## A DISSERTATION REPORT ON

## **Studying Effect of Different PVA-Based Formulations on Film Formation and Antimicrobial Performance**

Submitted in partial fulfillment of the requirement for the award of degree of

#### **MASTER OF TECHNOLOGY**

By

#### **ARVIND KUMAR YADAV**

17555003

Under the guidance of

Dr. ASHISH A. KADAM



# DEPARTMENT OF PAPER TECHNOLOGY INDIAN INSTITUTE OF TECHNOLOGY ROORKEE SAHARANPUR - 247001 (INDIA)

## **MAY-2019**

I hereby certify that the work which is presented in this project entitled, "Studying Effect of Different PVA-Based Formulations on Film Formation and Antimicrobial Performance", in fulfilment of the requirement for the award of the degree of Master of Technology in "Packaging Technology", submitted in Department of Paper Technology, Indian Institute of Technology, Roorkee is an authentic record of my work carried out under the guidance of Dr. Ashish A. Kadam, Assistant Professor, Department of Paper Technology, Technology, Indian Institute of Technology, Roorkee Saharanpur campus.

I have not submitted the matter embodied in the project for the award of any other degree.

Date: 24/05/2019

#### Arvind Kumar Yadav

Enrolment No. 17555003

This is certified that the above statement made by the candidate is correct to best of my knowledge.

Date: 24/05/2019

200

#### Dr. Ashish A. Kadam

(Assistant Professor) Department of Paper Technology

Indian Institute of Technology

Roorkee

I feel greatly honoured in presenting this project report in such an authentic form of altogether endurance and continuous efforts of inspiring excellence from various cooperation and sincere efforts drawn from all sources of knowledge. I express my sincere gratitude to **Dr. Ashish A. Kadam, Assistant Professor, Department of Paper Technology, Indian Institute of Technology Roorkee** for his valuable guidance and support for the completion of this project.

I extend my thanks to all my classmates and seniors who have given their full cooperation and worthwhile suggestion for providing me with the necessary assistance to carry out the accomplishment of my project.

Above all, my parents and my family members for their moral support and continuous encouragement while carrying out this study.

Date: 24/05/2019

 $\mathcal{L}_{\mathcal{L}}$ 

**Arvind Kumar Yadav** 

Enrolment No. 17555003

#### ABSTRACT

Active food packaging is encouraging and rapidly emerging technology in which the antimicrobial agents are incorporated into the packaging materials. It can provide the packed food high quality, safety, and long shelf life, normally by reducing or retarding the growth of microorganisms. Recently, there is great interest in active food packaging films made from biodegradable polymers because of serious environmental problems caused by conventional plastic food packaging material.

Antimicrobial PVA films of different concentrations of 1%, 2%, and 3% were prepared by incorporating cinnamaldehyde at a concentration of 15% and experiment has been performed. The growth of microbial species which is gram-negative bacteria E.coli was found to be significantly less in antimicrobial films when compared with reference film. Moreover CFU was decreased by increased CIN content in the films. The aim of this study was to develop active packaging films by incorporating CIN into PVA polymer and investigated their mechanical and physicochemical properties. The antimicrobial activity of the films in vapour phase was assessed by examining the microbiological analyses.



## **ABBREVIATIONS**

PVA -	Polyvinyl alcohol
CIN -	Cinnamaldehyde
E.coli-	Escherichia coli
EO(s) -	Essential oil(s)
DMSO -	Dimethyl sulfoxide
FT-IR -	Fourier-Transform Infrared Spectroscopy
FE-SEM-	Field emission-scanning Electron Microscope
ASTM-	American Society for Testing and Materials International
FDA-	Food and Drug Administration (USA)
GRAS-	Generally Recognized as Safe
MIC-	Minimum inhibitory concentrations
DSC-	Differential scanning calorimetry
EAB-	Elongation at break
TGA-	Thermogravimetric analysis
	CONTRACTION OF THE OWNER OWNER OF THE OWNER OW

## LIST OF FIGURES

Figure 1.1: Conventional vs. Antimicrobial Packaging System
Figure 1.2: Antimicrobial packaging systems
Figure 3.1: Chemical formula of CIN compound present maximum % in cinnamon oil6
Figure 3.2: Micrometre9
Figure 3.3: (a) FT-IR (b) FE-SEM10
Figure 3.4: Instron universal testing machine
Figure 3.5: AM testing setup by Headspace method14
Figure 4.1: TGA degradation curve16
Figure 4.2: DSC graph of samples17
Figure 4.3: FT-IR spectra of CIN, PVA and PVA+CIN
Figure 4.4: FT-IR spectrum for different samples20
Figure 4.5: FE-SEM pictures showing surface morphology of (a) 1% PVA, (b) 1% AM PVA,
(c) 2% AM PVA, (d) 3% AM PVA22
Figure 4.6: FE-SEM pictures showing Cross-sectional morphology of (a) 1% PVA,
(b) 1% AM PVA, (c) 2% AM PVA, (d) 3% AM PVA23
Figure 4.7: Tensile strength vs extension graph25
Figure 4.8: Total bacterial colony of E.coli for (a) 1% PVA, (a) 1% AM PVA,
(a) 2% AM PVA, (a) 3% AM PVA after 24 hours27
Figure 4.9: Bar graph display average CFU/ml for E. coli of samples after 24 hours
incubation
Figure 4.10: Total bacterial colony of E.coli for (a) 1% PVA, (a) 1% AM PVA, (a) 2% AM
PVA, (a) 3% AM PVA after 48 hours28
Figure 4.11: Bar graph display average CFU/ml for E. coli of samples after 48 hours
incubation
Figure 5.1: Application of the AM PVA film

## LIST OF TABLES

Table 2.1: Chief components of EOs which show antibacterial effect	4
Table 2.2: MICs of Antimicrobial agent that tested in vitro against different foodborne	
pathogens	5
Table 3.1: Description of samples	8
Table 4.1: Colour test values of the sample	.15
Table 4.2: Characteristic peaks of FTIR for PVA and CIN	19
Table 4.3. TS. Thickness, and EAB of the samples	.24



