A

Dissertation thesis on

Development of PVA Essential Oil Based Antimicrobial Packaging Film with Desirable Barrier Properties.

For the award of Degree in

MASTER OF TECHNOLOGY

IN

PACKAGING TECHNOLOGY

By

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(Saharanpur Campus)-2019

Declaration of the Candidate

I hereby certify that the work which is presented in this thesis entitled, "**Development of PVA Essential Oil Based Antimicrobial Packaging Film with Desirable Barrier Properties. "**, in partial 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 supervision of **Dr. Gaurav Manik, Department of Polymer Process Engineering & Dr. Ashish A. Kadam, Department of Paper Technology**, **Indian Institute of Technology, Roorkee.**

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

Date: 24/05/2019

Place: SAHARANPUR

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CERTIFICATE

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

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Abstract

PVA-essential oil based antimicrobial packaging film was developed for their effective usage for both food/ package (direct contact) system and food/headspace/package (indirect contact) system. The Antimicrobial package consists of PVA as a film substrate and cinnamaldehyde as an antimicrobial agent. The antimicrobial packaging film of PVA incorporating cinnamaldehyde (CIN) as with a different concentration of 15%, 30%, 45% (v/w) were fabricated by solvent casting method. The antimicrobial properties of the film were estimated by micro-atmosphere antimicrobial test. The antimicrobial film with a concentration of 45% CIN in PVA polymer matrix show total inhibition against *Escherichia Coli* at the dilution factor of 10^8. MD simulation was used to evaluate the diffusion coefficient oxygen gas and water vapor molecules in PVA-CIN polymer matrix. The diffusion coefficient was computed through a long run *NVE dynamics* for 1ns at the room temperature (300K). The computed diffusion coefficient of oxygen gas and water vapor show a similar trend with the reported literature. Surface morphology of PVA-CIN film was observed via FE-SEM show the irregularity and uneven surface with the increasing concentration of CIN. Tensile strength was reduced by 7.75% as compared to the pristine PVA film. WVTR of PVA first increased and then decreased with the increase in the concentration of the CIN. The FT-IR spectra confirmed the successful incorporation of CIN in the PVA polymer film.

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Abbreviations

AM	Antimicrobial
CIN	Cinnamaldehyde
EOs	Essential Oils
MD	Molecular Dynamics
D	Diffusion Coefficient
EAB	Elongation at break
DMSO	Dimethyl Sulfoxide
MAP	Modified Atmosphere Packaging
MSD	Mean square displacement
PVA	Poly-Vinyl Alcohol
E.Coli	Escherichia Coli
COMPASS	Condensed-phase optimized molecular potential for atomistic simulation
TS	Tensile Strength
WVP	Water vapor permeability
WVTR	Water vapor transmission rate
FT-IR	Fourier Transform-Infrared
FE-SEM	Field Emission -Scanning Electron Microscope

1. Introduction

With the increasing world population and simultaneously depletion of the world resources. The insurance of the food has emerged as an important concerned worldwide. According to the UN Food and Agriculture Organisation (FAO) Report, approximately one-third of the Food produced for the human consumption get lost and wasted[1].

In the field of developing a suitable food packaging material, lots of significant research have been carried out in the recent year. The main focus of such packaging material is the safeguards of food from both the physical and chemical deterioration and as well as from microbial degradation.

Nowadays, The Researcher moves more towards developing an active packaging in order to change the atmosphere of packaged food by incorporating active material into it .hence, improving the quality and increasing the shelf life of the product[1].

Recently, there is more focused on biodegradable polymer for making active food packaging because of the major environmental problem caused by conventional plastic food packaging.

Poly-Vinyl Alcohol (PVA) is a synthetic polymer which is being a biodegradable showed a large variety of other properties such as magnificent film forming ability, biocompatibility, excellent gas barrier properties especially outstanding oxygen barrier, as well as non-toxic in nature. With such excellent properties, it is used in various application including packaging material and drug delivery system[2].

In the latest year, lots of research on the development of antimicrobial packaging(AMP) film have been mentioned by incorporating with the different type of natural antimicrobial agent such as lysozyme, grapefruit seed extract, cinnamon oil into PVA.

The essential oils(EOs) which are derived from plants exhibit tremendous antimicrobial, antifungal, and antioxidant properties. US Food and Drug Administration categorized EOs as GRAS(Generally Recognized As Safe).AM films are formed by incorporating EOs in the PVA. These AM films for food packaging have been proven to effective in increasing the shelf life of

horticulture, bakery, dairy and flesh (i.e-meat, fish and poultry) product. Moreover, due to the brisk flavor of EOs, their usage is limited in Food trading industry.

In-Direct Contact Food packaging system, antimicrobial properties of EOs are used to create an environment around the food item for enhancing the shelf-life of food. Cinnamon oil, rosemary oil, clove oil are such example of EOs which have the potential to be used as AM agents in the food packaging system.

Molecular dynamics(MD) simulation are one of the most important tools to determines various properties of the polymer which have been burdensome by experimental method.

In order to study the diffusion of small penetrates gas molecules in the polymers have been reported in the literature to be the "hopping" mechanism. In hopping mechanism a penetrate molecules most of the duration lodge around minute cavities present inside the polymer matrix.[3]

Over a period of time, a minute channel develops, joining two of such cavities and allows the gas molecules to "hop" from one cavity to another.

1.1 Active packaging

The rapid changes in the demand of modern consumer and markets need leads to the introduction of active packaging. It is an innovative packaging technique in which the condition of the packaging changes in order to enhance the shelf-life and sensory properties of the food product apart from retaining the quality of the food. Active packaging has an extra edge over conventional packaging system due to its two main active function (i) active scavenging system which includes oxygen scavenger, moisture scavenger, ethylene scavenger. (ii) an active releasing system which includes CO_2 emitter, antimicrobial emitter.

1.2-Antimicrobial packaging

Antimicrobial packaging is the type of active packaging in which AM agents incorporate into the polymer matrix to conceal the action of the targeted microbes. In AM packaging, AM agents interact with both package food as well as package-head space depending upon food packaging

system The demand of customer for minimally processed and preservative free food item leads to the scrutiny of AM packaging for the food industry. Although tremendous research in the field of AM packaging, only a few products commercialized in the market yet.

The need for antimicrobial packaging

- Microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness.
- The transformation of nutrient and sensory characteristics of the food item which lead to unpleasing changes in appearance and its texture.

Uses:-

- Poultry, fish, meat
- Horticultural Produce

1.3 Barrier Properties of Packaging film

The barrier properties of the packaging film are measured by the permeability of the film. Its signify the resistance to both diffusion and sorption of the permeate molecules like oxygen gas or water vapor.

In order to increase the shelf of the food product item, the optimization of permeability is of the essential factor to be considered while developing a food package. The peculiar food qualities are changed when the food item interacts either with the packaging system (Packaging material & headspace) or with the exterior environment. This ultimately leads to the growth of microbial species on the food items.

Permeability in the literature defined as the quantification of the permeate (gas or vapor) transmission through the restricting material. Barrier properties and permeability of film are inversely proportional to each other[4]. Higher the barrier properties lower the permeability of the film and vice-versa.

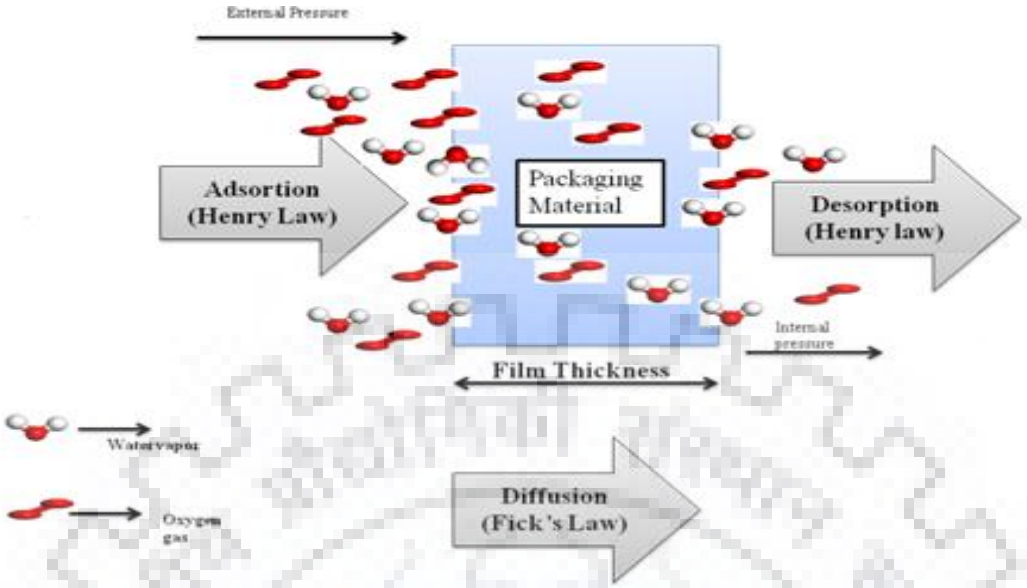

Figure 1: Oxygen and water vapor permeability mechanism through the food packaging material

The permeability coefficient (P) of the film is the combined effects of both the diffusion coefficient (D) which is governed by Fick's law and solubility parameter (S) which is governed by Henry Law.

The objective of my research work

1. Evaluate the antimicrobial properties of PVA-CIN packaging film.
2. Evaluate the diffusion coefficient of permeate molecules such as oxygen gas and water vapor through molecular dynamics simulation.
3. Characterization of PVA-CIN Antimicrobial film.
4. Proposed best optimal composition for development of PVA-CIN antimicrobial film on the basis of the above result.

2.1 Food packaging systems

Most food packaging systems represent either in these two most prominent ways represent below

1) A food/ package system
2) A food /headspace/package system.

