Chitosan Composites –Prospective Materials in Food Safety

Thesis submitted to
the University of Calicut in partial fulfilment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

Under the Faculty of Science

by

MUJEEB RAHMAN P.

Supervising teacher

Dr.V.M Abdul Mujeeb
Professor

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALICUT
KERALA 673635
INDIA
2016

DECLARATION

I declare that the work presented in the thesis **Chitosan Composites – Prospective Materials in Food Safety** is an authentic record of the original work done by me under the direct supervision and guidance of **Dr.V.M Abdul Mujeeb**, Professor, Department of Chemistry, University of Calicut in the partial fulfilment of the requirements for the award of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been included in any other thesis submitted previously for the award of any other degree of this university or any other university or institution.

MUJEEB RAHAMN .P

Dr.V.M.Abdul Mujeeb
Professor
Department of Chemistry
University of Calicut

CERTIFICATE

This is to certify that the dissertation entitled "**Chitosan Composites – Prospective Materials in Food Safety"** bound herewith is a bonafide work done Mr.Mujeeb Rahman under my supervision in the Department of Chemistry in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Chemistry of the University of Calicut, and that the work has not been included in any other thesis submitted previously for the award of any other degree.

Dr.V.M Abdul Mujeeb

ACKNOWLEDGEMENT

I would like express my deep sense of gratitude and sincere thanks to Dr.V.M Abdul Mujeeb, professor, Department of Chemistry, University of Calicut for his valuable guidance, precious suggestions and constant encouragement during the course of this investigation. He has not only provided wise advice but also been a source of inspiration. I am grateful to him for all the ways he has directed me to move forward in my research carrier.

I would like to express profound gratitude and indebtedness to Dr.K.Muraleedharan, Professor, Head of the Department of Chemistry, University of Calicut for providing all the necessary facilities and support throughout the course of period. I greatly acknowledge the valuable suggestions, encouragements and help that Dr.Muraleedharan provided during this period.

I express my sincere thanks to all the faculty members of the department of Chemistry, university of Calicut for their love and generous help throughout the course of this work. I would like to thank the technical and ministerial staff in the department of Chemistry for their kind help in the lab and office.

I express my gratitude to my friends in the department who have been extremely sincere to me and have provided me all sorts of help whenever needed. I would like to acknowledge gratefully the whole hearted cooperation and support rendered by lab mates in Physical Chemistry lab and other friends in Inorganic and Organic lab.

I extend my gratitude to the persons in the departments of Physics and life Sciences, University of Calicut, Department of Chemistry and Microbiology, MAMO college Mukkam, Department of Chemistry, St.Joseph's College, Devagiry, Department of Chemistry, NIT Calicut, Department of Chemical Engineering, NITC,STIC,CUSAT, Sree Chithira institute of medical Science, Thiruvanathapuram, Uni biosys Lab, Cochin for having kind enough to utilize their Instrumental, Analytical and Laboratory facilities.

I am thankful to my colleagues Dr. Mercy Raj, Mr.Saleem Kollam and all teaching and non teaching staff of Mannaniya college of Arts and Science for their sincere support and all sorts of help they rendered.

I would like to express my gratitude to University Grants Commission, UGC for providing Teacher fellowship for completing this work.

I would like to express my hearty thanks to my wife and children for their great support, endurance, prayer and encouragement during each stage of this work. I am extremely grateful to my parents, brothers, sisters and friends for their support and care.

Above all I am thankful to the Almighty God whose benevolence made this into a reality.

Mujeeb Rahman. P

Dedicated to

Richu, Rinu and Bava

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

PE	Polyethylene
ICL	imperial Chemical Limited
CPCB	Central Pollution Control Board
ASTM	American Society for Testing and Materials
PHA	Poly hydroxy alconate
PHBV	Poly hydroxy butyrate and hyroxyl valerate
OPC	Oxygen permeability Coefficient
WVP	Water vapour permeability
MAP	Modified Atmospheric Packages
WVTR	Water vapour transmission rate
OTR	Oxygen transmission rate
GRSM	Generally recognised safe materials
WHO	World Health Organization
PVC	Polyvinyl Chloride
PLA	Poly lactic acid
PHB	Polyhydroxy butyrate
CIFT	Central Institute of Fisheries Technology
PVA	Polyvinyl alcohol
UV	Ultra violet
ROS	Reactive Oxygen Species
Ev	electron Volt
GTE	Green tea extract
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray Diffraction
SEM	Scanning Electron Microscopy
E.Coli	Escherichia coli
S.aureus	Staphylococcus aureus
C	bare Chitosan
CN15	Chitosan-nano ZnO composite from 15% NaOH
CN30	Chitosan-nano ZnO composite from 30% NaOH
CN45	Chitosan-nano ZnO composite from 45% NaOH
TGA	Thermo Gravimetric Analysis
JCPDS	Joint Committee on Powder Diffraction Standards

β	Full width at half maximum
nm	nano meter
au	atomic unit
MTCC	Microbial Type Culture Collection
ATR	Attenuated total reflectance
DSC	Differential scanning calorimeter
FESEM	Field Emission Scanning Electron Microscopy
LCR HI tester	inductance (L), capacitance (C), and resistance (R) testing
p	Statistical probability
cm	Centimetre
C-0.5	Chitosan composite film prepared by adding 0.5g Zinc Acetate
C-1	Chitosan composite film prepared by adding 1.0g Zinc Acetate
C-1.5	Chitosan composite film prepared by adding 1.5g Zinc Acetate
C-2	Chitosan composite film prepared by adding 2.0g Zinc Acetate
TS	Tensile Strength
EB	Elongation at Break
Tg	Glass transition temperature
Pc	Percolation Threshold
cfu	colony forming units
OD	Optical Density
DPPH	2,2-diphenyl-1-picrylhydrazyl
LDPE	low density polyethylene
BHA	Butylated hydroxyl anisol
BHT	Butylated hydroxyl toluene
THBQ	tertiary butyl hydroquinone
CI	Chitosan composite film prepared by adding 0.1g GTE Powder
C2	Chitosan composite film prepared by adding 0.2g GTE Powder
C3	Chitosan composite film prepared by adding 0.3g GTE Powder
C4	Chitosan composite film prepared by adding 0.4g GTE

	Powder
	Chitosan composite film prepared by adding 0.5g GTE
C5	Powder
η	Micro
λmax	Wave length corresponding to maximum absorption

ABSTRACT

In spite of great advantages of synthetic polymers, they become now a serious threat to our environment. Discarded plastic products have caused irreparable damages to the environment and it led to a cry for an alternative for synthetic polymer products. The growing environmental concerns call for novel packaging materials having the attributes of environmentally benign nature and biocompatibility. So far, many biopolymers have been exploited for packaging applications, among them the most focussed material is Chitosan. Chitosan is the second most abundant biopolymer after cellulose. It is green, biocompatible and edible polymer. Chitosan is economically available from renewable resources like cell walls of crustaceans and crab shells. Even though Chitosan can form excellent films, their mechanical and barrier properties are not favourable for present packaging applications. This paved the way for research focussing to improve its physical, mechanical and barrier properties. Nowadays, incorporation of novel materials onto polymer matrix has been exploited to enhance overall performance of polymer materials. Hence, Chitosan composites have become thrust area of current packaging material research. Suitable functional molecules having characteristic properties were selected and incorporated with Chitosan so as to get desired packaging films.

In the present research, we aimed to develop smart packaging pouches of Chitosan composites to extend the shelf life of raw meat. Since meat is a rapidly perishable food due to contamination of various pathogens, it is packed with large amount of synthetic preservatives

and kept at refrigeration conditions. Growing health concerns of consumers over synthetic preservatives have become another issue in the international meat market. Hence, people are actively involved in developing “green” preservative molecules to improve the shelf life of raw meat. A smart packaging material can enhance shelf life and quality of raw meat by inhibiting microbial growth on the surface of packed meat. In view of this, we have synthesised two different Chitosan composites, were characterized and applied directly for shelf life studies.

The first prepared material was Chitosan-nano ZnO composite, the nano ZnO was selected due to its remarkable stability, facile synthetic methods and antimicrobial efficiency. According to U.S Food and Drug Administration protocol (21CFR182.8991), ZnO is considered as a generally recognized safe (GRAS) material. Initially, Chitosan-nano ZnO composite powder was prepared to elucidate the physical, chemical and antimicrobial properties of the new material. A one pot procedure was adopted with adequate care to follow green chemistry protocols. The composite powder was ably characterized to establish the existence of nano ZnO particles in the matrix. The XRD data revealed the true existence of ZnO particles in the nano regime, which was further confirmed by SEM analysis. UV-Vis analysis proved semiconductor behaviour of nanoparticles even in the matrix of Chitosan. Thermal investigation underlined the enhanced thermal stability of the composite as compared to bare Chitosan film. In addition to all enhanced physico-chemical properties, the composite materials exhibited surprisingly higher antimicrobial properties and it

was quantitatively investigated by well diffusion method and agar plate diffusion method. The standard and clinical samples of E-coli and Staphylococus were selected as model bacteria for the analysis. All experiments undeniably proved excellent antimicrobial properties of composites.

Chitosan-nano ZnO composite pouches have been prepared with required modification of above mentioned procedure. Composite films were prepared and optimised by different characterization techniques. The data obtained from FTIR, XRD, SEM TGA and DSC analysis were in agreement with that of composite powder. Antimicrobial efficacy of composite films was many folds greater than that of bare Chitosan film. Sensitive properties such as water sorption, water solubility and water vapour transmission rate were compared with that of pure Chitosan film. The incorporation of nano ZnO particles in the Chitosan matrix has significantly changed the water solubility, water transmission rate and water sorption capacity. Analysis of mechanical strength of the film was so encouraging that all composites exhibited excellent tensile strength than bare Chitosan film. Optimization of various properties of the film showed that C-2 film (equal amount precursors) has the highest antimicrobial activity with better mechanical and physical properties. Finally C-2 films were fabricated into small pouches for packaging studies of raw meat. The meat was collected from local market, washed well, packed as such without adding preservatives and stored at refrigerator temperature 4^0C.Polythene pouches, which is most commonly used to pack, store and transport meat between producers and consumers were selected for

comparatives studies. Equal amounts raw meat was packed in polythene pouches and stored at the same conditions for the comparative analysis. Shelf life investigation revealed that C-2 pouches have significantly inhibited the growth of microorganisms in raw meat whereas there had an exponential growth of the microorganism in the meat sample stored in polyethylene pouch. This investigation emphasized the property of composite bag to enhance the shelf life of raw meat.

The second type of composite material, we synthesised was Chitosan-green tea extract film. Green tea is one of the most common food additives having characteristic antioxidant and antimicrobial properties. There is a large cultivation of green tea leaves across Kerala, hence it is considered as economical for packaging applications. The composite films were prepared by simple one pot procedure without using any toxic chemicals or solvents. All composite films were well characterized by various physico-chemical methods. Mechanical strength, water content, water solubility and contact angle were measured prior to conducting packaging applications. It is well known that modified atmospheric condition inside the pack plays a crucial role in extending the shelf life of raw meat. The decisive factors of modified atmospheric condition vary with type of food and nature of packaging materials. Hence optimization of the film was done by preparing five different composite films. It was observed that mechanical and solubility properties of films have a rise and fall like trend, therefore C4 composite film was selected to fabricate pouches to extend the shelf

life of raw meat. Meat was collected from the local market and was washed well before doing packaging application. The shelf life analysis was repeated as in the preceding case. This composite film also exhibited excellent antimicrobial efficacy as compared to polyethylene packs and can be utilized as an efficient antimicrobial pouches.

Key words

Chitosan, Chitosan composites, nano ZnO, antimicrobial, antioxidant, dielectric properties, packaging applications, shelf life, Polythelene pouches, raw meat, polymer composite, green polymer,Green tea extract, composite pouches, modified atmospheric conditions, water transmission rate, contact angle, water content, sorption studies, tensile strength, elongation at break.

PCHAPTER 1
INTRODUCTION

Food safety is an important social and health priority in any developed society. It is vital for economic growth and progress as well. It has been a topic of major focus for researchers, policy makers and industrialists all over the world. In the era of globalisation every society depends upon import of food to cater their complete need as well. Hence, the emphasis on quality of food has taken the central stage in the global scenario of present food trade. It is well known that almost all packed food is vulnerable to serious contamination. The contamination of food is associated with its shelf life, methods of processing and storage conditions. A thorough understanding of harmless handling of food in all stages (preparation, packing and trading) is the key factors to provide safe food in our society. This will be possible only when researchers, policymakers and industrialists join hands together with a same goal.

1.1 Packed food

Packed foods have become an indispensable part of the daily requirements of the developed societies. The inception of modern packaging technologies has ensured availability of any food items in market breaking seasonal and energy barriers [1]. The notion of "packed food" was gradually developed with the demands of consumers, population growth and globalization of market. The current demands of packed foods are met with different packaging technologies. The effective packaging can be defined as a packaging, which ensures the quality and safety of foods without compromising on shelf life of foods[2]. With the advent of modern technology, there was a giant leap in packaging industry. The road map of development of packed food is given below [3].

Table-1 Road map of development of packaging applications

Year span	Developments
1800-1850	NicolasAppert in 1809 in France packed cooked food in sealed glass jars. Later PeterDurand used tin can for packing. In 1852, FrancisWolle of Pennsylvania, USA, industrialized the paper bag-making machine. [3].
1870-1880	Albert L. Jones in the USA patented (No. 122,023) the use of corrugated materials for packaging. In 1874, OliverLong patented (No. 9,948) the use of lined corrugated materials (Maltenfort, 1988). In1879, RobertGair of New York produced the first machine-made folding carton [3].
1880-1920	Era of folding paper boxes. Europeans widely used paper bottles, cartons, waxed paper wrappers for packaging applications[3].
1930 onwards	Era of synthetic polymers. In the early period of 1930s ethylene was first polymerized commercially by Imperial Chemical Industries (ICI) Ltd. Later, polyethylene (PE) was used in packaging since the 1960s. In 1990s invention of digital printing on packages revolutionized the packaging industry [3].

In the present decade, use of different polymer materials for packaging applications has revolutionized the current trend in food market. The use of polymer materials for packaging applications has significantly reduced the dependence on paper, glass and tin for packaging applications. The wide acceptability of polymers for packaging applications lies in its inherent properties like light weight, durability, cost effectiveness, etc. Today polymer is available in the form of flexible pouches, bags and envelopes; semi rigid trays or tubes; rigid bottles, tanks etc. Despite the huge advantages of polymer packaging materials, now they are identified as giant killers of our ecosystem. The discarded polymer materials at the end of use are appearing as litter in the environment. This calamity advocated a scientific society to practice a novel concept called green polymers. It is defined as any polymer material from natural origin, they are the part of living things and are metabolised by living things [4].

1.2 Green packages

The plastic revolution in food packaging has created irreparable damages to the ecosystem [5].

The rapid industrialization and population explosion has generated million tons of municipal wastes. As a specific example, India generates 5.6 million metric tons of plastic packaging waste annually. It was reported that more such wastes are generated in urban area as compared to rural area. According to Central Pollution Control Board (CPCB), Delhi, the capital of India creates municipality waste of 689.5 metric tons per day, the highest among other Indian cities. Currently, every Indian city generates eight times more municipal waste than in 1947. A recent study cited that fifty percentage of municipal waste in Indian cities is plastic carry bags. Most of the municipal solid wastes in India are dumped on land in an uncontrolled manner. Such indiscriminate discarding practices produced new threats, damaging human and animal health, which ultimately led to economic, environmental and biological losses [6]. As a country being focussed to emerge as a developed leading nation by 2020, we have to put together stringent efforts to mitigate the menace of plastic waste. The current efforts like recycling, incineration etc would not successfully meet the issues in terms of environmental impact as well as energy concerns [7].Hence there is always a demand for an alternative.

One of the recent alternative moves to alleviate the threat of plastic waste is the use of green plastics for packaging applications. Green plastics refer to the biodegradable plastic materials obtained from renewable sources. According to American Society for Testing and Materials (ASTM), a “biodegradable plastic” is liable to microbial attack in the presence of water molecules; such materials are compostable and would yield non toxic carbon dioxide, water and

inorganic compounds during its degradation process[8].According to the definitions there are three different classifications in green plastics.

(a) Plastic materials obtained directly from biomasses can be applied with or without modification. For example starch, cellulose etc.

(b) Polymer materials synthesised by microorganisms through large scale fermentation process. For example poly hydroxyl alcanoate (PHA), copolymer of Polyhyrodxy butyrate and hydroxyl valerate (PHBV).

(c) Polymers developed using naturally occurring monomers. For example Poly lactic acid obtained from the monomer, Lactic acid [8].

The concept of green plastic is slowly gaining momentum. So far it covers only 5-10% of current plastic market. The limited acceptability of bioplasitc materials for packaging applications is due to their inherent shortcomings in performance. Despite their eco-friendly quality they exhibit inadequate thermal, barrier and mechanical performances. The cost factor also holds back wide utilization of bio plastics for packaging applications. Fortunately, all these limitations open up new avenues of research in this challenging field. The incorporation of new materials to modify the overall performance of biomaterial is a threshold area of current research. Anyhow, in the near future these materials would find lucrative position in the international market of packaging materials, with an yearly plastics consumption of about 200 million tons and 7% average growth per year [9].

Instead of developing a mere biodegradable packaging material, now researches have focussed on novel type "intelligent or smart packaging systems", which have control over the overall performance of packed food.

1.3 Smart packaging

For a layman, Packaging material is defined as a barrier material wrapped around food to prevent it from the interaction with the outer environmental conditions and thus increases the shelf life of packed article. In current idea of "smart packaging", an innovative concept coined for those packaging systems, which does not act as a mere barrier but is actively involved in maintaining or improving the sensory, shelf life, nutritional and quality of packed food. This type of packaging would be possible if the material has intelligent barrier properties and specific positive interaction with packed articles[11]

1.4 Barrier properties of packaging films.

1.4.1Oxygen gas permeability.

Preventing food article from contact with the atmospheric oxygen is crucial for maintaining the freshness of food material notably perishable items (fruits, meat, ready to eat items). The ability of a film to prevent the permeation of oxygen was quantified by the term "Oxygen Permeability Coefficient" (OPC). It is defined as the amount of oxygen that permeates per unit area in unit time through a packaging material ($kgm^{-2}\ s^{-1}Pa^{-1}$)[10].

$$OPC = OTR \times (L/\Delta P) \quad (1)$$

OTR is the Oxygen Transmission Rate

L is the thickness of the film

ΔP is measure of the difference in partial pressure of Oxygen across the film

If oxygen gas permeates through the packaging film, the pressure of oxygen inside the pack would be considerably higher. This condition stimulates the undesirable oxidation of food articles and reduces the shelf life of food stuffs. Literature review pointed that synthetic polymer films have better oxygen barrier ability as compared to various biopolymer films[12].

Table-2. Oxygen permeability values of different packaging films.

Packaging films	Oxygen permeability ($ml.mm/m^2datm$)	References
Pectin	258.8	[13]
Chitosan	91.4	[13]
Pullulane	3	[13]
Pullulane/arabic gum	3.05	[13]
(Wheat) gluten	190	[13]
(Wheat) gluten	250	[13]
Fish proteins	56	[13]
Fish proteins	169	[13]
Na caseinate	77	[13]
Gluten-DATEM	153	[13]
Gluten-Beeswax	133	[13]
Na caseinate/Myvacet	83	[13]
MC/HPMC/fatty acids	46.6	[13]
MC and beeswax (bilayer)	4	[13]
Gluten-DATEM and	3	[13]

beeswax (bilayer)		
PET	1	[13]
Ethylene/Polyvinyl alcohol	4	[13]
Polyamide 6	9.8	[13]
polypropylene	44	[13]
Cellophane	55	[13]
Methylcellulose-palmitic acid	78.8	[13]

1.4.2 Water vapour permeability

Another vital property to be accounted seriously while selecting a packaging material is its water vapour permeability rate. The moisture content inside the pack has laudable role in deciding the shelf life of the food articles. The ability of any film to act as a barrier towards water vapours was quantified with the term water vapour permeability (WVP)[13].

$$WVP = WVTR \times (L/\Delta P) \quad (2)$$

WVTR is the water vapour transmission rate

L is the thickness of the film

ΔP is measure of the difference in partial pressure of water vapours across the film.

Literature survey revealed that all kinds of synthetic polymer films have lower water vapour permeability as compared to biopolymer films

Table-3.Water vapour permeability values of different biopolymer films

Films	Water vapour permeability (g/m^2/day) at 25^0 C	References
PHBV-6	13	[14]
PHBV- 12	21	[14]
Poly Lactic Acid (crystal)	82	[14]
Poly Lactic Acid	172	[14]
polycaprolactone	177	[14]
Bionolle	330	[14]
Cellulose Acetate Propionate	1700	[14]
Cellulose Acetate	2920	[14]

1.4.3 Carbon dioxide permeability.

The permeability study of CO_2 gas is very significant, since amount of CO_2inside the pack has pivotal role in deciding the shelf life of packed food article. Anomalous to the amount of oxygen gas, certain limit of CO_2 gas inside the pack is preferable. To accommodate this contradictory environmental conditions, researchers have coined a new term called “Modified-Atmospheric Packaging” (MAP)[15].It deals with a kind of packaging system which produces appropriate environmental condition inside the pack to improve shelf life of food.

Table-4.List of CO_2 gas permeability values of different polymer films

Film	CO_2 permeability ($\times 10^{18}$molmm^{-2}s^{-1}Pa^{-1}) at 25^{0}C	References
LDPE	4220	[15]
HDPE	972	[15]
Polyester	38	[15]
Methyl cellulose	29 900	[15]
Hydroxypropylcellulose	28 900	[15]
CronZein	216	[15]
Wheat gluten	7	[15]
Fish Mayofibrillerprotien	9	[15]
Pectin	21300	[15]
Chitosan	8010	[15]

The design of modified atmospheric packaging films became popular due to the availability of wide range of gas permeable films as well as absorbers for O_2, CO_2, water vapours etc. At present, such kind of modified packages are widely used in shipping containers, packages containing sliced food stuffs, fruits, drugs etc[16]. In addition to intelligent barrier properties, packages possessing additional qualities like antimicrobial and antioxidant properties are also thrust areas of the present research.

1.5 Antimicrobial packaging

Increasing demand of consumers for packed food has paved the way for food related health issues due to the consumption of spoiled packed food. Despite the new developments and innovations to monitor the quality of food in all stages, the incidents of serious food borne illness and accidents have become common in our society. The frequent out breaks of such tragedies grabbed the attentions of researches to strategise innovative ways to prevent food contamination by micro organism. The idea of using antimicrobial materials along with food to enhance shelf life has become a novel theme in current food industry. These antimicrobial substances inhibit microbial growth so as to extend the shelf life and maintain product quality and safety. A desirable antimicrobial material incorporated with packed food can be a broad spectrum antimicrobial material at low concentration and it shouldn't cause any unfavourable sensory effects in food. The selection of any such substance must abide with current food legislations. The researchers are focusing to develop new generation packaging materials, which would contain ideal antimicrobial properties by improving shelf-life period and reducing the risk of pathogens.

1.5.1 Different types of antimicrobial packaging methods

There are different approaches to develop antimicrobial packaging materials. They are summarised into three classes

(i). Use of sachets or pads incorporating volatile antimicrobial agents with food articles inside the packages.

(ii). Use of direct edible Coatings of antimicrobial substance on food surface

(iii). Use of antimicrobial packaging material as a whole to pack food [17].

Use of sachets or pads incorporating volatile antimicrobial agents with food articles inside the packages

It is a simple and convenient procedure for using antimicrobial agents for extending the shelf life of food. In this method, antimicrobial substances are enfolded with pads or sachets and are enclosed with food item inside the packets. The periodic releases of antimicrobial substances in the form of vapours would hinder the growth of pathogens in the food. Even though it is an easy approach, its use has been limited to volatile antimicrobial substances only. The major drawback of this method is the indiscriminate release of antimicrobial substance inside the pack may be causing undesirable changes in the sensory attributes of the food stuff [18,19].

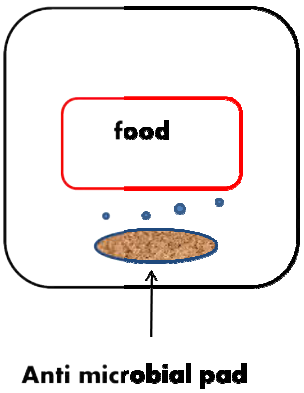


Fig-1. Schematic representation of the use of anti microbial pad

Edible Coating of antimicrobial substance onto food stuff

The use of edible antimicrobial coatings on the packed food stuff is an alternative approach to extend the shelf life of food. The protective coating on food was achieved by the direct dip into the antimicrobial substances. Such edible coatings generate an inert modified atmosphere, which can influence various changes in fresh and minimally processed foodstuff. Nowadays, coatings having antioxidant properties, colour, firmness, sensory quality improving and microbial growth inhibitions are widely used. Since the edible film is in direct contact with the food surface, they will act as a better barrier for microbial contamination. But the major demerit of this approach is the slow diffusion of antimicrobial substance from the surface of food into the bulk resulting objectionable changes and the loss of antimicrobial efficacy of the surface coatings [20].

Image of Potential Use of Edible Nano scale Coatings for Meat.
http://www.foodsafetymagazine.com

Use of antimicrobial packaging material to pack food

There are a few blessed polymers which have inherent antimicrobial activity and excellent film forming ability. Researchers have exploited these polymers to develop antimicrobial packages for extending the shelf life of food. Chitosan is the most promising polymer in this class. It is a linear polysaccharide consisting of (1, 4)-linked 2-amino-deoxy-b-D-glucan.Chitosan is obtained by the deacetylation of Chitin, the second most abundant polysaccharide found in nature after cellulose. The advantages of Chitosan are its nontoxicity, biodegradability, bio functionality, biocompatibility and its antimicrobial abilities [21].

The immobilization of antimicrobial functional molecules on the surface of packaging material is an innovative technique for the development of antimicrobial packages. Diverse surface modification techniques are employed by incorporating anti microbial substances to functionalise the packaging surface. Tailoring of surface to immobilise the antimicrobial substances depends solemnly on the nature of host material, time period of release and the nature of food. The common methods for immobilization of bioactive substances are either adsorption via electrostatic attraction or covalent attachment [22,23].The range of antimicrobial substances applied for packaging applications varies from synthetic antimicrobial material to natural antimicrobial materials. There are full-size lists of various anti microbial substances, which are considered as “Generally Recognised as Safe materials” (GRSM).Current research is heavily focused on natural antimicrobial substances to substitute all forms of synthetic antimicrobial molecules. The underlying mechanism behind the

antimicrobial activity of such composite package is the controlled release of active antimicrobial substance onto the food surface and direct interaction with microbes so as to inhibit the growth of microorganisms on its surface.

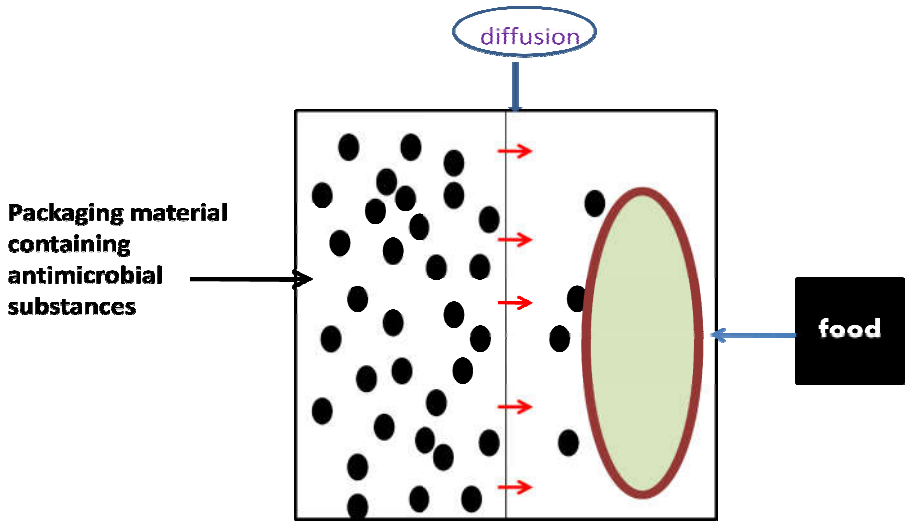


Fig-2. Slow diffusion of antimicrobial substances onto the food surface.

1.6 Green bioactive substances for packaging applications

The concerns of consumers over artificial food additives have motivated researchers to utilize green preservatives for packaging applications. The earliest attempt in this regard was the incorporation of anti microbial enzymes with packaging polymers for extending the shelf life of food. Different kinds of enzymes are incorporated with packaging films based on the nature of packed food. The active enzymes are installed in the packaging films in crude or semi crude or pure form. The impregnation of enzymes is achieved in packaging

material either by direct addition or in situ fermentation process. The impregnated enzymes would then be released in a controlled manner so as to prevent microbial growth on the surface of food. Antimicrobial efficacy of enzymes depends on various factors such as temperature, p^H and type of target bacteria. One of the most intensively studied antimicrobial enzymes is Lysozyme, which can inhibit the growth of different types of gram positive bacteria, but it is inactive towards gram negative bacteria. Recently it was incorporated with cellulose acetate to develop a smart packaging material [24]. The detailed study of its antimicrobial mechanism revealed that, packaging film releases active materials, which will interact with the cell wall of gram positive bacteria and interfere in its metabolic activities leading to the inhibition of microbial growth [25].

Another significant step in this context is the utilization of Bacteriocins, which are enzymes excreted by different types microorganisms. Among them, Nisin is substantially recognized and applied for food packaging applications and it is approved by GRAS and World Health Organization (WHO) [26–28]. Nisin has gained widespread commercial application since 1969. In the last decade this antimicrobial peptide has witnessed intensive research. There are different approaches to incorporate bacteriocins for packaging application. The easiest way is the direct addition of bio preservative as a food supplement as per current legislatives. Bacteriocins can be added either in the crude form or semi crude or purified form depending upon the nature of packed food. The recent advance in this scenario is the immobilisation bacteriocins on the surface packaging

material leading to their controlled release so as to protect the surface contamination of food [29].

Table -5. List of different bacteriocins

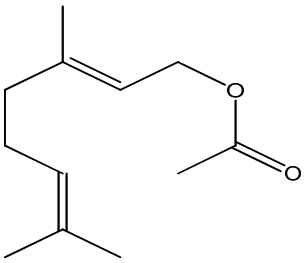
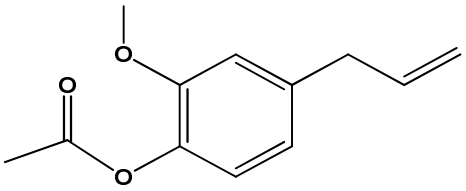
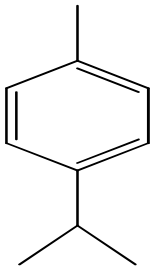
Bacteriocin	Bacteriocin Producing Strain	References
LactacinF	L. johnsoniispp (lactobacillus spp strain)	[30]
Lactocin 705	L. caseispp (Lactobacillus caseissp)	[30]
LactoccinG	L. lactisspp (Lactococcuslactisssp)	[24]
Lactococcin MN	Lactococcuslactisvarcremoris	[30]
Nisin	Lactococcuslactis spp.	[30]
LeucocinH	Leuconostocspp	[30]
Plantaricin	L. plantarumspp (Lactobacillus Plantarum)	[30]

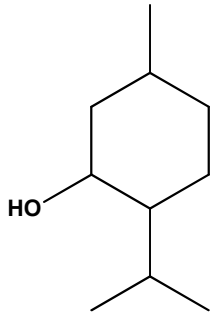
The search for new, efficient and cost effective green preservatives opened up the window of another class of functional materials called essential oils. Essential oils are natural organic extractions of plant origin. They are obtained from different parts of plants including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. The components of essential oils are separated by steam distillation technique and are characterized by spectroscopic techniques. Around 3000 varieties of essential oils were recognised and identified; most of them are chiefly used as flavouring and fragrance agents all over the world. Some of the essential oils are also well-known for their antimicrobial, antiviral and antioxidant properties. Recently, such

molecules are widely been exploited and incorporated with packaging materials for extending the shelf life of food. [31].

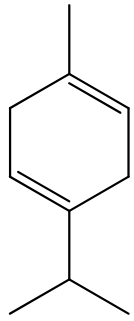
1.6.1 Structure of antimicrobial essential oil molecules.