## TABLE OF CONTENTS

1.	Introduction	
	1.1. Why antimicrobial packaging?	1
	1.2. Antimicrobial packaging system	.2
2.	Literature Review	4
	2.1. Essential oils	4
3.	Materials, Experimental Setup and Methodology	
	3.1. Materials	
	3.1.1. Polyvinyl alcohol	6
	3.1.2. Cinnamaldehyde (CIN)	6
	<ul><li>3.1.2. Cinnamaldehyde (CIN)</li><li>3.1.3. Dimethyl sulfoxide (DMSO)</li></ul>	7
	3.1.4. Nutrient Broth	.7
	3.1.5. Nutrient Agar	7
	3.2. Film Preparation	7
	3.3. Film Characterization	9
	3.3.1. Film Thickness	
	3.3.2. Fourier-transform infrared spectroscopy	.9
	3.3.3. Field emission scanning electron microscope	
	3.3.4. Mechanical Testing	11
	3.3.5. Statistical Analysis	11
	3.4. Antimicrobial Testing and Setup	
4.	Results and Discussion	15
	4.1. Film colour	15
	4.2. TGA	16
	4.3. DSC	17
	4.4. FT-IR Analysis	17
	4.5. FE-SEM	.20
	4.6. Mechanical properties.	23
	4.7. Antimicrobial activity	25
5.	Conclusion	
6.	References	

#### **1. INTRODUCTION**

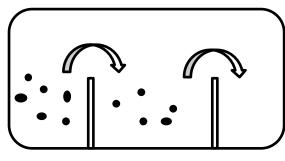
The main function of packaging is to save the product from any hazardous components present in the environment. The hazard component may be moisture, gases, odours, microorganisms, dust, and mechanical stresses because these elements lead to a decrease in the shelf-life of the food product ultimately affect consumer health. Moreover, with the rising population and exhausting resources, food safety has become a major issue at the world level nowadays due to the above concern food safety has become an equitably essential as food production. Therefore, great challenges for researches to innovate such food packaging material which can protect not only chemical and physical elements but also from microbes.<sup>[1]</sup>

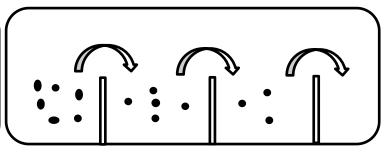
Active packaging can be referred to as interactive packaging which senses the internal or external environment and responds by changing the internal package environment by changing its own properties. The active function can be scavenging of odor, oxygen, moisture, or other unwanted substances, and emission of antimicrobial substances or other desired agents.

## 1.1 Why antimicrobial packaging?

Antimicrobial packaging is a subcategory of active packaging. It has accepted great attention due to its potential to enhance food safety, quality and improve the shelf-life of the packaged food. Apart from that the consumer demand for minimally processed and no preservatives used in food. It added several features which are absent in conventional packaging.

The basic difference between conventional as well as antimicrobial packaging is hurdle technology while both packaging technique must be satisfying other functions.<sup>[2]</sup> Conventional packaging does not inhibit the microbes whereas antimicrobial packaging inhibits microbes which are shown in Figure 1.1.





## Moisture Barrier Gas Barrier Moisture Barrier Gas Barrier Microbial Barrier

Conventional Packaging System

Antimicrobial packaging System

Figure 1.1: Conventional vs. Antimicrobial Packaging System

### 1.2 Antimicrobial packaging systems

There can be various forms of antimicrobial packaging system including:

- Addition of volatile antimicrobial pouch/pad in package
- Incorporating antimicrobial substances in polymers
- Coating antimicrobial agents on polymer films surfaces
- Immobilization
- The use of polymers which are naturally antimicrobial

There can be two types of antimicrobial packaging films:

- Food-packaging films which allow the migration of antimicrobial agents into the food. Figure (A) is a single layer packaging system which incorporated antimicrobial substances then releases steadily into the food. Fig (B) shows the identical idea however using another layer that could be used to control the release of antimicrobials. Fig (C) represents the single layer with a coating of the antimicrobial compound.<sup>[3]</sup>
- 2) Fig (D) Films which do not release antimicrobials and prevent microorganism growth

Direct contact of food is necessary, for both types of system, to improve the performance of the antimicrobial activity.

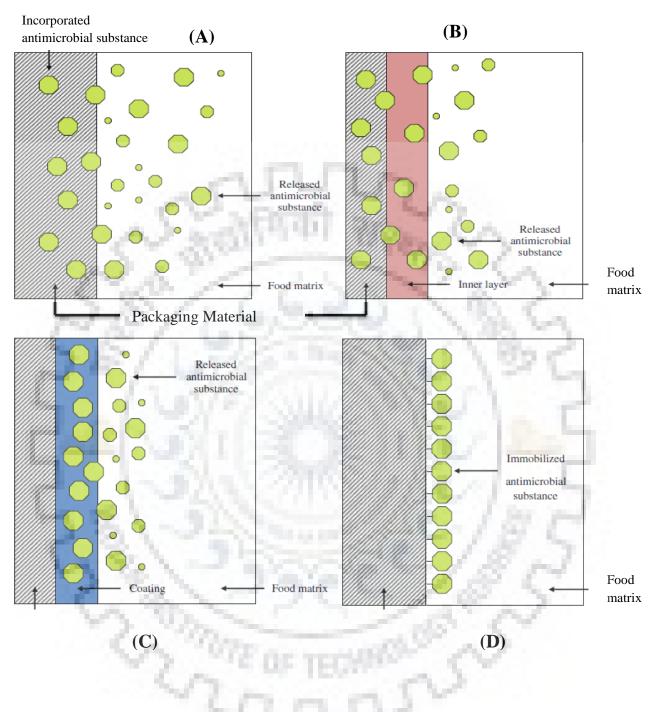


Figure 1.2: Antimicrobial packaging systems

#### **1. LITERATURE REVIEW**

#### **2.1** *Essential Oils*

In vitro study, the antibacterial activity of EOs and its component against bacteria grampositive (B. subtilis) and gram-negative (E. coli) have been studied. However, gram-negative bacteria are less susceptible than gram-negative. Essential oils are an aromatic oily volatile liquid which is extracted from numerous parts of the plants for example flowers, bark, seeds, leaves, fruits, wood, roots, etc. Natural antimicrobials draw attention to consumers and foodindustries because they are natural preservatives and safe or human health. Natural antimicrobials agents can be of from plant sources, animal, bacterial, algae/ mushrooms, etc. Apart from essential oils, there are several EO components have been recognized as effective antimicrobial substances. Table 2.1 and 2.2 show the antimicrobials and their MICs. MIC is defined as the lowest concentration required for complete inhibition of test bacterium equal to 48 hours incubation.<sup>[4]</sup>