2.1.1 A food/package system

A food/package system is the type of packaging system in which food product and packaging material are in direct contact with packaging material. No headspace exist between them. Some of the examples of the food/package system are RTE (ready to eat) product, *Sous-Vide* food product, etc. Main migration mechanism involved in this is the diffusion between the food and packaging material at the partition interface. Packaging material incorporated with the AM agents diffuse into the food item[5].

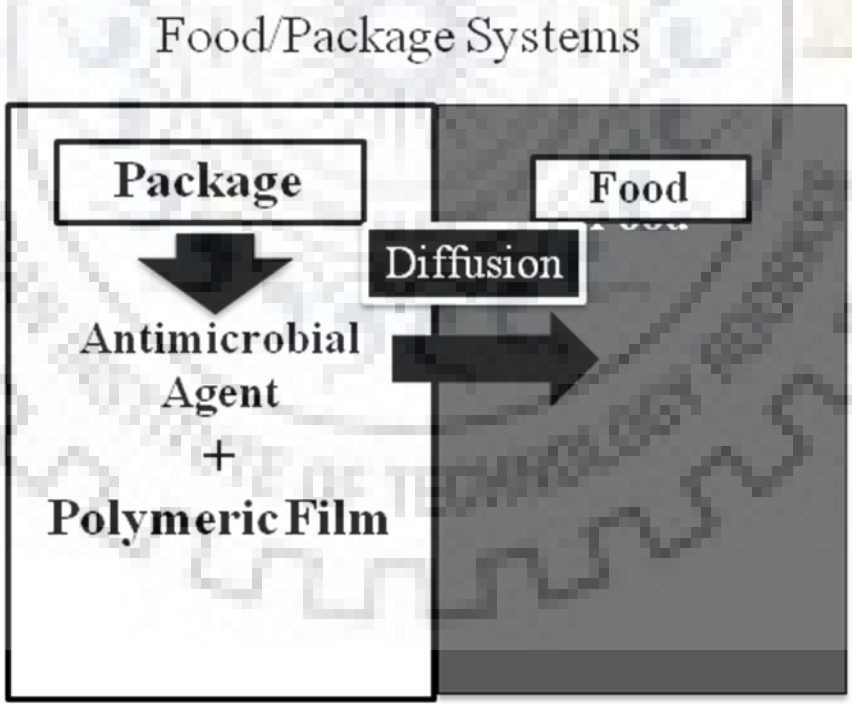

Figure 2: Food/ Package systems and behavior of active substances

2.2 A food/headspace/package system

A food/headspace/package systems are the type of packaging in which the packaging material in IDC with the food product and empty space between them is known as headspace. Majority of the flexible packaging as well as rigid packaging represent the food/headspace/package system. The headspace is generally filled with gas in order to develop the modified atmosphere packaging (MAP). Equilibrium sorption is the main migration phenomenon to evaluate the distribution of the AM agent. Volatile AM agents like EOs will be effectively used in such type system, where it can migrate through the headspace towards the food[4].

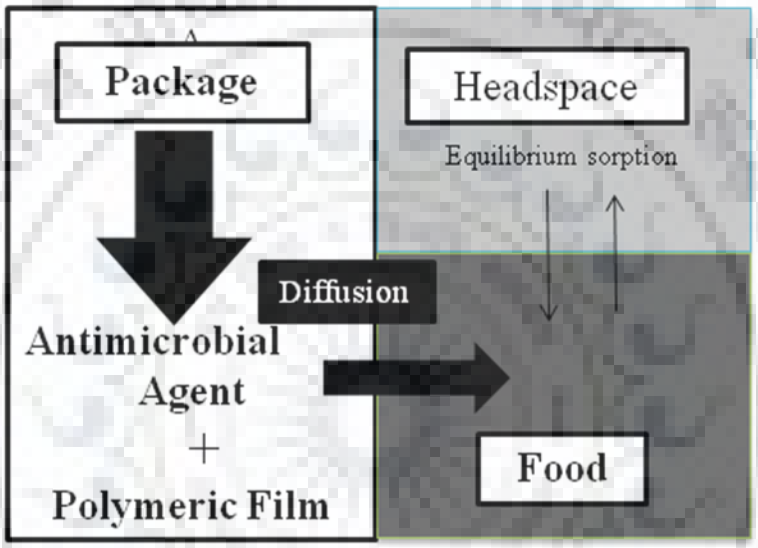

Figure 3: Package/Headspace/Food systems and behavior of active substances

2.4.Antimicrobial packaging system

The antimicrobial packaging system consists of two main components (i) AM Agents (ii) Substrate on which the AM Agents are incorporated. The substrate is used in AM packaging is mainly of polymer and paper. Polymer used as in general for packaging application is synthetic polymer but over a course of time and concerned regarding the sustainable environment, lots of research are carried to move towards biopolymer. On the other hand AM agents on the basis of origins are characterized into Organic acid or anhydride, Bacteriocins, natural extract like Essential oil, Metal substituted zeolite, etc. Some commercially available antimicrobial food packaging mention in the table below[6][7][8]:

Table 1: Commercially available AM Food Packaging

ANTIMICROBIAL AGENTS	Trade Name	Manufacturer Company	Packaging form
Silver substituite zeolite	Aglon™	Aglon Technologies LLC	Paper board carton,plastic or paper wrap
Triclosan	Microban®	Microban Products	Food Containers
Wasabi Extract -Allyisothio-cynate	WasaOuro	Lintec Corporation	Sheets ,Pressure sensitive label
Ethanol Vapor	Ethicap®	Freund	Sachets

The ways of incorporating AM agents into Food packages are as follows:

1. Addition of AM agents in the form of sachets/ pads into the package.
2. Incorporation of AM agents directly into the polymer.
3. Coating of AM agents onto the substrate surface.
4. Use to polymer in the package that inherits AM properties.
5. Immobilization of AM agents on the polymer means of ion or covalent linkage.

2.4.1.ESSENTIAL OIL AS ANTIMICROBIAL AGENT IN FOOD PACKAGING

EOs are aromatic volatile liquid derived from the different part (i.e leaves herbs, roots, bark) of the plant. The EOs are the amalgamation of more than hundreds of the aromatic compound. The extraction technique used to extract the EOs from the plants is steam distillation and cold pressing[5]. The key constituents of some EOs that exhibit AM properties mentioned in the table below[6][7][8]:

Table 2: Key components of the EOs that exhibits AM properties

Plant source of Eos.	Latin name	Key Components	Approx % composition
Cinnamon (bark)	*Cinnamomum verum*	Trans-Cinnamaldehyde	68.4
		Eugenol	4.4
Clove (bud)	*Syzygium aromaticum*	Eugenol	75-85
		Eugenyl acetate	8-15
Corriander (Seeds)	*Coriandrum sativum*	Linalool	70
		Camphor	6.5
Rosemary	*Rosemarinus officinallis*	α-pinene	11.8
		Bornyl acetate	17
		1,8-cineole	46.6

EOs have been extensively reported in the literature for having AM properties foodborne microbes which include mold, yeast, and both Gram-positive bacteria -Staphylococcus aureus, Listeria monocytogenes, as well as Gram-negative bacteria -E. Coli, Salmonella Enteritidis.

2.4.5Antimicrobial Testing

There are three main antimicrobial testing techniques which are used to provide the potential of the tested antimicrobial (AM)agents. These techniques categories into different method they are

(i) Diffusion Method

(ii) Serial Dilution Method

(iii) Micro-Atmosphere Method

(i) **Diffusion Method**: It is one of the widely used methods for antimicrobial testing. In this method screening of the antimicrobial activity is done. A region of no inhibition around the disk defines the intensity of antimicrobial activity. The size of this region depends upon the rates of diffusion of antimicrobial agent and cell growth of targeted microbes. The advantages of Diffusion method over other antimicrobial method is that it is simple and cost saving. And result obtained from this test is qualitative with a high degree of standardization.

(ii) **Serial Dilution Method**: It is one extensively used method to determine the minimum inhibitory concentration(MIC) of AM agents used. MIC is the lowest concentration of the AM agents inhibiting the visible growth of tested microbes after the specified incubation period. Hence, the MIC of cinnamon oil is in the range of **6.25-25** $\boldsymbol{\mu L m L^{-1}}$and the MIC of Cinnamaldehyde is in the range of **0.78-12.5** $\boldsymbol{\mu L m l^{-1}}$[7][8].

(iii**) Micro-Atmosphere Method-**This method is used for determining the AM activity of the volatile AM agents such as essential oil or components of the essential oil in vapor phase atmosphere. In this method, AM agents diffuse towards the nutrient agar in an inverted petri dish.

3. Literature Survey

Seenivasan et al., 2006 studied the in-vitro antimicrobial properties of several plant EOs against both the Gram-negative (E.Coli, Proteus Vulgaris)and Gram-positive (S. aureus, B. subtillis) bacteria[9]. The disc diffusion test is used to evaluate AM strength. Among the different EOs used, it was found out that five essential oil i.e. Cinnamon, clove, Rosemary shows AM resistance against more the one type of bacteria and out of all, cinnamon oil is showing the AM activity against both grams-positive bacteria as well as gram-negative bacteria even in low concentration. The main constituent of Cinnamon oil which is responsible for the AM activity is Cinnamaldehyde which comprises of 68% of the total composition.

C. Chen et al., 2018 studied the vapor phase AM properties and characterization of PVA-Clove oil based antimicrobial film in which it is found out that it shows effective AM properties in the vapor phase condition and it is suitable for food/headspace /package system also[2]. With the increases in the concentration of clove oil-oil droplets appeared in the surface of film when it was observed through FE-SEM. Tensile strength of the film reduced due to the poor PVA and clove oil interaction.WVTR value due to the increase in hydrophobicity of the film while OTR value of the film the decrease while due to creation microcavity due to PVA-clove oil interaction. The lightness of the film increases with increases in concentration, the redness value decreases while the yellowness value increases with increases in the concentration of the clove oil.