The structures and common names of selected antimicrobial essential oil molecules are given below**.**

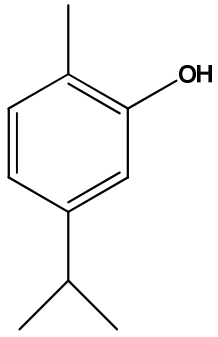
Structure	Common name
	Geranyl acetate
	Eugenyl acetate
	P-cymene



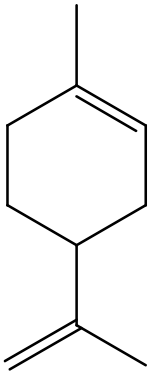
Menthol



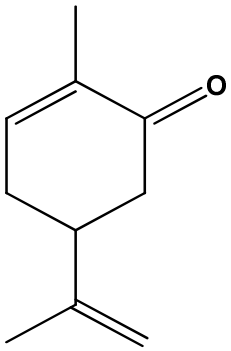
Terpinene



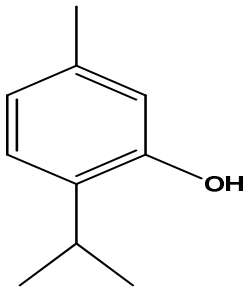
Cymenol



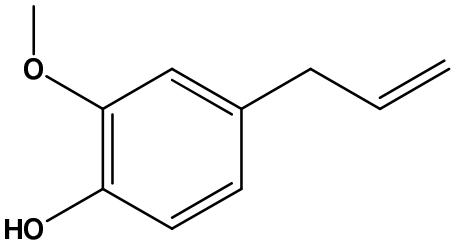
Limonene



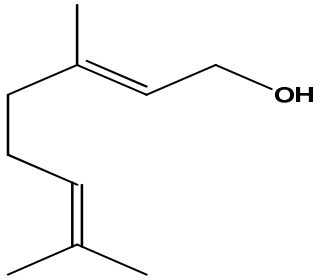
Carvone



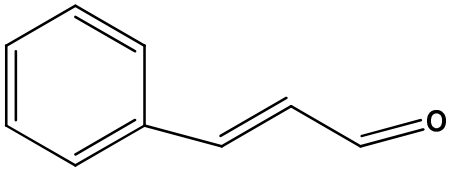
Thymol



Eugenol



Geraniol



Trans-Cinnamaldehyde

1.6.2 Essential oils and target microorganisms

Antimicrobial activity of different essential oil components was well studied and characterized. Unlike enzymes, most of them exhibit broad-spectrum antimicrobial activity and are effective against both gram negative and gram positive bacteria.

Table-6. List of essential oil components and target bacterial species.

Essential oil component	Species of bacteria	Reference
Terpineol	Escherichia Salmonella typhimurium Staphylococcus aureus Listeria monocytogenes Bacillus cereus	[31]
Carvacrol	E. coli 0.225– 5 S. typhimurium Staph. aureus L. monocytogenes	[31]
Citral	B. cereus E. coli 0.5 S. typhimurium Staph. aureus L. monocytogenes	[31]
Eugenol	E. coli S. typhimurium L. monocytogenes	[32]
Geraniol	E. coli S. typhimurium L. monocytogenes	[32]
Perillaldehyde	E. coli S. typhimurium L. monocytogenes	[32]
Thymol	E. coli S. typhimurium Staph. aureus L. monocytogenes B. cereus	[31]

The wide spread application of certain essential oils for food packaging purpose was limited due to its characteristic smell. When it was used as a packaging material, it would impart its characteristic smell to the food, changing its sensory attributes. The unpleasant odour and taste in the food when packed with essential oil made them awkward choice for packaging applications[33].This led to the exploitation of inorganic molecules for antimicrobial applications.

1.7 Anti microbial Metal nanoparticles for packaging applications

There are a few gifted metals and metal nanoparticles which exhibit inherent antimicrobial activity. Compared to antimicrobial organic molecules, metals are thermally stable, cost effective and show a broad-spectrum antimicrobial efficiency. Among the different metals, Silver has historical importance in antimicrobial applications. It was applied as an anti microbial material since ancient period. Even before the prehistoric period, silver pots were used for cooking, storing water and wine to prevent food contamination. Alexander the Great, used to drink from silver vessels. Silver nitrate, known in ancient times as "lapis infernalis", was applied for therapeutic purposes in the 8^{th} century. In 10^{th} century, scientist recorded and practiced the use of silver as a blood purifier and as heart tonic. In the 17^{th} and 18^{th} century, the use of silver and its compound became common practice for the treatment of fatal diseases like venereal infection, fistulae and abscesses. In the last century, researchers in different fields realized the wide applicability of silver and its sister compounds in the vast area of microbial contaminated diseases. [34–37].Despite the ample popularity of silver and sister compounds as antimicrobial agents, recent studies

have unravelled the adverse effects of silver nanoparticles. Now silver nanoparticles are identified as a toxicant. Recent studies underlined its "cytotoxic, genotoxic, and antiproliferative" potential. Moreover, the excess use of silver nano particles causes its deposition in human cells leading to adverse effects [38]. Hence the embracing of silver nanoparticles for biological applications should be done with utmost care and through research must be initiated in this field.

The essential metal, copper is another choice for antimicrobial applications. There are a few recent reports on anti microbial property of copper vessel or copper coated surface. The anti microbial packages of polyethylene or Chitosan containing copper have been developed to extend the shelf life of food. Copper metal has many advantages namely, homogeneous surface, wear resistance, durability etc. Compared to silver the antimicrobial potential of copper is lower, but still it is preferable due to its certain unique attributes such as easy mobilization of copper ions by simple chemical methods, durability and extended period of antimicrobial efficacy [39].The fabrication of anti microbial copper or copper oxide nanoparticles is achieved by different protocols. The easiest procedure to prepare copper nanoparticles is thermal or sonochemical reduction of copper hydrazine carboxylate complexes in aqueous media. Copper oxide nano particles were synthesised by direct reduction copper salt with sodium borohydride [40].

Titanium dioxide (TiO_2) is chiefly used as a photo catalytic material. However recently there are focussed researches on light induced biocidal activity of this material. Nowadays, surface coatings of nano

titania is used to develop self cleaning surfaces [41–43]. As an inherent photo catalyst, it can produce reactive oxygen species (ROS) in the presence of water, an essential radical component to inhibit the growth of micro organisms. It would promote peroxidation of the unsaturated poly phospholipids present in the cell wall of microorganisms and prevent their growth. Nano TiO_2 has been used with different polymers to fabricate antimicrobial packaging material. The recent report of TiO_2 coated packaging film to inhibit E. coli contamination on food surfaces has received wide attention. Researchers have adopted doping of different metal with TiO_2 to improve its antimicrobial efficacy. It was demonstrated that doping $TiO_2$with silver has enhanced its biocidal activity as compared to bare Titania. This combination was used with poly vinyl chloride (PVC) to develop antimicrobial packing material to extend the shelf life of food [44–46].

The incorporation of new materials in the polymer matrix can be achieved by different ways. Properties of composites are significantly changed depending on the size of fillers. In the era of nanotechnology researchers look forward on novel bionano composite films for packaging applications.

1.8 Bionano composite films; an innovative concept for food packaging

The term “nano” was coined to define any material under the scale of 10^{-9}m.The scientific community and industry have given immense interest to work with nano material ever since the historic opening of

the concept “nanotechnology” by Richard Feynman in 1959 in a meeting of American chemical society.

Richard Feynman (https://encrypted-tbn2.gstatic.com/images)

However, the term “polymer nano-composite” is relatively new and has been thrust of intense research by material chemist in last two decades. It is defined as a hybrid material of polymers incorporated with nano fillers. They may have different shapes but at least one dimension must be smaller than 100 nm. The filler components may be inorganic or organic molecules. The recent resurrection of interest in polymer nanocomposites can be attributed to many reasons. The hybridisation of two materials would create a synergistic interaction between them. This synergism will enhance properties of individual components and make the composite superior over bare components. Such hybridisations will open up the possibilities of development of new materials with specific properties. Since, nanoscale fillers are almost free from defects and their incorporation in polymer matrix

converts the material superior over traditional composites of polymers. Recently, the attention on polymer nanocomposite widened, resulted in the birth of new term "bionanocomposites" or bio-based nanocomposites. A bionano composite is defined as "Nanocomposite in which either the fillers or the polymer matrix or both has been obtained from biological resources". Such composites are biocompatible, eco-friendly and follow the principles of green chemistry.[47]. Physically, any composite material composed of more than one different phases in which at least one of phase is a continuous. Other components are dispersed in continuous phase and are held together in its matrix. The different components as well as continuous phases of a composite are separable and identifiable. Nowadays, plethoras of biocomposite materials are prepared and are categorized into two types; namely class-1 and class-2. In class-1 type composites fillers are randomly distributed in the matrix of polymers (continuous phase) and are weakly entrapped in the matrix by wader walls forces of attraction Fig-A.

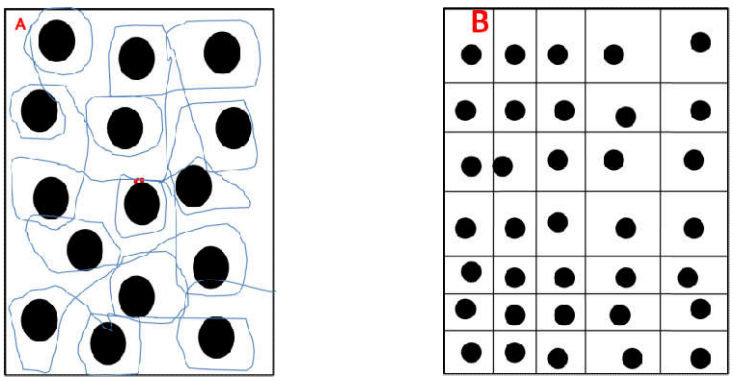


Fig-3 Illustration of nanocomposites (A) class -1 type, (B) class -11 type.

In class-2 type composites (Fig-B), fillers are more or less homogenously dispersed in the matrix of continuous phase. In this case continuous phase and fillers are held together by strong chemical bonds likely ionic or covalent [48].

Novel bio based composite films are exploited for food packaging applications. All these materials fall under the category of green packages. They are biodegradable, bio compatible, non toxic and are obtained from renewable resources. Majority of bio-based packaging materials served as edible protective coatings. The utilization of this eco friendly packaging materials has pivotal significance in the current scenario of synthetic polymer dominated packaging industries. A review of different biopolymer composite films are summarised below.

Table-7.List of different biopolymer Packages

Biopolymer	**Anti microbial substance**	**Packed food item**	**Reference**
starch	Clay nano particles	Culture media	[49]
Cellulose	Bacteriocin (pediocin)	meat	[27]
Cellulose derivative	Nisin	Cheese	[50]
k-carrageenan	Nisin & Lysozyme	fish	[51]
Na-alginate	Nisin & Lysozyme	Meat (beef)	[51]
Zein	Lysozyme	Culture media	[52]
Chitosan	Aceticacid/cinnamaldehyde / lauric acid Chitosan	Processed meat	[53]
Whey protein	Sorbic acid	Water – glycerol mixture	[54]

Cellulose	Bacteriocin	Meat	[55]
Ca-alginate	Organic acid	beef	[55]
Chitosan	Lysozyme	Culture media	[56]
Poly lactic acid	Nano cellulose & Silver nano particles	Culture media	[57]
PLA / Pectin	Nisin	Culture media	[58]
Soy protein- PLA film	Natamycin	Cheese	[59]
PLA	Limonene		[60]
PLA&Poly(hydroxybutyrate) PHB	Limonene	-	[61]
Nano cellulose based composite films	Organicacids/ bacteriocins/ Essential oils	-	[62]
Zein-PCL pouches		Carrots	[63]
Corn zein	Lysozyme/nisin	Culture media	[64]
Soy protein isolate	Lysozyme/nisin	Culture media	[64]

Unfortunately, the wide exploitation of various biodegradable polymers is limited due to the intrinsic disadvantages associated with their performance, processing and cost. Most of them have low barrier properties and are of poor mechanical strength. Polymers like starch and PLA are highly hydrophilic and water soluble. Most studied polymer Cellulose has lower ductility and unfavourable thermal properties. Some others are (alginate, Pectin, PHB etc.) neither cost effective nor efficient. Among the different biopolymers, Chitosan is the most suitable candidate for packaging applications due to its extra ordinary physico-chemical properties [61,65,66].

1.9 Chitin and Chitosan –potential of unlimited applications

Chitin (poly β-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide having enormous applications. This polymer was first identified in 1884 in nature as ordered crystalline micro fibrils, the major structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also produced by a number of other living organisms in the lower plant and animal kingdoms. Now Chitin is considered as the second most abundant organic molecule after the cellulose. Despite their widespread occurrence of Chitin the direct applications of Chitin is limited due to its unfavourable phisico-chemical features. Industrially Chitin is collected from the cell walls of crustaceans and crab shells; they are processed chemically to obtain Chitosan for versatile applications. Even though both Chitin and Chitosan have similar structural framework, the physical and chemical features of two are surprisingly diverse. Chitin contains hydroxyl groups (primary as well as secondary) and amide groups, whereas Chitosan retains same hydroxyl group but the amide group is deacetylated into highly reactive primary amine group, which seemingly makes Chitosan more reactive. It is almost impossible to obtain Chitosan with hundred percentage deacetylation, hence it is customary to indicate the degree of deacetylation with the name of Chitosan. The melting point of Chitosan was not reported accurately since it is decomposed before melting at higher temperature. One of the most significant differences in characteristics of Chitin and Chitosan is their solubility. Chitin is less soluble when compared to Chitosan. The number of suitable solvents for Chitin is relatively

smaller as compared to Chitosan. All aqueous acid solution (both organic and inorganic) can dissolve Chitosan, but the most commonly used are formic acid and acetic acid solutions. The chemistry of dissolution of Chitosan at low P^H is the formation of huge number of cationic sites in the place of primary amine group due to protonation, which enhances polarity and dissolution [67].

Table-8. List of solvents for Chitin and Chitosan.

Solvents for Chitin	Solvents for Chitosan	References
5% LiCl in dimethylacetamide, diethylacetamide LiCl in N-methyl-2-pyrrolidone $CaCl_2$- 2H $_2$O in saturated methanol Hexafluoroisopropyl alcohol Hexafluoroacetone sesquihydrate Mixture of 1,2-dichloroethane and trichloroacetic acid (35:65) Fresh saturated solution of lithium thiocyanate	Dilute aqueous organic or mineral acids below pH 6.5 Dimethylsulfoxide P-Toulene sulfonic acid Camphorsulfonic acid	[68–71]

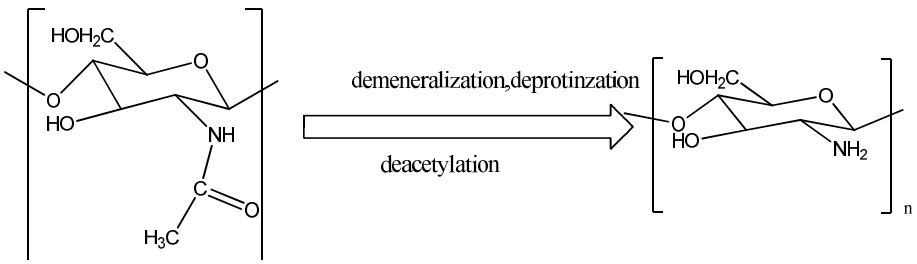
1.10 Conversion of Chitin to Chitosan

Chitosan is extracted from the cell walls of crustaceans by treatment using concentrated hydrochloric acid solution to dissolve calcium carbonate; subsequently alkaline extraction was carried out to solubilise undissolved proteins. Finally a decolourization step is done to remove colour of the product. The time period and concentration of solution of each treatment will vary with the source of Chitin. The

resulting Chitosan powder needs to be graded in terms of purity, colour and degree of deacetylation [72].

Table-9. Relative amounts of Chitin and calcium carbonate in different sources

Source	% of chitin	% of calcium carbonate	Reference
Crab cuticle	15–30	40–50	[73]
Shrimp cuticle	30–40	20–30	[48]
Krill cuticle	20–30	20–25	[48]
Squid pen	20–40	Negligible	[73]
Clam/oyster shell Insect cuticle	3–6 5–25	85–90 Negligible	[73]
Fungi cell wall	10–25	Negligible	[73]



Chitin **Chitosan**

Scheme -1. Illustration of conversion of Chitin to Chitosan

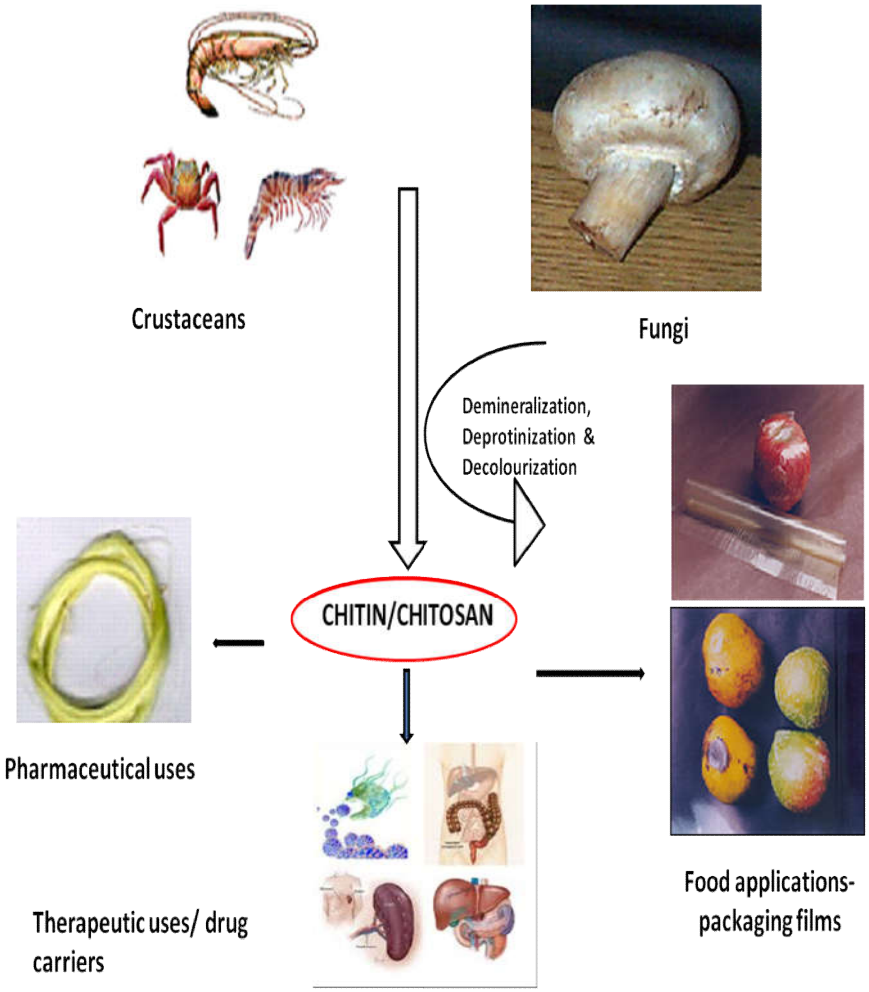
1.11 Chitosan market in India

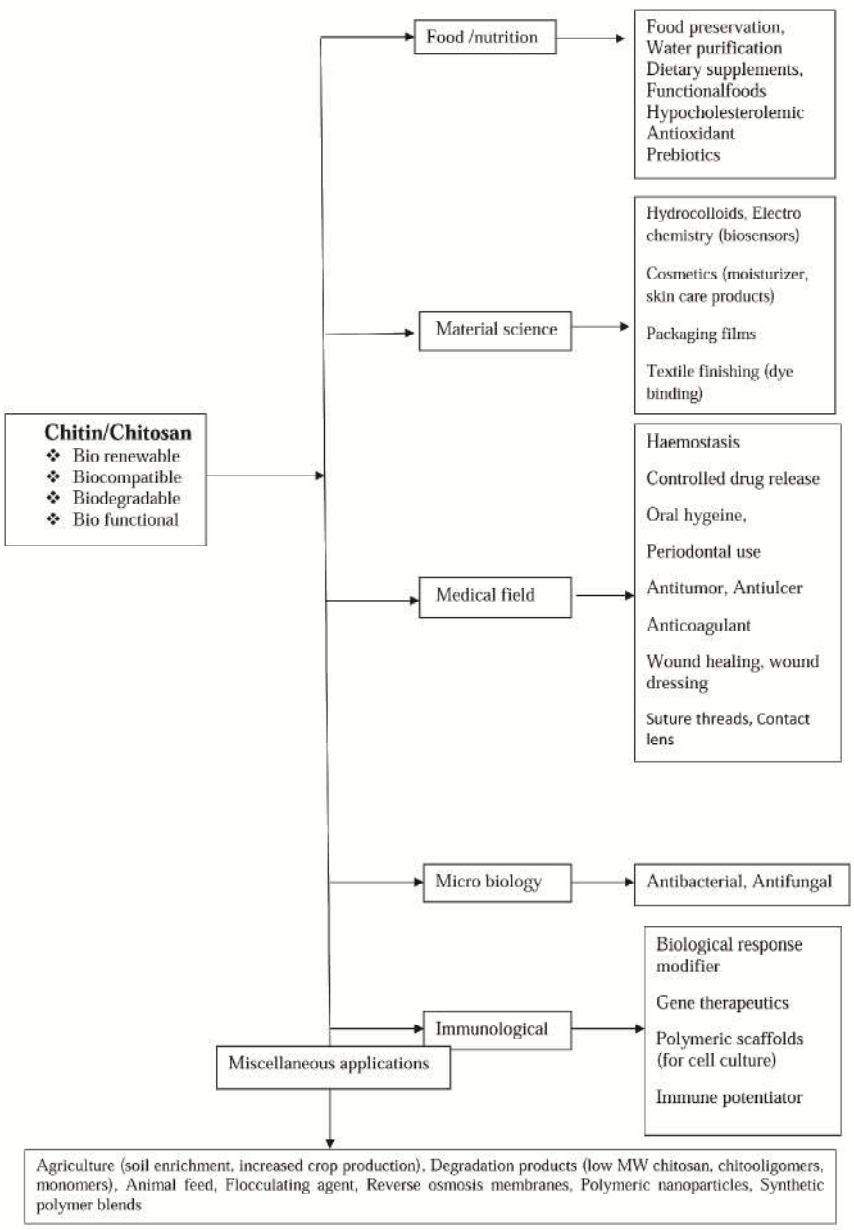
The shrimp processing industry in India pushes out more than 1.25 lakh tonnes of head and shell waste per annum. Until recently, it was creating enormous environmental pollution problems. Nearly 7000 tonnes of Chitin can be produced from the prawn shell which is thrown out as waste now. Another alternate source is squilla, which is a by-catch in shrimp trawlers, which is now discarded. Chitin is extracted from the prawn shell as this is the raw material for production of Chitosan and other value added products. Central Institute of Fisheries Technology (CIFT-Cochin) has developed and publicised technology for the production of Chitin, Chitosan and value added products. India has the potential of producing 10,000 tonnes of Chitin per annum from the prawn shell itself. Other untouched sources like squilla are also available. At present, the Chitin or chitosan industry in India is utilizing only less than 20% of the shell waste with an estimated Rs 100 cores annually. This has solved environmental problems significantly in addition to job and income generation. Our country has

enormous potential for meeting the increasing demand of Chitin or chitosan by utilising the unexposed resources.

1.12 Galore of Potential applications of Chitin and Chitosan

Chitin and Chitosan are used for a wide range of applications, such as in food, biotechnology, drug and pharmaceutical, material science etc. Due to specific physical and chemical advantages of Chitosan, it overweighs Chitin in versatility of applications. The adaptability of Chitosan for various applications owes to the presence of reactive functional groups; one primary amine, one primary alcohol and one secondary alcohol groups per monomer of Chitosan. The free amine group is easily protonated in aqueous acidic media and generate positive polarity throughout the polymer chain. The presence of large numbers of pendent groups offers scope for modification of bare Chitosan chain to fit for various characteristic applications. A schematic profile of potential applications of Chitin and Chitosan is given below [51].


Crustaceans
Fungi
Demineralization,
Deprotinization &
Decolourization
CHITIN/CHITOSAN
Pharmaceutical uses
Therapeutic uses/ drug
carriers
Food applications-
packaging films



Scheme-2. Illustration of applications of Chitosan

1.13 Antimicrobial applications of Chitosan

The microbial contamination and subsequent epidemic disease of packaged food incur great economic loses every year in different parts of the world. In order to preserve packaged food from spoilage, different techniques have been developed and practised. The most advanced protocol is the use of antimicrobial packaging films to pack and preserve food. Till date different span of polymer films; artificial, natural and semi synthetic; have been utilised with antimicrobial fillers as protective packages. The polymers include polyethylene (PE), polyvinyl chloride (PVC), polyvinyl alcohol (PVA), Poly lactic acid (PLA), nylon, and many others. The incorporation of anti microbial substances was achieved by simple to complicated methods depending on the nature of polymer matrix and anti microbial materials [74].Despite a handful of packaging material, recently Chitosan was chiefly focused owing to its unique features such as its bio origin, inherent anti microbial and anti fungal properties, ability to form chelating complexes with metal atoms and the presence of reactive functional groups. Moreover, Chitosan is generally considered as safe materials(GRSM) and thus it can be consumed together with the preserved food [21].

1.14 Mechanism of antimicrobial activity of Chitosan

A clear account of mechanism of anti microbial activity of Chitosan was not recognised so far, moreover, various proposed mechanisms put forward contradictory explanations [75].This diverse and complex anti microbial behaviour of Chitosan is due to the inter play of various

factors, which effect the anti microbial ability in different ways. Following are the crucial factors which affect the anti microbial activity of Chitosan [76].

- The type of Chitosan
- The degree of deacetylation
- Molecular weight of Chitosan
- The target organism and the conditions of the medium for the preservation of food
- P^H and Temperature
- Ionic strength
- Physical state of Chitosan (e.g. Solubility)

There are different mechanisms proposed for anti microbial properties of Chitosan, the most common explanations are summarised below.

- Chitosan itself form a surface membrane to cover micro organism, which act as a barrier and inhibit the entering of nutrients into the cells of microorganism resulting their ultimate death [77].
- Low molecular weight Chitosan polymers penetrate in to the cell wall of microorganism and interact with negatively charged substances in the cell wall and ultimately destroy them [78].

- Chitosan forms chelating complexes with essential metal ions present in the cell walls of microorganism and inhibit its metabolism [79].

Solubility of Chitosan has important effect on antimicrobial property of Chitosan, normally Chitosan is soluble in low P^H aqueous media but lower molecular weight Chitosan is soluble in water even at neutral P^H. Recently a surprising mechanism was proposed for the antimicrobial activity in terms of solubility of Chitosan. Water soluble CFhitosan, at P^H 7 exhibits significantly lower antimicrobial efficacy as compared to water insoluble Chitosan at P^H 7.This can be envisaged as the ability of insoluble Chitosan to interact with bacterial surface molecules and get coagulated around its surface, paving the way for impermeable membrane on the bacterial cells and inhibits its growth. But water soluble Chitosan cannot generate such interaction and subsequent coagulation due to its solubilised condition resulting low anti microbial efficacy [80,81].

1.15 Chitosan based packaging materials

One of the best qualities of any active packaging film is the inhibition of microbial contamination of food during its shelf life. In this century of green packages, Chitosan is the most suitable candidate to develop bio active packaging film due to its excellent Physico-chemical properties in addition to its antimicrobial and antioxidant character. Since Chitosan is non toxic, biocompatible and safe material, it can be used as a direct food coating material to prevent microbial spoilage of food. The properties of Chitosan vary with the source, preparation

technique, target microorganism, type of packed food etc. Hence, in the present decade there is a high inclination to Chitosan for its enhanced performance. The efficiency of Chitosan can be tuned by blending it with different active components in micro or nano size. The functional molecules used to hybridise with Chitosan include enzymes, essential oils, metals etc. Quite a huge quantum of quest and research are devoted to the synthesis and application of Chitosan based composite films. The search for novel and efficient Chitosan based film is continuing as a need of hour [21,53,64,76,82,83].

1.16 Conclusion

The environmental catastrophe occurred by indiscriminate disposal of petroleum based polymer packaging materials led to hue and cry from all walks of life for green polymers. Governments and industries all over the world are funding heavily to materialise this dream. Researchers are immensely focussing to develop green packaging materials to save our ecosystem. But the development of such material is a great challenge for any researcher in this field. Different factors such as cost effectiveness, availability of resources, durability etc are the hurdles in wide exploitation of major biopolymers. Hence the developments in this field are quite slow but steadily increasing. Currently immense attention is paid on the biopolymer Chitosan due to its intriguing properties. In near future this blessed polymer may find a prominent place in international market of green polymers.

1.17 Reference

[1] G. Kennedy, G. Nantel, P. Shetty, Globalization of food systems in developing countries: a sythesis of country case studies, 2004. doi:17-05-2011.

[2] International Trade Centre, Packaging for organic foods, (2012) 1–83.

[3] R. Coles, M.J. Kirwan, D. McDOWELL, Food Packaging Technology, (2003).

[4] G. Scott, `Green' polymers, Polym. Degrad. Stab. 68 (2000)1–7. doi:10.1016/S0141-3910(99)00182-2.

[5] U. Arena, M.L. Mastellone, F. Perugini, Life Cycle assessment of a plastic packaging recycling system, Int. J. Life Cycle Assess. 8 (2003) 92–98. doi:10.1007/BF02978432.

[6] M. Sharholy, K. Ahmad, G. Mahmood, R.C. Trivedi, Municipal solid waste management in Indian cities - A review, Waste Manag. 28 (2008) 459–467. doi:10.1016/j.wasman.2007.02.008.

[7] R.S. Stein, Polymer recycling: opportunities and limitations., Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 835–838. doi:10.1073/pnas.89.3.835.

[8] Z. Kuruppalil, Green Plastics: An Emerging Alternative for Petroleum-Based Plastics, Int. J. Eng. Res. Innov. 3 (2011) 59–64.http://ijeri.org/IJERIArchives/issues/spring2011/IJERIVol3N1Spring2011final1.PDFpage 61.

[9] V. Siracusa, Food packaging permeability behaviour: A report, Int. J. Polym. Sci. 2012 (2012). doi:10.1155/2012/302029.

[10] M. Schmid, A. Benz, C. Stinga, D. Samain, K.P. Zeyer, Fundamental investigations regarding barrier properties of grafted PVOH layers, Int. J. Polym. Sci. 2012 (2012). doi:10.1155/2012/637837.

[11] V. Siracusa, P. Rocculi, S. Romani, M.D. Rosa, Biodegradable polymers for food packaging: a review, Trends Food Sci. Technol. 19 (2008) 634–643. doi:10.1016/j.tifs.2008.07.003.

[12] S. Guilbert, N. Gontard, L.G.M. Gorris, Prolongation of the Shelf-life of Perishable Food Products using Biodegradable Films and Coatings, LWT - Food Sci. Technol. 29 (1996) 10–17. doi:10.1006/fstl.1996.0002.

[13] D.L.D.-L. Randolph M. Beaudry, Arthur C. Cameron, Ahmad Shirazi, Modified-atmosphere packaging of blueberry fruit: effect of temperature on package O2 and CO2, J. Am. Soc. Hortic. Sci. 117 (1992) 436–441. http://journal.ashspublications.org/content/117/3/436.short.

[14] R. Shogren, Water vapor permeability of biodegradable polymers, J. Environ. Polym. Degrad. 5 (1997) 91–95. doi:10.1007/bf02763592.

[15] D. Zagory, A. a. Kader, Modified Atmosphere Packaging of Fresh Produce.pdf, Food Technol. 42 (1988) 70–74 & 76–77.

[16] S. Guilbert, B. Cuq, N. Gontard, Recent innovations in edible and/or biodegradable packaging materials., Food Addit. Contam. 14 (2009) 741–751. doi:10.1080/02652039709374585.

[17] P. Appendini, J.H. Hotchkiss, Review of antimicrobial food packaging, Innov. Food Sci. Emerg. Technol. 3 (2002) 113–126. doi:10.1016/S1466-8564(02)00012-7.

[18] M.N. Akhtar, Antmicrobial Packaging, a Step towards Safe Food: A Review, 15 (2015).