Table 2.1: Chief components of EOs which show antibacterial effect

EOs	Major component	Approximate % composition
Cinnamon	Cinnamaldehyde	64%
Clove	Eugenol	74–85%
Thyme	Thymol	10–64%
Coriander	Linalool	70%
Rosemary	a-pinene	2 –25%
Oregano	Carvacrol	80%

Table 2.2: MICs of Antimicrobial agent that tested in vitro against different foodborne pathogens

Agents	Bacteria	MIC, approximate range (µl/ml)	
Cinnamon oil	E. coli	6.25-25	
cinnamaldehyde	E. coli	0.78-12.5	
Clove	E. coli	0.41-2.5	
Thyme	L. monocytogenes	0.3	
Turmeric	E. coli	>0.2	
Rosemary	E. coli	4.5–10	
Oregano	E. coli	0.48-1.2	
Carvacrol	E. coli	0.25–5	

EOs consists of a huge number of compounds and it is generally their mode of action implicates numerous goals in the bacterial cell. The EOs permeates into the lipid of the cell membrane and mitochondria due to their hydrophobic nature which leads to leakage of cell constituents. Physical conditions that can enhance the action of essentials oils are low temperature, low pH and low oxygen levels. Essentials oils are identified for ensuring antimicrobial properties, Moreover EOs or their components shows antiviral, antioxygenic, insecticidal, antimycotic and other properties as well.

There are various types of polymers which can be used as a potential application for antimicrobial food-packaging. Unconventional polymers are mostly non-biodegradable that can be accumulated in the environment which can lead to serious environmental concerns. <sup>[5]</sup> After consuming food, disposal is also an important concern of food consuming materials. Therefore, polyvinyl alcohol which is biodegradable polymer was used in active food packaging material. <sup>[6]</sup>

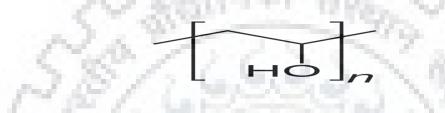
#### 2. MATERIALS, EXPERIMENT SETUP, AND METHODOLOGY

#### 3.1 *Materials*

Materials were purchased from Hi-Media Laboratories Private Limited, India.

#### 3.1.1 Polyvinyl alcohol

It is a synthetic water-soluble polymer with the formula of  $[CH_2 CH (OH)]_{n}$ . It is white in color and odorless. Here, partially hydrolyzed PVA with a degree of hydrolysis is (86.5-89) mol %.



#### 3.1.2 Cinnamaldehyde (CIN)

It has a chemical formula  $C_9H_8O$  and its molecular weight is 132.16 g/mol. It is the major component in cinnamon oil and it has more effective in antimicrobial activity, and able to kill E. coli, C. albicans and S. aureus, than cinnamon essential oil constituents.<sup>[7]</sup> Apart from antimicrobial, CIN is also exhibited antifungal, antiviral and effective control of growing mould and east as well. The MIC of CIN and the cinnamon essential oil is in a range of 0.78–12.5 and 6.25–25 µL/ml respectively.

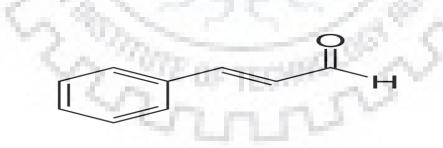
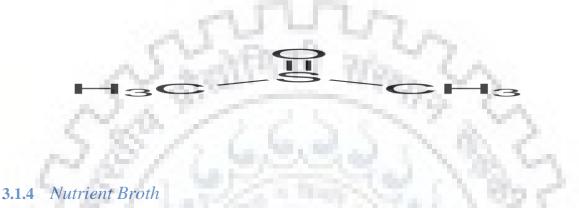


Figure 3.1: Chemical formula of CIN compound present maximum % in cinnamon oil

#### 3.1.3 Dimethyl sulfoxide (DMSO)

This one is an organic compound which contains sulfur with formula  $(CH_3)SO$  and it's molar mass is 78.12 g/ mol. Polar and nonpolar compounds can dissolve in DMSO. Most importantly, this is miscible in water in addition to a broad range of organic solvents as well. Another reason to use this solvent because of essential oils has low water solubility. Hence, DMSO enhances the solubility of hydrophobic compounds.



# This is used to prepare bacterial culture for growth and cultivation for the production of fastidious microorganisms. This is a liquid medium that contains ingredients such as tryptone, yeast powder, and other ingredients. Blood or additional biological liquids can be augmented for less fastidious microorganisms. It can be suspended 1.3 % (w/v) in distilled water. It can be sterilized by autoclaving at 1.0342 bar pressure 121 °C for 15 minutes.

#### 3.1.5 Nutrient Agar

It is used for the isolation and preservation of bacterial culture. It can be suspended 2.8 % (w/v) of distilled water. It can be dissolved completely by autoclaving at 1.0342 bar pressure 121 °C for 15 minutes sterilization process. If wanted, this medium can be enriched with 5-10 % blood or biological fluids.

#### 3.2 Sample Preparation

The PVA film samples were prepared incorporated with CIN by solution casting methods. For neat PVA films, The PVA solutions were prepared via dissolving PVA (1% w/v) in distilled water at 80 °C. The solution was stirred about 4 hours for proper dissolution. Thereafter, the calculated quantity of the solution was poured into a Petri plate and allowed them to dry at 55 °C for 24 h.