R. Priyadarshi et al., 2018 developed the antimicrobial film with the incorporation of kernel oil on chitosan in which AM strength of the film determined by the bacterial serial dilution method. The incorporation of the kernel oil confirmed by FT-IR spectra. The incorporation of kernel oil results in the enhancement of the mechanical properties of the chitosan film[1].

Meunier et al., 2018 evaluated the diffusion coefficient of the gas molecules in polybutadiene by molecular dynamics simulation. in order to validate the chain length of the PBD, the density and the solubility parameter are estimated with different chain length ranging from n=5,10,15,20,25,30. till the saturation of density and solubility parameter achieved[3]. The PBD with a chain length of 30 is used to estimate further properties. The diffusion coefficients of gas

molecules were the estimated by *NPT dynamics run* of 3ns different temperature. The computed diffusion coefficient compared with the experimented diffusion coefficient.

S. Sethi et al., 2018 studied the compatibility of Poly (dimethyl siloxane) and PVAc blend.In order to study the compatibility of blends, The density(ρ) and solubility parameter(δ) of PVA show saturation beyond the chain length (n=20).The average simulated density $\rho_{PVA=1.12}\ g/cm^3$and solubility parameter $\delta_{PVA=20.975(J/cm^3)^{0.5}}$ show quite close literature reported density is $\rho_{PVA=1.19}\ {}^{g}/_{cm^3}$ and solubility parameter $\delta_{PVA=22.9(J/cm^3)^{0.5}}$ [10].

4. Material & Software Selection

For Film Fabrication:

1. Poly (vinyl alcohol) (PVA) fully hydrolyzed (degree of hydrolysis: 98-100) purchased from Himedia laboratories used for polymeric matrix.
2. Cinnamaldehyde (CIN) (98%) Mol. wt-136 g/mol purchase from MERCK(erstwhile Sigma-Aldrich) used essential oil.
3. Dimethyl Sulfoxide (DMSO) Mol. wt-78.13 g/mol purchased from MERCK (Erstwhile Sigma-Aldrich) as aprotic Solvent.

For Antimicrobial Test:

Bacterial Strain of gram-negative bacteria, Escherichia Coli (E. coli) purchased from MTCC, Chandigarh. For Bacterial broth and culture preparation of bacterial strain, Nutrient Agar purchased from Hi-Media Laboratories, India. For Agar Plating, Nutrient Agar was used purchased from Hi-media Laboratories.

For Molecular Simulation:

Material Studio 7.0 software purchased from BIOVIA^{TM}, Dassault System used for performing MD simulation on the forcite software package to evaluate the diffusion coefficient of penetrating molecules like O_2 and H_2O molecules.

5. Methodology

5.1 Film Fabrication

The solvent casting method is used for casting the PVA -CIN Film. It is majorly used for casting film for heat-sensitive antimicrobial agents which require low temperature for mixing.1% (w/v) of PVA dissolve in distilled water with continuous stirring at 330-360 rpm at magnetic stirrer and heating at a temperature of 80℃ for time duration of 4 hours ,then the aqueous solution of PVA is gradually slowed down to the temperature of 40℃ ,then the solution of different concentrations CIN (15%, 30%, 45%) (v/w PVA) with 1% (v/v) of DMSO to PVA solution is added to the previously made PVA solution with continuous stirring and heating at 40℃ for 30-60 min depending upon different concentration of CIN. The flow process of the casting of the film is mention below in figure:

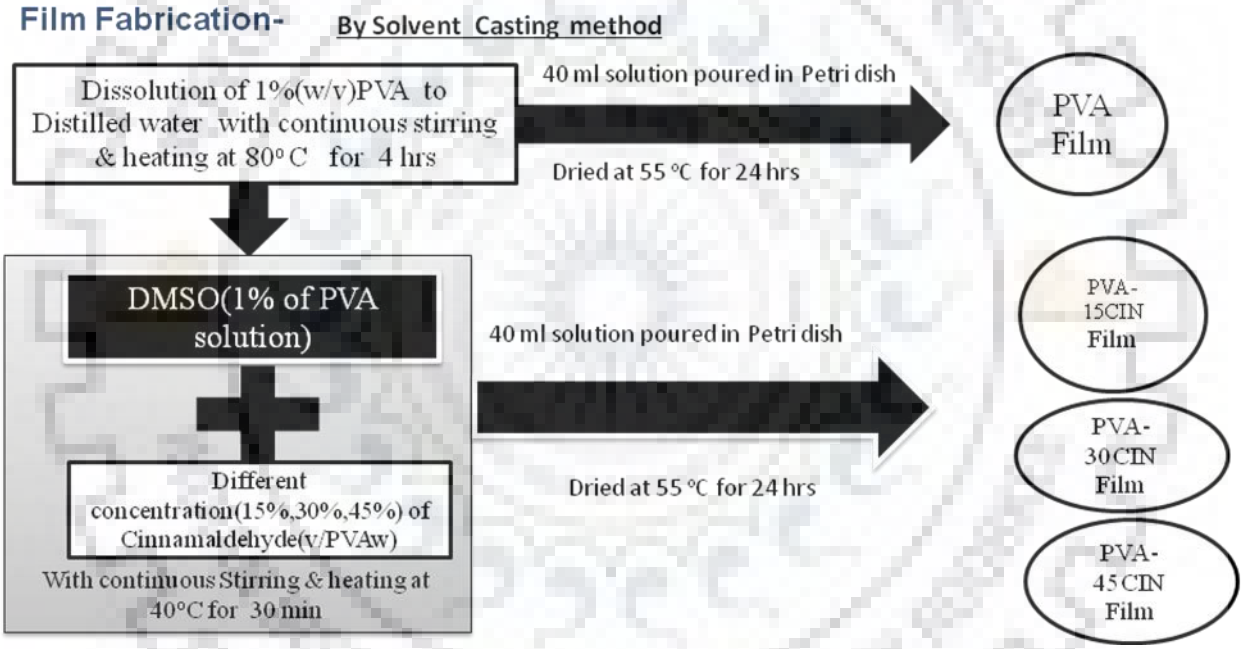

Figure 4: Flow Chart of fabrication of PVA and PVA-CIN Films

5.2 Antimicrobial Testing

Microatmosphere method is used for evaluating AM properties of the film against the Gram-negative bacteria -Escherichia Coli(E.Coli). There three main steps are involved in AM testing they are as follows:

1 *Culture Media Preparation:*

In culture media preparation, E.coli strain from the master plate was inoculated with freshly prepared and sterile *Nutrient broth*. Bacterial Nutrient broth solution was incubated at 32℃ with continuous shaking for a time duration of 18 hrs. The color of the bacterial broth changes from light yellowish to turbid yellow during incubation.

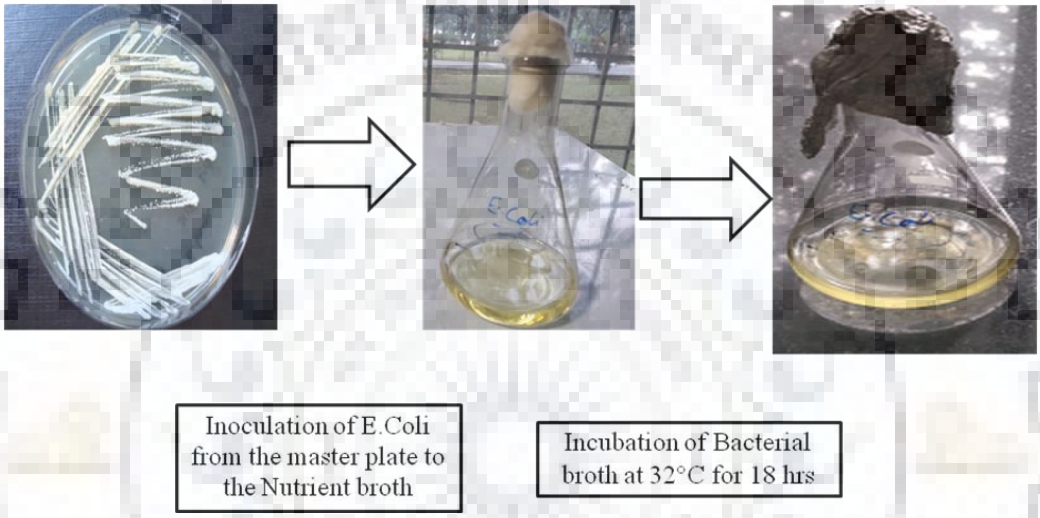

Figure 5: Image of Culture media (bacterial broth) before and after incubation

2. *Bacterial broth serial dilution:*

Bacterial broth solution of E. coli strain is diluted after the incubation for 18 hrs. The bacterial broth diluted with the dilution factor of 10^8. The reason behind these dilution level is to decrease the colony count on an agar plate which is uncountable on dilution factor10^1 to the countable count of dilution factor of 10^8. The viable count of microbes is estimated in term of "CFU/ml" which is calculated by formula:

$$\frac{CFU}{ml} = \frac{Bacterial\ plate\ count\ \times dilution\ factor}{Innoculum\ Quantity}$$