[19] F.K. K??ferstein, Y. Motarjemi, D.W. Bettcher, Foodborne Disease Control: A Transnational Challenge, Emerg. Infect. Dis. 3 (1997) 503–510. doi:10.3201/eid0304.970414.

[20] V. Falguera, J.P. Quintero, A. Jiménez, J.A. Muñoz, A. Ibarz, Edible films and coatings: Structures, active functions and trends in their use, Trends Food Sci. Technol. 22 (2011) 292–303. doi:10.1016/j.tifs.2011.02.004.

[21] P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for Chitosan based antimicrobial films in food applications, Food Chem. 114 (2009) 1173–1182. doi:10.1016/j.foodchem.2008.11.047.

[22] J.M. Goddard, J.H. Hotchkiss, Polymer surface modification for the attachment of bioactive compounds, Prog. Polym. Sci. 32 (2007) 698–725. doi:10.1016/j.progpolymsci.2007.04.002.

[23] U. Metin, Preparation of Controlled Release Antimicrobial Food Packaging Materials, Izmir Inst. Technol. Thesis. (2009) 1–85.

[24] IDem Mecitolu, A. Yemeniciolu, A. Arslanolu, Z.S. Elmaci, F. Korel, A.E. Etin, Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging, Food Res. Int. 39 (2006) 12–21. doi:10.1016/j.foodres.2005.05.007.

[25] S. Gemili, A. Yemeniciolu, S.A. Altinkaya, Development of cellulose acetate based antimicrobial food packaging materials for controlled release of lysozyme, J. Food Eng. 90 (2009) 453–462. doi:10.1016/j.jfoodeng.2008.07.014.

[26] G. Mauriello, E. De Luca, a. La Storia, F. Villani, D. Ercolini, Antimicrobial activity of a nisin-activated plastic film for food packaging, Lett. Appl. Microbiol. 41 (2005) 464–469. doi:10.1111/j.1472-765X.2005.01796.x.

[27] X. Ming, G.H. Weber, J.W. Ayres, W.E. Sandine, Bacteriocins applied to food packaging materials to inhibit Listeria monocytogenes on meats, J. Food Sci. 62 (1997) 413–415. doi:10.1111/j.1365-2621.1997.tb04015.x.

[28] E. Jones, V. Salin, G. Williams, NISIN and the Market for Commercial Bacteriocins, Reports. (2005). http://afcerc.tamu.edu/publications/Publication-PDFs/CP 01 05 Nisin Report.pdf.

[29] E.M. Balciunas, F.A. Castillo Martinez, S.D. Todorov, B.D.G.D.M. Franco, A. Converti, R.P.D.S. Oliveira, Novel biotechnological applications of bacteriocins: A review, Food Control.32(2013) 134–142. doi:10.1016/j.foodcont.2012.11.025.

[30] M.P. Zacharof, R.W. Lovitt, Bacteriocins Produced by Lactic Acid Bacteria a Review Article, APCBEE Procedia. 2 (2012) 50–56. doi:10.1016/j.apcbee.2012.06.010.

[31] M.H. Gracia-Valenzuela, C. Orozco-Medina, C. Molina-Maldonado, Efecto antibacteriano del aceite esencial de orégano (Lippia berlandieri) en bacterias patógenas de camarón Litopenaeus vannamei, Hidrobiologica. 22 (2012) 201–206. doi:10.1016/j.ijfoodmicro.2004.03.022.

[32] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, Biological effects of essential oils - A review, Food Chem. Toxicol. 46 (2008) 446–475. doi:10.1016/j.fct.2007.09.106.

[33] W.X. Du, R.J. Avena-Bustillos, R. Woods, A.P. Breksa, T.H. McHugh, M. Friedman, et al., Sensory evaluation of baked chicken wrapped with antimicrobial apple and tomato edible films formulated with cinnamaldehyde and carvacrol, J. Agric. Food Chem. 60 (2012) 7799–7804. doi:10.1021/jf301281a.

[34] H.J. Klasen, A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver, Burns. 26 (2000) 131–138. doi:10.1016/S0305-4179(99)00116-3.

[35] R. Bhattacharya, P. Mukherjee, Biological properties of "naked" metal nanoparticles, Adv. Drug Deliv. Rev. 60 (2008) 1289–1306. doi:10.1016/j.addr.2008.03.013.

[36] X. Chen, H.J. Schluesener, Nanosilver: A nanoproduct in medical application, Toxicol. Lett. 176 (2008) 1–12. doi:10.1016/j.toxlet.2007.10.004.

[37] R. Vaidyanathan, K. Kalishwaralal, S. Gopalram, S. Gurunathan, Nanosilver-The burgeoning therapeutic molecule and its green synthesis, Biotechnol. Adv. 27 (2009) 924–937. doi:10.1016/j.biotechadv.2009.08.001.

[38] B. Uygur, G. Craig, M.D. Mason, A.K. Ng, Cytotoxicity and genotoxicity of silver nanomaterials, Tech. Proc. 2009 NSTI Nanotechnol. Conf. Expo, NSTI-Nanotech 2009. 2 (2009) 383–386. doi:10.1021/nn800596w.

[39] J.O. Noyce, H. Michels, C.W. Keevil, Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing, Appl. Environ. Microbiol. 72 (2006) 4239–4244. doi:10.1128/AEM.02532-05.

[40] A. Llorens, E. Lloret, P. a. Picouet, R. Trbojevich, A. Fernandez, Metallic-based micro and nanocomposites in food contact materials and active food packaging, Trends Food Sci. Technol. 24 (2012) 19–29. doi:10.1016/j.tifs.2011.10.001.

[41] A.G. Agrios, P. Pichat, State of the art and perspectives on materials and applications of photocatalysis over TiO2, J. Appl. Electrochem. 35 (2005) 655–663. doi:10.1007/s10800-005-1627-6.

[42] B. Xi, L.K. Verma, J. Li, C.S. Bhatia, A.J. Danner, H. Yang, et al., TiO 2 thin films prepared via adsorptive self-assembly for self-cleaning applications, ACS Appl. Mater. Interfaces. 4 (2012) 1093–1102. doi:10.1021/am201721e.

[43] M. Montazer, S. Seifollahzadeh, Enhanced Self-cleaning , Antibacterial and UV Protection Properties of Nano TiO 2 Treated Textile through Enzymatic Pretreatment, (2011) 877–883. doi:10.1111/j.1751-1097.2011.00917.x.

[44] K.L. Yeung, W.K. Leung, N. Yao, S. Cao, Reactivity and antimicrobial properties of nanostructured titanium dioxide, Catal.Today.143(2009)218-224.doi:10.1016/j.cattod.2008.09.036.

[45] H. a. Foster, I.B. Ditta, S. Varghese, A. Steele, Photocatalytic disinfection using titanium dioxide: Spectrum and mechanism of antimicrobial activity, Appl. Microbiol. Biotechnol. 90 (2011) 1847–1868. doi:10.1007/s00253-011-3213-7.

[46] H.M.C. De Azeredo, Nanocomposites for food packaging applications, Food Res. Int. 42 (2009) 1240–1253. doi:10.1016/j.foodres.2009.03.019.

[47] G. Siqueira, J. Bras, A. Dufresne, Cellulosic bionanocomposites: A review of preparation, properties and applications, Polymers (Basel). 2 (2010) 728–765. doi:10.3390/polym2040728.

[48] S. Gross, D. Camozzo, V. Di Noto, L. Armelao, E. Tondello, PMMA: A key macromolecular component for dielectric low-?? hybrid inorganic-organic polymer films, Eur. Polym. J. 43 (2007) 673–696. doi:10.1016/j.eurpolymj.2006.12.012.

[49] R. Zhao, P. Torley, P.J. Halley, Emerging biodegradable materials: Starch- and protein-based bio-nanocomposites, J. Mater. Sci. 43 (2008) 3058–3071. doi:10.1007/s10853-007-2434-8.

[50] R.O. Benech, E.E. Kheadr, C. Lacroix, I. Fliss, Antibacterial activities of nisin Z encapsulated in liposomes or produced in situ by mixed culture during Cheddar cheese ripening, Appl. Environ. Microbiol. 68 (2002) 5607–5619. doi:10.1128/AEM.68.11.5607-5619.2002.

[51] D. Su Cha, J.H. Choi, M.S. Chinnan, H.J. Park, Antimicrobial Films Based on Na-alginate and κ-carrageenan, LWT - Food Sci. Technol. 35 (2002) 715–719. doi:10.1006/fstl.2002.0928.

[52] M. Jin, Sustained release of lysozyme encapsulated in zein micro- and nanocapsules, (2008).

[53] B. Ouattar, R.E. Simard, G. Piett, a Bégin, R. a Holley, Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with Chitosan., Int. J. Food Microbiol. 62 (2000) 139–148. doi:10.1016/S0168-1605(00)00407-4.

[54] M. Ozdemir, J.D. Floros, Analysis and modeling of potassium sorbate diffusion through edible whey protein films, J. Food Eng. 47 (2001) 149–155. doi:10.1016/S0260-8774(00)00113-8.

[55] J.-W. Rhim, P.K.W. Ng, Natural biopolymer-based nanocomposite films for packaging applications., Crit. Rev. Food Sci. Nutr. 47 (2007) 411–33. doi:10.1080/10408390600846366.

[56] S.I. Park, M. a Daeschel, Y. Zhao, Functional properties of antimicrobial lysozyme-chitosan composite films, J. Food Sci. 69 (2004) M215–M221. doi:10.1111/j.1365-2621.2004.tb09890.x.

[57] E. Fortunati, I. Armentano, Q. Zhou, a. Iannoni, E. Saino, L. Visai, et al., Multifunctional bionanocomposite films of poly(lactic acid), cellulose nanocrystals and silver nanoparticles, Carbohydr. Polym. 87 (2012) 1596–1605. doi:10.1016/j.carbpol.2011.09.066.

[58] C. Guo, L. Zhou, J. Lv, Effects of expandable graphite and modified ammonium polyphosphate on the flame-retardant and mechanical properties of wood flour-polypropylene composites, Polym. Polym. Compos. 21 (2013) 449–456. doi:10.1002/app.

[59] A. González, C.I. Alvarez Igarzabal, Soy protein - Poly (lactic acid) bilayer films as biodegradable material for active food packaging, Food Hydrocoll. 33 (2013) 289–296. doi:10.1016/j.foodhyd.2013.03.010.

[60] M.P. Arrieta, J. Lpez, S. Ferrndiz, M. a. Peltzer, Characterization of PLA-limonene blends for food packaging applications, Polym. Test. 32 (2013) 760–768. doi:10.1016/j.polymertesting.2013.03.016.

[61] M.P. Arrieta, J. Lpez, A. Hernndez, E. Rayn, Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications, Eur. Polym. J. 50 (2014) 255–270. doi:10.1016/j.eurpolymj.2013.11.009.

[62] A. Khan, T. Huq, R. a. Khan, B. Riedl, M. Lacroix, Nanocellulose Based Composites and Bioactive Agents for Food Packaging, Crit. Rev. Food Sci. Nutr. 8398 (2012) 120904065059008. doi:10.1080/10408398.2011.578765.

[63] G. Mensitieri, E. Di Maio, G.G. Buonocore, I. Nedi, M. Oliviero, L. Sansone, et al., Processing and shelf life issues of selected food packaging materials and structures from renewable resources, Trends Food Sci. Technol. 22 (2011) 72–80. doi:10.1016/j.tifs.2010.10.001.

[64] D.S. Cha, M.S. Chinnan, Biopolymer-Based Antimicrobial Packaging: A Review, Crit. Rev. Food Sci. Nutr. 44 (2004) 223–237. doi:10.1080/10408690490464276.

[65] R.N. Tharanathan, Biodegradable films and composite coatings: Past, present and future, Trends Food Sci. Technol. 14 (2003) 71–78. doi:10.1016/S0924-2244(02)00280-7.

[66] A. Sorrentino, G. Gorrasi, V. Vittoria, Potential perspectives of bio-nanocomposites for food packaging applications, Trends FoodSci.Technol.18(2007)84–95.doi:10.1016/j.tifs.2006.09.004.

[67] V.K. Mourya, N.N. Inamdar, Chitosan-modifications and applications: Opportunities galore, React. Funct. Polym. 68 (2008) 1013–1051. doi:10.1016/j.reactfunctpolym.2008.03.002.

[68] G. Berth, H. Dautzenberg, M.G. Peter, Physico-chemical characterization of chitosans varying in degree of acetylation, Carbohydr. Polym. 36 (1998) 205–216. doi:10.1016/S0144-8617(98)00029-0.

[69] C.K.S.Pillai, W. Paul, C.P. Sharma, Chitin and chitosan polymers: Chemistry,solubility and fiber formation, Prog. Polym.Sci.34(2009)641-678.doi:10.1016

[70] R. Ravindra, K.R. Krovvidi, a. a. Khan, Solubility parameter of chitin and chitosan, Carbohydr. Polym. 36 (1998) 121–127. doi:10.1016/S0144-8617(98)00020-4.

[71] K. V. Harish Prashanth, R.N. Tharanathan, Chitin/chitosan: modifications and their unlimited application potential-an overview, Trends Food Sci. Technol. 18 (2007) 117–131. doi:10.1016/j.tifs.2006.10.022.

[72] M. Rinaudo, M.R. Ã, Chitin and chitosan: Properties and applications, Prog. Polym. Sci. 31 (2006) 603–632. doi:10.1016/j.progpolymsci.2006.06.001.

[73] K. Kurita, Chitin and chitosan: Functional biopolymers from marine crustaceans, Mar. Biotechnol. 8 (2006) 203–226. doi:10.1007/s10126-005-0097-5.

[74] F. Shahidi, J.K.V. Arachchi, Y.J. Jeon, Food applications of chitin and chitosans, Trends Food Sci. Technol. 10 (1999) 37–51. doi:10.1016/S0924-2244(99)00017-5.

[75] L.Y. Zheng, J.F. Zhu, Study on antimicrobial activity of chitosan with different molecular weights, Carbohydr. Polym. 54 (2003) 527–530. doi:10.1016/j.carbpol.2003.07.009.

[76] M. Aider, Chitosan application for active bio-based films production and potential in the food industry: Review, LWT - Food Sci. Technol. 43 (2010) 837–842. doi:10.1016/j.lwt.2010.01.021.

[77] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: A state of the art review, Int. J. Food Microbiol. 144 (2010) 51–63. doi:10.1016/j.ijfoodmicro.2010.09.012.

[78] Y.C. Chung, C.Y. Chen, Antibacterial characteristics and activity of acid-soluble chitosan, Bioresour. Technol. 99 (2008) 2806–2814. doi:10.1016/j.biortech.2007.06.044.

[79] F. Devlieghere, a Vermeulen, J. Debevere, Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables, Food Microbiol. 21 (2004) 703–714. doi:10.1016/j.fm.2004.02.008.

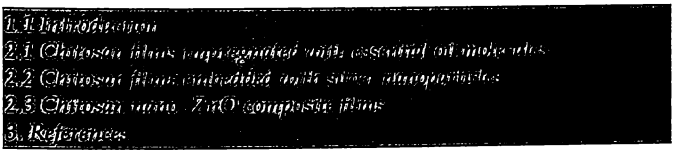
[80] R.C. Goy, D. De Britto, O.B.G. Assis, Antibacterialchitosan, 19 (2009) 241–247. doi:10.1590/S0104-14282009000300013.

[81] C. Qin, H. Li, Q. Xiao, Y. Liu, J. Zhu, Y. Du, Water-solubility of chitosan and its antimicrobial activity, Carbohydr. Polym. 63 (2006) 367–374. doi:10.1016/j.carbpol.2005.09.023.

[82] P.K.W.N.G. Erry, Preparation and Characterization of Chitosan-Based Nanocomposite Films with Antimicrobial Activity, (2006) 21–23.

[83] F. Chemistry, Diffusion of Acetic and Propionic Acids from Chitosan-based Antimicrobial Packaging Films, J. Food Sci. 65 (2000) 768–773. doi:10.1111/j.1365-2621.2000.tb13584.x.

CHAPTER 2
LITERATURE REVIEW



1.1 Introduction

In the last decade there has been an increasing surge of interest in the field of chitosan based composite materials. The hybridisation of Chitosan with biocompatible and nontoxic functional molecules will lead to novel smart materials. These molecules have tremendous applications in various fields, especially in designing packaging materials. The most attractive aspect of this hybridisation is synergistic interaction between Chitosan and functional molecules to improve their individual properties. The composite materials exhibit enhanced mechanical properties and physico-chemical characteristics. Many synthetic routes adopted for the preparation of Chitosan composite materials were simple and cost effective. Several such composite materials are finding remarkable applications in packaging of different kind food articles ranging from perishable fruits to the meat items. The discussion in this chapter covers most of available reports of Chitosan composites published in last decade.

2.1 Chitosan films impregnated with essential oil molecules

Hybridisation of Chitosan with bioactive molecules has been a trend to improve its properties. The novel composite materials of Chitosan will exhibit better performance in all respect. There are abundant of recently published literature works on modification of Chitosan for packaging applications. One of the most cited studies in this field was the incorporation of bioactive molecules like garlic oil, potassium sorbate and nisin in the Chitosan matrix. The incorporation of these molecules with Chitosan matrix has enhanced antimicrobial activity of Chitosan

without compromising its mechanical properties [1].Despite the improvement in antimicrobial properties, the amalgamation of essential oils with Chitosan matrix has also enhanced certain other crucial characteristics like barrier properties, antioxidant capacity etc [2].The edible coating of composite film containing Chitosan and bergamot essential oil was developed to improve the shelf life of cold store grapes. It was reported that in the presence of coating microbial growth has been reduced significantly and shelf life was improved [3]. Recently, edible biocomposite films entrapped with clove essential oil was prepared and applied to enhance the shelf life period of fish. The authors reported periodical release of active molecules onto the fish surface so as to inhibit the growth of micro organisms leading to enhanced shelf life [4,5].It is well known that, fruit Strawberry has low post harvest shelf life due to fast fungal decay. There were lot of attempts from researchers to exploit the biocide activity of Chitosan and essential oil composite films to increase post harvest shelf life of strawberry fruits. Recently Chitosan-lemon essential oil composite film has been coated on strawberry to improve its shelf life period[6]. It was generally agreed that barrier properties of any packaging film has crucial role in deciding the shelf life period of packed food. As a packaging film Chitosan exhibits commendable oxygen and carbon dioxide barrier property. But it has significantly lower water barrier property due to the presence of large number of hydrophilic groups. The incorporation of essential oil molecules with Chitosan has solved this concern satisfactorily. The hydrocarbon groups present in essential oil molecules tune hydrophilic Chitosan matrix into sufficiently hydrophobic nature. Recently, Chitosan composite films with required

water barrier properties have been prepared with the incorporation of different essential oil molecules[7].

2.2 Chitosan films embedded with silver nanoparticles

Chitosan-nano silver composite films were widely acclaimed for packaging applications. Different protocols including green synthesis routes were adopted for the synthesis of Chitosan-nano silver composites[8,9]. Antimicrobial efficacy of nano silver particles is well known and has been widely investigated. Generally nano form of silver is prepared by the reduction of silver salts in the presence of strong reducing agents and stabilizing molecules. Most of the preparation methods involve indiscriminate use of toxic chemicals and environmentally objectionable approaches[10]. Recently direct reduction of silver salt using Chitosan suspension is reported. There are many reports on utilization of Chitosan nano silver composites other than packaging applications such as drug carrier molecules, removal of toxic material from atmosphere etc. [11–23].

2.3 Chitosan nano -ZnO composite films

Probably nano ZnO is one of the most studied inorganic metal oxides owing to its unique properties such as wide band gap energy (3.37 eV), high surface area, high catalytic efficiency, non toxicity, chemical stability, antibacterial character and UV protection ability. The literature survey reveals that among the various properties, antimicrobial property of nano ZnO was less exploited. A few researchers tried to improve antimicrobial efficacy of nano ZnO particles by the hybridisation of nano particles with Chitosan. This

hybridisation leads to novel green composite materials due to synergistic interaction between Chitosan and nano ZnO particles having enhanced anti microbial efficiencies. So far, a few studies on anti microbial properties of Chitosan nano ZnO composites were reported. Li-Hua et al synthesised Chitosan –nano composite membranes by sol-cast protocol and ably characterized. The mechanical strength as well as elongation at break of all prepared films was determined. According to their investigation all composite films exhibited excellent mechanical strength compared to naive Chitosan film. The antimicrobial behaviour of composite films was evaluated against three pathogens, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus. The results were encouraging as all composite films demonstrated higher antimicrobial behaviour than bare Chitosan film [24]. Asmaa Farouk et al synthesised Chitosan nano ZnO composite sols and coated on textile materials to obtain antimicrobial textile finishing. In this method previously prepared nano ZnO particles were mixed with Chitosan solution to get nano sols of Chitosan -ZnO composite. Cotton fabrics were immersed in the composite sol to adsorb anti microbial composite material on the surface of cotton fabrics[25]. Authors Muhammad Shafiq et al synthesised Chitosan nano ZnO composite powder by direct addition of nano ZnO particles into Chitosan solution. Complete structural, thermal and anti microbial features of composite powder were determined and compared with pure Chitosan powder. They investigated one of the least exploited properties, that is current-voltage relationship of composite powder. The result of their investigation was hopeful as current -voltage relation was linearly connected to the amount of nano ZnO particles[26].One of

the excellent works in Chitosan-nano ZnO composite was the synthesis of flexible and microporous Chitosan hydrogel/nano ZnO composite bandages for wound dressing applications. The direct application of this novel composite hydrogel containing nano ZnO particles for wound healing purpose was the reflection of mutual enhancement of anti microbial properties of individual components present in composite material. The authors were successful to carry out in vitro as well as in vivo analysis to support wound healing ability of composite material [27].To widen the horizon of applications of Chitosan –nano ZnO composite, authors Yuvaraj Haldorai and Jae-Jin Shim synthesised Chitosan nano ZnO composite powder for photo catalytic activity. The prepared composite material was introduced into the dye solution and kept under UV irradiation to catalyse the photo degradation of dye molecules. The result was promising and comparable with existing photo catalyst materials [28].The notion of photo catalytic performance of Chitosan nano ZnO composite was further expanded by preparing a ternary composite of Chitosan, nano ZnO and TiO_2 molecules. The addition of TiO_2 molecules enhanced catalytic activity and decolourised methyl orange in presence of simulated sunlight. This ternary composite material had reusability and showed activity in sunlight. Since the composite was prepared in the film form, it was easy to remove from the reaction mixture after the use [29].

Literature survey showed that all researchers have adopted same protocol for the synthesis of Chitosan nano ZnO composite material. Pre synthesised nano ZnO particles were added in to Chitosan solution to get desired composite. They tuned the properties of composite

material by varying either the amount of nano ZnO particles or amount of Chitosan. Recently two researchers S. Anandhavelu, S. Thambidurai prepared Chitosan nano ZnO composite in a novel way. They adopted one pot procedure to synthesise composite material from precursor chitin and Zinc Chloride molecules and this is the easiest method ever reported to prepare Chitosan-nano ZnO composite materials. [30].

One of the profoundly studied properties of Chitosan was its utilization as adsorbent for purification of water. There are a few recent reports comparing the adsorption capacity of pure Chitosan with Chitosan ZnO composite powder. Authors Shahram Moradi Dehaghi et al applied the composite to remove harmful pesticide Permethrin from waste water. The composite beads were prepared and investigated adsorption capacity. They reported 100 times enhancement of adsorption as compared to bare Chitosan beads, more over composite bead maintained removal capacity up to 56% even after three cycles of uses [31]. Yangshuo Liu, Hyung-Il Kim prepared genipin cross linked biocompatible Chitosan/poly(ethylene glycol)/ZnO/Ag nano composites for wound healing applications. In this investigation they successfully synthesised composite hydro gel and its anti microbial activity was tested against four pathogens Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis. The high light of this report is superior antimicrobial efficacy composite hydrogel than Chitosan – nano ZnO composite [32]. P. Bhadra et al published a different report on anti microbial mechanism of Chitosan –nano ZnO composite. The authors could successfully explain the nature of interaction between E-coli and composite

material. According to the report Chitosan capped ZnO nano rods got attached to the outermost cell membrane of bacteria through —NH_2 group of Chitosan and permeated into cell membrane. The permeation of composite materials into the cell membrane leads to leakage of cell cytoplasm and complete destruction of bacteria [33].The authors Krishnaveni Rajendran and Thambidurai Sivalingam reported a significant relevance of Chitosan nano ZnO composite with cotton fabrics for anti microbial finishing applications. They chemically cross linked composite materials on cotton fabrics and its antimicrobial and thermal stability were evaluated. The result was promising and would open up new avenues of research to fabricate bio finished cotton with anti microbial efficiencies[34]. There is a report from Raju Khan et al on application of Chitosan-nano ZnO composite as a biosensor. They fabricated novel Chitosan -nano ZnO composite electrode using indium-tin-oxide (ITO) glass plate. The enzyme Cholesterol oxidase was immobilized on the electrode surface by simple physisorption method. The sensor electrode responded even in the detection limit 5$mgdl^{-1}$with sensitivity 1.41×10^{-4} $Amgdl^{-1}$. The value of Michaelis–Menten constant of composite material is 8.63$mgdl^{-1}$. This novel cholesterol sensor is expected to find applications for the estimation of cholesterol in serum samples [35].In addition to pesticide adsorption studies, a few authors, Raziyeh Salehi et al explored in detail the dye adsorption capacity of Chitosan nano ZnO composite powder. They selected two most common textile dyes Direct Blue 78 and Acid Black, as model compounds for adsorption studies. The mechanism of adsorption was investigated with the Langmuir, Freundlich and Tempkin isotherm models. The result was hopeful to use for as dye

removal applications [36]. Li-Hua Li et al reported ever first chitosan – ZnO-Ag ternary composite material for highly enhanced anti microbial properties. The synthesis route was relatively new sol-cast transformation method. The co-existence of ZnO and Ag in the matrix of Chitosan was characterised and confirmed by various methods. The composite exhibited excellent anti microbial properties against B. subtilis, E. coli, S. aureus, Penicillium, Aspergillus, Rhizopus and yeast. The notable feature of composite film was its ability to maintain initial colour of film even in the presence of two different nano particles [37].

The fabrication of novel sensors is always the thrust area of material research owing to its wide applicability and ever changing demands. The researchers Wei Zhang et al reported fabrication of new type of DNA sensor electrode using combination of Chitosan-Multi wall Carbon nano tubes and nano ZnO on glassy carbon electrode. They successfully immobilised DNA probe material on the composite electrode and investigated sensing ability using differential pulse voltammetry [38].The researches working on Chitosan –nano ZnO composite materials agreed unanimously the enhancement of anti microbial efficacy of this hybridmaterial, but no one of them put forth a satisfactory mechanistic explanations of anti microbial property. Recently Yan Wang et al published a detailed mechanism based study of anti microbial property of Chitosan –nano ZnO composite. Time-dependent fluorescence microscopies of Chitosan and Chitosan-nanoZnO were used to follow the mechanism. The suggestive mechanisms of synergistic anti microbial efficacy of composite explain

following possibilities. Through the electrostatic attraction, Chitosan, ZnO nanoparticles, and the released Zn^{2+} ions are accumulated and adhered to the surface of microbial cell membrane, which would lead to denaturation of membrane proteins and membrane permeability change leading to further destruction of microbial cell wall structure. Reactive Oxygen Species (ROS) produced by ZnO nanoparticles in the presence of UV or Visible light disrupted the microbial cell membrane, causing leakage of intercellular fluids. After nanoparticles pass through the membrane, nano-ZnO would interfere with the intercellular metabolism to perturb the function of proteins and DNA. On the other hand, the penetrated Chitosan could combine with intracellular DNA and RNA molecules to block the genome replication [39].Low solubility of chitosan at neutral p^H limited its applications, to overcome this hurdle people prepared various derivatives of chitosan including water soluble carboxy methyl Chitosan.Two researchers A. El.Shafei, A. Abou-Okeil synthesised carboxymethyl chitosan- nano ZnO composite to fabricate anti microbial and UV protection cotton fabrics [40].

3. References

[1] Y. Pranoto, S.K. Rakshit, V.M. Salokhe, Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin, LWT - Food Sci. Technol. 38 (2005) 859–865. doi:10.1016/j.lwt.2004.09.014.

[2] Zivanovic, S., Chi, S., Draughon, Antimicrobial Activity of Chitosan, Science (80-.). 70 (2005) 45–51.

[3] L. Sanchez-Gonzalez, C. Pastor, M. Vargas, A. Chiralt, C. Gonzalez-Martinez, M. Chafer, Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes, Postharvest Biol. Technol. 60 (2011) 57–63. doi:10.1016/j.postharvbio.2010.11.004.

[4] J. Gmez-Estaca, a. Lpez de Lacey, M.E. Lpez-Caballero, M.C. Gmez-Guilln, P. Montero, Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation, Food Microbiol. 27 (2010) 889–896. doi:10.1016/j.fm.2010.05.012.

[5] J.. L.D.L. Gómez-Estaca A.; Gómez-Guillén, M. C.; López-Caballero, M. E.; Montero, P., Antimicrobial Activity of Composite Edible Films Based on Fish Gelatin and Chitosan Incorporated with Clove Essential Oil, J. Aquat. Food Prod. Technol. 18 (2009) 46–52. doi:10.1080/10498850802581252.

[6] Perdones, L. Sánchez-González, a. Chiralt, M. Vargas, Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry, Postharvest Biol. Technol. 70 (2012) 32–41. doi:10.1016/j.postharvbio.2012.04.002.

[7] L. Snchez-Gonzlez, M. Chfer, A. Chiralt, C. Gonzlez-Martnez, Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on Penicillium italicum, Carbohydr. Polym. 82 (2010) 277–283. doi:10.1016/j.carbpol.2010.04.047.

[8] Y.-K. Twu, Y.-W. Chen, C.-M. Shih, Preparation of silver nanoparticles using chitosan suspensions, Powder Technol. 185 (2008) 251–257. doi:10.1016/j.powtec.2007.10.025.

[9] D. Wei, W. Sun, W. Qian, Y. Ye, X. Ma, The synthesis of chitosan-based silver nanoparticles and their antibacterial activity, Carbohydr. Res. 344 (2009) 2375–2382. doi:10.1016/j.carres.2009.09.001.

[10] M. Yadollahi, S. Farhoudian, H. Namazi, One-pot synthesis of antibacterial chitosan/silver bio-nanocomposite hydrogel beads as drug delivery systems, Int. J. Biol. Macromol. 79 (2015) 37–43. doi:10.1016/j.ijbiomac.2015.04.032.

[11] P. Kaur, A. Choudhary, R. Thakur, Synthesis of Chitosan-Silver Nanocomposites and their Antibacterial Activity, Int. J. Sci. Eng. Res. 4 (2013) 869–872.

[12] M. Potara, E. Jakab, A. Damert, O. Popescu, V. Canpean, S. Astilean, Synergistic antibacterial activity of chitosan-silver nanocomposites on Staphylococcus aureus., Nanotechnology. 22 (2011) 135101. doi:10.1088/0957-4484/22/13/135101.

[13] A.S. Dehnavi, A. Aroujalian, A. Raisi, S. Fazel, Preparation and characterization of polyethylene/silver nanocomposite films with antibacterial activity, J. Appl. Polym. Sci. 127 (2013) 1180–1190. doi:10.1002/app.37594.

[14] M. Bin Ahmad, M.Y. Tay, K. Shameli, M.Z. Hussein, J.J. Lim, Green synthesis and characterization of silver/chitosan/polyethylene glycol nanocomposites without any reducing agent, Int. J. Mol. Sci. 12 (2011) 4872–4884. doi:10.3390/ijms12084872.

[15] S. Govindan, E. a K. Nivethaa, R. Saravanan, V. Narayanan, a Stephen, Synthesis and characterization of chitosan–silver nanocomposite, (2012) 299–303. doi:10.1007/s13204-012-0109-5.

[16] S. Agnihotri, S. Mukherji, S. Mukherji, Antimicrobial chitosan–PVA hydrogel as a nanoreactor and immobilizing matrix for silver nanoparticles, Appl. Nanosci. 2 (2012) 179–188. doi:10.1007/s13204-012-0080-1.

[17] X.L. Cao, C. Cheng, Y.L. Ma, C.S. Zhao, Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities, J. Mater. Sci. Mater. Med. 21 (2010) 2861–2868. doi:10.1007/s10856-010-4133-2.

[18] C. Guo, L. Zhou, J. Lv, Effects of expandable graphite and modified ammonium polyphosphate on the flame-retardant and mechanical properties of wood flour-polypropylene composites, Polym. Polym. Compos. 21 (2013) 449–456. doi:10.1002/app.