For PVA + CIN films, 15% CIN (v/w) of PVA on the dry basis was added into 1% DMSO (v/v) of the final volume of solution and mix well. After that, add CIN solution in PVA solution and continue stirring for another 15 minutes at 45 °C. Thereafter, a miscellaneous solution was homogenized at 5000 revolutions per minutes for 5 minutes with IKA T25 Ultra Turrax homogenizer. After that films were cast of required volume into Petri dishes. After drying, the film samples were peeled off from Petri dishes and wrapped in the aluminium foil for further analysis. Sample IDs, compositions and other important parameters designated in Table 3.1.

Sample Code	Composition	Average Drying Time in hours
1% PVA Film	1% PVA (w/v) of distilled water: dry weight basis/final volume of solution	24
1% AM PVA Film	1% PVA (w/v) of distilled water - dry weight basis/final volume of solution, 1% DMSO (v/v) - Vol. of DMSO/ final volume of solution, and 15 % CIN (v/w) - CIN volume/ dry weight of PVA	24
2% AM PVA Film	2% PVA (w/v) of distilled water - dry weight basis/final volume of solution, 1% DMSO (v/v)-Vol. of DMSO/ final volume of solution, and 15 % CIN (v/w) - CIN volume/ dry weight of PVA	12
3% AM PVA Film	2% PVA (w/v) of distilled water - dry weight basis/final volume of solution, 1% DMSO (v/v)-Vol. of DMSO/ final volume of solution, and 15 % CIN (v/w) - CIN volume/ dry weight of PVA	9

Table 3.1: Samples Description

#### 3.3 Film Characterization

#### 3.3.1 Film thickness

The thickness of each film was measured at five arbitrary points with a micrometre (Lorentzen & Wettres, Sweden) that least count is 0.005 mm and the average thickness was determined for each film samples.



#### 3.3.2 Fourier-transform infrared spectroscopy

This analysis was done by attenuated total reflection (ATR) mode with Perkin-Elmer spectrophotometer, USA and it was shown in figure 5 (a).

#### 3.3.3 Field Emission Scanning Electron Microscope

TESCAN Mira 3 SEM was used for the observation of morphologies of surface as well as cross-section under high vacuum. For cross-sectional morphology observation, samples were

immersed into liquid nitrogen then subsequently fractured. Prier the analysis, a thin layer of gold coating was done for evading of electrical charging.



Figure 3.3: (a) FT-IR (b) FE-SEM

#### 3.3.4 Mechanical properties

ASTM standard D882-18 was used to measure tensile strength and extension with Instron UTM, and it has the 5KN capacity. The samples were cut in rectangular strips with width and length of (15mm×90mm) correspondingly. The initial distance between grips and cross-head speed were 50 mm and 50 mm/min respectively.

At each film type, the average values of at least three samples were used to calculate the TS and percentage EAB.



Figure 3.4: Instron universal testing machine

#### 3.3.5 Statistical Analysis

Origin Pro 8.5 was used for plotting of graphs and MS Excel for statistical study. Wherever required to mean  $\pm$  standard deviation ( $\mu \pm \sigma$ ) was calculated.

#### 3.4 Antimicrobial Testing and Setup

There are three types of antimicrobial testing methods namely the agar diffusion method, serial broth dilution method, and micro-atmosphere method (headspace method).

#### **1.** Agar diffusion Method

This technique is most broadly used for antimicrobial testing. The region of "no growth" on disc defines the level of antimicrobial activity. The size of the inhibition area depends on the active ingredient also the spread of the development of microbial cells. Advantages of the agar diffusion method are simple and cost-saving actability. This type of test gives qualitative results. Microbes are usually called "highly susceptible," "intermediate," or else "resistant," depending on the diameter of the inhibitory area.

#### 2. Broth dilution method

This method is commonly used to find out the MIC of oil-based compounds which prevents the development of a microbial after a specific incubation period, usually 48 hours.

#### **3.** Micro atmosphere method (Headspace method)

This technique is used to determine the antimicrobial activity of EOs or their components in a vapour phase. In this mode, the antimicrobial agents diffuse towards the agar plating where culture is inoculated. In the Petri plate case, Microbial maturity can be seen visually.<sup>[8]</sup>

There are five steps for testing of antimicrobial activity as follows

#### **Step 1: Sample preparation**

Sample preparation, I have already discussed in section 3.2.

#### **Step 2: Culture preparation**

Take nutrient broth 1.3% (w/v) of distilled water in a conical flask. Mix well, cover it with cotton and paper also rubber band is applied over it. Subsequently, put the solution into an autoclave for sterilization. It should be placed at 125 °C for 15 minutes to kill any microbes present. After that take out the solution and put into the fume hood, switch on UV

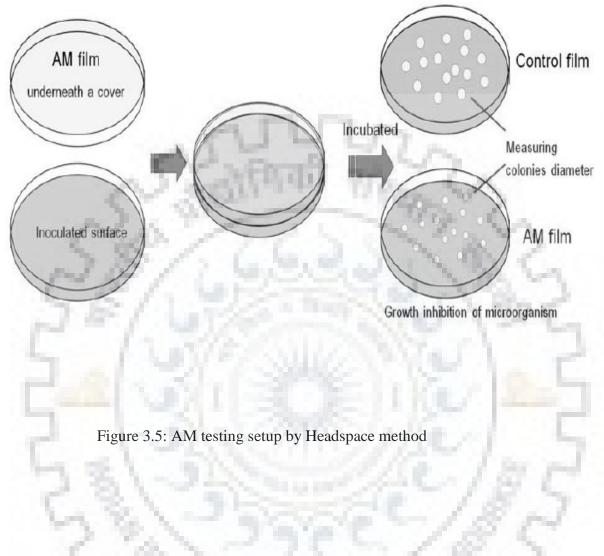
sterilization for 2- 5 minutes and ensure there is no master plate or any other sophisticated components. Dispense one colony of E. coli from the master plate and mix well.

After all, put the solution into the incubator at 32 °C, which is the optimum temperature of E. coli to grow, for 18 hours.

#### Step 3: Serial dilution

It is the stepwise dilution; each step dilution factor is constant, of a substance in solution. It is used to decrease the concentration of bacteria of solution. These are widely used in microbiology, biochemistry, etc. Here, I used 10 fold serial dilutions which are also known as logarithmic or log dilution.