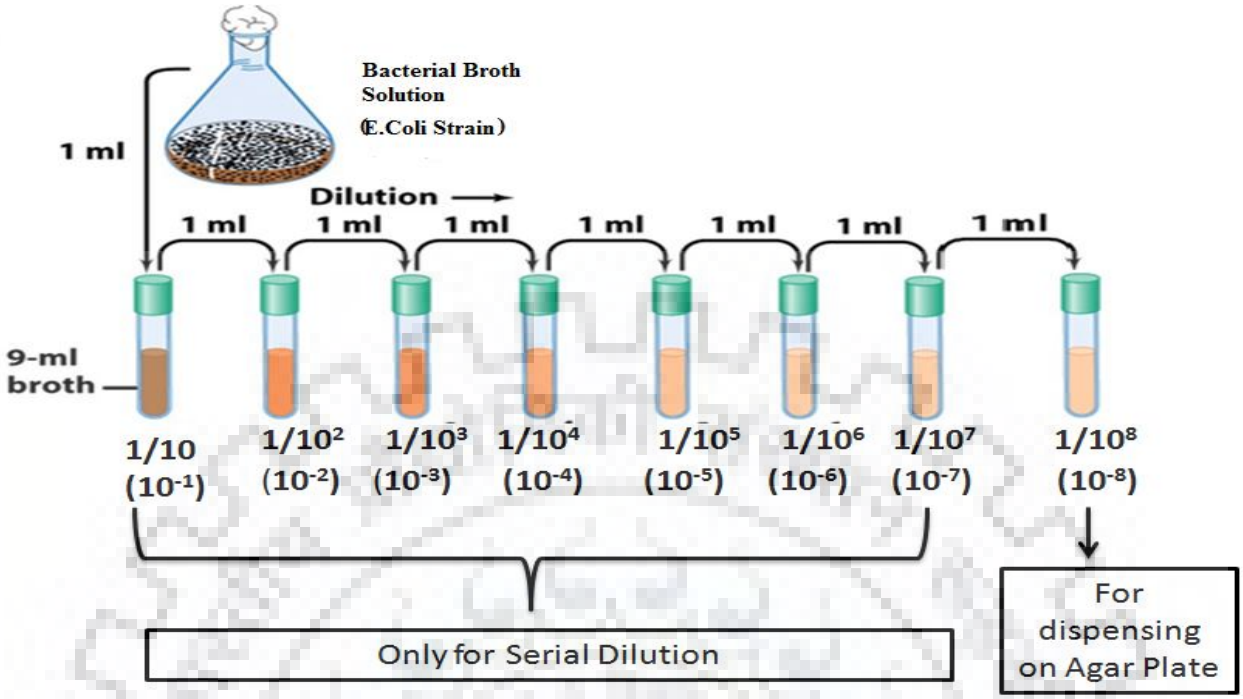

Figure 6: Bacterial Serial dilution Method

3. Vapour Diffusion Assay:

AM Film solution prepared while film fabrication is poured on the cover of glass Petri plate kept for drying at 55℃ for 24 hrs. 1μl of bacterial broth of dilution factor of 10^8 is dispensed on the agar surface which is spread with the help of L-shaped spreader.Then petri plate enclosed with AM film cover which is sealed with Parafilm^{TM}. Then the sample kept in the incubator at 32℃ for dua the ration of 48hrs and checking the growth of bacteria after every 24 hrs.

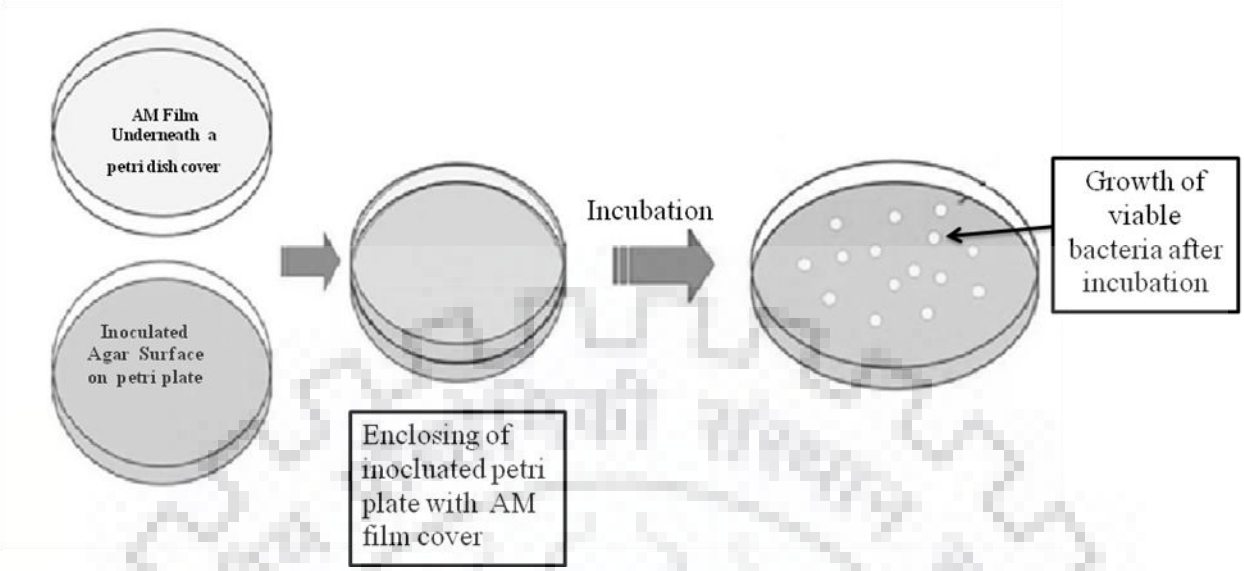

Figure 7: Schematic of Vapor diffusion Assay

5.3 Simulation Strategies

Molecular Simulations were performed on the Amorphous Cell and FORCITE module software package of Material Studio 7.0. COMPASS (Condensed-Phase Optimized Molecular Potential for Atomistic Simulation Studies) ForceField is used to calculate interactions between the atoms. This forcefield facilitates the prediction of both gas phase properties and condensed phase properties for a wide spectrum of molecules. Berendsen algorithm and Andersen Thermostat are used to maintaining and controlling pressure (NPT ensembles) as well as temperature (NVT ensembles) in all the simulation run. Group-based approach is used to calculate the Non-binded interaction with the cutoff distance of 12.50 $°A$. The flow chart of simulation protocols used to estimated the diffusion coefficient of permeating moleclues mentioned in the figure:

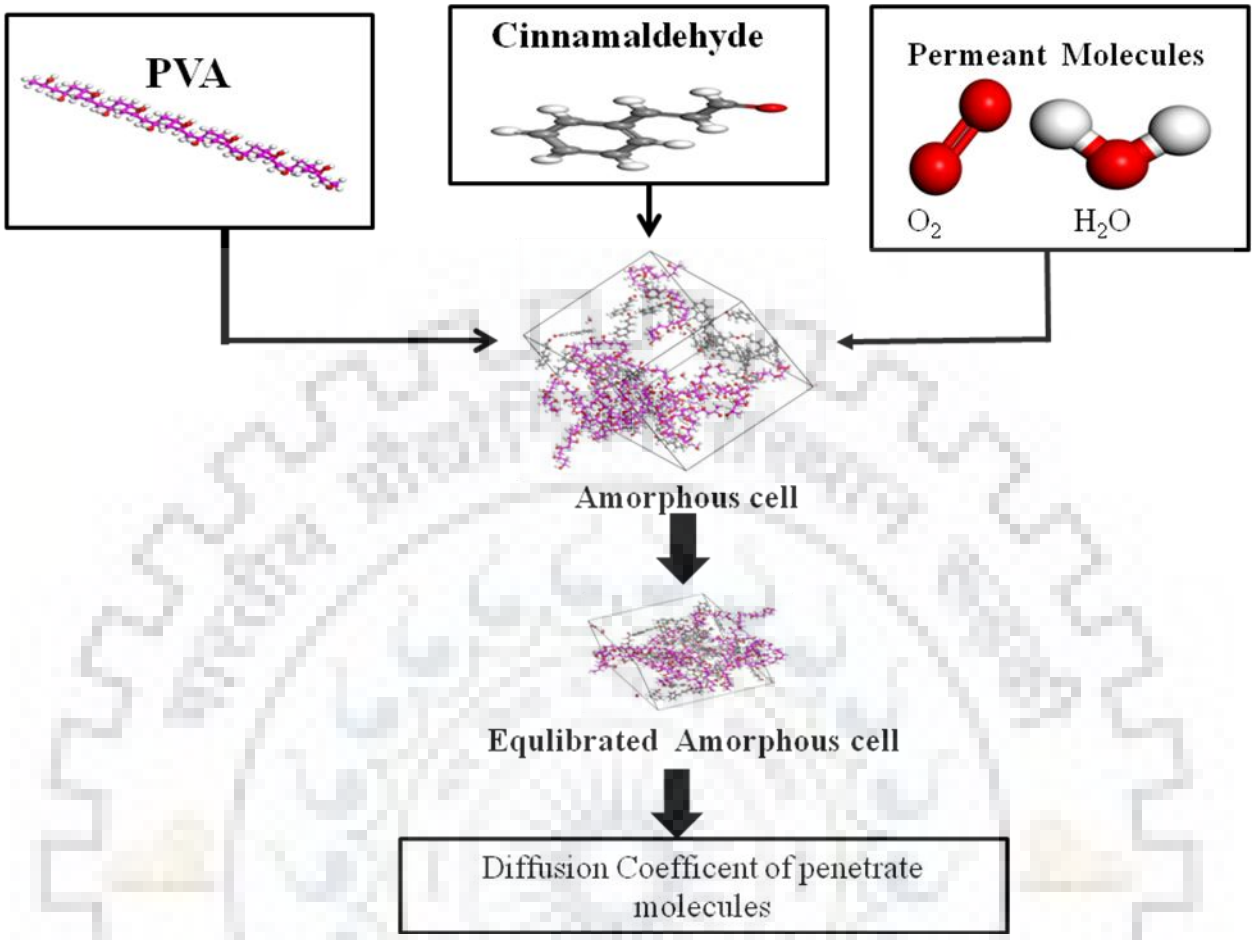

Figure 8: Flow Chart of simulation strategy to evaluate diffusion coefficient of permeant molecules.