[19] D.K. Boanić, L. V. Trandafilović, A.S. Luyt, V. Djoković, "Green" synthesis and optical properties of silver-chitosan complexes and nanocomposites, React. Funct. Polym. 70 (2010) 869–873. doi:10.1016/j.reactfunctpolym.2010.08.001.

[20] K. Vimala, Y.M. Mohan, K.S. Sivudu, K. Varaprasad, S. Ravindra, N.N. Reddy, et al., Fabrication of porous chitosan films impregnated with silver nanoparticles: A facile approach for superior antibacterial application, Colloids Surfaces B Biointerfaces. 76 (2010) 248–258. doi:10.1016/j.colsurfb.2009.10.044.

[21] K.H. Yang, Y.C. Liu, C.C. Yu, B.C. Chen, Fabrication of chitosan/silver nanocomposites based on electrochemical methods for removing formaldehyde in air, Mater. Chem. Phys. 126 (2011) 993–997. doi:10.1016/j.matchemphys.2010.09.069.

[22] M.M.G. Fouda, M.R. El-Aassar, S.S. Al-Deyab, Antimicrobial activity of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles, Carbohydr. Polym. 92 (2013) 1012–1017. doi:10.1016/j.carbpol.2012.10.047.

[23] K.S. V Krishna Rao, P. Ramasubba Reddy, Y.I. Lee, C. Kim, Synthesis and characterization of chitosan-PEG-Ag nanocomposites for antimicrobial application, Carbohydr. Polym. 87 (2012) 920–925. doi:10.1016/j.carbpol.2011.07.028.

[24] L.-H. Li, J.-C. Deng, H.-R. Deng, Z.-L. Liu, L. Xin, Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes, Carbohydr. Res. 345 (2010) 994–998. doi:10.1016/j.carres.2010.03.019.

[25] A. Farouk, S. Moussa, M. Ulbricht, T. Textor, ZnO Nanoparticles-Chitosan Composite as Antibacterial Finish for Textiles, Int. J. Carbohydr. Chem. 2012 (2012) 1–8. doi:10.1155/2012/693629.

[26] N.M. Barkoula, B. Alcock, N.O. Cabrera, T. Peijs, Fatigue properties of highly oriented polypropylene tapes and all-polypropylene composites, Polym. Polym. Compos. 16 (2008) 101–113. doi:10.1002/pc.

[27] P.T. Sudheesh Kumar, V.-K. Lakshmanan, T.V. Anilkumar, C. Ramya, P. Reshmi, a. G. Unnikrishnan, et al., Flexible and Microporous Chitosan Hydrogel/Nano ZnO Composite Bandages for Wound Dressing: In Vitro and In Vivo Evaluation, ACS Appl. Mater. Interfaces. 4 (2012) 2618–2629. doi:10.1021/am300292v.

[28] Y. Haldorai, J.-J. Shim, Chitosan-Zinc Oxide hybrid composite for enhanced dye degradation and antibacterial activity, Compos. Interfaces. 20 (2013) 365–377. doi:10.1080/15685543.2013.806124.

[29] H. Zhu, R. Jiang, Y. Fu, Y. Guan, J. Yao, L. Xiao, et al., Effective photocatalytic decolorization of methyl orange utilizing TiO 2/ZnO/chitosan nanocomposite films under simulated solar irradiation, Desalination. 286 (2012) 41–48. doi:10.1016/j.desal.2011.10.036.

[30] S. Anandhavelu, S. Thambidurai, Effect of zinc chloride and sodium hydroxide concentration on the optical property of chitosan-ZnO nanostructure prepared in chitin deacetylation, Mater. Chem. Phys. 131 (2011) 449–454. doi:10.1016/j.matchemphys.2011.10.003.

[31] S. Moradi Dehaghi, B. Rahmanifar, A.M. Moradi, P.A. Azar, Removal of permethrin pesticide from water by chitosan-zinc oxide nanoparticles composite as an adsorbent, J. Saudi Chem. Soc. 18 (2014) 348–355. doi:10.1016/j.jscs.2014.01.004.

[32] Y. Liu, H. Il Kim, Characterization and antibacterial properties of genipin-crosslinked chitosan/poly(ethylene glycol)/ZnO/Ag nanocomposites, Carbohydr. Polym. 89 (2012) 111–116. doi:10.1016/j.carbpol.2012.02.058.

[33] P. Bhadra, M.K. Mitra, G.C. Das, R. Dey, S. Mukherjee, Interaction of chitosan capped ZnO nanorods with Escherichia coli, Mater. Sci. Eng. C. 31 (2011) 929–937. doi:10.1016/j.msec.2011.02.015.

[34] R. Krishnaveni, S. Thambidurai, Industrial method of cotton fabric finishing with chitosan-ZnO composite for anti-bacterial and thermal stability, Ind. Crops Prod. 47 (2013) 160–167. doi:10.1016/j.indcrop.2013.03.007.

[35] R. Khan, A. Kaushik, P.R. Solanki, A. a. Ansari, M.K. Pandey, B.D. Malhotra, Zinc oxide nanoparticles-chitosan composite film for cholesterol biosensor, Anal. Chim. Acta. 616 (2008) 207–213. doi:10.1016/j.aca.2008.04.010.

[36] R. Salehi, M. Arami, N.M. Mahmoodi, H. Bahrami, S. Khorramfar, Novel biocompatible composite (Chitosan–zinc oxide nanoparticle): Preparation, characterization and dye adsorption properties, Colloids Surfaces B Biointerfaces. 80 (2010) 86–93. doi:10.1016/j.colsurfb.2010.05.039.

[37] L.-H. Li, J.-C. Deng, H.-R. Deng, Z.-L. Liu, X.-L. Li, Preparation, characterization and antimicrobial activities of chitosan/Ag/ZnO blend films, Chem. Eng. J. 160 (2010) 378–382. doi:10.1016/j.cej.2010.03.051.

[38] W. Zhang, T. Yang, D.M. Huang, K. Jiao, Electrochemical sensing of DNA immobilization and hybridization based on carbon nanotubes/nano zinc oxide/chitosan composite film, Chinese Chem. Lett. 19 (2008) 589–591. doi:10.1016/j.cclet.2008.03.012.

[39] Y. Wang, Q. Zhang, C.L. Zhang, P. Li, Characterisation and cooperative antimicrobial properties of chitosan/nano-ZnO composite nanofibrous membranes, Food Chem. 132 (2012) 419–427. doi:10.1016/j.foodchem.2011.11.015.

[40] a. El.shafei, a. Abou-Okeil, ZnO/carboxymethyl chitosan bionano-composite to impart antibacterial and UV protection for cotton fabric, Carbohydr. Polym. 83 (2011) 920–925. doi:10.1016/j.carbpol.2010.08.083.

CHAPTER-3

SCOPE AND OBJECTIVE OF THE WORK

Research on Chitosan based packaging materials are popular due to their many advantages [1,2].Chitosan is an economically available biopolymer, it has specific properties and it can be extended to smart packaging applications. The selection of Chitosan for packaging applications is supported by many merits like ecofriendly nature (green chemistry aspect), economic (cost factor), and modifiable thermal, physical and chemical features (technological requirements). Chitosan is available in many grades differing degree of deacetylation and molar mass. It has excellent film forming ability, moderate antimicrobial and antioxidant ability [3].

In the present work, Chitosan has been modified by incorporating two functional materials, namely nano ZnO and green tea extract powder. Among the various inorganic metal compounds, ZnO was preferably used for the synthesis of Chitosan - nanocomposite owing to its unusual properties such as wide band gap energy (3.37 eV), high surface area, high catalytic efficiency, non toxicity, chemical stability, biocidel activity and UV protection ability. In this context, it was our objective to fabricate Chitosan–nano ZnO composite packaging films, optimise its mechanical, physical, dielectrical, antimicrobial and antioxidant properties. Finally, we crafted the film into flexible pouches and investigated its ability to extend the shelf life of raw meat.

Another functional material used to incorporate Chitosan matrix was green tea extract powder (GTE). Green tea is abundantly available in the state of Kerala, hence it is cost effective. Green tea is also available in different varieties depending growth conditions, plant varieties, processing methods, etc. Traditionally it was used in foods and drinks

and it is well known for its health effects and antioxidant properties. In the present investigation, we incorporated GTE powder with Chitosan to develop a packaging material which is absolutely ecofriendly and follows green protocol. The film was ably characterized and subjected to various studies for optimisation of properties. Finally film was crafted into pouch and its efficiency to extend the shelf life of raw meat was investigated.

In the final phase of work, two types of films were compared in all respects, including synthesis protocols, merits of properties and extend of applications.

1.2 The objective of this work

The development of "*Chitosan Composites-Prospective Materials for food saftey*"

1.2 Stagewise objectives

To accomplish this final objective, following stages has to be covered.

1. Synthesise and characterization of Chitosan nano ZnO composite powder-gather a deep understanding of interaction between Chitosan and nano ZnO molecules, chemical environment and antimicrobial properties.

2. Fabrication and characterization of Chitosan-nanoZnO composite film.

3. Development of composite pouches and evaluation of ability of pouches to extend the shelf life of raw meat

4. Synthesis and characterization of Chitosan –GTE powder films

5. Evaluation of physical, mechanical, antimicrobial and antioxidant properties Chitosan-GTE powder film

6. Application study of Chitosan-GTE powder film for extending the shelf life of raw meat.

7. Comparison of properties of two kinds of synthesised films.

Reference

[1] H.K. No, S.P. Meyers, W. Prinyawiwatkul, Z. Xu, Applications of chitosan for improvement of quality and shelf life of foods: A review, J. Food Sci. 72 (2007). doi:10.1111/j.1750-3841.2007.00383.x.

[2] F. Shahidi, J.K.V. Arachchi, Y.J. Jeon, Food applications of chitin and chitosans, Trends Food Sci. Technol. 10 (1999) 37–51. doi:10.1016/S0924-2244(99)00017-5.

[3] P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for chitosan based antimicrobial films in food applications, Food Chem. 114 (2009) 1173–1182. doi:10.1016/j.foodchem. 2008.11.047.

CHAPTER-4
MATERIALS AND CHARACTERISATION TECHNIQUES

1.1 Chemicals

The list major chemical employed for the studies have been listed here. A facile one pot procedure was adopted for the preparation of composite films. Adequate care and attention were taken to ensure that the chemicals and procedures adopted for the investigation followed green chemistry protocols.

Table-1 List of chemicals and solvents

SI No	Chemicals	Source /company	purification
1	Chitosan from shrimp shells (practical grade)	Sigma aldrich	Used as received
2	Chitosan powder	Marin Chemicals-Cochin	Used as received
3	Zinc Acetate -Dihydrate	Merck	Used as received
4	Sodium hydroxide	Merck	Used as received
5	Acetic acid	Merck	Used as received
6	2,2-Diphenyl-1-Picrylhydrazyl (extra pure)	SRL	Used as received
7	Nutrient Agar	Himedia	Used as received
8	Agar powder	Himedia	Used as received
9	Green tea extract powder	Medizen labs, Bangalure	Used as received
10	Raw meat	Local market	Washed with pure water
11	Green tea leaves	Local market	Refluxed
12	Phosphate Buffer(p^H-7.2)	Sigma	Used as received
13	E-coli (MTCC737)	Cultured in the lab	
14	S-aureus (MTCC(1687)	Cultured in the lab	

2. Characterization techniques

2.1 Attenuated Total Reflection (ATR) Technique

A reflection spectrum in the Infra Red region was used to characterise film samples. The principle underlined in the analysis is called Attenuated Total Reflection (ATR).The instrument contains an ATR crystal made up of highly refractive index materials (e.g ZnSe, Ge etc). In this technique IR radiations are internally reflected between sample and ATR crystal. This internal reflection creates an evanescent wave which can extend beyond the surface of crystal. At this interface, the electric field of reflected IR radiations (evanescent wave) enter into the sample. The penetration of wave leads to the absorption at the characteristic wave length region and causes attenuation effect on the incident wave, detected and recorded as ATR-IR spectrum. During the calibration of ATR spectra following precautions must be undertaken[1–3].

a) The sample must have sufficient contact with the ATR crystal, since the evanescent wave can protrude only above the crystal 0.5 μ - 5 μ.

b) The refractive index of sample and crystal must differ significantly

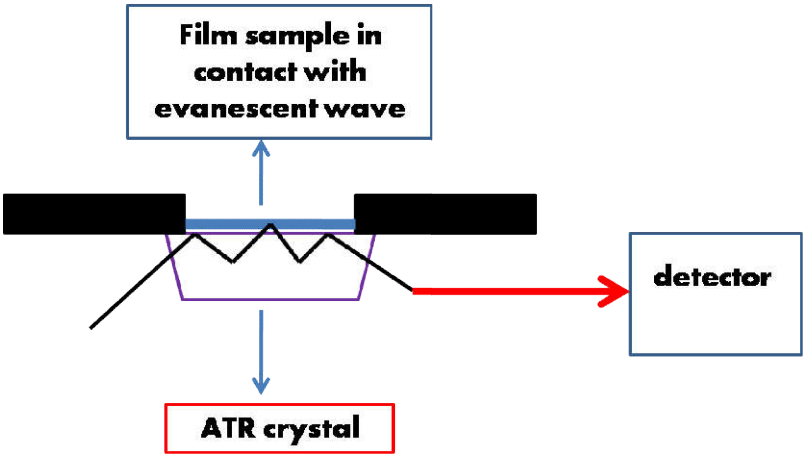


Fig-1 Illustration of ATR spectroscopy

2.2 X-ray diffraction studies

X-ray diffraction patterns of film samples have taken to compare crystallinity, size of nano particles and the nature of interaction between polymer and filler molecules. The principle of XRD is based on Bragg's Diffraction law.

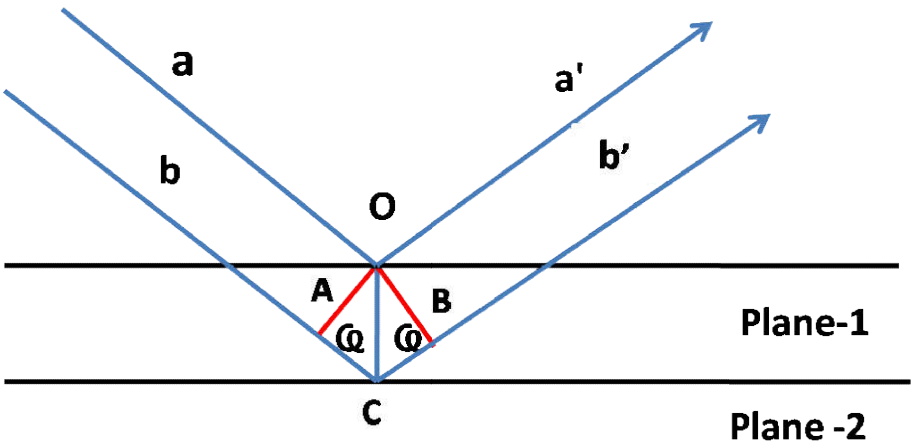


Fig-2 Illustration of X-ray diffraction

The Bragg's law is

$$n\lambda = 2d \sin\theta \quad \text{..... (1)}$$

Where

n = 1,2,3 etc

n = order of diffraction

λ = wave length of X-ray (0.15406nm)

d = inter planar distance

θ = angle between incident ray and lattice plane

XRD diffractogram was used to determine average size of crystallites with the help of Scherrer equation

$$D = k\lambda / \beta\cos\theta \quad \text{..... (2)}$$

D = size of particle in nm

β = full width at maximum

k = constant (0.94)

The XRD patterns of samples were compared with JCPDS (Joint Committee on Powdered Diffraction Standards) data base. As it known, JCPDS is non profit scientific organisation of dedicated researches which maintain this data base with proper update. Currently the database contains X-ray diffraction data of more than five lakh compounds[4].

2.3 UV –Vis spectroscopy

UV-Vis spectroscopy was used to evaluate optical properties of the sample. As it is already known, when a beam of light incidents on a film sample following phenomena will take place (fig-3). The light may be reflected, transmitted, diffused, absorbed, refracted or polarized. According to Beer-Lambert law[5]

Abs = log(1/T)

T = Transmittance

%Abs = 100%-%R-%T

R = Reflectance

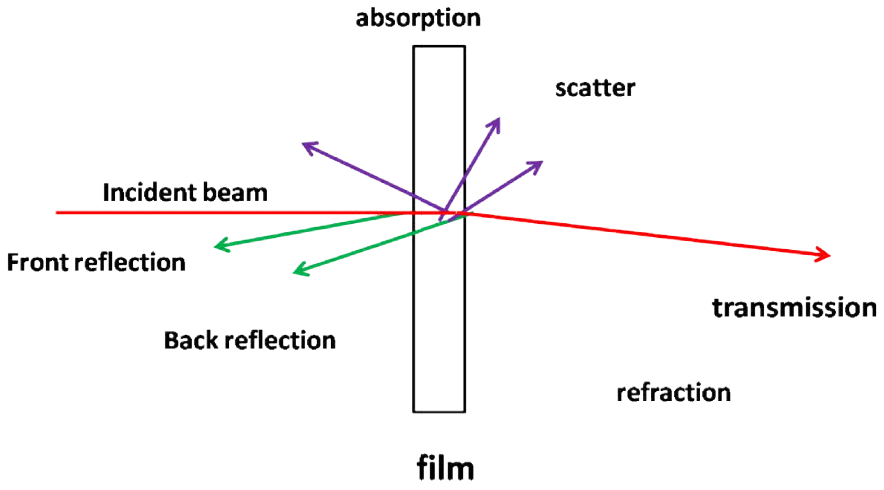


Fig-3 Illustration of interaction of light with film sample

The evaluation of absorption properties of films gives lucid idea regarding the optical barrier capacity of films. It is obvious that a

packaging film shall have enough UV-vis light blocking ability to improve shelf life of packed food.

The Transparency and opacity of films were determined using following equations [6].

$$T_{600} = -\log \%T / b$$

Opacity = absorbance at 500 nm X film thickness (mm)

Where %T is percentage transmittance and b is the film thickness (mm)

In the case of powdered sample diffuse reflectance spectra (DRS) was taken for the evaluation of absorbance phenomena. Diffuse reflectance is the type of reflectance of light taking place in all directions of the powdered sample. It differs from specular reflectance, where reflected beam has same angle as that of incident beam. The band gap energy of composites powder can be measured from absorption spectra using the following equation:

$$(\alpha h\gamma)^2 = E_D(h\gamma - E_g)$$

Where **α** is the optical absorption coefficient, $h\gamma$ is the photon energy, E_g is the direct band gap and E_D is a constant. The Y intercept of the linear plot of $(\alpha h\gamma)^2$ versus $h\gamma$ will give the value of direct band gap energy (E_g) [7,8].

2.4 Thermal analysis

Thermo gravimetric analysis (TGA) of samples was carried out to examine thermal properties. The sample was heated under nitrogen flow with constant heat rate while the difference of the mass during this process was recorded by balance. The mass loss indicates the occurrence of various thermally induced changes in the sample. The plot of percentage of mass loss (Y-axis) verses temperature (X-axis)was obtained for each sample[9].

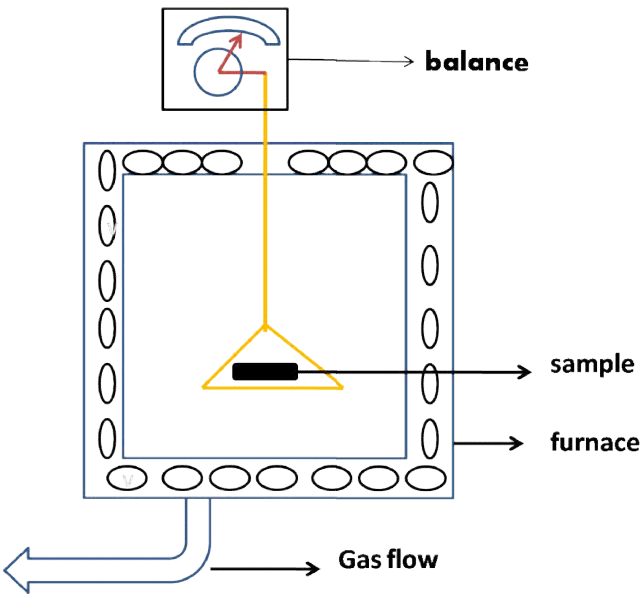


Fig-4 Schematic representation of TGA

Another most important thermal characterization technique is Differential Scanning Calorimetry (DSC).In this technique, film sample and reference (empty pan) were heated at constant heat flow. The difference in heat flow into sample and reference was measured as

the function of sample temperature.Since the Differential Scanning Calorimeter is operating at constant pressure, heat flow is equal to enthalpy changes

i. e $(dq/dt)_p = (dH/dt)$

Here dH/dt is the heat flow measured in m cal per sec.

The heat flow difference between the sample and the reference is given as $\Delta(dH/dt) = (dH/dt)_s - (dH/dt)_r$

and this value can be either positive or negative. In an endothermic process heat is absorbed and, therefore, heat flow to the sample is higher than that to the reference. Hence $\Delta dH/dt$ is positive. Where as in an exothermic process, heat is released from the sample and $\Delta dH/dt$ is negative. The DSC curve was plotted by taking heat flow (Y-axis) verses temperature (X-axis).

Thermal studies of samples were conducted to compare thermal stability of samples and to obtain glass transition temperature values[10].

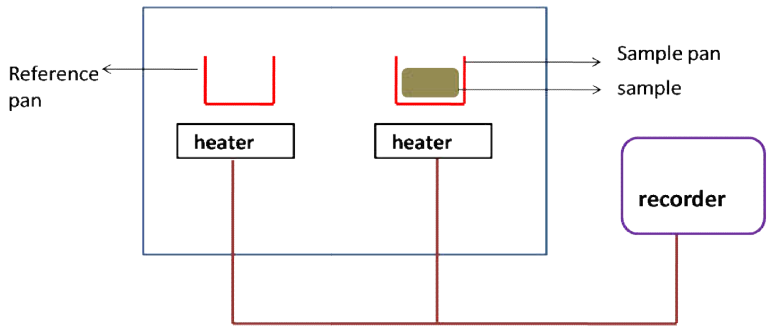


Fig-5 Schematic representation of DSC

2.5 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used to evaluate the surface morphology of samples. The change in surface morphology of chitosan in the presence of fillers was ably characterised by SEM photographs. The size and mode of distribution of nanoparticles in polymer surface were accurately determined by this technique. The instrument comprises an electron gun at the top, which produces electron beam. The high energy electron beam is focussed by a series of magnetic lenses. The focussed beam is used to scan the sample surface from left to right; this process is kwon as rastering. The scanning of surface produces corresponding images and displayes on the computer screen same way as in television.

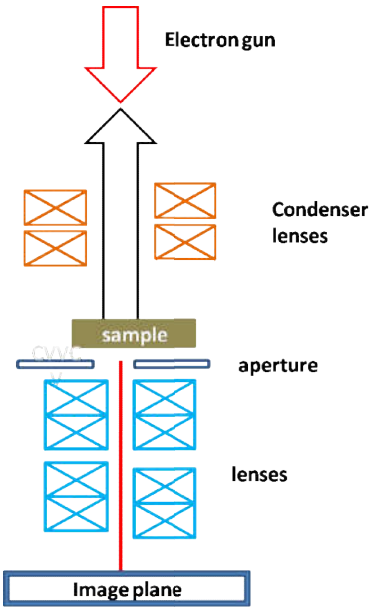
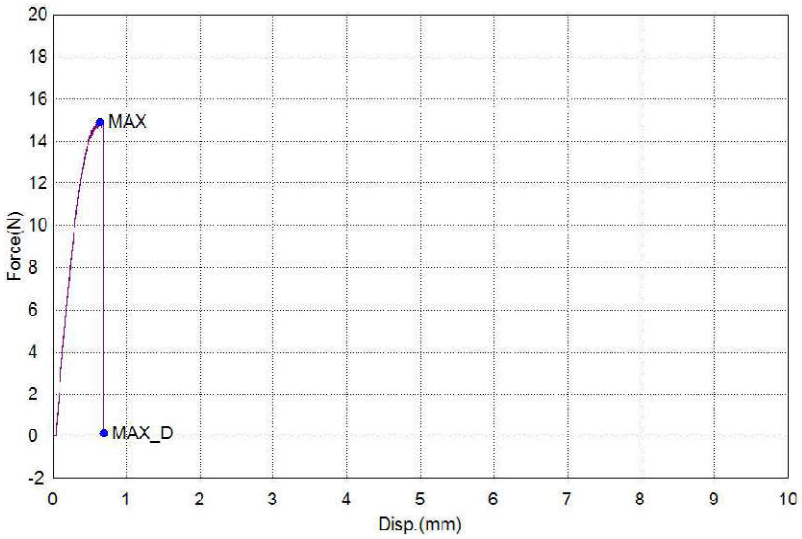


Fig-6 Schematic representation of SEM

2.6 Mechanical properties

Mechanical properties of film sample were evaluated using two measurements namely, tensile strength and elongation at break. Tensile strength is the ability of film material to withstand against external force. It was obtained from stress-strain diagram. The film sample was cut into dumbbell shape and positioned vertically in the grips of the testing machine. The grips were tightened so as to avoid the slippage of specimen. During the measurement, continuous stress is applied and corresponding strain is recorded. From the load value at the time of rapture of film, tensile strength and elongation at break is calculated.



Tensile strength = force (load) / cross sectional area or (width X thickness)

Elongation at break = change in length at break/ initial length

2.7 Antimicrobial analysis

Antimicrobial efficacy of samples was evaluated using following methods. The selection of different methods is based on nature of specimen and test conditions.

2.7.1 Agar well diffusion method

It is the most common method to evaluate the antimicrobial activity. In this method, one millilitre (mL) of bacterial inoculums is uniformly applied over the entire agar surface. Then, a hole/well with a diameter of 8 mm is made aseptically with a sterile well borer. Then 0.5 mL of the antimicrobial agent solution at desired concentration is introduced into the well. Then, agar plates with antimicrobial substances are incubated at 37^0C for 24hrs. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microorganisms. The efficiency of antimicrobial substances is linearly related to the diameter of inhibition Zone [11].

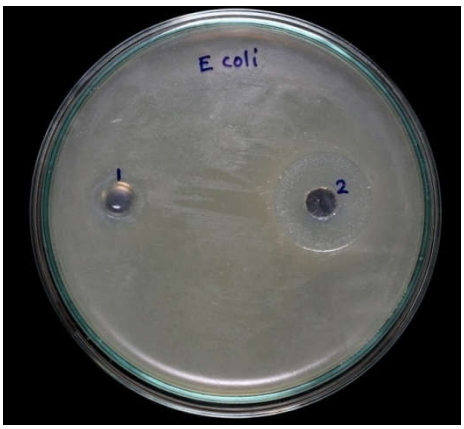


Fig-7. **Image of agar diffusion of chitosan composite against E-coli (standard sample MTCC 1687)**

2.7.2 Disc diffusion method

In this method antimicrobial disc was prepared using whatman No-40 filter paper. The paper was cut into disc shape (7mm) and dipped in sample suspension, dried and used as antimicrobial disc. The dried disc was placed on the inoculated agar surface. The whole system, agar plate with paper disc was incubated at ambient temperature for 24hrs. The efficiency of disc to prevent the growth of microorganism is directly related to the diameter of inhibition zone around the disc. This method emphasizes the ability of composite material to release and diffuse through the medium [12].

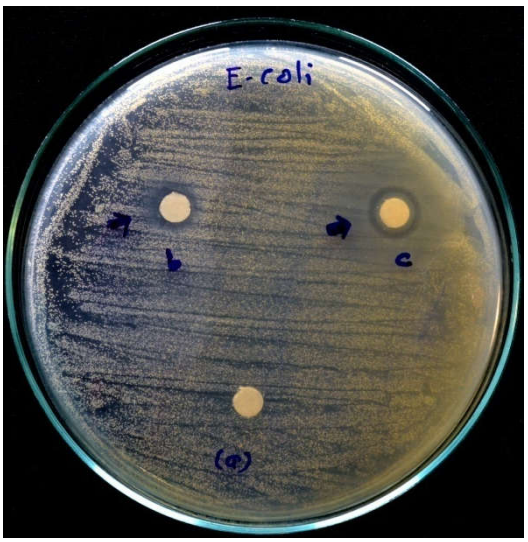


Fig-8 Image of agar diffusion of chitosan composite against E-coli collected from a patient

2.7.3 Colony Forming Unit (CFU) method

Colony Forming Unit (CFU) refers to individual groups of bacteria, which are growing together to form a colony. It was used to

enumerate the number of microorganism present in a media. The value was reported as CFU per volume of sample. To determine the number of colony forming units, the antimicrobial film samples were treated with suspension containing microorganism, the treated suspension serially diluted (fig-9), poured uniformly on the surface of an agar plate and incubated at appropriate temperature for 24 hours. The colonies formed were counted and reported as.

Colonies Forming Unit (CFU) per millilitre (CFU/ml) = (number of colonies × dilution factor)/ (Volume of culture plate) [13].

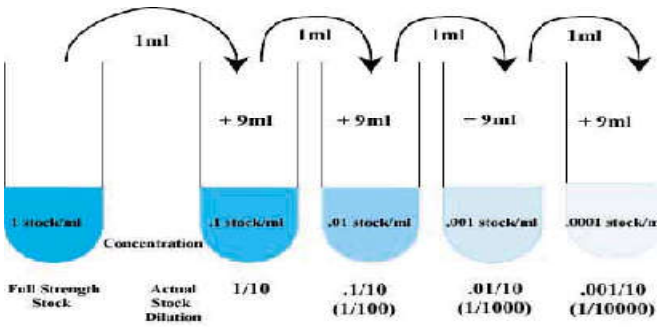


Fig-9. Illustration of serial dilution

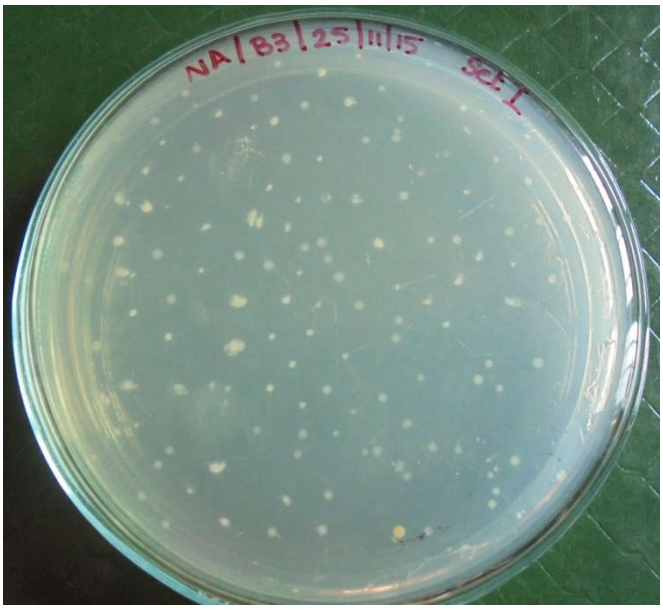


Fig-9. Image of CFU formed by bacteria present in meat sample.

2.7.4 Optical density (OD) method

It is a quick and efficient method for evaluating antimicrobial efficacy of film samples. The method is based on the relation between turbidity of medium and its transmittance. The transmittance of light through a medium is inversely proportional to its turbidity. As it is known that, microbial growth develops turbidity in the medium and the turbidity of such medium is directly proportional to amount of bacteria in the medium. Transmittance of medium was determined using spectrophotometer at the wavelength 600nm at different time intervals and converted to optical density. The intensity of light reaches at recorder through clear medium is taken as reference and OD value is considered as 0. When same monochromatic radiation passes through bacterial medium, a significant portion of light was scattered and the

intensity reaches at recorder is considerably lowered. The difference in absorption was converted into optical density.