#### **Step 5: Incubation**

Put the solution into an incubator at 32 °C which is the optimal temperature of E. coli to grow then it can be monitored and counted visually.

#### 4. RESULTS AND DISCUSSION

#### 4.1 *Colour test of the film*

Colour is another important property of the food packaging material. It was measured by the instrument KONICA MINOLTA Spectrophotometer CM-3630, Japan. Colour parameters of the food-packaging films have been changed by incorporation of essential oils and it depends on the amount and type of essential oils and their components used. After incorporation of CIN, films appeared slightly yellowish, increase Chroma, decrease hue and less whiteness when compared to the neat PVA film.

Value of colour difference ( $\Delta E$ ) was calculated by below-given equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$
(4.1)

Where,  $L^* = Lightness$  [0 represents for black and 100 represents for white]

 $a^* = \text{Redness} [\text{from green} (-) \text{ to red} (+)]$ 

 $b^* =$  Yellowness [from blue (-) to yellow (+)]

Values of all the films sample are given in Table 4.1.

Similar results were also informed by integrating clove essential oil into PVA.<sup>[9]</sup>

Sample	L*	a*	b*	ΔΕ*
Standard	96.79	-0.09	0.30	3
1% PVA	94.87 ±0.06	-0.17 ±0.04	0.21 ±0.03	1.92 ±0.03
1% AM PVA	94.66 ±0.07	-0.25 ±0.06	0.41 ±0.06	2.13 ±0.02
2% AM PVA	94.32 ±0.06	-0.33 ±0.05	0.46 ±0.04	2.48 ±0.02
3% AM PVA	93.85 ±0.08	-0.38 ±0.06	0.55 ±0.06	2.91 ±0.04

Table 4.1: Colour test values of the samples

#### 4.2 *TGA*

This analysis was done by thermogravimetric analysis (TGA) with TGA55, USA. For all the samples, the weight of each sample was about 5 mg used and heating range from 30 °C to 700 °C. The heating rate was 10 °C/ min and a nitrogen flow rate was 40 ml/min.

All samples pure, as well as CIN, incorporated decomposed similar three-step process. The temperature range of the first step is 30 °C to 195 °C and weight loss was about 9% because of water loss as well as CIN. Second degradation step, the temperature range is from 190 °C to 360 °C and weight loss about 64 % due to side chain breakdown. Third degradation step, the temperature range is from 360 °C to 560 °C caused by main chain breakdown which is shown in figure 4.1.

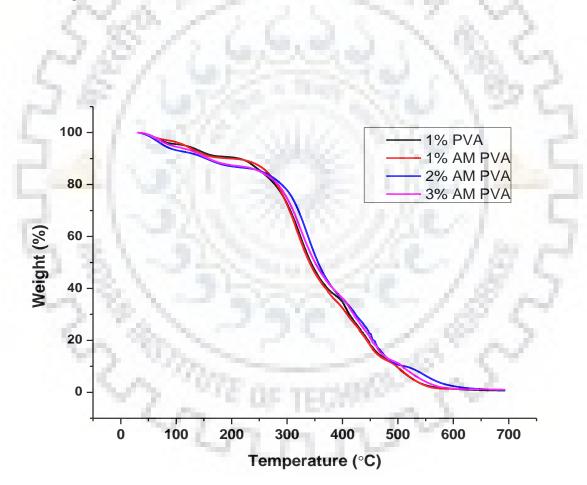


Figure 4.1: TGA degradation curve

#### 4.3 DSC

Differential Scanning calorimetry analysis gives information like as glass transition (Tg) and melting point (Tm) temperature. <sup>[10]</sup> For all the samples, the weight of each sample was about 5 mg used and heating range from 30 °C to 300 °C. The heating rate was 10 °C/ min and a nitrogen flow rate was 40 ml/min. From the figure 4.2 the first downward peak tells the glass transition temperature which is around 88 °C and second downward peak tells the melting point which is around 195 °C. These peaks are similar for all the samples reason could be that small amount of CIN used in the samples.

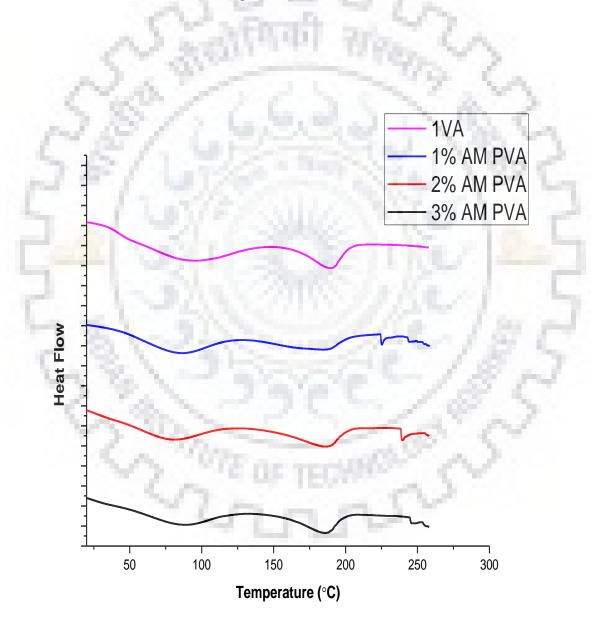


Figure 4.2: DSC graph of samples

#### 4.3 FT-IR Analysis

This analysis was used to identify the existence of particular chemical groups of PVA and cinnamaldehyde. The O-H stretching vibration of the hydroxyl group was detected nearby 3318 cm<sup>-1</sup> while the peak of asymmetric stretching of  $CH_2$  was detected at 2920 and others peaks are 1718, 1421, 1242, 1083 and 836 cm<sup>-1</sup> caused by mainly C=O carbonyl, C-H wagging, C-H bending, C-O from acetyl groups, C-C Stretching respectively. <sup>[11]</sup>

The spectra of PVA incorporated with CIN were observed and found almost similar peaks other than one new peaks situated at 1560 caused by the C=C from the aromatic ring because neat PVA consist of single bond while aromatic ring present only in CIN. These results have been distributed in Table 4.2 and figure 4.2 & 4.3. In another report, PVA incorporated with cinnamon essential oil (CO) explained that CO molecules did not make chemical bonds with PVA polymer matrix but physically diffused over PVA matrix. <sup>[12]</sup>