The following steps involve in evaluating the diffusion coefficient of O_2 and water vapor are as follows:

1. Construction of initial structure

The initial structure of PVA with different chain lengths (n=5, 10, 15, 20, 25), Cinnamaldehyde, O_2 and H_2O were constructed. The geometrical optimization of structures of PVA with 1000 iteration so that the energy of the material's structure is brought to stable geometry.

2. Selection of the critical chain length for PVA

To select the critical chain length of PVA for MD simulation, The series of dynamics (NVT, NPT ensembles) performed on the geometrically optimized structure of PVA with different chain length(n=5, 10, 15, 20, 25) to determined density and solubility parameter which is plotted with

repeated chain length. The stable saturated value obtained from the graph confirmed that this chain length (n) is suitable for a simulation run.

3. Construction of amorphous cell

The amorphous cells (AC) are built with 10 chains of 20 monomeric unit of PVA with different composition of CIN (15%, 45%, 30%) and 10 molecules of permeating molecules (O_2/H_2O) in the amorphous cell.

Table 3: Loading of PVA, CIN and permeant molecules in Amorphous Cell.

Composition	Loading of Chain/Molecules(%) in Amorphous Cell		
	PVA	CIN	O_2/H_2O
PVA	10 (98%)	-	10 (2%)
PVA-15CIN	10 (82.2%)	12 (14.8%)	10 (3%)
PVA-30CIN	10 (67.3%)	30 (30.2%)	10 (2.4%)
PVA-45CIN	10 (53.8%)	55 (44.3)	10 (1.9%)

4. Equilibration of amorphous cell

The initially created ACs have exposed to energy minimization by using Geometry optimization Forcite module which is governed by Smart Minimizer for 1000 iteration. The optimized energy structure is then subjected to Annealing at an initial temperature of 300K and mid-temperature of 500K to make the system to obliterate the initial configuration bias. Once initial configuration bais forget by the system, NVT ensembles were performed at 300K for 100 ps, in order to maintain a constant temperature. The generated frame of NVT ensembles is subjected to the constant pressure of 1 atm at 300 K for the duration of 200 ps in order to equilibrate the density. The equilibrate A C has been used to predict various properties of the molecules or the PVA.

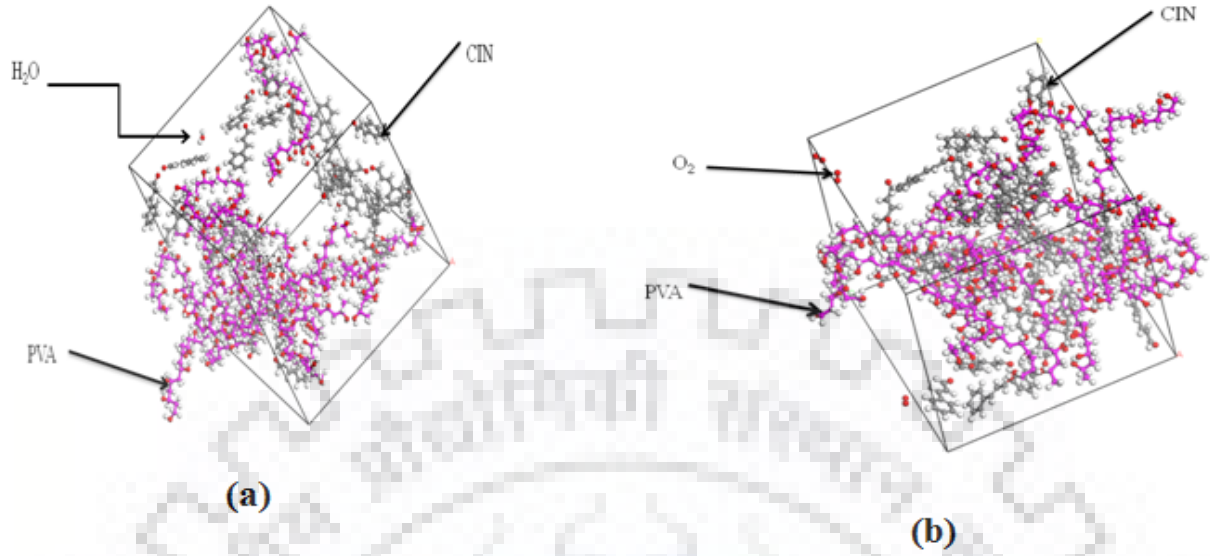

Figure 9: Snapshot of Equilibrated Amorphous cell of (a) PVA-30 CIN with water vapor molecules (b) PVA-30CIN with Oxygen gas molecules

5. Diffusion Coefficient of Permeating molecluces

In order to evaluate the diffusion coefficient of the penetrating molecules production run of 1000 ps of *NVE dynamics* at the temperature of 300 K have performed. for a small time duration, The penetrate gas molecules like O_2, water vapor collides inside the cavity of the free volume, but for the longer time duration penetrates molecules jump from the cavity of one free volume to another cavity of free volume. This resulted jump is the diffusion which is characterized by an MSD. The increase of MSD with time is given by,

$$D_\alpha = \frac{1}{6N_\alpha} \lim_{t\to\infty} \frac{d}{dt} \sum_{i=1}^{N_\alpha} \langle |r_t(t) - r_i(0)|^2 \rangle$$

The diffusion coefficient of the penetrating molecules is given by $\frac{1}{6}$ *of the slope of MSD as function of the time graph.*

Where r_i *and* r_t *are the position vector of permeant molecules at time* $t = o$ & $t = t$.

5.4 Film Characterization

5.4.1 Film Thickness

The Film thickness was measured by a manual gauge meter with a least count of 0.005 mm at six random points of each film sample. The average film thickness was determined.

5.4.2 Film Color

The Film color was measured by using a Konica Minolta CM-3630 spectrophotometer. The white standard plate with a specification of L^*=96.79, a*=-0.09, b*=-0.30 was taken for the calibration of the spectrophotometer and also as the background of polymeric film for measuring film color. The film color is expressed by the CIELab color space.

Figure 10: Image of Spectrophotometer (a) and white standard plate (b).

The total color difference (ΔE *) was determined by using an equation mentioned below:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \text{.....E.Q.(5.4.2)}$$

Where L^* resembles lightness which is 0 for black and 100 for white; a^* resembles redness in which (-) sign is for green and (+) is for red;b* resembles yellowness in which (-) sign is for blue and (+) sign is for yellow.

The opacity of the film performed by using a UV spectrophotometer. A rectangular strip of dimension (10×40mm) of the film sample was taken. The rectangular strip then placed in the cuvette. The empty cuvette was taken as reference.

5.4.3 Field Emission-Scanning electron microscope(FE-SEM)

The Surface morphologies of the film samples were observed by using a TESCAN Mira 3 Scanning TESCAN Mira 3 Scanning Electron Microscope operating at 5 kV acceleration under moderate vacuum. Prior to the observation of film morphologies, all the film sample were made conductive by sputtering of golden layer over it.

5.4.4 Mechanical Properties

The tensile properties of film samples were estimated according to ASTM D882-18. The film sample was cut into a rectangular strip in the dimension of $15mm(Width) \times 100\ mm(length)$ and clamped in the device. The stretching speed and initial grip distance were taken as 50 mm/min and 50 mm respectively. The three film sample replicate were used to perform the test.

5.4.5 Water Vapor barrier properties

The Gravimetric cup method was employed to determine the water vapor barrier properties of the AM film samples in which a glass beaker with an internal diameter of 20 mm and 314.159 mm^2 was used. Silica gel was used as a desiccant. It was activated by heating at $100°C$ at the oven in order to remove all moisture present in it. 1g of dried dessicant is filled inside the glass beaker, and sealed with film sample . After joting down the weight of film sealed glass beaker, it is placed inside the dessicator containing tap water .T hen the dessicator was put inside the oven which is set at $27°C$.

Figure 11: Experimental setup to evaluate water barrier properties.

With the help of this setup, inside the beaker, 0% RH (relative humidity) was obtained by using silica gel and 99% RH was maintained externally using tap water. The changes in the weight of the beaker were weighed and recorded in the regular spell of 24hrs.

The water vapor permeability (WVP) and water vapor transmission(WVTR) of film samples were estimated using the following equation:

$$WVTR = \frac{w}{A \times t} \qquad \& \qquad WVP = \frac{WVTR \times d}{p \times (R_1 - R_2)}$$

Where "w" is the weight gained, "t "was the time elapsed, "A" is the exposed Film area for the water vapor transmission, "d" is film thickness and "R_1" and "R_2" was the relative humidity internal and external of the beaker and "p" water vapor pressure.

6.1 Antimicrobial Testing

The antimicrobial activity of the pristine PVA and PVA incorporated with CIN was studied against the gram-negative bacteria *Escherichia Coli* was taken from experiments reported by S. Kardam, 2017[11]. The control samples were empty petri plates i.e. without PVA film and cinnamaldehyde. The bacteria growth reduced as the concentration of the CIN in the PVA polymer matrix increase. The bacterial growth for after 24 hours and 48 hours of the incubation has been seen from figure depicted below. PVA-45CIN shown of bacteria both after the incubation of 24 hours and 48 hours.