Absorbance (O.D.) = 2 - log Transmittance[14]

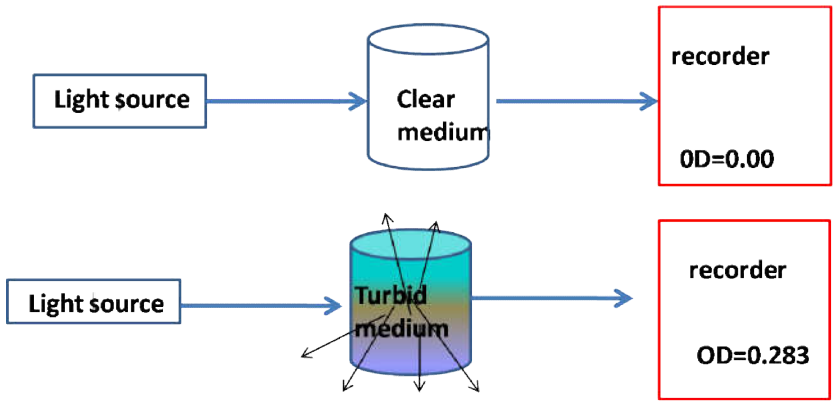
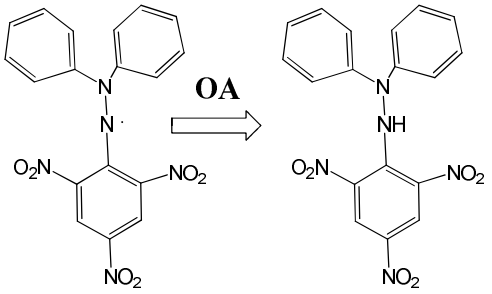


Fig-10 Illustration of OD method

2.8 Antioxidant property

Antioxidant property of film was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. It is a stable radical and widely used for antioxidant studies. The structure of DPPH shows the presence of an odd electron and can be reduced by an oxidising agent. The solution of DPPH radical has purple colour and its odd electron gives a strong absorption band at 517 nm. The strong purple colour turns to yellow as the molar absorptivity of the DPPH radical solution at 517 nm reduces from 9660 to 1640 when the odd electron of radical is paired with hydrogen donated by a free radical scavenging antioxidant. The intensity of decolourization is related to number of

electrons captured , in turn the ability of film to reduce the radical [15,16].



Purple colour radical form

Yellow colour reduced form

3 References

[1] PerkinElmer, FT-IR Spectroscopy Attenuated Total Reflectance (ATR), PerkinElmer Life Anal. Sci. (2005) 1–5. http://www.utsc.utoronto.ca/~traceslab/ATR FTIR.pdf.

[2] J. Fahrenfort, Attenuated total reflection, Spectrochim. Acta. 17 (1961) 698–709. doi:10.1016/0371-1951(61)80136-7.

[3] K.L.A. Chan, S. V. Hammond, S.G. Kazarian, Applications of attenuated total reflection infrared spectroscopic imaging to pharmaceutical formulations, Anal. Chem. 75 (2003) 2140–2146. doi:10.1021/ac026456b.

[4] B.J. Wuensch, Standard x-ray diffraction powder patterns, 1970. doi:10.1524/zkri.1970.131.1-6.159.

[5] M.C. Gómez-Guillén, M. Ihl, V. Bifani, A. Silva, P. Montero, Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (Ugni molinae Turcz), Food Hydrocoll. 21 (2007) 1133–1143. doi:10.1016/j.foodhyd.2006.08.006.

[6] J.D. Torres, E. a. Faria, J.R. SouzaDe, a. G.S. Prado, Preparation of photoactive chitosan-niobium (V) oxide composites for dye degradation, J. Photochem. Photobiol. A Chem. 182 (2006) 202–206. doi:10.1016/j.jphotochem.2006.02.027.

[7] C. Tams, N. Enjalbert, The Use of UV/Vis/NIR Spectroscopy in the Development of Photovoltaic Cells, PerkinElmer. (2009). http://www.perkinelmer.com/pdfs/downloads/APP_UseofUVVisNIRinDevelopmentPV.pdf.

[8] S. Anandhavelu, S. Thambidurai, Effect of zinc chloride and sodium hydroxide concentration on the optical property of chitosan-ZnO nanostructure prepared in chitin deacetylation, Mater. Chem. Phys. 131 (2011) 449–454. doi:10.1016/j.matchemphys.2011.10.003.

[9] C.G.T. Neto, J.A. Giacometti, A.E. Job, F.C. Ferreira, J.L.C. Fonseca, M.R. Pereira, Thermal analysis of chitosan based

networks, Carbohydr. Polym. 62 (2005) 97–103. doi:10.1016/j.carbpol.2005.02.022.

[10] M.J. Zohuriaan, F. Shokrolahi, Thermal studies on natural and modified gums, Polym. Test. 23 (2004) 575–579. doi:10.1016/j.polymertesting.2003.11.001.

[11] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, J. Pharm. Anal. 6 (2016) 71–79. doi:10.1016/j.jpha.2015.11.005.

[12] A. Nostro, M.P. Germanò, V. D'angelo, A. Marino, M. a Cannatelli, M. Germano, et al., Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity, Lett. Appl. Microbiol. 30 (2000) 379–384. doi:10.1046/j.1472-765x.2000.00731.x.

[13] T. Maneerung, S. Tokura, R. Rujiravanit, Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing, Carbohydr. Polym. 72 (2008) 43–51. doi:10.1016/j.carbpol.2007.07.025.

[14] F. Widdel, Theory and measurement of bacterial growth, Di Dalam Grundpraktikum Mikrobiol. (2007) 1–11. doi:10.1017/CBO9781107415324.004.

[15] R.H.. Garcia, E.J ; Oldoni, T.L.C. ; Alencar, S.M ; Reis, A ; D.Loguercio, A ; Grande, Antioxidant Activity by DPPH Assay of Potential Solution to be Applied on Bleached Teeth, Brazil Dent. J. 23 (2012) 22–27.

[16] A. Prakash, F. Rigelhof, E. MIller, Antioxidant Activity, Eur. Rev. Med. Pharmacol. Sci. 15 (2011) 376–378. doi:10.1016/j.bmcl.2010.12.025.

CHAPTER 5

SYNTHESIS AND CHARACTERIZATION OF NOVEL CHITOSAN-NANO ZnO COMPOSITE POWDER

1.1 Introduction

The aim of this work was to strategize an easy and efficient method to prepare the Chitosan-nanocomposite and explore its physico-chemical characteristics so as to utilize them as antimicrobial material in future packaging applications. A one pot procedure was adopted, where ZnO nanoparticles were immobilized on the Chitosan matrix by an in situ sol–gel conversion in a single step process. Three different composites were prepared by varying the concentration of sodium hydroxide. The composites were characterized by FTIR, UV–Visible spectra, and XRD. In the IR spectra of composites, the significant reduction of band width corresponding to OH and NH_2 groups is ascribed to the decrease of hydrogen bond due to the presence of ZnO nanoparticles. The direct evidence of the immobilization of nano ZnO particles in the Chitosan matrix was obtained by SEM. The average particle size was determined using Debye–Scherrer equation. Optical studies of composites proved that all of them have characteristic band gap energies and are in agreement with the reported values. Antimicrobial analysis underlined excellent antimicrobial activity against Gram negative bacteria Escherichia coli (E. coli) and Gram positive bacteria Staphylococcus aureus (S. aureus). Based on the above studies, the biocompatible, eco-friendly and low-cost composite material could be employed in smart packaging applications with required modifications.

2 Experimental

2.1 Materials

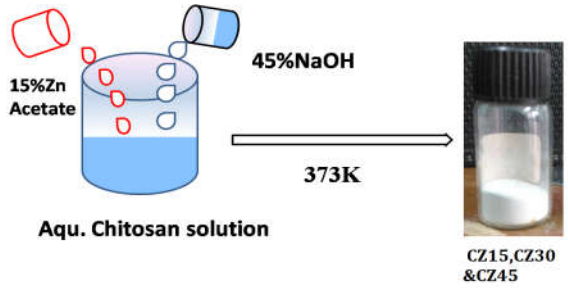
Chitosan with 85% degree of deacetylation was purchased from Sigma Aldrich Co. Ltd (USA). Acetic acid, sodium hydroxide, and zinc acetate di hydrate were purchased from Merck (Germany). Mineral salt broth and nutrient agar are obtained from Himedia Chemicals (India). Escherichia coli (E. coli) and Staphylococcus aureus (S.aureus) were grown at the microbiology laboratory, Department of Life Science, University of Calicut. Clinical sample of E. coli and S.aureus were obtained from Santhi hospital, Omessary, Calicut, India. Deionized water was used to prepare all the solutions. All the chemicals were of analytical grade reagents and used without further purification

2.2 Preparation

Chitosan solution was prepared by dissolving 0.5g of Chitosan in 2% acetic acid solution at room temperature. The solution was then filtered to remove undissolved particles and added 30 mL of 15% zinc acetate dihydrate solution and shaken well. To this solution, 30 mL of 45 % sodium hydroxide solution was added slowly at a temperature of 70^0C and the entire mixture was stirred at 70^0C for 4 h. The white precipitate formed was allowed to settle by keeping the mixture for 24 h. The supernatant solution was discarded, the precipitate was rinsed with distilled water for several times to remove excess of NaOH present (if any), filtered and dried by keeping in an air oven maintained at a temperature of 50^0C for 12h. The white dried precipitate was powdered well in a mortar by using a pestle and this sample was designated as

CZ45. The above process was repeated with decreased concentration of sodium hydroxide, 30 and 15%, the corresponding composites formed were designated as CZ30 and CZ15 respectively scheme-1.

Scheme-1 Preparation of composites



2.3 Characterization

The FTIR spectra of the samples were recorded in KBr pellets using Fourier Transform IR Spectrometer (Model: JASCO FTIR 4108). The absorption spectra of powder was recorded by using a UV-Visible spectrophotometer (Model: JASCO V-550) in the wavelength range of 200-800nm. X-ray diffraction (XRD) patterns of composites were recorded with a X-ray diffractometer (Model: Rigaku Minifex 600) using Cu Kα radiation (kα = 0.15406nm) at a scanning rate of 1^{o} min^{-1}. The thermal stability and degradation behavior of composites were assessed using a thermo gravimetric analyzer (TGA) (Model: TGA/DTA851) under flowing air (at a flow rate of 60 μL min^{-1}) in the temperature range, 40 to 780^{0}C at a heating rate of 10^{0}C min^{-1}. The SEM images of Chitosan and composites were recorded using a Field

Emission Scanning Electron Microscope (Model: HITACHI SU—6600 FESEM).

2.4 Antibacterial activities

The antimicrobial activity of Chitosan and Chitosan-based nanocomposite powders was evaluated by Agar well diffusion method. Gram-negative bacteria E.coli (MTCC 1687) and Gram-positive bacteria S.aureus (MTCC 737) were used as test organisms. Sterile NA plates were prepared and 0.1 mL of the inoculums of the test organism was spread uniformly over it. Wells were prepared by using a sterile borer of diameter 6mm and the samples of pure Chitosan C, CZ15, CZ30 and CZ45 were added in each well separately. The plates were incubated at 35-37^{o}C for 24 h, a period of time sufficient for the growth. The zone of inhibition of microbial growth around the well was measured in mm. The antimicrobial efficacy of samples against clinical bacteria (E.coli and S.aureus) was evaluated by the disc diffusion method. The antimicrobial disc was prepared as described earlier. Whatman No.1 filter paper was cut into disc shape using a circular knife and autoclaved. This paper discs were soaked separately into 5mL of 1% acetic acid solution containing 0.05g of C, CZ45, CZ30 and CZ15 and dried at room temperature to adsorb the composite on the paper surface. Antibacterial properties of filter paper disc treated with bionanocomposites were measured by the inhibition zone method. The presence of any clear zone that formed around the disc on the plate medium was recorded as an indication of inhibition against the microbial species.

3.1 Results and discussion

3.2 FTIR spectroscopy

Fig. 1 shows the FTIR spectra of pure Chitosan and Chitosan-nano ZnO composites powder. The characteristic broad band at 3413 cm^{-1} observed in Fig. 1a is attributed to the stretching vibrations of $–NH_2$ and –OH groups of Chitosan. The bands at 2930 and 2847 cm^{-1} are due to the asymmetric stretching vibration of $–CH_3$ and $–CH_2$ groups and the peaks observed at 1647 and 1468 cm^{-1} are designated respectively to the stretching vibrations of C=O and scissoring vibrations of $–NH_2$ groups of Chitosan. The band observed at 1019 cm^{-1} is due to the stretching vibration of –C-O-C- of glycosidic linkage of the polymer [2].

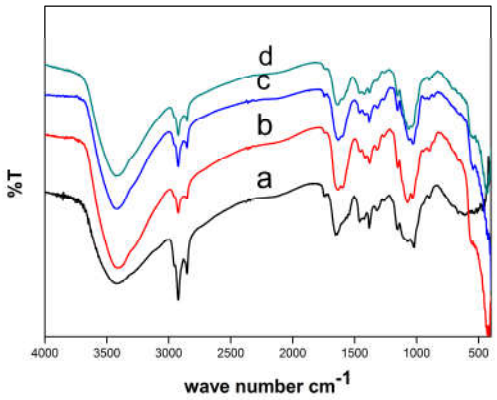


Fig. 1. FTIR spectra of pure Chitosan (a), CZ 15 (b), CZ 30(c), CZ 45(d)

The FTIR spectrum of ZnO composites (Figs 1-b, c and d) are similar to that of pure Chitosan but in contrast to Fig. 1a, the band width

corresponding to stretching vibrations of $–NH_2$, and –OH are reduced significantly in composites, possibly due to the reduction of inter molecular hydrogen bonds in the presence of nanoparticles. During the formation of composites, the strong bands corresponding to $–NH_2$, and –OH are found to be shifted to lower regions (3387 cm^{-1}). Similarly, the band corresponding to C=O group is found to be shifted to a lower wave number (1633 cm^{-1}) and the band observed at 1468 cm^{-1} is found to be shifted to 1385 cm^{-1}. The above changes observed in the IR spectra of composites emphasize the development of favorable interaction between various groups of Chitosan and ZnO nanoparticles. In addition to this, all composites show a characteristic intense band at 398, 419 and 433 cm^{-1} which were ascribed to the stretching mode of O-Zn-O group (Figs. 1b, c and d) [3].

3.3 XRD analysis

The XRD was used to examine the crystalline state of Chitosan and Chitosan-ZnO composites (shown in Fig. 2). The Chitosan showed a characteristic peak at 19.9^o (Fig. 2a) which clearly indicates its semicrystalline nature. This property is attributed to the presence of strong intermolecular hydrogen bonds in Chitosan. Whereas this typical peak is not observed in Chitosan nano –ZnO composites (Figs 2b, 2c and 2d), which may be due to the loss of semicrystalline nature of Chitosan in the presence of nanoparticles.

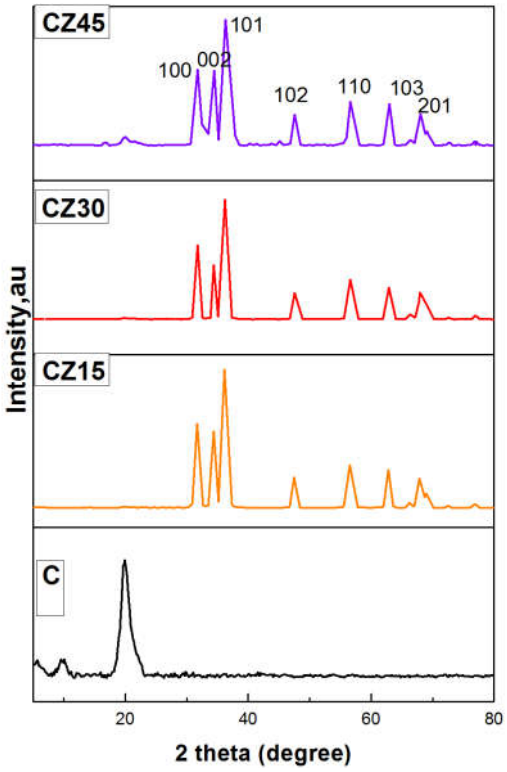


Fig. 2. The XRD patterns (a) pure Chitosan powder (C) (b) CZ15 (c) CZ30 and (d) CZ45

The peaks observed at 31.78, 34.44, 36.27, 47.5, 56.56, 62.87 and 67.96^0 in the X-ray diffractograms of Chitosan –nano ZnO composites were assigned to the diffractions from the planes (100), (002), (101), (102), (110), (103) and (201) respectively. Similar planes are observed in pure ZnO particles with hexagonal structure and the results are in consistent with the data base of (JCPDS No 36-1451) [3]. The average size of particles (D) was calculated using Debye-Scherrer equation [31].

$$D = K\lambda/\beta \cos\theta \qquad (1)$$

Where the value of K is equal to 0.89, λ is the wave length of X-ray ($1.54A^0$), β is the full width at half maximum and θ is the half of the diffraction angle. The values of D obtained for CZ45 is 13.5 and 18 nm for both CZ 30 and CZ 15.

3.4 Thermo gravimetric analysis

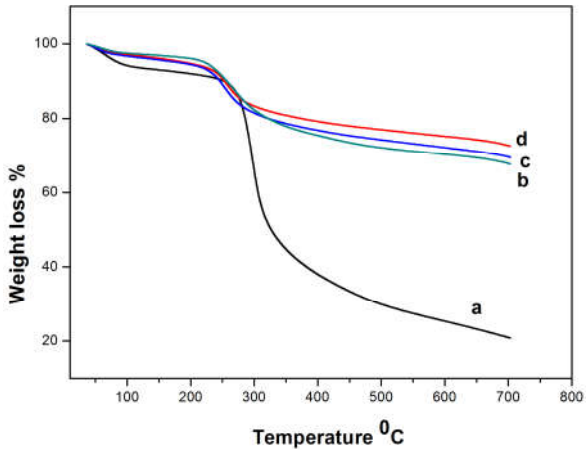


Fig. 3. TGA curves (a) pure Chitosan powder (C) (b) CZ15 (c) CZ30 and (d) CZ45

Thermo gravimetric analysis was used to compare thermal properties of Chitosan and prepared composite materials. As shown in Fig. 3, Chitosan exhibited significantly different decomposition trend as compared to the composite powder. However, the three composite materials show same fashion of thermal decomposition. Pure Chitosan polymer exhibited a two-step mass loss; the first stage of mass loss (observed up to 140°C) was attributed to the loss of hydrated water and the second stage of mass loss (observed in the temperature range 290-500°C) was due to the degradation of the polymer [4,5]. The residual mass percentage for C, CZ15, CZ30 and CZ45 at the temperature of 750^0C are 20, 69, 73 and 79% respectively and the excess residual

percentage values of composite indicate the presence of enormous amount of ZnO nanoparticles in the polymer matrix**.**

3.5 UV-Visible spectral analysis

The UV-Visible absorption spectra of pure Chitosan and Chitosan nano- ZnO composite powder are shown in Fig. 4. It is observed that pure Chitosan powder has lower absorbance (Fig. 4a) and the maximum absorption was exhibited below 300 nm. All composite materials have shown significant increase in absorbance in the UV region (Figs 4b, 4c and 4d) indicating the presence of nano zinc oxide particles in the Chitosan matrix. The λ_{max} values shown by composites are at 348, 335 and 338 nm respectively for CZ15 (b), CZ30 (c) and CZ45 (d) and these values are lower than that of microcrystalline ZnO particles (λ_{max} is 372 nm) [6]. It has been reported that UV-Vis absorbance of ZnO particles is a size dependent property; when the size of nanoparticle is decreased, reduction in absorbance and blue shift into lower wavelength region are observed [7,8].

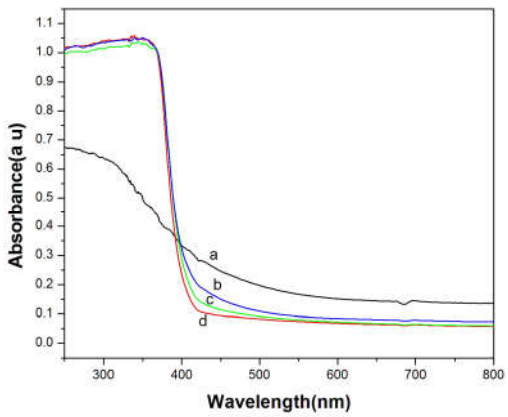


Fig.4 UV-Visible absorbance spectra; (a) pure Chitosan (b) CZ 15 (c) CZ 30 and (d) CZ45

3.6 Energy band gap of composites

One of the major applications of semiconductor metal oxides is their ability to function as photo catalyst. An ideal photo catalyst would have band gap energy in the visible region and long resistivity towards the photo corrosion reactions. The band gap energy of semiconductor materials depend upon the size of the particles. The structure and size of nano semiconductor particles can be tuned by varying the synthesis methods and reaction conditions. Similarly photo corrosion can be reduced by dispersing the nanoparticles in the polymer matrix [9,10].

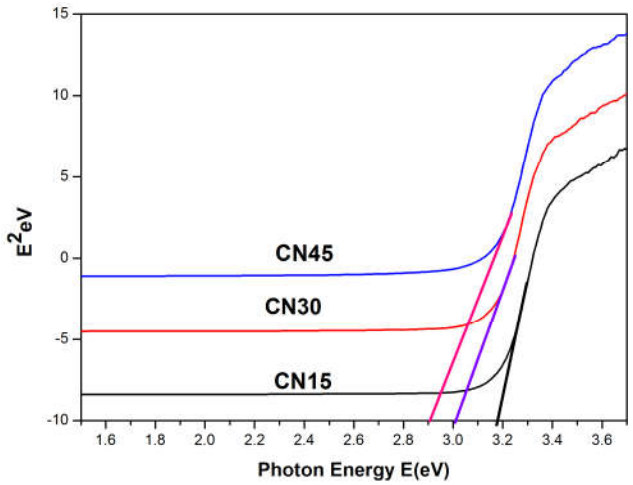


Fig.5 Energy band gap of three Chitosan–ZnO nanocomposite powders

The band gap energy of Chitosan-nanoZnO composites powder was measured from absorption spectra using the following equation:

$$(\alpha h\gamma)^2 = E_D(h\gamma - E_g) \qquad (2)$$

where **α** is the optical absorption coefficient, $h\gamma$ is the photon energy, E_g is the direct band gap and E_D is a constant. The Y intercept of the linear plot of $(\alpha h\gamma)^2$ versus hγ will give the value of direct band gap energy (E_g). As shown in Fig. 5 it is interesting to note that nano ZnO particles in all the three composites has the different values of band gap energy (Fig.5), which agrees very well with the reported values [11, 12].

3.7 SEM analysis

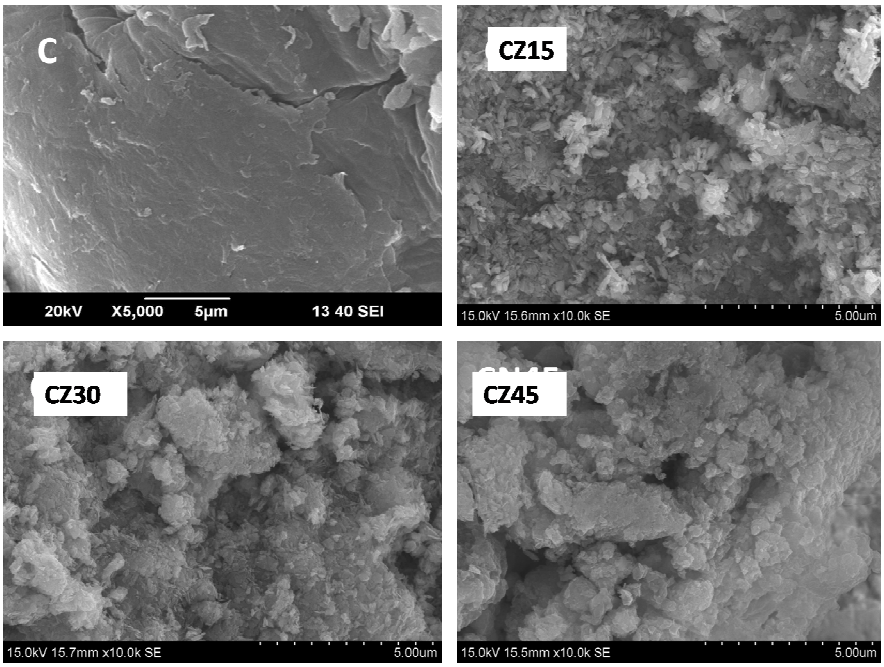


Fig-6.SEM images

The direct evidence of the immobilization of nano-ZnO particles in the Chitosan matrix was given by SEM images. Fig. 6 shows the surface morphology of pure Chitosan powder and its composites, CZ 45, CZ 30 and CZ 15. As shown in Fig 6a, the surface of the Chitosan was

found to be smooth. However, during the in situ formation of composites, ZnO particles accumulate and disperse over the surface of Chitosan and the smoothness of the surface get disappeared. SEM images shown in Figs. 6b-d clearly show the formation of nano ZnO particles, but the nature of distribution of particles is different when concentration of NaOH was changed. In CZ 45 and CZ15 agglomeration of rod like particles was observed whereas in CZ 30 ZnO nanorods are found with well defined boundaries.

3.8 Antimicrobial properties

The antibacterial properties of composites were measured by Agar well diffusion method. The test was done against Gram negative bacteria (E.coli) and Gram positive bacteria (S.aureus). Fig-7 shows the antimicrobial activity of pure Chitosan (C), CZ 45, CZ 30 and CZ 15 against E.coli and S.aureus respectively. The results of zone inhibition diameter of pure Chitosan and its composites against two pathogens E.coli and S.aureus were given in Table 1. Fig-8 shows the antimicrobial activity of composites against clinical pathogens.

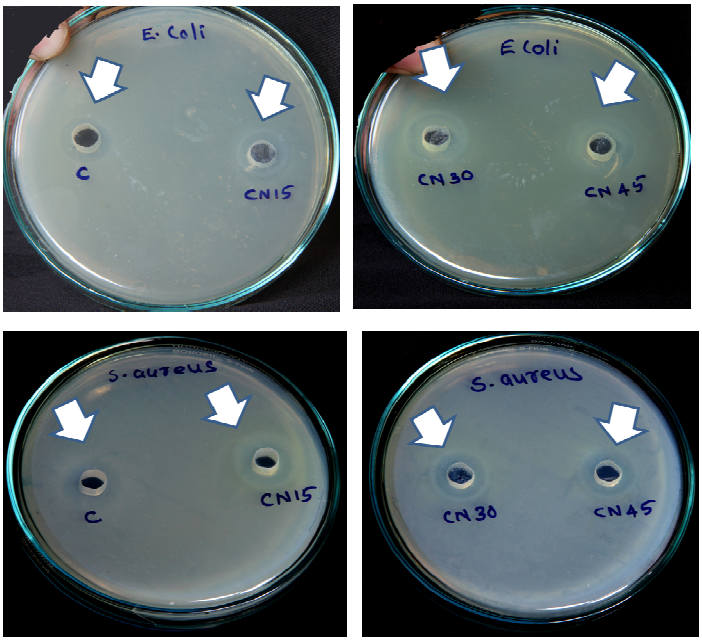


Fig-7. Images antimicrobial activity-well diffusion method.

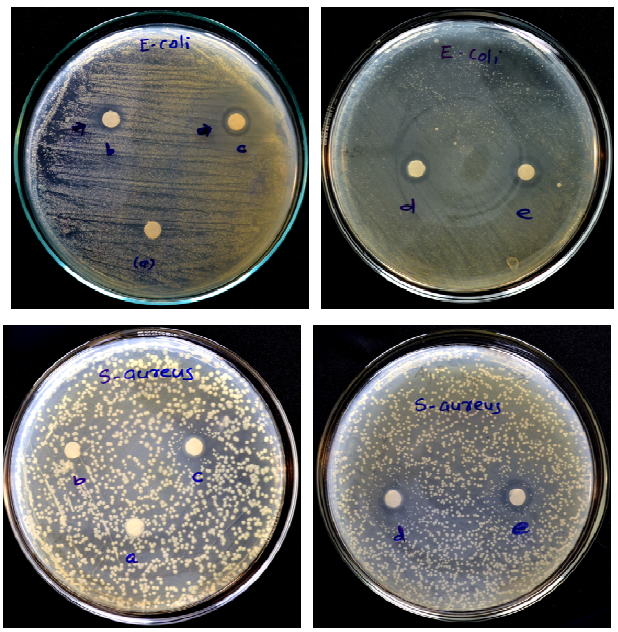


Fig-8. Images antimicrobial activity (disc diffusion method for clinical sample)

Table-1 Zone inhibition diameter well diffusion method

sample	Inhibition Zone diameter (mm)	
	E-coli	S-aureus
C	7	5
CZ 15	13	10
CZ30	12	11
CZ45	15	12

The data show that Chitosan ZnO composite has enhanced antimicrobial activity as compared to pure Chitosan. Inherent anti microbial activity of Chitosan was explained by its ability to attach the negatively charged cell membrane of microbes using its positively charged amino groups. The presence of nanoparticles in Chitosan matrix is expected to promote this columbic interaction with the outer cell wall of microorganisms and leads to increased antimicrobial activity [13]. It has been reported that nano ZnO particles can produce the ROS (reactive oxygen species), which also results in increased antimicrobial activity of the composites. The composites show higher activity towards E.coli, compared to S.aureus, which may be due to the presence of a thick layer of peptide glycans in the cell wall of S.aureus [14]. Figs 7 and 8 also show that the composites are active against standard and clinical samples as well, but low diameter observed in the disc diffusion method may be due to the lack of diffusion through the media.

4.1 Conclusions

Chitosan/ nano-ZnO composite powder was prepared through a simple and facile in situ method. The immobilization of nano ZnO on the Chitosan matrix was endorsed by FTIR and UV-Visible spectrum. The thermal stability of the composite was examined by TGA. The particle size and the structure of ZnO were determined by XRD analysis. The existence of particles and their sizes was determined from SEM images. Band gap energy of the composite was determined from UV-Visible spectral analysis. The antibacterial efficacy of composites against E.coli and S.aureus was tested using Zone inhibition method. It was proved that this composite has excellent antibacterial activity and could be used for antimicrobial applications.

5. References

[1] A.Farouk, S.Moussa, M.Ulbricht, T.Textor, ZnO Nanoparticles-Chitosan Composite as Antibacterial Finish for Textiles, Int. J. Carbohydr. Chem. 2012(2012), Article ID 693629

[2] A. Pawlak, M. Mucha, Thermogravimetric and FTIR studies of chitosan blends, Thermochim. Acta 396 (2003) 153–166.

[3] P. Bhadra , M.K. Mitra, G.C. Das, R. Dey, S. Mukherjee, Interaction of chitosan capped ZnO nanorods with Escherichia coli, Mater. Sci.Eng.C 31(2011) 929–937.

[4] A.P. Martinez-Camacho, M.O. Cortez-Rocha, J.M. Ezquerra-Brauer, A.Z. Graciano-Verdugo, F. Rodriguez-Felix, M.M. Castillo-Ortega, M.S. Yepiz-Gomez, M. Plascencia-Jatomea, Chitosan composite films: Thermal, structural, mechanical and antifungal properties, Carbohydr. Polym. 82 (2010) 305–315 .

[5] H. Zhu, R. Jiang, Y. Fu, Y. Guan, J. Yao, L. Xiao, G. Zeng, Effective photocatalytic decolorization of methyl orange utilizing TiO_2/ZnO/chitosan nanocomposite films under simulated solar irradiation, Desalination 286 (2012) 41–48 .

[6] A.El. Shafei, A. Abou-Okeil, ZnO-carboxymethyl Chitosan bionano-composite to impart antimicrobial and UV protection for cotton fabric, Carbohydr. Polym. 83 (2013)920-925.

[7] E.G. Goh, X. Xu, P.G. McCormick, Effect of particle size on the UV absorbance of zinc oxide nanoparticles, Scripta Materialia 78,79 (2014) 49–52.

[8] S.S. Kumar, P. Venkateswarlu, V.R. Rao, G.N. Rao, Synthesis, characterization and optical properties of zinc oxide nanoparticles, Int. J. Nano Lett. 3 (2013) 30.

[9] J. Ru, Z. Huayue, L. Xiaodong, X. Ling, Visible light photocatalytic decolourization of C. I. Acid Red 66 by Chitosan capped CdS composite nanoparticles, Chem. Eng. J. 152 (2009) 537–542

[10] N. Soltani, E. Saion, W.M.M. Yunus, M. Erfani, M. Navasery, G. Bahmanrokh, K. Rezaee, Enhancement of visible light photocatalytic activity of ZnS and CdS nanoparticles based on organic and inorganic coating, Appl. Surf. Sci. 290(2014) 440–447.

[11] J.D. Torres, E.A. Faria, J.R. SouzaDe, A.G .S. Prado, Preparation of photoactive Chitosan–niobium (V) oxide composites for dye degradation, J. Photochem. Photobio. A: 182 (2006) 202–206 .