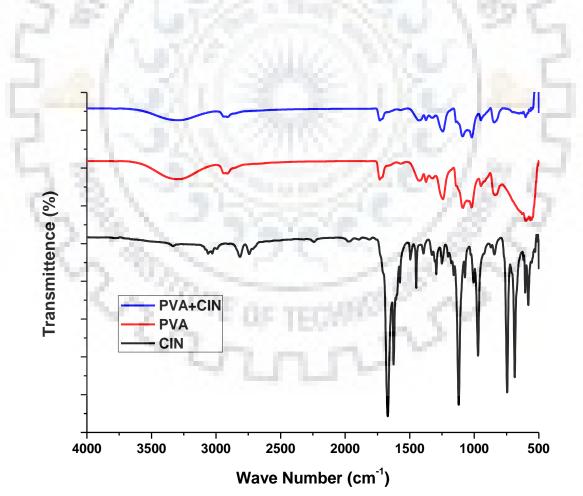


Figure 4.3: FT-IR spectrum for CIN, PVA and PVA+CIN

## Table 4.2: Characteristic peaks of FTIR for PVA and CIN

Assignments	The detected Wavenumber of PVA	The detected wavenumber of CIN
O-H stretching from a hydroxyl group	3400-3200	-
C=O stretching in acetate functional group	1750-1735	22
C-H stretching from alkyl group	2840-3000	2.00
C=C stretching		1622
C=O carbonyl stretching		1671
C-H str <mark>etching</mark> in aldehyde		2812



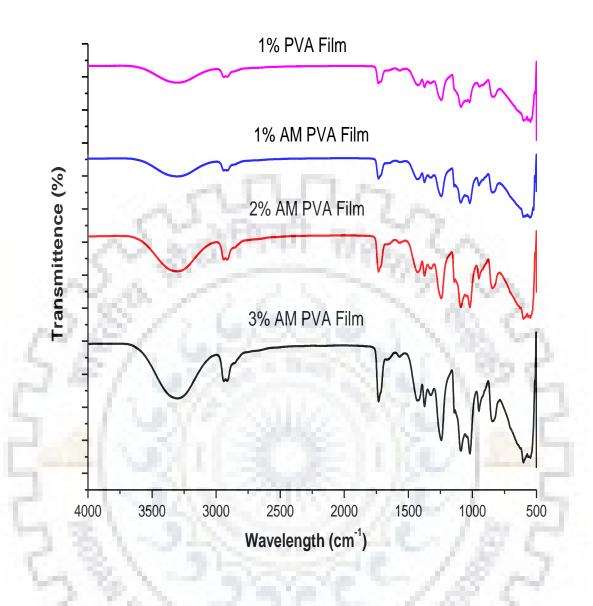


Figure 4.4: FT-IR spectrum for different samples

#### 4.4 *FE-SEM*

Surface and cross-section FE-SEM micrographs are presented in figure 4.4 and 4.5 correspondingly of pure PVA as well as PVA + CIN. The surface and cross-section of the 1% PVA film which is a neat film and taken as reference were plane surface, homogeneous and without any discontinuities. Whereas Surface, as well as a cross-section of 1% AM PVA film, was a little bit uneven. It can be seen heterogeneity increased when the CIN content increased in films.

When compared to the reference film, irregularity and heterogeneity increased 2% AM PVA and 3% AM PVA film correspondingly reason might be that oils droplets were entrapped into polymer matrix. No CIN droplets were detected on the surface as well as a cross-section of the film the cause might be that CIN well embedded in the PVA matrix.<sup>[9]</sup> Another reason might be that use of a small percentage of CIN.

Hence, uniformity was decreased as CIN content increased in the PVA polymer matrix.



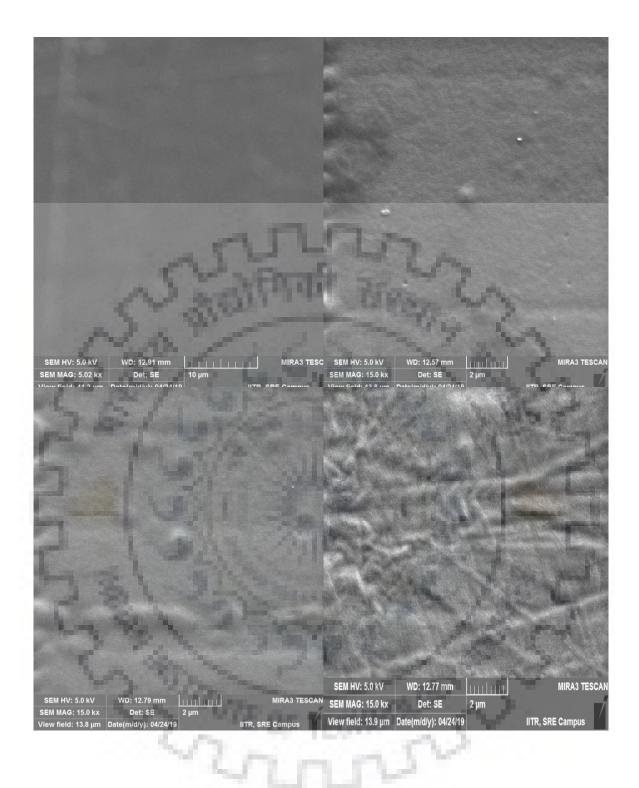


Figure 4.5: FE-SEM pictures showing surface morphology of (a) 1% PVA, (b) 1% AM PVA, (c) 2% AM PVA, (d) 3% AM PVA



Figure 4.6: FE-SEM pictures showing Cross-sectional morphology of (a) 1% PVA,

(b) 1% AM PVA, (c) 2% AM PVA, (d) 3% AM PVA

#### 4.5 Mechanical properties

Mechanical properties are important for food packaging films because they must withstand from any physical hazards during transportation such as compressive, tensile, vibrational, and impact stresses.