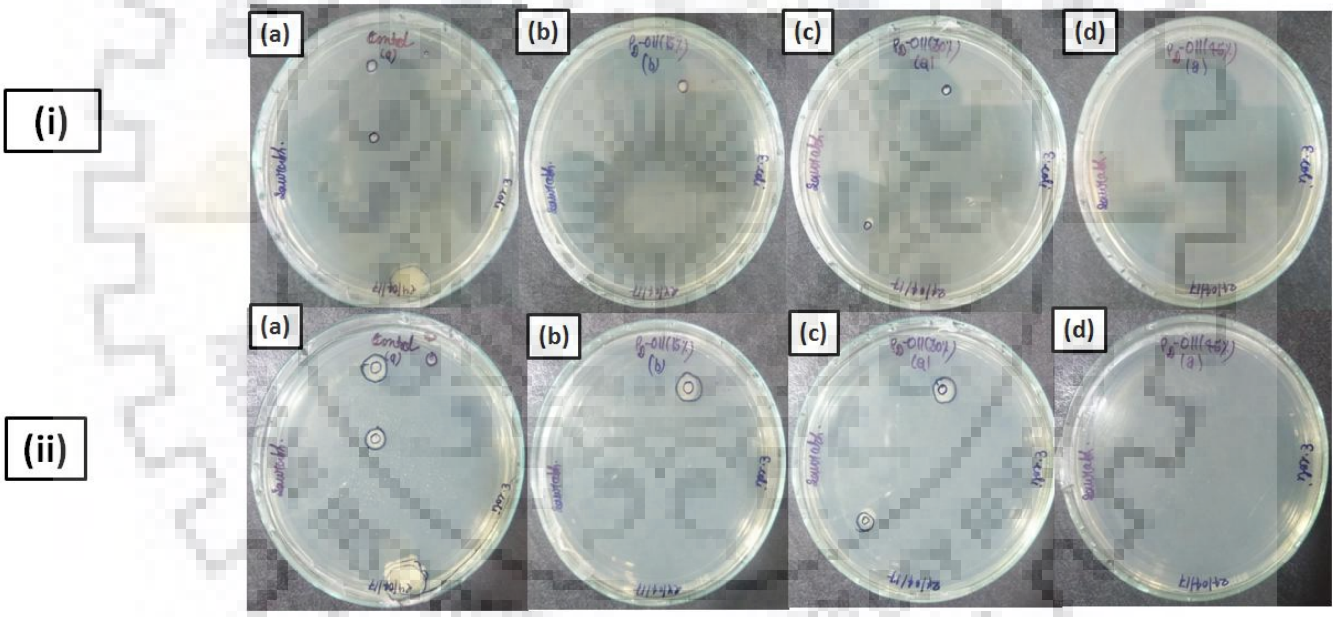

Figure 12: E.coli bacterial colony growth after (i) 24 hours (ii)48 hours of incubation for (a) Control (b) PVA-15CIN (c) PVA-30CIN (d)PVA-45CIN

The E. coli bacterial colony count for (a) Control, (b) PVA-15CIN, (c) PVA-30CIN, (d) PVA-45CIN are 3×10^{10} CFU/ml, 1×10^{10} CFU/ml, 2×10^{10} CFU/ml and 0×10^{10} CFU/ml respectively.

The antimicrobial properties of the PVA-CIN are due to the aldehyde group which dissolves the membrane lipids on interacting with it, hence resulting in the rupture and leakage of the intracellular liquid which caused microbes cell death.

6.2 Molecular Dynamics Simulation

6.1.1 Selection of Critical Chain Length (n_C) of PVA

The graph obtained from plotting repeated unit v/s density (ρ) and repeated unit v/s solubility parameter (δ) show the saturation beyond the chain length n=20 of the PVA for MD simulation. For the chain length for n= 20, the value of density and solubility parameter are $\rho_{PVA=1.12}\ g/cm^3$ and $\delta_{PVA=20.975(J/cm^3)^{0.5}}$ which is quite close to the reported literature value of PVA that is $\rho_{PVA=1.19}\frac{g}{cm^3}$ and $\delta_{PVA=22.9(J/cm^3)^{0.5}}$ [10].

As the simulation time increase with the increasing chain length, Thus the use of $n_{c=}$ 20 for simulation to replicate the PVA property is justified.

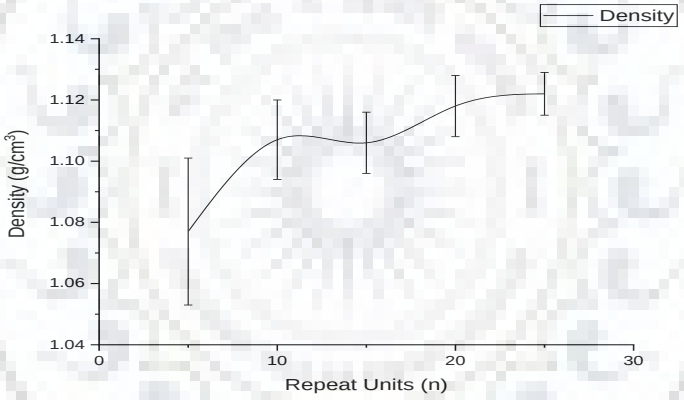

Figure 13: Density of PVA as a function of chain length

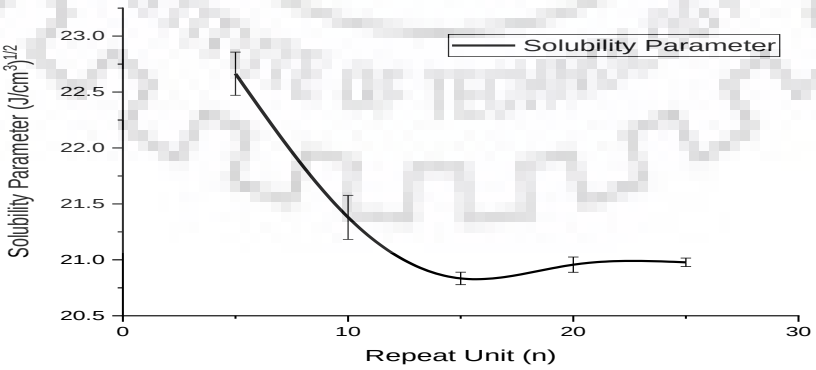

Figure 14: Solubility parameter of PVA as a function of chain length.

6.2.2 Diffusion Coefficient of Permeate molecules

The diffusion coefficient of O_2 gas and water vapor in PVA-CIN is computed at room temperature of 27℃. The diffusion coefficient of permeate molecules computed using the 1/6 of the slope of the MSD as a function of time graph. The MSD as a function of the time graph mentioned in figure 15.

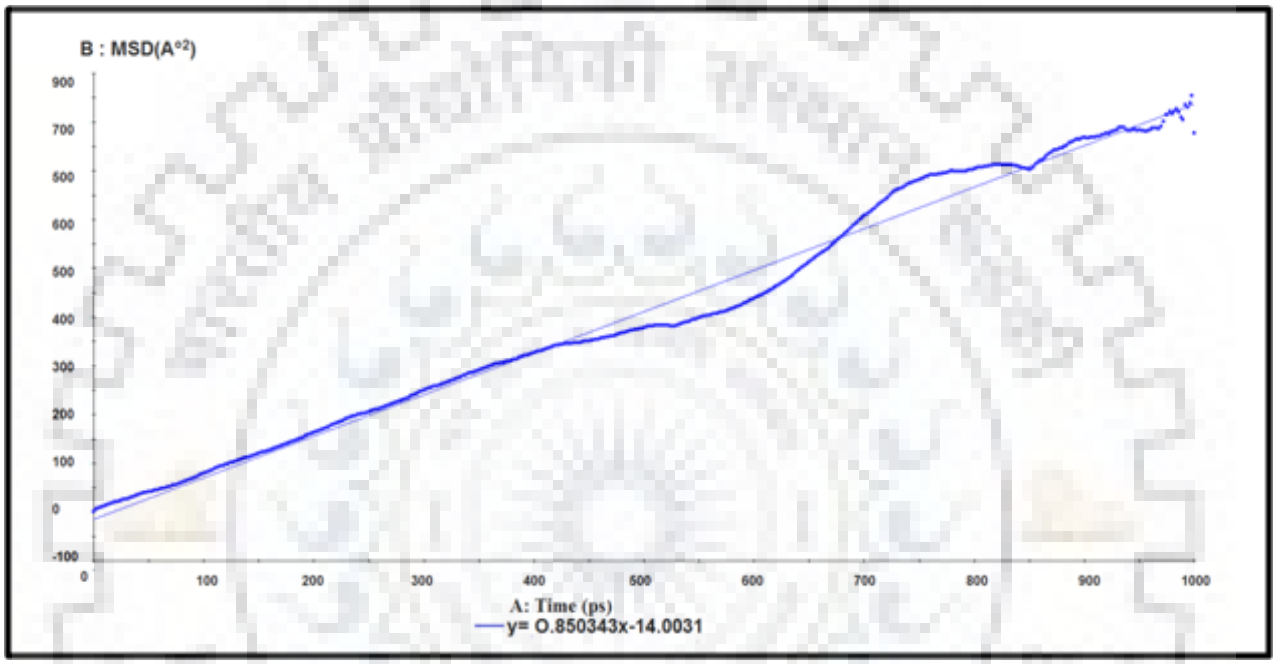

Figure 15: Oxygen MSD as Function of Time

Diffusion Coefficient of Oxygen gas in PVA-CIN matrix

With the above-stated procedure, the diffusion coefficient of oxygen with different composition of CIN is computed shown in Figure 16. It was found out that with the increase in the concentration of the CIN in PVA the diffusion coefficient of oxygen gas increases significantly. With the incorporation of the CIN in the PVA matrix, the interaction of the hydrophobic CIN with the hydrophilic PVA is very less. Due to low interaction between the PVA-CIN void is created which enables the O_2 molecules to pass through PVA-CIN matrix. A similar trend of increase in permeability with the increasing concentration of the EOs is reported by (C. Chen et al., 2018)[2].

