospun mats with 0.1% Triton X-100, 12% PVA concentration and 10% thymol addition being the optimum in both fiber mats production and fiber morphology. Second, the moisture triggered system was confirmed by the different release profiles thymol in RH of 0, 75 and 100% over 11 days. The rate of release of thymol was found to increase with relative humidity: very low at 0% RH, somewhat higher at 75% RH, and much higher at 100% RH. The results also suggest the possibility to achieve controlled release packaging by controlling the RH. Third, thymol encapsulated electrospun mats were applied on to fresh tomatoes. It was shown that the moisture produced by the respiration of fresh tomatoes was enough to trigger the release of thymol and the released thymol was enough to extend the shelf life of fresh tomatoes by at least 5 days without causing unacceptable sensory changes.

All in all, this study provides quantitative and qualitative data from the construction of thymol encapsulated electrospun mats to the application of the mats on the fresh tomatoes to extend their shelf life. The system could also be applied onto other fresh produces provided the fresh produces create RH conditions enough to trigger the release of active compounds. The positive results further showed the potential to use electrospinning as a

technique to construct nanofibers to encapsulate antimicrobial or antioxidant active compounds for the controlled release of the compounds to reduce post-harvest losses or other purposes.

## 6. FUTURE WORK

From the encapsulation itself, as thymol is volatile which caused the losses during production in this study, double encapsulation could be used to encapsulate thymol within a primary shell and then into the nanofiber. For example, beta-cyclodextrin could be one of candidates in the double encapsulation system. In addition, by double encapsulate the active compound, it is possible to achieve a more controlled release system. To suit the needs of different applications or different release profiles, methods include double or triple layer the mats with different materials or active compounds of different concentrations could also be studied in the future.

In this study, the release profiles of thymol were measured in the RH of 0%, 75% and 100% environments. However, no study was done to measure the release profile of thymol triggered by the moisture from real food system. For the next stage, the release profile could be measured in real food systems.

For the modeling of the release, more data could be collected to establish an equation to estimate the release under different RH throughout the application of the mats. A more complicated modeling could also be established taking temperature, oxygen/carbon dioxide level and the package into consideration. The establishment of the models could help us predict the headspace concentration and release profile of the active compounds during its application to maximize the effectiveness of the method.

In addition, instead of shelf life extension study, more quantitative antimicrobial experiments could be conducted to understand exactly to what extend the thymol encapsulated mats are able to inhibit the growth of microbes. Spoilage microbes include *pseudomonas* spp. and pathogenic microbes such as *salmonella* spp. could be inoculated onto plates or real food to measure the antimicrobial effectiveness.

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