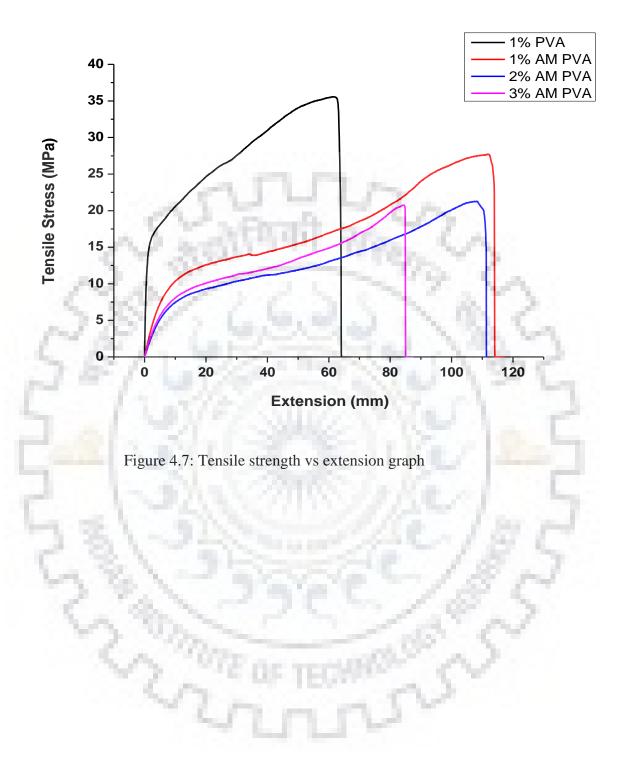
As compared to the reference film which is 1% PVA, due to the addition of CIN into PVA films tensile strength decreased evidently for samples as CIN content increased significantly. Tensile strength of 1 %, 2 %, and 3% AM PVA film decreased 23.53 %, 38.23 %, and 41.18 % respectively when compared to the 1% PVA film. The reason could be that replacement of weaker PVA- CIN interfaces in its place of PVA-PVA intermolecular interfaces.

Whereas EAB increased initially and then declined as the CIN proportion increased significantly. For 1% AM PVA film, EAB increased by 74.24 % the reason might be that non-uniformity and discontinuity in film decreased resistibility as well.

Tensile strength, average thickness and per cent elongation at break of the samples were given in Table 4.3 and graph was plotted in figure 4.6 respectively.

Samples	Average thickness (µm)	Tensile Strength (MPa)	EAB (%)
1% PVA	31.7 ±3.53	34 ±1.85	132 ±3.23
1% AM PVA	32.6 ±3.36	26 ±1.71	230 ±3.42
2% AM PVA	32 ±3.08	21 ±1.95	224 ±4.58
3% AM PVA	32.4 ±2.51	20 ±1.04	170 ±6.38

Table 4.3: TS, Thickness, and EAB of the samples



#### 4.6 Antimicrobial Activity

The AM activity of different samples was calculated on Escherichia coli which is classified as gram-negative bacteria. These types of bacteria can grow-up even the absence of oxygen. This is also classified as a facultative anaerobe.

Bacterial growth can be seen in Fig. 4.7 and 4.9 of different incubation period respectively. Bacterial growth and number of colonies are lagging as CIN content improved in the film the reason could be that reduces the drying time of the film.

CFU stands colony-forming unit and count of total viable bacterial cells. For the sake of simplicity, results could be calculated in CFU/mL for liquids.

The CFU/ml (colony-forming units per millilitre) can be expressed as:

CFU/ml = Number of colonies × dilution factor Volume of culture plate

From figure 4.8, observed that populations of E. coli 1% PVA film which is control film and 1% AM PVA were  $52 \times 10^{10}$  CFU/ ml and  $46 \times 10^{10}$  CFU/ml, correspondingly. There is not too much difference between these two samples because of the low proportion of CIN in 1% AM PVA film. While 2% AM PVA and 3% AM PVA film total bacterial counts were  $9 \times 10^{10}$  and  $7 \times 10^{10}$  CFU/ml respectively. The reason is that the maximum existent of antimicrobial agents in the films. If compared to the reference and 3% AM PVA film, CFU is decreased from 52 to  $7 \times 10^{10}$  CFU/ml that means drying time of films greatly affect the antimicrobial performance.

Moreover, after 48 hours of incubation, it was found that the number of colonies and its diameter increased to some extent which was shown in figure 4.9 and 4.10.

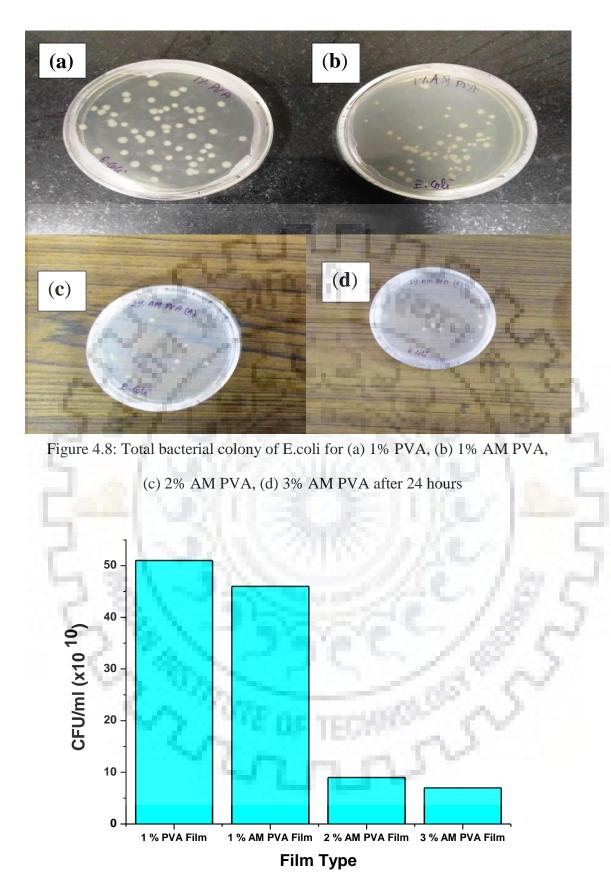


Figure 4.9: Bar graph display average CFU/ml for E. coli of samples after 24 hours incubation