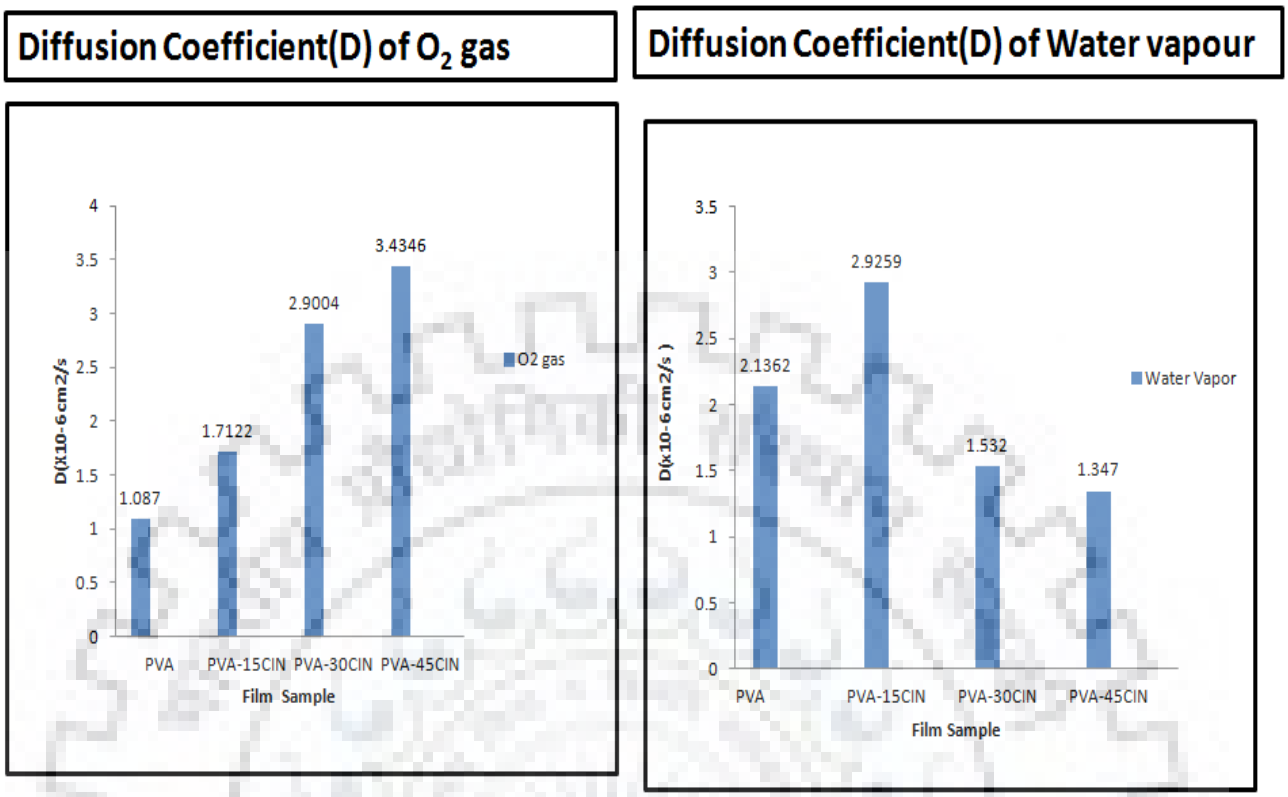

Figure 16: Diffusion Coefficient of Oxygen gas and water vapour of PVA-CIN.

Diffusion Coefficient of water vapor in PVA-CIN matrix

The diffusion coefficient of the water vapor in PVA-CIN matrix shown in figure 16. With the increase in the concentration of CIN in the PVA, the diffusion coefficient of water vapor increase for PVA-15CIN (1.7122 cm^2/s) as compared to PVA (1.087 cm^2/s).The reason behind this is the hydrophobic nature of CIN disperse in the PVA matrix which creates the pore or cavity. Similar trends are shown in experimentally calculated water vapor permeability of PVA-CIN and also the same trend reported by (Gao et al., 2017)[12]. But the diffusion coefficient of water vapor for PVA-30CIN and PVA-45CIN decreases as compared to the D of PVA-15 CIN. The similar trend was shown in the experimentally calculated water vapor permeability and also similar trend reported.

6.3 Film Characterization

6.3.1. Film Color

The all films color were listed below in the table , the lightness value (L^*) value of the film slightly decreases when the incorporation of the cinnamaldehyde increases with compared to pristine PVA,.While the redness value (a^*) decreases drastically as the concentration of cinnamaldehyde increases with respect to pristine PVA film, which means color of the film moves towards greenish in nature with the incorporation of cinnamaldehyde in the antimicrobial film. While the yellowness value increases significantly with the incorporation of cinnamaldehyde which means yellowness intensity increases as we increase the concentration of cinnamaldehyde. Thus, The incorporation of the CEOs in the PVA films results in lower chroma, less whiteness and higher yellowish in appearance.

The transmittance (%) of the film shown the film was highly transparent. But the transparency of the film decreases with the increases in the concentration of the CIN.

Table 4: Film color and transmittance(%) comparison of PVA and PVA-CIN Film

Film Sample	L^*	a^*	b^*	ΔE	Transmittance (%) At 600nm
PVA	94.54±0.040	-0.12±0.016	0.13±0.025	2.29±0.039	99.60
PVA-15CIN	94.02±0.085	-0.24±0.025	0.21±0.014	2.82±0.021	99.03
PVA-30CIN	93.62±0.025	-0.45±0.015	0.78±026	3.36±0.047	98.34
PVA-45CIN	93.17±0.030	-0.69±0.040	1.22±0.036	3.97±0.018	97.85

6.3.3. Film Structure

The FE-SEM images of the pristine PVA in figure (17a) that it shows an even and uniform surface without any significant irregularities. and insoluble particles of PVA observe with the dimension of 0.24 μm. With the increase in the concentration of the cinnamaldehyde, uneven

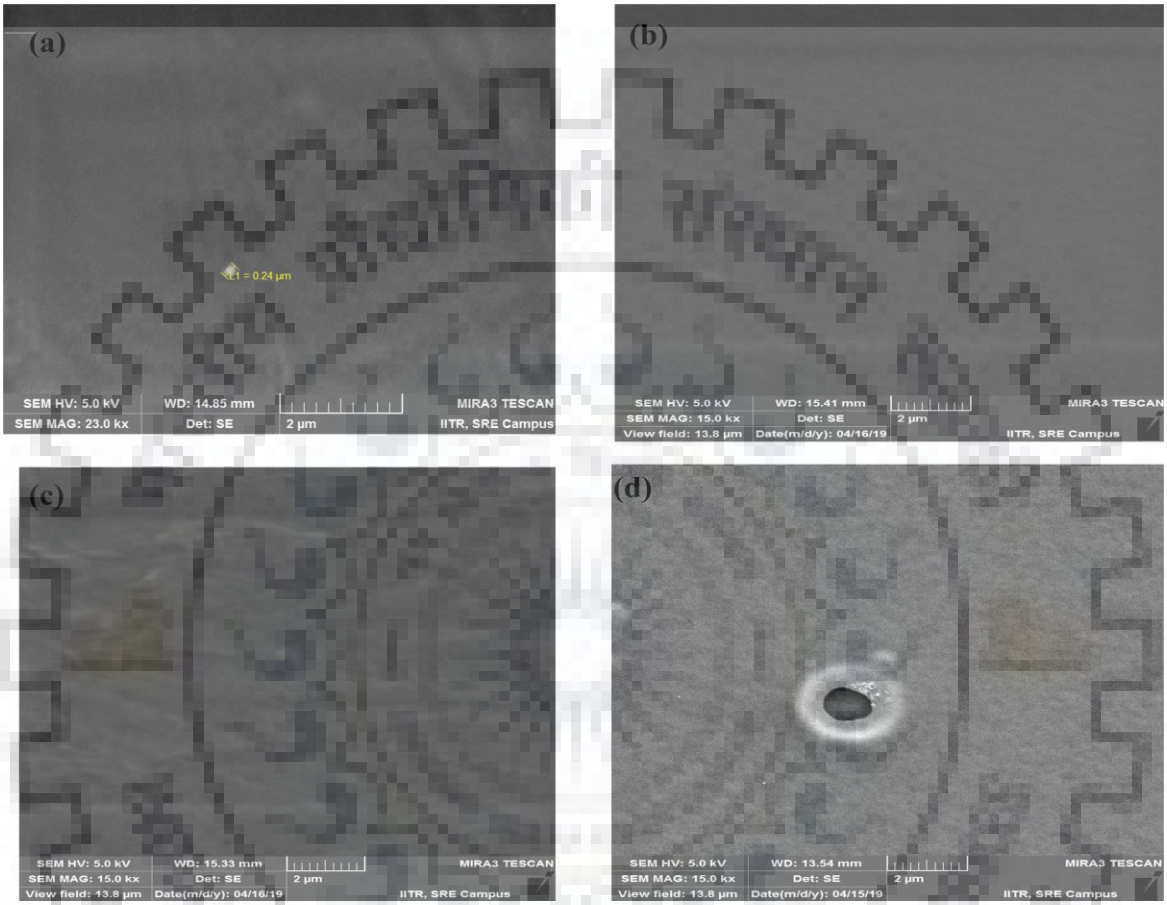

Figure 17: FE-SEM of Surface of Film (a) PVA (b) PVA-15CIN (c) PVA-30 CIN (d) PVA-45 CIN

surfaces are observed in figure (17).The hole like struture appear in PVA-45CIN which resembles the droplets of CIN entraped on the polymer structure of PVA .Th e smoothness and the uniformity of the film topology is accord with the increasing cinnamaldehyde concentration in the antimicrobial film.

6.3.4 Mechanical Properties

The tensile properties of the film samples are mentioned in the table below. As the concentration of CIN in the PVA polymer film increases the tensile strength decrease by 7.75% for PVA-45CIN as compared to the pristine PVA. Elongation at break (EAB%) increase by 55.98% for

PVA-45CIN as compared to pristine PVA. It is due to weaker PVA-CIN interaction at PVA-CIN film as compared to strong PVA intermolecular interaction at PVA film.