[12] S. Anandhavelu, S. Thambidurai, Effect of zinc chloride and sodium hydroxide concentration on the optical property of chitosan–ZnO nanostructure prepared in chitin deacetylation, Mat. Chem. Phys. 131 (2011) 449–454.

[13] I. Leceta, P. Guerrero, I. Ibarburu, M.T. Duenas, K. de la Caba, Characterization and antimicrobial analysis of chitosan-based films, J. Food Eng. 116 (2013) 889–899

[14] P.T. Sudheesh Kumar, V.K Lakshmanan, T.V. Anilkumar, C. Ramya, P. Reshmi A.G. Unnikrishnan, S.V. Nair, R. Jayakumar, Flexible and Microporous Chitosan Hydrogel/Nano ZnO Composite Bandages for Wound Dressing: In Vitro and In Vivo Evaluation, ACS Appl. Mater. Interf. 4(5) (2012)2618−29.

CHAPTER 6

DEVELOPMENT OF FLEXIBLE CHITOSAN-NANO ZnO COMPOSITE POUCHES FOR EXTENDING SHELF LIFE OF RAW MEAT.

1.1 Introduction

In the previous chapter enhanced antimicrobial property of Chitosan-nano ZnO composite material was well established. The next objective was exploitation of this antimicrobial property to extend the shelf life of raw meat. As it is well known, meat is highly prone to decay and hence there is always a cry for an efficient packaging material for meat. Unfortunately traditional methods of preserving meat from the effect of microbial contamination comprising of drying, freezing, refrigerating, and adding antimicrobial agents or salts are neither cost effective nor efficient. Vulnerable growth of microbes on the meat surface stands as a major hurdle in its packaging. This microbial growth unanimously affects the shelf- life of meat. The popular methods of using antibacterial sprays or dips have limited benefits since the active substances are neutralized on contact or will diffuse rapidly into the bulk of food, away from the surface [1,2]. Hence the foremost criterion for an ideal smart packaging system is that it must have antimicrobial efficacy. Therefore the notion of packaging films incorporated with antimicrobial agents is a novel focal theme in the development of new generation smart packaging materials. Apart from this, it must have portability, optimal water sorption capacity, high mechanical strength, physico-chemical stability, microbial safety, environmentally benign and cost effective [3-9]. However, the idea of synthetic polymers with an antimicrobial agent is totally obsolete in the present scenario of environmental issues. Hence, the fabrication of packaging material using Chitosan-nano ZnO composite has great importance in the era of new generation packaging films [10-13].

Nano ZnO particles are emerging as a popular additive in the class of Chitosan based bionano composite films. According to U.S Food and Drug Administration protocol (21CFR182.8991), ZnO is considered as a generally

recognized safe (GRAS) material [14]. The remarkable stability and antibacterial activity of ZnO have under lined its status as a suitable blend for Chitosan matrix [15].Herein, we present the first report on Chitosn-ZnO based portable pouches that have the capacity to extend the shelf life of raw meat by complete inhibition of bacterial growth. In this study, Chitosan-ZnO nano composite film was synthesized by a simple one pot procedure and was adequately characterized using XRD, FTIR, SEM etc. The influence of noted factors such as water sorption capacity, water solubility, and thermal stability were investigated in detail. The antimicrobial test of the synthesized films revealed their efficiency in controlling bacterial growth. We believe that the pouches developed can be conveniently employed as a packaging material for raw meat which creates significant advances in packaging industry.

2. Experimental

2.1. Materials

Chitosan with 85 percent degree of deacetylation was purchased from Sigma Aldrich Co. Ltd (USA).Acetic acid, Sodium hydroxide and Zinc acetate dihydrate were obtained from Merck (Germany).Mineral salt broth and Nutrient agar were purchased from Himedia Chemicals (Mumbai, India). Two bacterial strains Escherichia coli (E-Coli, MTCC737) and Staphylococcus Aureus (S-Aureus, MTCC 1687) were cultured in the UniBiosys Biotech Research Lab, Cochin. Deionized water was used to prepare all solutions. All the chemicals were analytical grade and used without further purification

2.2. Preparation of films

Chitosan nano ZnO composite films were prepared by solution casting technology. 2g of Chitosan flakes were dissolved in 100mL of 2% (v/v) aqueous acetic acid using ultra sonication for 2h at room temperature. Subsequently, 2g of zinc acetate dihydrate was added into this solution and

the reaction mixture was again sonicated for 1h to form a homogeneous dispersion. To obtain even films, 25mL of the viscous mixture were casted in to a circular glass dish and was dried at room temperature. Glass plates with dried thin films were immersed in 0.2molL^{-1} sodium hydroxide solution for wet phase separation. After coagulation, the solidified films were kept in a constant temperature oven kept at 60±0.2°C for 4h. The resulting nanocomposite films (C-2) were then washed with methanol for three times and was dried. The scheme of preparation of film was illustrated in Figure 1.

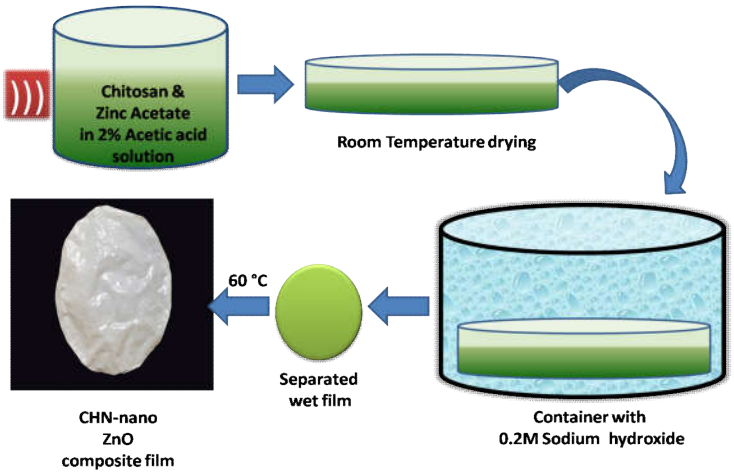


Fig.1 Schematic illustration of synthesis of composite films

Three more nanocomposite films were prepared by adopting the same procedure with different amount of zinc acetate (1.5g, 1.0g and 0.5g); named as C-1.5, C-1 and C-0.5. A pure Chitosan film was prepared without zinc acetate and named as C.

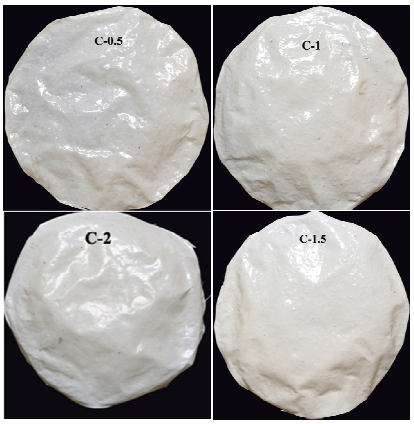


Fig.2. Photographs of composite films

2.3 Characterization of films

FT-IR Spectra of the films were calibrated using an Attenuated Total Reflection (ATR) Technique (Model, Perkin Elmer Inc.USA), Films were cut into a disc shape (5mm x 5mm) and placed gently on diamond ATR crystal. Prior to the analysis, the film was locked with the pressure arm to maintain an ample contact between the sample and the crystal. The absorption was measured between 400 and 4000cm^{-1}.X-ray diffraction (XRD) patterns of the samples were obtained in the scanning range of 20–75° by a X-ray diffractometer (Model: Rigaku Minifex 600 diffractometer) with Cu Kα radiation (λ = 0.15406nm). Surface morphology of samples was observed by scanning electron microscopy (SEM) (Model: Hitachi SU-6600 FESEM); during the measurement samples were sputtered with a layer of gold to prevent the charging effect. Dielectric properties were measured at room temperature using an impedance analyzer. The impedance was determined using the fully automated instrument (HOIKY 3532-50 LCR HI-Tester). The dielectric constant and AC conductivity were calculated from the measured data using the thickness of the film.

2.4 Thermal stability

Thermal stability of films was investigated using Thermo gravimetric analysis (TGA) (Model: TGA/DTA851e) and Differential Scanning Calorimetry. During TGA, each sample was heated from 25 to 800 °C at a scanning rate of 2°C min^{-1} under a dynamic nitrogen atmosphere. Differential Scanning Calorimetry (Perkin Elmer Model DSC 7) was performed from room temperature to 400^0C at a scanning rate of 10°C min^{-1} under dynamic nitrogen atmosphere.

2.5 Mechanical properties

Mechanical properties of films were evaluated by Instron Universal Testing Machine (Model-3345), at a test speed of 1.0 mm/min at room temperature (23-27^0C). Specimens were taken in dumbbell shapes for the analysis. The values of tensile strength (TS) and percentage of elongation at break (%E) were reported as the average of three replicates.

2.6 Water sorption studies of nanocomposite films

Water sorption study gives a lucid idea regarding the ability of a packing material to remove the water molecules from the surface of packed food. Moreover this ability of films is unanimously related to its efficiency to prevent microbial contamination on the surface of food. The experiment was carried out at room temperature. Prior to the measurement, the specimens were dried in vacuum desiccators over anhydrous $CaCl_2$ at room temperature for about 24hours.The experiment was done by immersing the previously weighed (2cm×2cm) films in double distilled water (30mL), removing at regular intervals (the excess water was wiped off from the surface) and weighing until equilibrium sorption was achieved. The result was expressed as moles of water adsorbed according to the Eq. (1).

$$Q_t = [(W_t / M_s)/W_p] \times 100 \quad (1)$$

Where W_t is the mass of the solvent adsorbed at a given time, M_s is the molar mass of water; W_p is the initial weight of films. Sorption curves were obtained by plotting Q_t values as a function of square root of time [16].

2.7 Measurement of Water solubility

The solubility of the films in water was determined as follows. Rectangular pieces (7.5 × 15 mm) were cut from each film and dried under vacuum in an oven at 105 °C for 24 h sufficient time to achieve constant weight and the initial dry weight was measured. Subsequently, the films were immersed in 50mL of distilled water at room temperature (23-27 ^{0}C) with gentle agitation. After 24h of immersion, the specimens were recovered from distilled water and dried to constant weight at 105 °C. The solubility was expressed using Eq. (2).

$$\text{Solubility (\%)} = [(M_i - M_f)] / (M_i) \times 100 \quad (2)$$

Where M_i and M_f are the initial dry weight and final mass of films respectively [17].

2.8 Water vapour transmission rate

The water vapour transmission rate (WVTR) of films was analysed according to ASTM E96-95 method with required modifications. Circular Plexiglass cups with inner diameter 35 mm and height 45 mm were chosen for the analysis. 25mL of distilled water was taken in each test cup and cup mouth was sealed perfectly by films having uniform thickness. The distance between water level and the film was 20 mm. The effective area of water transmission through the film was 961.26 mm^2. All the assemblies were placed in desiccators at 25^{0}C containing small lumps of anhydrous calcium

chloride. The amount of water vapours transferred through the films was measured as the mass loss of the test cup at a time interval of 1h.WVTR (Water vapour transmission rate, $gh^{-1}m^{-2}$) was obtained by dividing the slope of the graph (weight loss of test cup verses time) with the area of the film (m^2). The experiment was repeated three times and average of values was reported.

2.9 Antimicrobial property

The antimicrobial activity of films was evaluated against two different pathogens; Gram negative bacteria Escherichia Coli (E-Coli, MTCC 1687) and Gram positive bacteria Staphylococcus Aureus (S-Aureus, MTCC 737) by Colony Forming Units (CFU/mL) method. The test organisms were grown in a Nutrient Agar Broth media at 35-37^{0}C for 24h, a period of time sufficient for the growth of microorganisms. 1.0 mL of culture was diluted with 99mL of the same broth solution to meet required bacterial population.10.0mL of this diluted media was taken separately in five different dishes and the previously autoclaved rectangular pieces of films (20mm×20mm) were introduced separately in to the dish. The dishes were then shaken gently at 50rpm for 24h at room temperature. One millilitre (1mL) of culture was taken out and diluted serially nine times to count the number of colonies. Three replicate experiments were conducted and the result was reported as the average of three sets of values.

2.10 Antioxidant properties

The ability of films to prevent oxidation of packed food was evaluated by the DPPH radical scavenging method. The procedure was adopted from previous reports with required modification. Film specimens (25mm X 25mm) were introduced into separate beaker containing 50ml distilled water. 1mL sample solution was taken out in different time intervals and mixed with previously

prepared 4ml 150ηM DPPH solution in methanol. The mixture was kept in the dark for half an hour sufficient time to react with DPPH radicals. The absorbance of the mixture was measured at 517nm using UV-Visible spectrometer. The free radical scavenging ability of film specimens was determined by the following equations.

DPPH radical scavenging activity (%) = $[(Ac- A_s) / A_c] \times 100$ (5)

A_S is the absorbance of test sample

A_c is the absorbance of control solution (4ml DPPH plus 1ml distilled water)

Statistical analysis

All experiments were repeated in three times and average values with standard errors were reported. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with Tukey's test using software originPro8 (Origin Lab corporation). A statistical difference at $p < 0.05$ was considered to be significant.

3. 1Results and discussion

3.2 FT-IR

Figure 3a represents the FT-IR spectra of pure Chitosan. It exhibited a broad band at 3322cm^{-1} which can be indexed to the stretching vibrations of pendant groups like -NH_2 and -OH on the Chitosan. The band at 2872cm^{-1} corresponds to the asymmetric stretching vibrations of -CH_2- groups of Chitosan chain. Stretching vibration of C=O and C-N groups in Chitosan is responsible for the bands at 1564 and 1370cm^{-1}. The band obtained at 1014cm^{-1} arises due to the stretching vibrations of –C-O-C- linkages [18]. It is seen the spectra of pure Chitosan showed the low intensity bands which

can be attributed to insufficient contact between the film and the crystal surface [19].

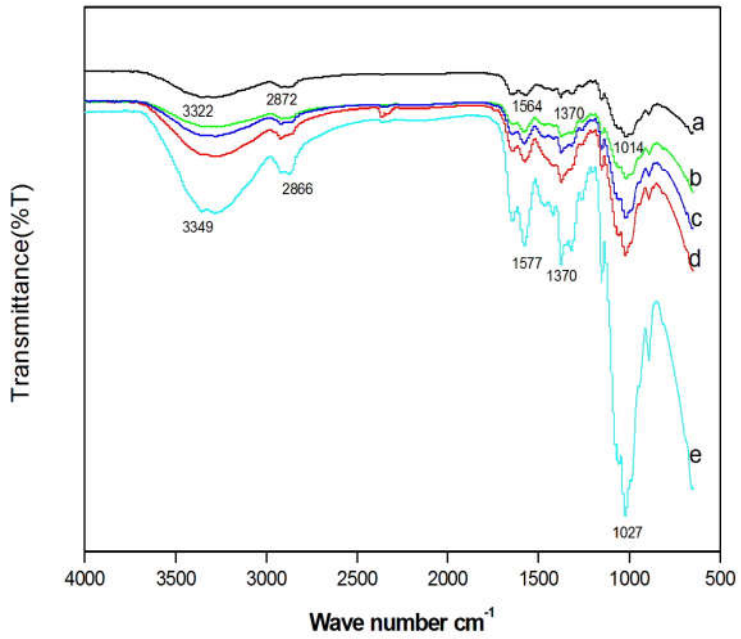


Fig. 3. The IR spectra of (a) pure Chitosan film (b) C-0.5 film (c) C-1 film (d) C-1.5film(e)C-2 film

The IR spectra of composite films are shown in Fig. 3b-e.The incorporation of ZnO has greatly modified the IR spectra. It is evident from the spectra that the width of the bands corresponding to $–NH_2$ and –OH groups is inversely related to the amount of ZnO particles in the Chitosan matrix. This can be accounted by the reduction of hydrogen bonds between $–NH_2$ and –OH groups with the incorporation of ZnO particles onto the Chitosan matrix [20]. As compared to IR spectra of pure Chitosan film, considerable shift in the position of bands towards lower and higher wave number region is clearly

visible in the IR spectra of composites (-NH_2 & -OH from 3322 to 3355; -CH_2 from 2872 to 2866; C=O from 1564 to 1577; -C-O-C- from 1014 to1027 cm^{-1}).This confirms stronger interaction between the functional groups and ZnO particles. The incremental increase of intensities of IR bands at; 3349, 2866, 1577, 1370, 1027 cm^{-1}, is the indication of formation of coordination bonds between various groups of Chitosan and Zn^{2+} ions of nanoparticles, hence it was concluded that ZnO particles would be located in between Chitosan chains connecting through the functional groups Fig-4 [21].

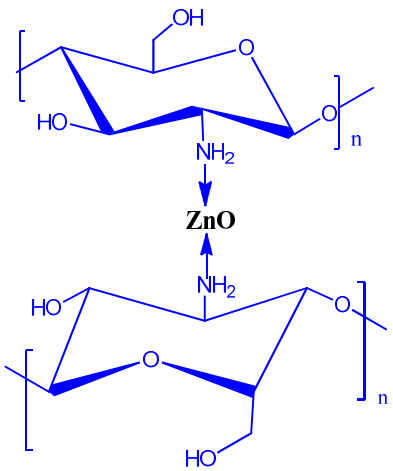


Fig.4 Illustration of linkages nanoparticles with Chitosan

3.3 XRD results.

The XRD patterns of pure Chitosan and composite films are depicted in Fig 4. The diffraction peaks observed in the composite films at various 2θ values 33, 36, 40.35, 54.3, 61 and 71.8^0 can be indexed to the position of the planes of ZnO particles (002), (100), (101), (110), (103) and (201) respectively. All these data were in well agreement with the data base of ZnO particles (JCPDS No.1436-51) [22]. The average particle size of ZnO crystallites was calculated using Sherrer equation, each composite films comprised of different sized nanoparticles i.e. C-0.5, C-1, C-1.5 and C-2 have particle size

6.5nm, 28.3nm, 9.66nm and 7.73nm respectively. The XRD pattern of pure Chitosan exhibited a sharp peak at 20^0, which can be correlated to the semicrystalline nature of Chitosan. The semicrystalline property of Chitosan arises from the compact arrangement of polymer molecules in the presence of strong inter molecular hydrogen bonds[23].The XRD patterns of composite films showed an interesting linear relationship between the intensities of major peaks and amount of ZnO nanoparticles. Moreover there was a notable shift in the positions of peaks. The variation of intensities and peak positions is due to the differences in size of incorporated ZnO nanoparticles and also due to the lattice strain developed in the composite because of the presence of nanoparticles [24] . Hence XRD data underlines the dispersion of crystalline ZnO nanoparticles on the Chitosan matrix

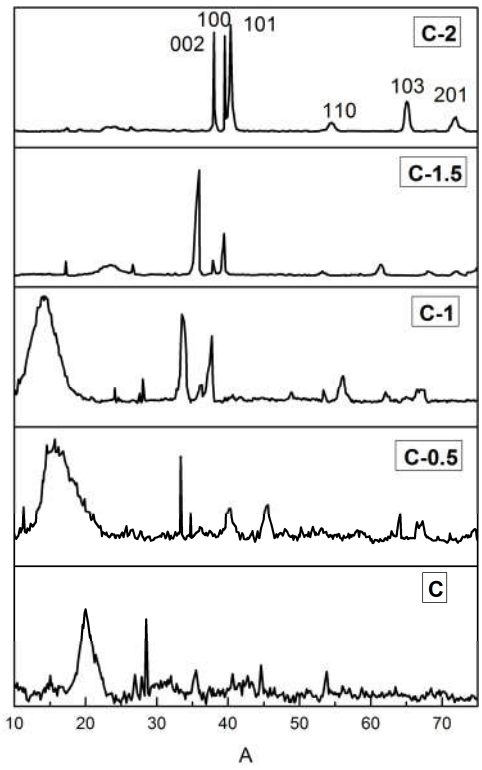


Fig.5. XRD patterns of films

3.4 SEM analysis

The surface morphology of C, C-0.5, C-1, C-1.5 and C-2 films was evaluated using scanning electron microscopy. In the SEM photographs, presence of nano ZnO particles on the polymer surface was spotted as white coloured images. As showed in Fig. 6a, the surface of pure Chitosan film is rather smooth, compact and homogenous, whereas in composite films (Figs 6b-d) the incorporation of nanoparticles modified the Chitosan surface to coarse and heterogeneous. In the SEM images of composite films, agglomeration of nanoparticles was observed and the mode of distribution of nanoparticles was different in different films. The image of C-1film exhibited the distribution of equal sized clusters of particles throughout the surface but high degree of agglomeration of nanoparticles was observed in C-2 films. These nanoparticles were strongly adhered to the Chitosan matrix and modified physical and chemical features of Chitosan.

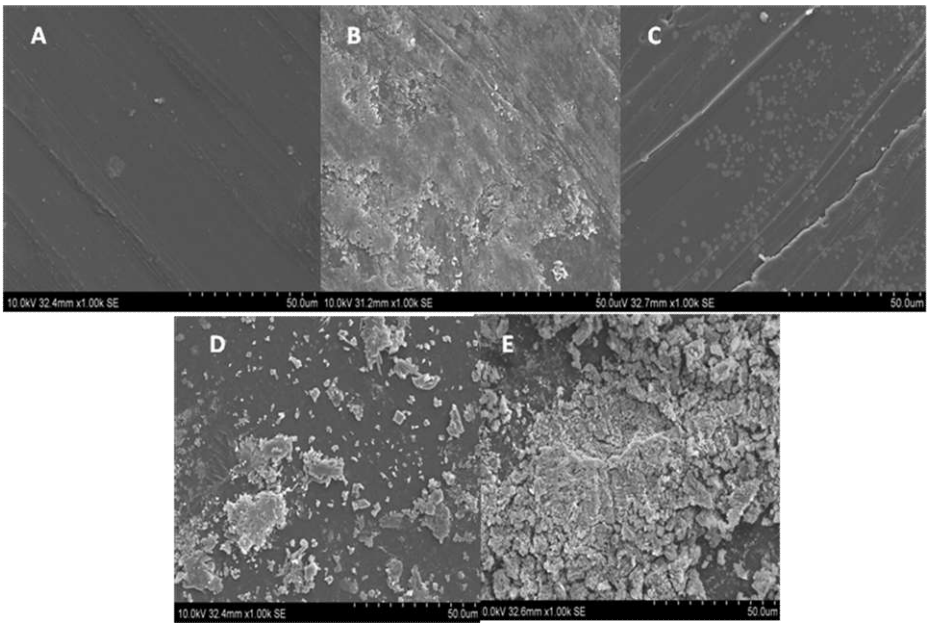


Fig. 6 SEM images of (A) pure Chitosan film (B) C-0.5 film (C) C-1 film (D) C-1.5 film (E) C-2 film

3.5 Dielectric and Conductivity measurements

Dielectric property is the very less exploited feature of Chitosan nano metal oxide composite films. Here we adopted this characterization technique to unravel the true existence of semiconductor nano ZnO on the Chitosan matrix. The dielectric property of flexible Chitosan composite materials is technologically inspiring due to many reasons such as cost effectiveness, biocompatibility and processability. It can also find applications in new generation actuators, sensors, fuel cells, capacitors and self regulating heaters. Flexible biocompatible films with tunable dielectric property can act as artificial muscle in future medical treatment and expected to have a major role in the realization of 'smart skins' in medical field [25–28]. The dielectric property of Chitosan composite materials depends on various complex factors and it is unexploited. There are only a few reports on dielectric properties of certain synthetic polymer composites[26].

The percolation theory is commonly used to explain the dielectric property of polymer composites. According to this theory, gradual addition of metal oxides onto the polymer matrix increases its dielectric values. At a critical amount, a swift in dielectric value will be shown and it is designated as percolation threshold (Pc). The amount of a substance corresponding to this sharp change in dielectric value is considered as percolation value[25]. In the present case, all composite films exhibited higher dielectric values compared to bare Chitosan film (Fig-7). The enhancement of dielectric properties of composite films is assigned to development of different types of polarizations in composite films. The interfaces of composite materials contain large numbers of defects, which result unequal charge distributions and pave the way to space charge polarizations when an electric field is applied. When the amount of ZnO particles increases, space polarization also increases in the interface of composite materials leading to enhancement of dielectric values.

The continuous decrease of dielectric constant with an increase in frequency is quite common for all dielectric substances. It is well known that, when frequency of electric field is increased the mechanism of polarization cannot be able to follow the change in the electric field and therefore, the contribution of polarization to the dielectric constant will be lowered[28].

The conductivity values of composite films were compared to that of pure Chitosan film (Fig-8). The C-2 film exhibited highest conductivity, whereas pure Chitosan film displayed lowest values of conductivities in all frequencies. The variation of conductivities of bare Chitosan film at different frequencies was negligible but significant changes are shown by the composite films. The smart conductivity exhibited by C-2 films at all frequencies justifies the development of smooth conduction pathway in the presence of nanoparticles. In C2,as showed in its SEM images, a compact and connected array of semiconducting ZnO particles are framed in the polymer matrix leading to continuous carrier way or tunneling for charges in the composite film[29–31].

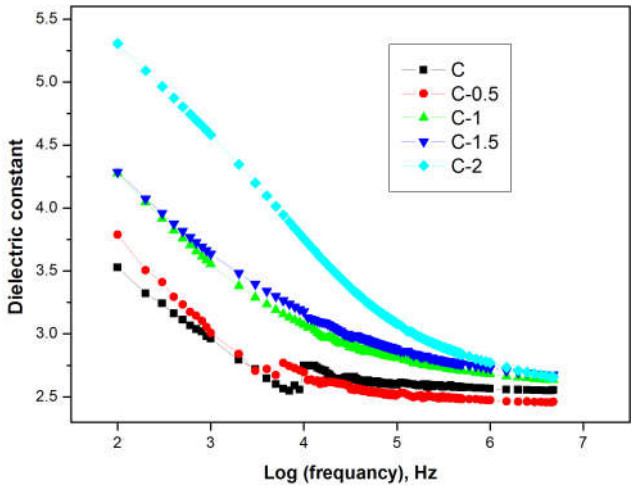


Fig 7. Variation of dielectric constant with frequency

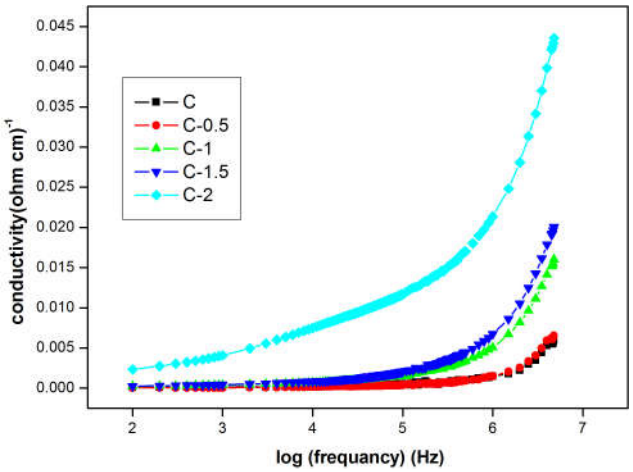


Fig 8. Variation of conductivity with frequency

3.6 Thermal properties

3.6.1 Thermo gravimetric analysis.

Thermal stability of films was evaluated by thermo gravimetric analysis (TGA) and results is shown in fig-9.Thermal properties of pure Chitosan films were already reported and well documented[32]. As showed in the figure, all films exhibited the same thermal decomposition trend. There was no any weight loss below the 100^0C, and the slope between 100^0C and 223^0C is attributed to the loss of water molecules from the polymer films. In the case of bare Chitosan film, the significant weight loss appeared at 223^0C is correlated to the thermal degradation of polymer chains. Whereas the onset of thermal degradation of composite films is observed at higher temperature 240^0C indicating enhanced thermal stability of composite films. The residual mass percentage of Chitosan and composite films was determined at 486^0C and recorded as 26%, for Chitosan(C) and 36% for (C-0.5). Three other films (C-1, C-1.5 and C-2) recorded higher residual mass percentage (49%) at the same temperature. In contrast to composite films, pure Chitosan film didn't show any plateau above 500^0C probably due to its complete thermal

degradation above this temperature. All these data vindicate enhanced thermal stability of composite films.

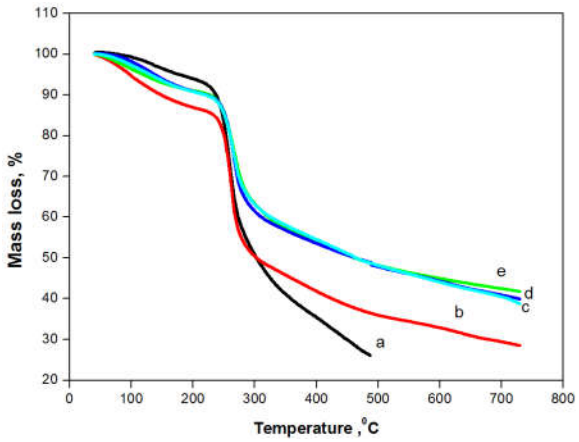


Fig-9.TGA curves of (a) pure Chitosan film (b) C-0.5 film (c) C-1 film (d) C-1.5 film (e) C-2 film

3.6.2 Differential Scanning Calorimetric analysis

The thermal stability and glass transition temperature values of composite films were confirmed by DSC studies. Here we have compared the thermal behavior of our novel composite film with existing popular synthetic packaging polymers. It was well evident from the DSC data that, all the composite films exhibited enhanced thermal stability and higher glass transition temperature over common synthetic polymers [33].The DSC curves of bare Chitosan and composite films exhibited two distinct peaks Fig-10. The first endothermic peak appeared in the lower temperature region (323K - 383K) corresponding to the dehydration of water molecules from the polymer lattice. Chitosan would adsorb the water molecules due to the presence of hydrophilic groups in the polymer, these water molecules being loosely bound to polymer lattice are removed by heating in the temperature range 323K - 383K. Generally water molecules present in the biopolymer matrix can be classified into two types, namely “freezing and non freezing water”.

The former type of water molecules could be removed easily by heating and would exhibit the bulk property of water molecules [34]. The shifts in the peak values corresponding to the dehydration (C at 84.31,C-0.5 at 79.46 , C-1 at 81.20 , C-1.5 at 76.82 and C-2 at 77.50 °C) can be ascribed to the amount of water molecules in the matrix of different films[35]. The second major thermal behavior was registered as distinct exothermic peaks, corresponding to the onset of thermal degradation of polymers and the glass transition temperature T_g values. All the composite films exhibited significantly higher T_g values over the naïve Chitosan film (C - 272.73,C-0.5 - 282.23 , C-1 - 279.98 , C-1.5 - 279.31and C-2 - 277.67 °C[36]. These noteworthy differences in T_g values hints the remarkable changes in the structural features of the polymer matrix due to the presence ZnO particles. Moreover enhancement of T_g values portrays the presence of ZnO nanoparticles in the Chitosan matrix, paves the way for superior thermal stability and mechanical property of the composite films [37].

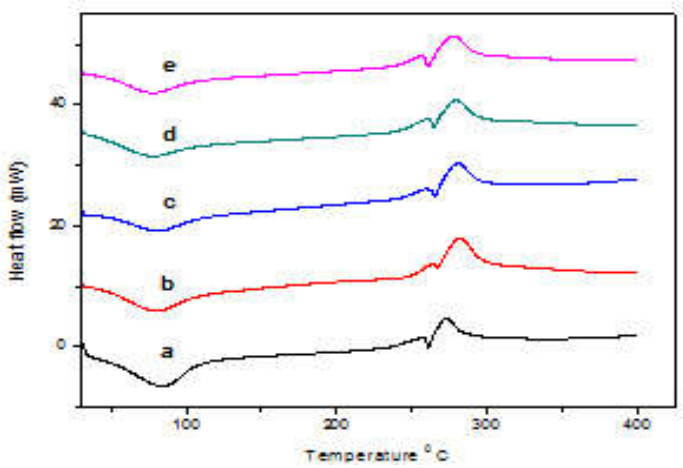


Fig.10. DSC curves of (a) Bare Chitosan film (b) C-0.5 film (c) C-1 film (d) C-1.5 film (e) C-2 film

3.7 Mechanical properties

The wide range of applicability of any film as a packaging material is significantly pertained to its mechanical properties such as tensile strength and elongation at break. According to previous reports, mechanical properties of Chitosan based films are affected by different factors such as degrees of deacetylation, molecular weight, amount of Chitosan and fillers[38].