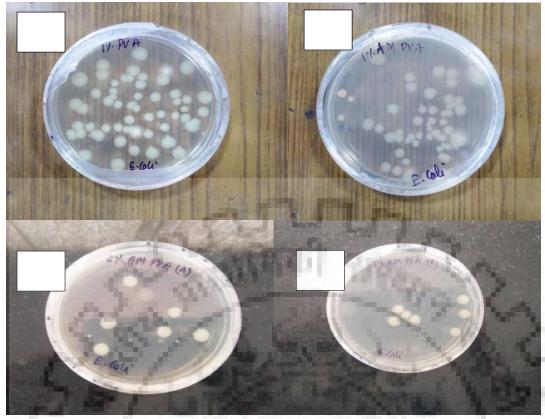


Figure 4.10: Total bacterial colony of E.coli for (a) 1% PVA, (b) 1% AM PVA, (c) 2% AM PVA, (d) 3% AM PVA after 48 hours

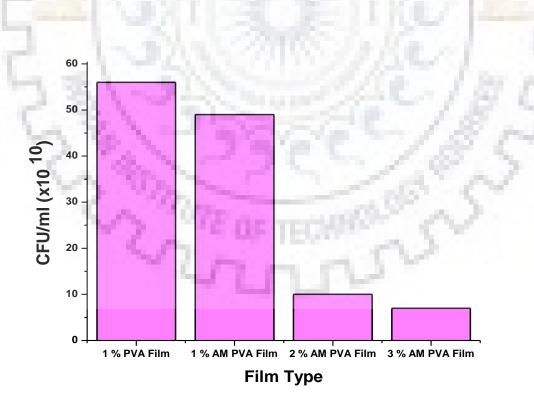
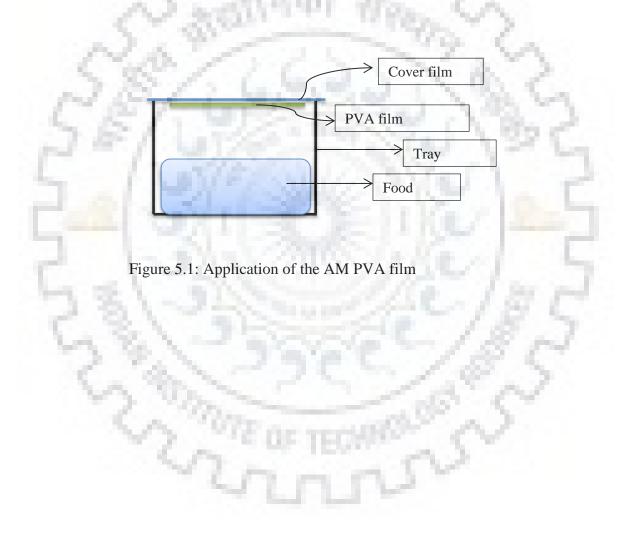


Figure 4.11: Bar graph display average CFU/ml for E. coli of samples after 48 hours incubation

## **5.** CONCLUSION

Active packaging can play an essential role in decreasing the threat of pathogens infection and extending the shelf-life of packaged foods. It is a rapidly emerging technology.

Therefore, more research is needed to recognize food-type which could be profit the maximum from this. The objective of this study is to use the AM packaging film with the non-direct contact of food applications. The release kinetics (control release) of an antimicrobial agent from an extremely hydrophilic polymeric matrix (PVA) can be controlled, to a certain extent, by adjusting the degree of a cross-link of the polymeric matrix.



#### **6. REFERENCES**

[1] Schaefer, D., & Cheung, W. M. (2018). Smart Packaging: Opportunities and Challenges. *Procedia CIRP*, 72, 1022-1027.

[2] Ahvenainen, R. (Ed.). (2003). Novel food packaging techniques. Elsevier.

[3] Bastarrachea, L., Dhawan, S., & Sablani, S. S. (2011). Engineering properties of polymeric-based antimicrobial films for food packaging: a review. *Food Engineering Reviews*, *3*(2), 79-93.

[4] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, 94(3), 223-253.

[5] Priyadarshi, R., Kumar, B., Deeba, F., Kulshreshtha, A., & Negi, Y. S. (2018). Chitosan films incorporated with Apricot (Prunus armeniaca) kernel essential oil as active food packaging material. *Food Hydrocolloids*, 85, 158-166.

[6] Priya, B., Gupta, V. K., Pathania, D., & Singha, A. S. (2014). Synthesis, characterization and antibacterial activity of biodegradable starch/PVA composite films reinforced with cellulosic fibre. *Carbohydrate Polymers*, *109*, 171-179.

[7] Gao, H., & Yang, H. (2017). Characteristics of poly (vinyl alcohol) films crosslinked by cinnamaldehyde with improved transparency and water resistance. *Journal of Applied Polymer Science*, *134*(38), 45324.

[8] Suppaku, P. (2012). Alternative technique for the quantitative determination of antimicrobial activity of antimicrobial packaging film containing lipophilic materials. Food Packaging: Procedures, Management and Trends. 93-116.

[9] Chen, C., Xu, Z., Ma, Y., Liu, J., Zhang, Q., Tang, Z., ... & Xie, J. (2018). Properties, vapour-phase antimicrobial and antioxidant activities of active poly (vinyl alcohol) packaging films incorporated with clove oil. *Food control*, *88*, 105-112.

[10] Guirguis, O. W., & Moselhey, M. T. (2012). Thermal and structural studies of poly (vinyl alcohol) and hydroxypropyl cellulose blends. *Natural Science*, *4*(1), 57.

[11] Kharazmi, A., Faraji, N., Hussin, R. M., Saion, E., Yunus, W. M. M., & Behzad, K. (2015). Structural, optical, opto-thermal and thermal properties of ZnS–PVA nanofluids synthesized through a radiolytic approach. *Beilstein journal of nanotechnology*, *6*(1), 529-536.

[12] Jo, H. J., Park, K. M., Min, S. C., Na, J. H., Park, K. H., & Han, J. (2013). Development of an anti-insect sachet using a polyvinyl alcohol– cinnamon oil polymer strip against Plodia interpunctella. *Journal of food science*, 78(11), E1713-E1720.