Table 5: Mechanical properties of the film

Film Sample	Film Thickness (µm)	TS (Mpa)	EAB (%)
PVA	34.998±2.205	27.3682±0.6533	136.66±.00367
PVA-15CIN	38.887±2.017	26.6098±0.5459	152.71±.0604
PVA-30CIN	38.348±2.471	25.9405±0.4054	184.15±.07213
PVA-45CIN	39.8873±2.0171	25.2765±0.9827	213.17±0.1101

6.3.5 Water vapor Barrier Properties

The water vapor barrier properties of PVA-CIN Film is found out by the gravimetric cup method. The WVTR and WVP of the PVA-CIN matrix shown in the figure. With the increase in the concentration of CIN in the PVA, WVTR and WVP increase for PVA-15CIN as compared to PVA. The reason behind this is the hydrophobic nature of CIN disperse in the PVA matrix which creates the pore or cavity. Similar trends are shown in Simulated D of water vapor permeability of PVA-CIN and also the same trend reported by (Gao et al., 2017)[12]. But the WVTR for PVA-30CIN and PVA-45CIN decreases as compared to the WVTR of PVA. The similar trend is shown in the experimentally calculated Water vapor permeability and also similar trend reported[2].

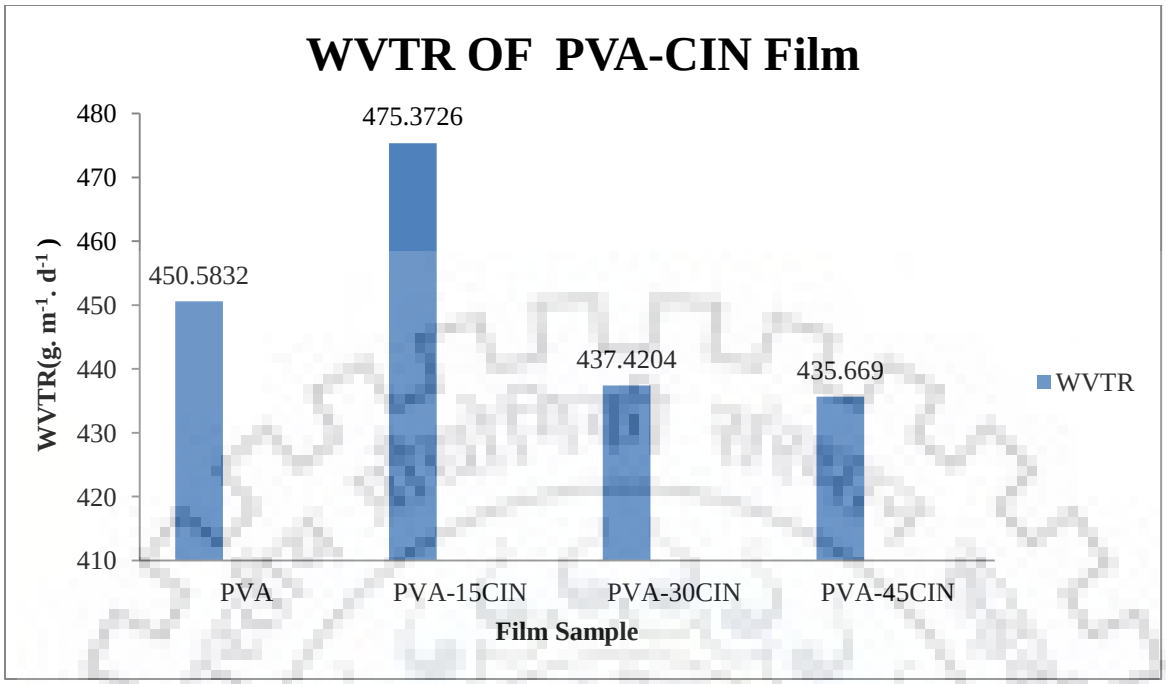

Figure 18: WVTR of PVA-CIN Film

Table 6: WVTR and WVP of PVA-CIN Film

Film Sample	Weight gained/day (g/day)	WVTR (g m^{-2} d^{-1})	WVP (x10^{-13} g m m^{-2} s^{-1} Pa^{-1})
PVA	0.14148±0.00049	450.583	5.9025
PVA-15CIN	0.14927±0.00552	475.373	6.0712
PVA-30CIN	0.13735±0.00596	437.420	5.7293
PVA-45CIN	0.13681±0.00592	435.669	5.7055

6.3.5 FT-IR

The FTIR spectra of (a) PVA, (b) PVA-15CIN, (c) PVA-30CIN and (d) PVA-45CIN film are shown in figure 19. For PVA film, the absorbance band obtained around the 3094-3694 cm^{-1} resembles the -OH bond. The absorption peak around 2901 resembles stretching of -C-H- in the-C-O-H- bond where absorption bands at 1434 represent stretching vibration of -C-O- in the -C-O-H-.

For the PVA-CIN film, the Absorption band of 2935 resembles the stretching and bending of -C-H-in the aldehyde group in Cinnamaldehyde.

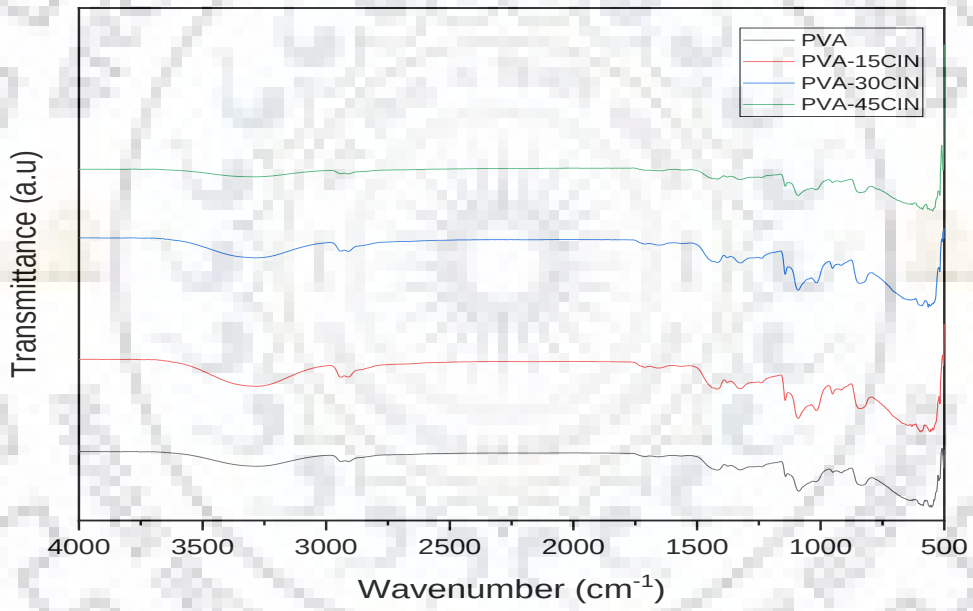

Figure 19:FT-IR spectra (a) PVA Film, (b) PVA-15CIN, (c) PVA-30CIN Film and (d) PVA-45CIN Film.

7. Conclusion

The PVA-45CIN Film and PVA-30CIN show the total inhibition and 89.47% against the gram-negative bacteria E. coli even after the incubation period of 48 hours. The diffusion coefficient of oxygen gas in PVA-CIN matrix increase with an increase in the concentration of CIN. The diffusion coefficient of water vapor, it first increases the decrease with the concentration of CIN and show similar trends with experimental results and also with the reported literature. The WVP of the PVA-45CIN and PVA-30 film reduced by 3.3% and 2.934% as compared to pure PVA films, which signifies an increase in the moisture barrier properties. The tensile strength of PVA-45CIN film and PVA-30 films lightly decrease by 7.64% and 5.21% as compared to PVA Film.

On the basis of above antimicrobial testing, diffusion coefficient, WVP and tensile properties the best optimal composition is PVA-30CIN Film.

8. Reference

[1] R. Priyadarshi et al., Chitosan incorporated with Apricot kernel essential oil as active food packaging material.Food hydrocolloids.2018.

[2] C. Chen et al., Properties , vapour-phase antimicrobial and antioxidant activities of active poly(vinyl alcohol) packaging films incorporated with clove oil.2018.

[3] M. Munier, Diffusion coefficient of small gas molecules in amorphous cis-1,4-polybutadiene estimated by molecular dynamics simulation ,The journal of Chemical Physics, 2005.

[4] Siracusa, Food Packaging Permeability Behaviour: A Report, Journal of Polymer Science, 2012.

[5] Calo et al., Essential oils as antimicrobials in food systems e A review,Food Control,2015.

[6] P. Appendini et al., Review of antimicrobial food packaging.Innovative food sciences and emerging technology, 2002.

[7] Perez et al., Incorportation of Antimicrobial agents in Food Packaging Film and coating,Advances in food and biotechnology, 2006.

[8] Kung F. et al, Enhancement of the antimicrobial activity of cinnamon essential oil-loaded electrospun nanofilm by the incorporation of lysozyme,Royal society of chemistry,2016.

[9] Seenivasan et al., In Vitro Antibacterial Activity of some plant essential oil. BMC Complementary and Alternative Medicine, 2006.

[10] S. K. Sethi et al,Component compatibility study of poly (dimethyl siloxane) with poly (vinyl acetate) of varying hydrolysis content: An atomistic and mesoscale simulation approach,Journal of Molecular Liquid, 2018.

[11] S. Kardam et al., Packaging Material with natural (plant) based antimicrobial substances, M.Tech Thesis, Department of Paper Technology, IIT Roorkee, 2017.

[12] Gao et al., Characteristics of poly(vinyl Alcohol) films cross linked by cinnamaldehyde with improved transparency,Journal of Applied Polymer science,2012.

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