The fashion of change of tensile strength (TS) and elongation at break (EB) is given in (Table-1). Bare Chitosan film exhibited TS and EB values 12.84±0.62 MPa and 30.42±0.95 % respectively. The variation of tensile strength of composite films with the amount of ZnO particles is quite different. The values of TS increased initially, attained a maximum value at C-1 film and set to decrease. Yet all composite films maintained higher TS values as compared to bare Chitosan films. The enhancement of TS of composite films is explained as follows. When nano ZnO particles are incorporated, they exist in between polymer chains and an intermolecular cross linking effect is generated. This observation is inconsistent with FTIR and SEM analysis.

In the case of EB, when nano ZnO particles is incorporated a reverse trend was observed except in C-1.The EB values sharply lowered from 30.42±0.95% in C to 13.36±0.69 % in C-2. But C-1 has showcased anomalously higher EB value 32.44±0.52 indicating its distinctive mechanical feature. The characteristic mechanical property of C-1 was again justified by its unique WVTR values and a similar trend also observed in Chitosan/ lignin composite film[39].

Table-1. Variation of Tensile strength and elongation at break

Sample	Tensile strength (MPa)	Elongation at break (%)
C	12.84 ± 0.62^{a}	30.42 ± 0.95^{a}
C-0.5	26.1 ± 0.96^{b}	22.62 ± 0.577^{b}
C-1	41.73 ± 0.48^{c}	32.44 ± 0.52^{c}
C-1.5	36.21 ± 0.28^{d}	14.71 ± 0.37^{d}
C-2	28.55 ± 1.28^{e}	13.36 ± 0.69^{e}

Each value indicates means of three set of samples at identical conditions

Superscript letters (a–e) within the column indicate significant differences between mean values ($P < 0.05$)

3.8 Water adsorption studies

Water molecules at the surface of meat are very sensitive for microbial contamination. Hence packing materials which can quickly adsorb water molecules have a significant role in extending the shelf life of raw meat [40].The adsorption capacity of all the films in aqueous media was examined and the results are shown in Fig. 11. Water adsorption ability of Chitosan is due to the presence of large numbers of hydrophilic groups in the polymer chain[41]. Intake of water molecules increased initially reached the maximum limit and set down to a constant value indicating the equilibrium state of sorption. In contrast to pure Chitosan films, all composite films exhibited higher adsorption capacity and is linearly proportional to the amount of ZnO particles.

The incorporation of ZnO nanoparticles onto the Chitosan matrix increases the heterogeneity of the composite films. The formation of cavities as a result of introduction of ZnO particles in the films paves the way for more adsorption of water molecules. Another notable feature exhibited by all kinds

of films was that the maximum limit of sorption was achieved as soon as the contact with water was established.

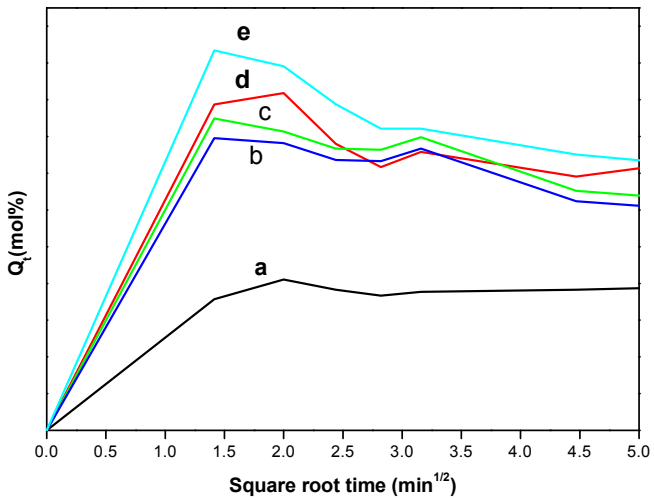


Fig.11. Water sorption ability of (a) pure Chitosan film (b) C-0.5 film (c) C-1 film (d) C-1.5 film (e) C-2 film

3.9 Solubility of films

Solubility is the index of the stability of packaging films in the presence of solvent molecules present on the food surface. If the packaging film is soluble, it will detoriate the quality of food [40]. Since we aim to fabricate packaging pouches for raw meat which possess a large amount of water molecules on its surface, we examined the solubility of our packaging film in distilled water at room temperature and the result is expressed as percentage of solubility. The trend of solubility of films with respect to the amount of ZnO particles is shown in Table-2. When the amount of ZnO increases solubility decreases but not significantly different ($P>0.5$) as there is a

decrease in the availability of hydrophilic groups for interaction with solvent molecules, since ZnO particles cross links Chitosan chains connecting through their hydrophilic functional groups [42].

Table-2. Comparison of solubility of films

Sample (23 -27 ^{0}C)	Solubility (%)
C	5.65 ± 0.5^a
C-0.5	3.5 ± 0.53^b
C-1	2.6 ± 0.63^a
C-1.5	2.1 ± 0.76^c
C-2	1.5 ± 0.05^a

Each value indicates means of three set of experiments

Same superscript letter indicates no significant differences between mean values ($P > 0.05$)

3.10 Water vapour transmission rate

Water vapour transmission rate (WVTR) is the measure of ease of moistures to penetrate and cross any film, which is an important property to be accounted for packaging applications. Excess water molecules inside the pack will lead to undesirable changes in foods, whereas moderate amount of water molecules is tolerable to transfer active molecules from the packaging films to the food surface. Hence, depending upon food and mechanisms involved in enhancement of its shelf life, the specificity of WVTR of a packaging film will be changed. The values of WVTR are given in the (Table-3). As showed in the result, all composite films exhibited lower WVTR values compared to bare Chitosan film except C-1. It is found that WVTR of films was inversely related to the amount of ZnO particles. When nano ZnO particles are incorporated in Chitosan matrix, segmental motion and porosity in the

polymer surface are reduced leading to low water vapour transmission. The exceptionality of C-1 film is attributed to its unique feature as revealed by mechanical property analysis.

Table-3. Comparison of WVTR of films

Sample	WVTR (g/hm^2)
C	22.2 ± 0.67^{a}
C-0.5	21.7 ± 0.41^{b}
C-1	22.53 ± 0.31^{c}
C-1.5	21.63 ± 0.11^{d}
C-2	20.57 ± 0.17^{e}

Each value indicates means of three set of samples at identical conditions

Superscript letters (a–e) within the column indicate significant differences between mean values ($P < 0.05$)

3.11 Anti microbial property

Antimicrobial activity of bare Chitosan and against S.aureus and E.coli was investigated by colony counting method. The results of the study are shown in Table-4.Control Chitosan didn't show any appreciable antimicrobial activity towards both strain when compared to composite films. The investigation also revealed that the antimicrobial activity is linearly related to the amount of nano ZnO particles in the composite films. Hence C-2 is the most efficient composite film against both E-coli and S-aureus and was taken the optimized film for packaging of raw meat. Literature survey hints to the

fact that the commendable antimicrobial activity of the composite relays on the presence of ZnO nanoparticles in the matrix. It is believed that the ZnO nanoparticles release reactive oxygen species (ROS). The ROS together with Zn^{2+} ions attack the negatively charged cell wall and will lead to leakage and ultimately in death of bacteria [43].Moreover, the addition of ZnO, enhances the positive charge on the amino group of Chitosan. This results in easier interaction with anionic components on cell wall and composite films Fig-12. The decreased activity of all kinds composites towards S.aureus bacteria is due to the presence of a thick layer of peptide glycans in its cell wall [44,45].

Table-4. Plate counts (cfu/g) values of films against two bacteria (E-Coli, MTCC 1687) and (S-Aureus, MTCC 737).

Sl.No	Sample	E. coli (cfu/g)	S. aureus (cfu/g)
1	C	$9.5 \pm 0.336^{a} \times 10^{9}$	$10.4 \pm 0.303^{a} \times 10^{9}$
2	C-0.5	$5 \pm 0.421^{b} \times 10^{9}$	$6.3 \pm 0.378^{b} \times 10^{9}$
3	C-1	$4 \pm 0.298^{c} \times 10^{9}$	$5 \pm 0.301^{c} \times 10^{9}$
4	C-1.5	$2.4 \pm 0.315^{a} \times 10^{9}$	$3.5 \pm 0.273^{a} \times 10^{9}$
5	C-2	$2.5 \pm 0.421^{d,e} \times 10^{7}$	$9 \pm 0.367^{d,e} \times 10^{7}$

Each value indicates means of three set of samples at identical conditions

Same superscript letter indicates no significant differences between mean values ($P > 0.05$)

[e] C-2 sample was serially diluted for seven times only due to its high antimicrobial activity.

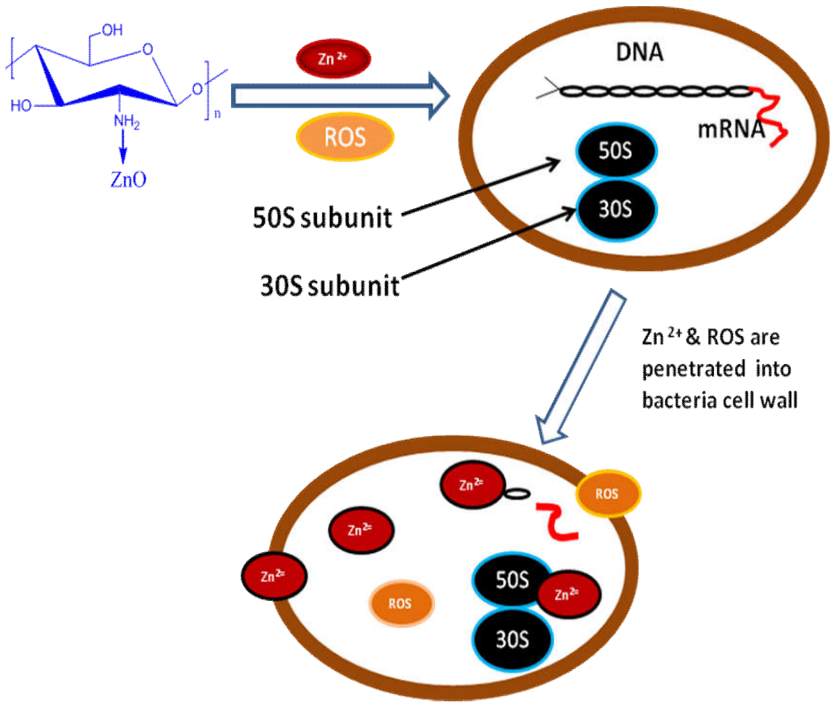


Fig.12.Schematic illustration of antibacterial activity of composite film

3.12 Antioxidant properties

Table-5 Scavenging activity of films

Sample	%of Scavenging activity	
	After one day	After three days
C	5.76	10.23
C-0.5	2.32	3.02
C-1	1.39	10.04
C-1.5	2.79	8.37
C-2	1.86	4.65

As it is shown in table-5, composite films have a little antioxidant activity in aqueous media. This is attributed to low solubility of Chitosan at the neutral

p^H. When ZnO was incorporated solubility is further decreased, hence Antioxidant property is again declined. The limited antioxidant activity of Chitosan was justified as the reaction of radical species with hydroxyl group at the C-6 position and amino group at C-2 position of Chitosan chain. These functional groups can transfer hydrogen to the unstable radical species and will form macromolecular radicals. Since oxidation of meat depends upon oxygen permeability of packaging material, there for composite material with excellent gas barrier property can prevent the lipid oxidation of meat.

4. 1Packaging applications

The efficiency of the optimized C-2 film as a packaging material for raw meat was examined. Prior to the analysis, the C-2 films were kept in air tight poly ethylene bags for six months. C-2 films were stitched into flexible pouch like bags using cotton yarn by an in-house weaving machine Fig.13.

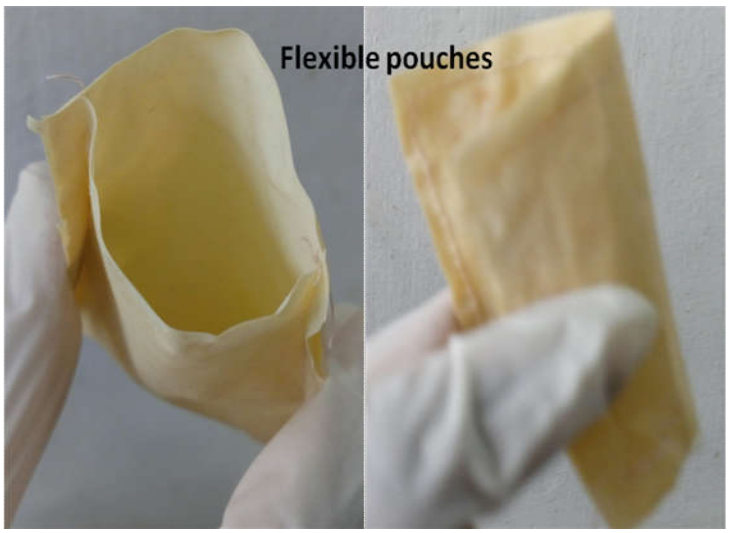


Fig.13. Image of flexible Anti microbial C-2 pouch

The shelf life efficiency of the composite bag was compared with synthetic plastic bag of low density polythene, which is popularly used to pack, store and

transport meat and related items. The composite bags and plastic bags were sterilized by autoclaving at higher temperature for 15 minutes. A meat sample were collected from the local market and was washed well with distilled water to remove blood stains; equal amount of meat was placed in separate sets of bags, each set consisting of three duplicates of C-2 bag namely A1 SET and polythene bag namely B1 SET and incubated at 4°C for 4 days. Following the 4th day of incubation, aerobic plate count was performed using the procedure described below. This protocol was repeated for A2 SET &B2 SET and incubated for 5 days, followed for A3 SET &B3 SET and incubated for 6 days Fig.14.

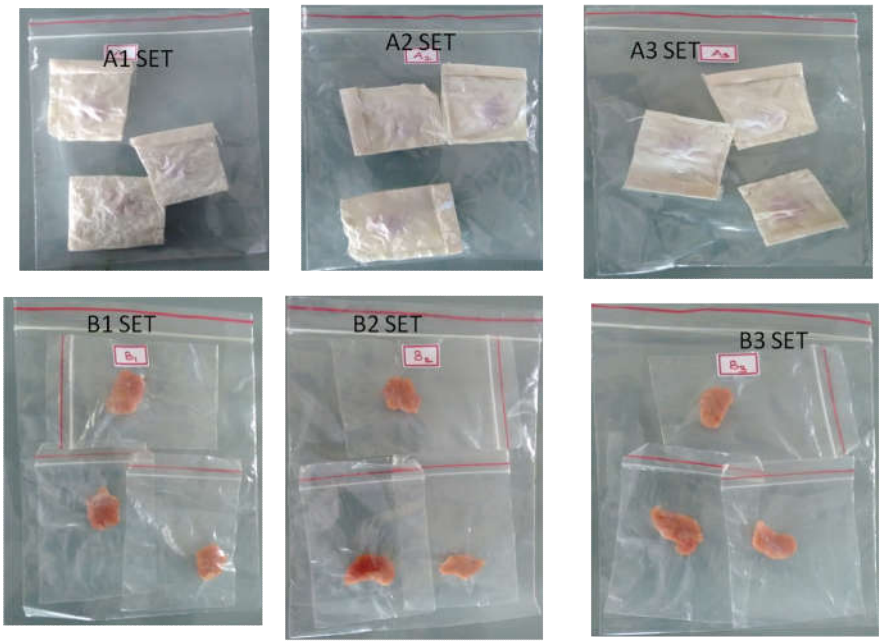


Fig.10.Image of packed meat samples in C-2 pouches (A1, A2&A3) and plastic bags (B1,B2 &B3)

4.2 Standard plate count procedure

After four days of incubation, sample sets A1&B1 were taken out to carry out the plate count procedure. One gram1g of homogenized meat sample from each packet was separately transferred to different test tubes containing 9ml sterile saline blank (nutrient agar in distilled water) to get 10^{-1} dilution. This 10^{-1} dilution was then shaken for a few minutes to distribute the bacteria and break up any clumps. Immediately after proper dilution, 1ml of the mixture was aseptically transferred to a second 9ml saline blank to get 10^{-2} dilution. This serial dilution was repeated until to get 10^{-7} dilution. Then 0.1 ml of the solution from the finally diluted tube was transferred to a Petri plate containing nutrient agar media and incubated at 37°C for 24 hours, a sufficient time for bacterial growth. The enumeration of microbes present in the meat sample was counted and represented as Colonies Forming Unit (CFU) per milliliter.CFU/ml = (number of colonies × dilution factor)/ (Volume of culture plate).Similar procedure was adopted for A2 SET &B2 SET after 5 days of incubation and A3 SET &B3 SET after 6 days of incubation. The results CFU counts are given as the average three bags of each set of Table-6.

Table-6 Aerobic plate count of microbial growth in the meat samples stored in different days at 4^0 C (expressed as colony forming unit (cfu) per ml)

Number of days	C2 bag	Aerobic plate count (cfu /ml)	plastic bag	Aerobicplate count (cfu /ml)
4^{th} day	A1 (SET 1,2&3)	$13 \pm 1.14^{a} \times 10^8$	B1 (SET 1,2&3)	$67 \pm 2.3^{a} \times 10^8$
5^{th} day	A2 (SET 1,2&3)	$4.56 \pm 0.23^{b} \times 10^8$	B2 (SET 1,2&3)	$110 \pm 4.11^{b} \times 10^8$
6^{th} day	A3 (SET 1,2&3)	N.G.D [x]	B3 (SET 1,2&3)	$160 \pm 6.45 \times 10^8$

Each value indicates means of three set of samples at identical conditions

Different superscript letter indicates significant differences between mean values ($P < 0.05$)

[x] No growth detected

In each set samples, the C-2 pouches undoubtedly exhibited higher antimicrobial activity than polyethylene bag. On the fourth day, meat sample in a polyethylene bag showed bacterial growth five times greater than that in C-2 bag Fig-15.

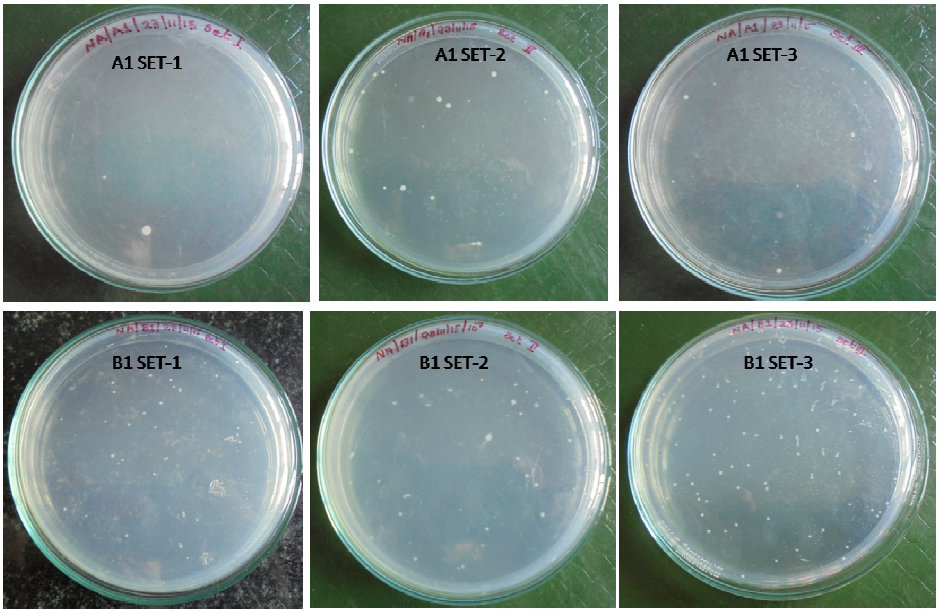


Fig.15. Images of colony forming units (cfu) of bacteira after 4days incubation,C-2 bags (A1) and plastic bags (B1).

On the fifth day, the meat sample in a polyethylene bag exhibited a bacterial growth which was nearly 25times greater than in C-2 bag Fig-16.

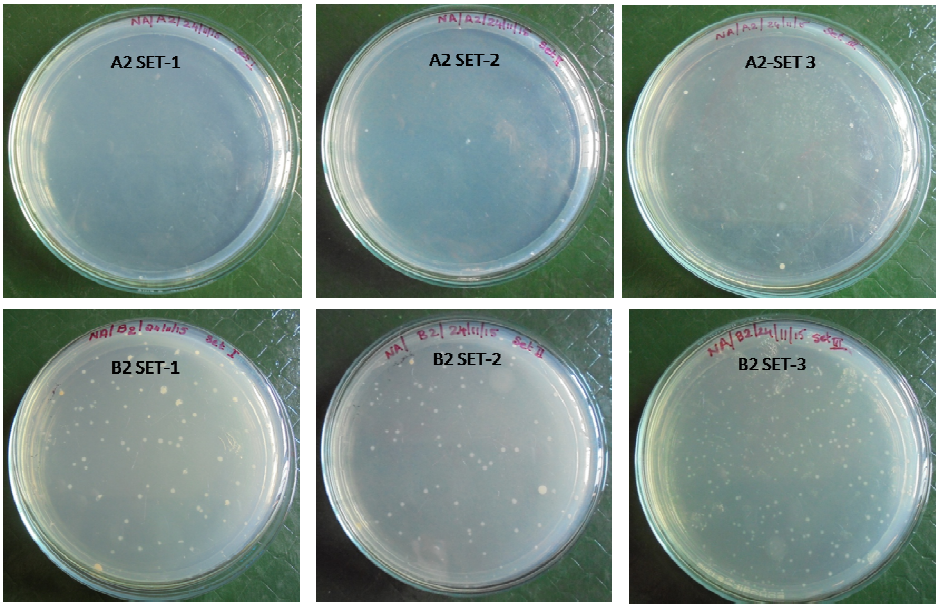


Fig.12. Images of colony forming units (cfu) of bacteira after 5 days incubation,C-2 bags (A2) and plastic bags (B2).

To our surprise, on the sixth day, there was no bacterial growth in the meat sample kept in C-2 bag indicating complete inhibition of microbes. On contradictory, the number of bacteria present in meat sample stored in polythene bag was exponentially increased. Maximum colonies were found on the sixth day storage in polythene bag Fig-17.

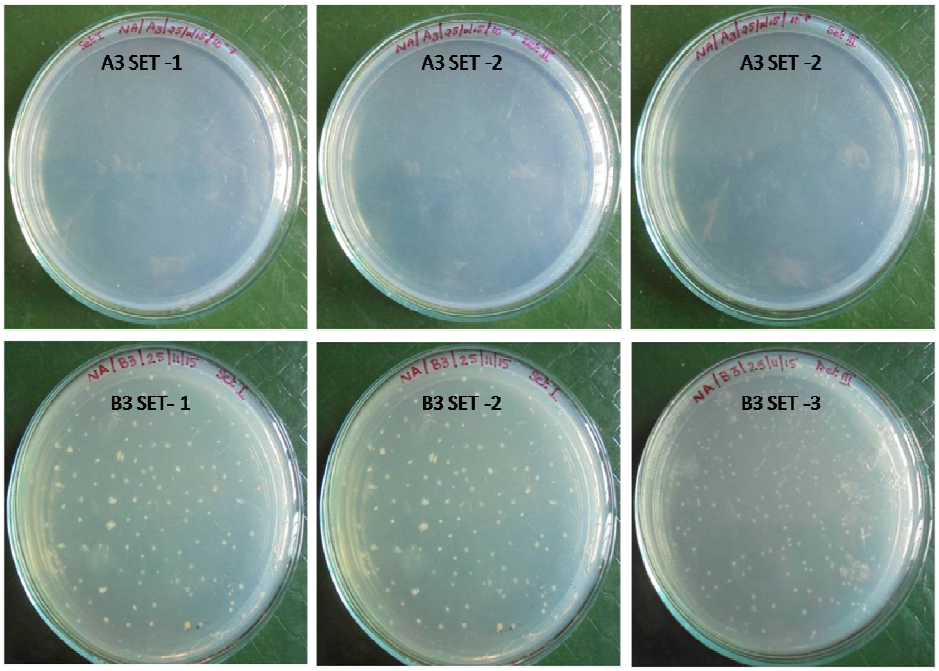


Fig.13. Images of colony forming units (cfu) of bacteira after 6days incubation,C-2 bag (A3) and plastic bags (B3).

Hence we concluded that the composite bag acted as an ideal packaging material by gradually inhibiting the growth of microorganism in the meat sample surface. This result would be a break through to turn up our novel composite film as future smart antimicrobial packaging material to store and transport meat and related items. To the best of our knowledge, till date no report exists on the utilization of Chitosan ZnO nanocomposite films as a packaging material for raw meat that would efficiently ensure complete inhibition of bacterial growth on the meat surface.

5.1 Conclusion

Four different Chitosan/ nano ZnO composite films were synthesized by a simple one pot procedure. Presence of nano ZnO particles in the Chitosan matrix was proved well by FT-IR (ATR), XRD and SEM. Enhanced thermal

stability of composite films was established by the TGA and DSC techniques. Water sorption ability of films was calibrated and compared with pure Chitosan film. Solubility tests proved that all composite films were less soluble than bare Chitosan film and the solubility of composite films varied inversely with the amount of ZnO particles. The usefulness of composite films to substitute current polythene bags to store meat and related items was analyzed. The C-2 bag came out to be a star candidate by ensuring a total inhibition of bacterial growth. The proposed film exhibited a great potential in the future of packing pouches for meat via promising its impact on extending the shelf-life of raw meat and controlling spoilage through complete inhibition of bacterial growth.

6. 1 References

[1] S. Quintavalla, L. Vicini, Antimicrobial food packaging in meat industry, Meat Sci. 62 (2002) 373–380. doi:10.1016/S0309-1740(02)00121-3.

[2] B. Ouattar, R.E. Simard, G. Piett, a Bégin, R. a Holley, Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan., Int. J. Food Microbiol. 62 (2000) 139–148. doi:10.1016/S0168-1605(00)00407-4.

[3] F. Chemistry, Diffusion of Acetic and Propionic Acids from Chitosan-based Antimicrobial Packaging Films, J. Food Sci. 65 (2000) 768–773. doi:10.1111/j.1365-2621.2000.tb13584.x.

[4] Y.X. Xu, K.M. Kim, M. a. Hanna, D. Nag, Chitosan–starch composite film: preparation and characterization, Ind. Crops Prod. 21 (2005) 185–192. doi:10.1016/j.indcrop.2004.03.002.

[5] R. Jiang, H. Zhu, X. Li, L. Xiao, Visible light photocatalytic decolourization of C. I. Acid Red 66 by chitosan capped CdS composite nanoparticles, Chem. Eng. J. 152 (2009) 537–542. doi:10.1016/j.cej.2009.05.037.

[6] a. P. Martínez-Camacho, M.O. Cortez-Rocha, J.M. Ezquerra-Brauer, a. Z. Graciano-Verdugo, F. Rodriguez-Félix, M.M. Castillo-Ortega, et al., Chitosan composite films: Thermal, structural, mechanical and antifungal properties, Carbohydr. Polym. 82 (2010) 305–315. doi:10.1016/j.carbpol.2010.04.069.

[7] P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for chitosan based antimicrobial films in food applications, Food Chem. 114 (2009) 1173–1182. doi:10.1016/j.foodchem.2008.11.047.

[8] R. Jiang, H. Zhu, J. Yao, Y. Fu, Y. Guan, Chitosan hydrogel films as a template for mild biosynthesis of CdS quantum dots with highly efficient photocatalytic activity, Appl. Surf. Sci. 258 (2012) 3513–3518. doi:10.1016/j.apsusc.2011.11.105.

[9] R. Salehi, M. Arami, N.M. Mahmoodi, H. Bahrami, S. Khorramfar, Novel biocompatible composite (Chitosan–zinc oxide nanoparticle): Preparation, characterization and dye adsorption properties, Colloids Surfaces B Biointerfaces. 80 (2010) 86–93. doi:10.1016/j.colsurfb.2010.05.039.

[10] J.Y. Chen, P.J. Zhou, J.L. Li, Y. Wang, Studies on the photocatalytic performance of cuprous oxide/chitosan nanocomposites activated by visible light, Carbohydr. Polym. 72 (2008) 128–132. doi:10.1016/j.carbpol.2007.07.036.

[11] D. Dehnad, H. Mirzaei, Z. Emam-Djomeh, S.-M. Jafari, S. Dadashi, Thermal and antimicrobial properties of chitosan–nanocellulose films for extending shelf life of ground meat, Carbohydr. Polym. 109 (2014) 148–154. doi:10.1016/j.carbpol.2014.03.063.

[12] I. Perelshtein, E. Ruderman, N. Perkas, T. Tzanov, J. Beddow, E. Joyce, et al., Chitosan and chitosan–ZnO-based complex nanoparticles: formation, characterization, and antibacterial activity, J. Mater. Chem. B. 1 (2013) 1968. doi:10.1039/c3tb00555k.

[13] E. Yan, C. Wang, S. Wang, L. Sun, Y. Wang, L. Fan, et al., Synthesis and characterization of fluorescent chitosan-ZnO hybrid nanospheres, Mater. Sci. Eng. B. 176 (2011) 458–461. doi:10.1016/j.mseb.2011.01.005.

[14] P.J.P. Espitia, N.D.F.F. Soares, J.S.D.R. Coimbra, N.J. de Andrade, R.S. Cruz, E.A.A. Medeiros, Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications, Food Bioprocess Technol. 5 (2012) 1447–1464. doi:10.1007/s11947-012-0797-6.

[15] El.shafei, Abou-Okeil, ZnO/carboxymethyl chitosan bionano-composite to impart antibacterial and UV protection for cotton fabric, Carbohydr. Polym. 83 (2011) 920–925. doi:10.1016/j.carbpol.2010.08.083.

[16] S. Mali, L.S. Sakanaka, F. Yamashita, M.V.E. Grossmann, Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect, Carbohydr. Polym. 60 (2005) 283–289. doi:10.1016/j.carbpol.2005.01.003.

[17] C. Qin, H. Li, Q. Xiao, Y. Liu, J. Zhu, Y. Du, Water-solubility of chitosan and its antimicrobial activity, Carbohydr. Polym. 63 (2006) 367–374. doi:10.1016/j.carbpol.2005.09.023.

[18] J. Brugnerotto, J. Lizardi, F.M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, An infrared investigation in relation with chitin and chitosan characterization, Polymer (Guildf). 42 (2001) 3569–3580. doi:10.1016/S0032-3861(00)00713-8.

[19] Pawlak, M. Mucha, Thermogravimetric and FTIR studies of chitosan blends, Thermochim. Acta. 396 (2003) 153–166. doi:10.1016/S0040-6031(02)00523-3.

[20] S.E.S. Saeed, M.M. El-Molla, M.L. Hassan, E. Bakir, M.M.S. Abdel-Mottaleb, M.S. a Abdel-Mottaleb, Novel chitosan-ZnO based nanocomposites as luminescent tags for cellulosic materials, Carbohydr. Polym. 99 (2014) 817–824. doi:10.1016/j.carbpol.2013.08.096.

[21] Y. Haldorai, J.-J. Shim, Chitosan-Zinc Oxide hybrid composite for enhanced dye degradation and antibacterial activity, Compos. Interfaces. 20 (2013) 365–377. doi:10.1080/15685543.2013.806124.

[22] N. Padmavathy, R. Vijayaraghavan, Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study, Sci. Technol. Adv. Mater. 9 (2008) 035004. doi:10.1088/1468-6996/9/3/035004.

[23] Y.T. Prabhu, K.V. Rao, X-Ray Analysis by Williamson-Hall and Size-Strain Plot Methods of ZnO Nanoparticles with Fuel Variation, World J. Nano Sci. Eng. 4 (2014) 21–28. doi:10.4236/wjnse.2014.41004.

[24] H. Sarma, K.C. Sarma, X-ray Peak Broadening Analysis of ZnO Nanoparticles Derived by Precipitation method, Ijsrp. 4 (2014) 1–7.

[25] R. Aepuru, H.S. Panda, Electric-Potential-Driven Pressure-Sensing Observation in New Hollow Radial ZnO and Their Heterostructure with Carbon, J. Phys. Chem. C. 120 (2016) 4813–4823. doi:10.1021/acs.jpcc.6b00123.

[26] C.K. Chiang, R. Popielarz, Polymer composites with high dielectric constant, Ferroelectrics. 275 (2002) 1–9. doi:10.1080/00150190214285.

[27] L. Cordero-Arias, S. Cabanas-Polo, O.M. Goudouri, S.K. Misra, J. Gilabert, E. Valsami-Jones, et al., Electrophoretic deposition of ZnO/alginate and ZnO-bioactive glass/alginate composite coatings for antimicrobial applications, Mater. Sci. Eng. C. 55 (2015) 137–144. doi:10.1016/j.msec.2015.05.034.

[28] S.B. Kondawar, a D. Dahegaonkar, V. a Tabhane, D. V Nandanwar, Thermal and frequency dependance dielectric properties of conducting polymer / fly ash composites, 5 (2014) 360–365. doi:10.5185/amlett.2014.amwc.1036.

[29] D.R. Tobergte, S. Curtis, No Title No Title, J. Chem. Inf. Model. 53 (2013) 1689–1699. doi:10.1017/CBO9781107415324.004.

[30] A. Qureshi, A. Mergen, M.S. Eroğlu, N.L. Singh, A. Güllüoğlu, Dielectric Properties of Polymer Composites Filled with Different Metals, J. Macromol. Sci. Part A. 45 (2008) 462–469. doi:10.1080/10601320801977756.

[31] J.B. Marroquin, K.Y. Rhee, S.J. Park, Chitosan nanocomposite films: Enhanced electrical conductivity, thermal stability, and mechanical properties, Carbohydr. Polym. 92 (2013) 1783–1791. doi:10.1016/j.carbpol.2012.11.042.

[32] P.M. Aneesh, K. a. Vanaja, M.K. Jayaraj, Synthesis of ZnO nanoparticles by hydrothermal method, Nanosci. Eng. 6639 (2007) 66390J–66390J. doi:10.1117/12.730364.

[33] S. Tripathi, G.K. Mehrotra, P.K. Dutta, Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications, Int. J. Biol. Macromol. 45 (2009) 372–376. doi:10.1016/j.ijbiomac.2009.07.006.

[34] H.N. Lim, N.M. Huang, C.H. Loo, Facile preparation of graphene-based chitosan films: Enhanced thermal, mechanical and antibacterial properties, J. Non. Cryst. Solids. 358 (2012) 525–530. doi:10.1016/j.jnoncrysol.2011.11.007.

[35] K.R. Beck, R. Korsmeyer, R.J. Kunz, H. Company, W.J. Ave, N. Il, An Overview of the Glass Transition Temperature of Synthetic Polymers, J. Chem. Educ. 61 (1984) 1–3.

[36] J. Ratto, T. Hatakeyama, R.B. Blumstein, Differential scanning calorimetry investigation of phase transitions in water/ chitosan systems, Polymer (Guildf). 36 (1995) 2915–2919. doi:10.1016/0032-3861(95)94340-Y.

[37] A. Lazaridou, C.G. Biliaderis, Thermophysical properties of chitosan, chitosan-starch and chitosan-pullulan films near the glass transition, Carbohydr. Polym. 48 (2002) 179–190. doi:10.1016/S0144-8617(01)00261-2.

[38] P.-P. Zuo, H.-F. Feng, Z.-Z. Xu, L.-F. Zhang, Y.-L. Zhang, W. Xia, et al., Fabrication of biocompatible and mechanically reinforced graphene oxide-chitosan nanocomposite films., Chem. Cent. J. 7 (2013) 39. doi:10.1186/1752-153X-7-39.

[39] L. Chen, C. Tang, N. Ning, C. Wang, Q. Fu, Q. Zhang, Preparation and Properties of Chitosan/Lignin Composite Films, Chinese J. Polym. Sci. 27 (2009) 739–746. doi:10.1142/S0256767909004448.

[40] M. Pereda, G. Amica, N.E. Marcovich, Development and characterization of edible chitosan/olive oil emulsion films, Carbohydr. Polym. 87 (2012) 1318–1325. doi:10.1016/j.carbpol.2011.09.019.

[41] M. Zhai, L. Zhao, F. Yoshii, T. Kume, Study on antibacterial starch/chitosan blend film formed under the action of irradiation, Carbohydr. Polym. 57 (2004) 83–88. doi:10.1016/j.carbpol.2004.04.003.

[42] M. Mucha, A. Pawlak, Thermal analysis of chitosan and its blends, Thermochim. Acta. 427 (2005) 69–76. doi:10.1016/j.tca.2004.08.014.

[43] Z.-Y. Zhang, H.-M. Xiong, Photoluminescent ZnO Nanoparticles and Their Biological Applications, Materials (Basel). 8 (2015) 3101–3127. doi:10.3390/ma8063101.

[44] I. Leceta, P. Guerrero, I. Ibarburu, M.T. Dueñas, K. de la Caba, Characterization and antimicrobial analysis of chitosan-based films, J. Food Eng. 116 (2013) 889–899. doi:10.1016/j.jfoodeng.2013.01.022.

[45] P.T. Sudheesh Kumar, V.-K. Lakshmanan, T.V. Anilkumar, C. Ramya, P. Reshmi, a. G. Unnikrishnan, et al., Flexible and Microporous Chitosan Hydrogel/Nano ZnO Composite Bandages for Wound Dressing: In Vitro and In Vivo Evaluation, ACS Appl. Mater. Interfaces. 4 (2012) 2618–2629. doi:10.1021/am300292v.

CHAPTER -7

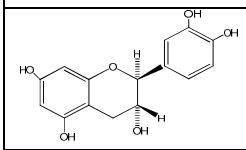
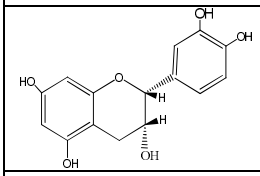
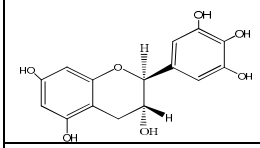
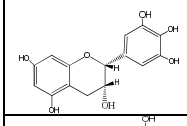
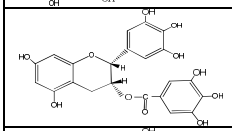
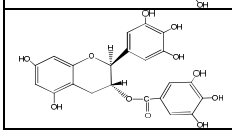
CHITOSAN–GREEN TEA EXTRACT POWDER COMPOSITE POUCHES FOR EXTENDING THE SHELF LIFE OF RAW MEAT

1.1Introduction

It was our target to exploit naturally occurring functional molecules for extending the shelf life of raw meat.In the limited gallery of diverse natural bio-active substances one of the least utilized substance for packaging applications is Green tea extract powder.It is a good source of polyphenolic compounds, has been exploited as outstanding antioxidant substance [1,2]. But, its excellent antimicrobial efficacy against common food spoilage microorganisms was less explored. The potential of inhibiting microbial growth and reducing radical concentration is attributed to characteristic structures of various catechin molecules (tea polyphenols) present in GTE powder Table-1.Hence, the exploitation of tea extract powder-Chitosan composite film for extending the shelf life of raw meat has immense importance.

As it is well known,many synthetic preservatives such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary butyl hydroquinone (THBQ) are directly mixed with raw meat for extending the shelf life. The increased concerns of consumers over artificial preservatives urged researchers to fabricate healthier and eco friendly technologies for meat storage [3]. Recently, much attention has been paid to develop biodegradable packaging films incorporated with active naturally occurring functional materials for extending the shelf life of raw meat.

Table-1. Structure of various tea polyphenol molecules.

Structure	Name
	Catachin (C)
	Epicatechin (EC)
	Gallocatechin (GC)
	Epigallocatechin (EGC)
	Epigallocatechin gallate (EGCG)
	Gallocatechin gallate (GCG)

Herein, we present the first report on Chitosan-Green tea extract powder based portable pouches having potential to extend the shelf life of raw meat. The incorporated green tea extract will diffuse slowly onto the meat surface and will create an ambient atmosphere inside the pack. The presence of diffused molecules will prevent lipid oxidation and microbial out grow, leading to extended shelf life of raw meat. In this study, Chitosan-green tea extract composite film was synthesized by a simple one pot procedure and was ably characterized using XRD,

FTIR, SEM etc. The influence of the noted factors such as water content, water solubility and thermal stability was investigated in detail. The antimicrobial and antioxidant potentials of the synthesized films were investigated. Finally, an optimized composite film was crafted into a pouch like bag for packing raw chicken meat. The efficiency of this smart pouch to extend shelf life of raw meat was compared with the common synthetic packaging material of low density polyethylene (LDPE). The pouches developed can be conveniently employed as a packaging material for raw meat which would create significant advances in the packaging industry.

2. Experimental

2.1. Materials

Chitosan with 85 percent degree of deacetylation was purchased from Sigma Aldrich Co. Ltd (USA).Green tea extract (GTE) powder was obtained from Medizen Labs Pvt. Ltd. (Bangalore, India).Acetic acid, sodium hydroxide and zinc acetate dihydrate, methanol and ethanol were obtained from Merck (Germany).2,2-Diphenyl-1-Picrylhydrazyl(DPPH) extra pure was purchased from SRL (India).Mineral salt broth and Nutrient agar were purchased from Himedia Chemicals (Mumbai, India). Two bacterial strains Escherichia coli (E-Coli, MTCC737) and Staphylococcus Aureus (S-Aureus, MTCC 1687) were cultured in the UniBiosys Biotech Research Lab, Cochin. Deionized water was used to prepare all solutions. All the chemicals used in this study were analytical grade and were used without further purification.

2.2. Preparation of composite films

A facile one pot procedure was adopted for the preparation of composite films. Adequate care and attention were taken to ensure that the chemicals and procedures adopted for the investigation followed green chemistry protocols. Chitosan flakes (2g) were dissolved in 100mL of 2% (v/v) aqueous acetic acid using ultra sonication for 2h at room temperature. Previously prepared solution of 0.1g Green tea Extract powder (GTE) in 10ml distilled water, added to the Chitosan solution. Composite solution was magnetically stirred for 1h to get a homogenous dispersion. To obtain even films, 25mL of viscous mixture was cast into a circular glass dish and was dried at room temperature. The dried films (C1) were peeled out and washed well with ethanol (Fig-1). Four more composite films were prepared by adopting the same procedure with different amount of GTE (0.2, 0.3g, 0.4g and 0.5g); named as C2, C3, C4, and C5. A pure Chitosan film was prepared without green tea extract powder and named as C. All prepared films were stored in airtight polyethylene packets for further analysis.

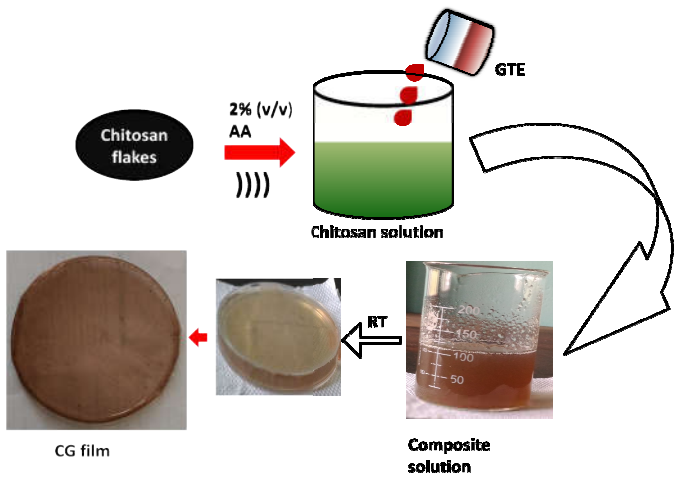


Fig-1.Scheme of preparation of Chitosan –GTE powder composite films.

2.3 Characterization of films

FT-IR Spectra of the films were calibrated using an Attenuated Total Reflection (ATR) Technique (Model, Perkin Elmer Inc. USA), Films were cut into disc shape (5mm x 5mm) and placed gently on a diamond ATR crystal. Prior to the analysis, the film was locked with the pressure arm to maintain an ample contact between the sample and the crystal. FT-IR Spectra of Green tea extract powder was recorded in a KBr pellet form using Fourier Transform IR Spectrometer (Model: JASCO FTIR 4108). X-ray diffraction (XRD) patterns of the films were obtained in the scanning range of 20–75° by an X-ray diffractometer (Model: Rigaku Minifex 600 diffractometer) with Cu Kα radiation (λ = 0.15406nm). Thermo gravimetric analysis (TGA, Model: TGA/DTA851e) was performed at a scanning rate of 2°C min^{-1} under dynamic nitrogen atmosphere to investigate the thermal stability. The absorption spectra of composite films were recorded by using UV–visible spectrophotometer (Model: JASCO V-550) in the wavelength range of 200–800 nm. Surface morphology of the samples was observed by scanning electron microscopy (SEM) (Model: Hitachi SU-6600 FESEM); during the measurement samples were sputtered with a layer of gold to prevent the charging effect.

2.4 Measurement of tensile strength films

Tensile strength of films was measured by Instron Universal Testing Machine (Model-3345) to analyze mechanical strength of films. Calibration was done at a test speed of 2.0 mm/min at room temperature. Previously sliced dumbbell shaped specimens were used

for the analysis. The values of tensile strength (TS) were reported as the average of three replicate experiments.

2.5 Water Vapour Transmission Rate

The Water Vapor Transmission Rate (WVTR) of the Chitosan and composite films was analyzed according to the ASTM E96-95 method with required modifications. Circular Plexi glass cups with inner diameter 35mm and height 45mm were taken for the analysis. 25mL of distilled water was taken in each test cup and cup mouth was sealed perfectly by films having uniform thickness. The distance between the water level and the film was 20mm; the effective area of water transmission through the film was 961.26mm^2.All the assemblies were placed in desiccators at Room Temperature containing small lumps of anhydrous calcium chloride. The amount of water vapours transferred through the films was measured as the weight loss of test cup at the interval of 1h. WVTR, ($gh^{-1}m^{-2}$) was obtained by dividing the slope of the graph (weight loss of test cup verses time) with the area of the film (m). The values were reported as the average of three replicate experiments.

2.6 Water content of films

The equilibrium moisture content of the prepared films was calibrated by weight loss method [4]. Initial mass (W_i) of equal sized film samples (20mm X 20mm) was determined and final mass (W_f) of dried sample was measured after heating at 110^0 C in an oven until constant weight is obtained.

Percentage of water content = $[(W_i - W_f)/ (W_f)]X\ 100$ (1)

2.7 Measurement of Water solubility

Rectangular pieces (7.5 × 15 mm) were cut from each film and dried under vacuum in an oven at 105 °C for 24 h sufficient time to achieve constant weight and the initial dry weight was measured. Subsequently, the films were immersed in 50mL of distilled water at room temperature (23 -27 ^{0}C) with gentle agitation. After 24h of immersion, the specimens were recovered from distilled water and dried to constant weight at 105 °C. The solubility was expressed using Eq. (2).

$$\text{Solubility } (\%) = [(M_i - M_f)] / (M_i) \times 100 \quad (2)$$

where M_i and M_f are the initial dry weight and final mass of films respectively.

2.8 Contact angle measurement

Surface wettability of films was studied using contact angle measurements. Lower the contact angle higher the hydrophilicity [5].It was measured using sessile drop method using a video-based Goniometer (Kruss G10, Germany).A drop of distilled water was carefully placed by micro syringe at temperature,25–27^{0}C on the smooth side of the films, water drop forms equilibrium cap resting on the film. The angle between the film surface and the tangent line at the point of contact of water droplet with the surface was recorded immediately after the water drop was deposited (Fig-2). For each film five measurements were carried out and average values were analyzed.

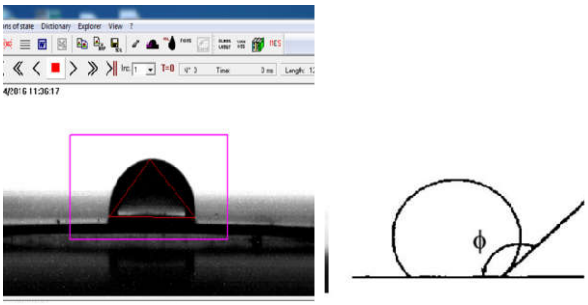


Fig-2.Image of contact angle measurement

2.9 Measurement of optical barrier properties

The ability of films to block light was measured using previously described method with slight modifications [6]. Each film specimen, having thickness 1mm were cut into rectangular pieces (10x 40mm) and placed directly in the test cell. The absorption spectrum of each film was obtained in the wavelength region 200-800nm, using air as reference. The measurements were repeated three times and the average of spectra was calculated. Transparency and opacity of the films were determined by following equation [7].

$$T_{600} = -\log \%T / b \quad (3)$$

$$\text{Opacity} = \text{absorbance at 500 nm X b} \quad (4)$$

Where %T is percentage transmittance and b is the film thickness (mm)

2.10 Antioxidant properties of films

The ability of films to prevent oxidation of packed food was evaluated by the DPPH radical scavenging method. The procedure was adopted from previous reports with required modifications [8,9]. Film specimens (25mm X 25mm) were introduced into separate beaker containing 50mL distilled water. 1mL sample solution was taken out in different time intervals and mixed with previously prepared 4ml 150ηM DPPH solution in methanol. The mixture was kept in the dark for half an hour sufficient time to react polyphenol molecules and DPPH radicals. The colour of DPPH solution changes from dark violet to pale yellow indicating the scavenging activity of the film sample solutionin aqueous media. The absorbance of the mixture was measured at 517nm using UV-Visible spectrometer. All calibrations were repeated for three times. The free radical scavenging ability of film specimens was determined by the following equations.

DPPH radical scavenging activity (%) = $[(Ac- A_s) / A_c] \times 100$ (5)

A_S is the absorbance of test sample

A_c is the absorbance of control solution (4mL DPPH and 1mL distilled water)

2.11 Antimicrobial property

Antimicrobial efficacy of composite films was evaluated against two common food pathogens, E-coli and S-areas using optical density (OD) procedure. Using aseptic techniques, a single colony of test bacteria was carefully transferred into a 100 mL nutrient broth, capped and

placed in an incubator overnight at 37°C.1mL fresh culture of bacteria was inoculated separately into fresh broth medium containing film samples (10mm x 10mm) and incubated with shaking bed (100rpm) at 37°C for 40 hours. During the incubation OD of the medium was calibrated at 600nm using spectrophotometer at different time intervals.

2.12 packaging applications

The potential of optimized C-4 film as a packaging material for raw meat was investigated by plate count procedure. The C-4 films were stitched into flexible pouches like bags using cotton yarn by an in-house weaving machine. The shelf life efficiency of C4 pouches was compared with low density polyethylene (LDPE) bag, which is popularly used to pack, store and transport meat and related items. The composite bags and polyethylene bags were sterilized prior to the analysis. Meat samples of chicken were collected from the local poultry market and were washed well with distilled water to remove blood stains. Equal amount of meat was packed in separate set of bags, each set consisting of three duplicates of C-4 bags (A-1, A-2 and A-3) and polythene bags (B-1, B-2 and B-3) and incubated at 4°C. Following the 1st day of incubation, aerobic plate count was performed using the procedure described below. This protocol was repeated after 2 and 6 days of storage.

2.13 Standard plate count procedure

After one day of incubation, total bacterial count in the packed meat sample was enumerated. 1g sample from each packet was separately

transferred to different test tubes containing 9mL phosphate buffer solution (p^H 7.2). This mixture was then shaken for a few minutes to distribute the bacteria and break up any clumps. Immediately after proper dilution, 1mL of the mixture was aseptically transferred to a second 9mL phosphate buffer solution to get 10^{-2} dilution. The serial dilution was repeated until to get 10^{-3} and 10^{-4} dilutions. Then 1mL sample from the finally diluted solution was transferred to a Petri plate containing nutrient agar media and incubated at 37°C for 24 hours. The enumeration of microbes present in the meat sample was counted and represented as Colonies Forming Unit (CFU) per mL.

CFU/mL = (number of colonies × dilution factor)/ (Volume of culture plate).

2.12. Statistical analysis

All experiments were repeated in three times and the average values with standard errors were reported. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with Tukey's test using software originPro8 (Origin Lab corporation). A statistical difference at $p < 0.05$ was considered to be significant.

3.1 Results and discussions

3.2 Appearance and film thickness

All composite films are visually smooth, shiny and homogenous. The colour of prepared film is light brown and intensity of colour is linearly proportional to the amount of GTE powder incorporated. Films have

no brittle areas or bubbles and can be peeled out easily from the casting plate. Film surface is fairly soft and even, shows the lotus leaf effect (Fig-3) in the presence of water drop. All films were prepared with uniform thickness 1± 0.18 mm and it was observed that even the addition of 0.5g GTE powder didn't change the average thickness of films significantly ($P > 0.05$).

Fig-3. Lotus leaf effect-Image of water drop on the film surface

3.3 FT-IR

FT-IR spectroscopy was used to analyze the nature of interaction between Chitosan and green tea extract powder. The FT-IR spectra of pure Chitosan, green tea extract powder (GTE) and composite films were calibrated and compared (Fig-4). The spectrum of pure Chitosan film (Fig-4a) is in accordance with previous reports. The broadband at

3355cm^{-1} can be indexed to the stretching vibrations of pendant groups -OH, which overlaps with -NH is the stretching vibrations in the same region. The small band at 2887cm^{-1} is due to asymmetric vibrations of -CH_2- groups in pyranose ring. The peak at 1666cm^{-1} is assigned to the stretching vibrations of C=O (amide I), the sharp peak at 1539cm^{-1} can be attributed to –NH bending vibrations (amide II). The band at 1413cm^{-1} can be correlated to C-N axial deformation of amine groups. The distinct and clear band at 1091cm^{-1} is due to the stretching vibration of C–O–C linkages in the polymer chain [10–12].

Green tea extract powder is a mixture of different polyphenolic compounds containing different functional groups (Table-1). The IR spectra of extract powder (Fig-4g) showed characteristic bands corresponding to various characteristic functional groups. The broadband in the region 3561-3454cm^{-1} is assigned to –OH stretching vibrations. The distinctive bands observed at1620cm^{-1} and 1520cm^{-1} are due to the aromatic ring quadrant stretching and the aromatic semicircle stretching vibrations respectively. The peak at 1152cm^{-1} can be attributed to C–O stretching of aromatic alcohols. The two typical peaks at 676 and 604 cm^{-1} are due to the “out of plane bending vibrations” of C-H present in substituted aromatic ring [13].

The IR spectra of composite films are shown in (Fig-4 b-f). The incorporation of Green tea extract powder into Chitosan matrix has modified the positions and intensities of characteristic peaks, reflecting the successful incorporation of GTE powder in the Chitosan matrix. In the spectrum of composite films, the width and intensities of the bands corresponding to $–NH_2$ and –OH groups have increased linearly with

the amount of GTE indicating the interactions between polyphenol molecules and Chitosan. Even though the intensity has decreased, the sharp and obvious peak observed at 1152cm^{-1} in the spectrum of GTE (Fig-4g) is still present in all composite films. It is also observed that peak at1539cm^{-1}corresponding to –NH bending becomes more visible with the increase in the amount of GTE, indicating the interaction of GTE molecules through the pendant groups (–NH_2 and –OH) of Chitosan [14]. When the GTE is added, peak at 1091cm^{-1} became more intense and shifted to lower region 1046cm^{-1}indicating changes in polymer chains in the presence of tea GTE powder [15].

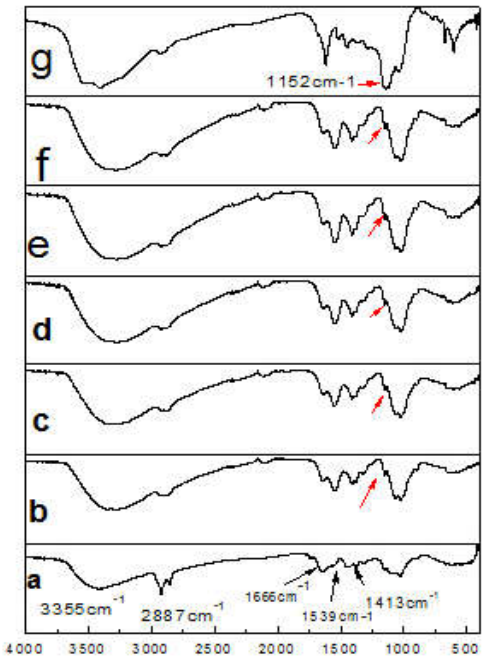


Fig-4. FTIR spectra (a)C, (b) C1, (c) C2, (d) C3, (e) C4, (f) C5, (g) GTE

3.4 XRD results

XRD diffractograms of composite films were compared with pure Chitosan film to examine its crystallinity in the presence of GTE powder. Lower the crystallinity, higher the flexibility of films [16]. Flexible but mechanically strong films are necessary for the fabrication of packaging materials. The pure Chitosan film has crystalline behavior due to strong inter molecular hydrogen bond between the polymer chains and it is evidenced by characteristic peaks at 11.2, 17.7, 21 and 28.9^0 (Fig-5a). The two characteristic peaks at 11.2 and 21^0 can be indexed to two different planes corresponding to unit cells, namely unit cell-1(a = 7.76, b = 10.91, c = 10.30, and interfacial angle value at 90^0) and unit cell-2 (a = 4.4, b = 10.0, c = 10.30, and interfacial angle value at 90^0) [17–21].In contrast to pure Chitosan films, the number of diffraction peaks observed in composite films is inversely related to the amount of GTE powder. In C1 (fig-5b) and C2 (fig-5c), the trend of declining of peak is clearly visible, whereas in C3 (fig-5d), C4(fig-5e) and C5(fig-5f) there are only two peaks justifying the loss of crystallinity in presence of GTE powder. When GTE powder was added, they occupy in between polymer chains and forms hydrogen bonds with the functional groups of Chitosan. These new interactions may weaken the attraction between the polymer chains and improves its flexibility (Fig-6). Moreover, in C3, C4 and C5, the excess GTE powder underwent agglomeration and deposited in the Chitosan matrix (in consistent with SEM analysis) leading to decline in crystallinity.

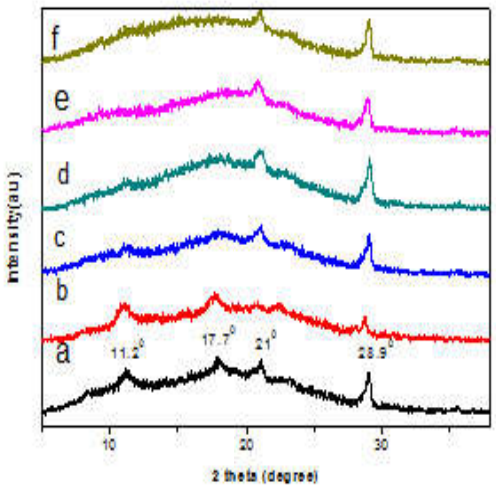


Fig-5. XRD patterns (a) C, (b) C1, (c) C2, (c) C2, (d) C3, (e) C4, (f) C5

Fig-6. Folded images of flexible composite films

3.5 Optical properties

One of the essential features of any packaging film is that it should block the entry of light, especially UV radiations inside the pack. The presence of such harmful radiations will trigger undesired changes in the packed food [7]).To evaluate the light blocking ability of composite films, the absorbance of each film was recorded in UV-Visible region (Fig-7). As shown in the figure, pure Chitosan film exhibited lowest absorbance in all wavelength, lowest opacity or highest transparency (Table-2).Incorporation of GTE powder has greatly affected on optical properties of composite films. All films have excellent absorbance particularly in the UV region due to the presence of different catechins and epicatechin molecules of GTE powder[22].It was already reported that individual poly phenols present in GTE powder has characteristic λmax in UV region [23]. The incorporation of 0.1mg of GTE powder has improved the absorbance of films four folds higher than bare Chitosan films at the wave length 300nm. Moreover, absorbance of composite films is linearly related to the amount of GTE powder incorporated. The excellent absorbance of composite films in the UV region extols its virtue to prevent light-induced lipid oxidation when it will be used to pack raw meat [24].

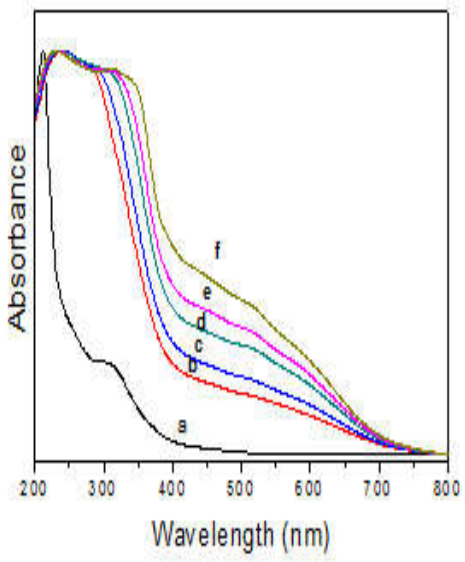


Fig-7. UV-Vis Absorption spectra (a)C, (b) C1, (c) C2, (d) C3, (e) C4, (f) C5

Table-2. Transparency and opacity value different films

Film	Opacity	Transparency
C	0.00642 ± 0.0003[a]	1.96± 0.2 [a]
C1	0.14473 ± 0.02 [b]	1.55± 0.18 [b]
C2	0.19 ± 0.04 [c]	1.44± 0.24 [c]
C3	0.27 ± 0.05 [d]	1.25± 0.19 [d]
C4	0.3122 ± 0.07 [e]	1.11± 0.22 [e]
C5	0.386 ± 0.1 [f]	0.94± 0.28 [f]

All values are average three replicate experiments with slandered deviation

Superscript letters (a–f) within the column indicate significant differences between mean values ($P < 0.05$).

3.6 Thermal stability

Thermo Gravimetric Analysis (TGA) unravels thermal behavior and thermal stability of prepared films [25].The TGA analysis showed that all films has displayed similar thermal behaviour when subjected to programmed heating (Fig-8). The characteristic weight loss occurred at 40-145^{0}C is owing to dehydration of the films[26]. It was reported that Chitosan can retain 5% water even at elevated temperature due to the presence of hydrophilic groups in its chain [27].The second slop onset from 200^{0}C and ended at 450^{0}C corresponds to thermal degradation of Chitosan and release of volatile molecules. Pyrolysis of Chitosan starts by a random split of glycosidic bonds, followed by decomposition and leading to release of smaller volatile molecules having carbon atoms C_2 to C_6 [28]. As shown in figure (Fig-8), all composite films exhibit a higher weight loss in the region corresponding to evaporation of water. This behavior vindicates lower hydrophilicity of polymer in the presence of GTE powder and it was confirmed further by contact angle measurements. When GTE powder was incorporated, new interaction between poly phenol molecules and hydrophilic groups of Chitosan are developed, which decreases prospect of hydrogen bonds between Chitosan and water molecules and facilitate dehydration. Similarly, higher weight loss of composite films in the polymer decomposition region (200^{0}C-450^{0}C) justifies the possibility of GTE powder assisted pyrolysis of Chitosan.

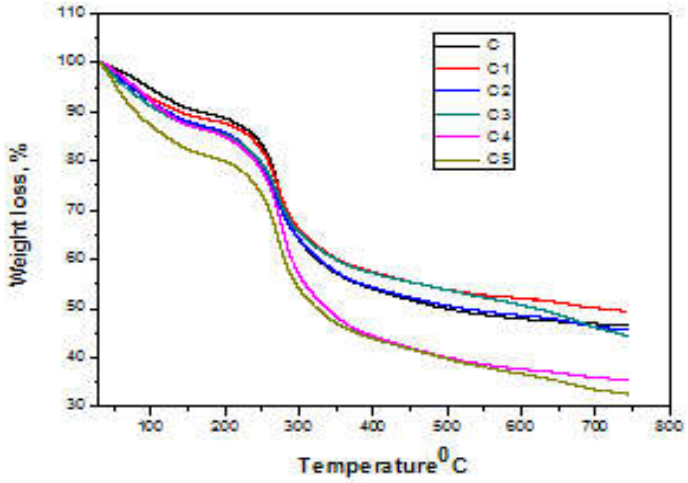


Fig-8. TGA curves of pure and composite films.

3.7 SEM analysis

SEM images give a clear and logical depiction of the surface changes occurred when GTE powder was incorporated. In the case of C1 and C2, the added GTE molecules snugly fit in between the Chitosan chains without any visible surface changes. Hence their addition didn't produce any discontinuity or heterogeneity on the surface. As shown in (Fig-9), surface roughness increases gradually with an increase in the amount of GTE incorporated, the maximum surface roughness were founded in C5 film. The surface heterogeneity is attributed to the accumulation of GTE powder on the surface and lead to lowering of crystallinity of film as observed by XRD analysis. The GTE molecules adhere on to the surface by intermolecular hydrogen bonding and extensively modify the properties of Chitosan.

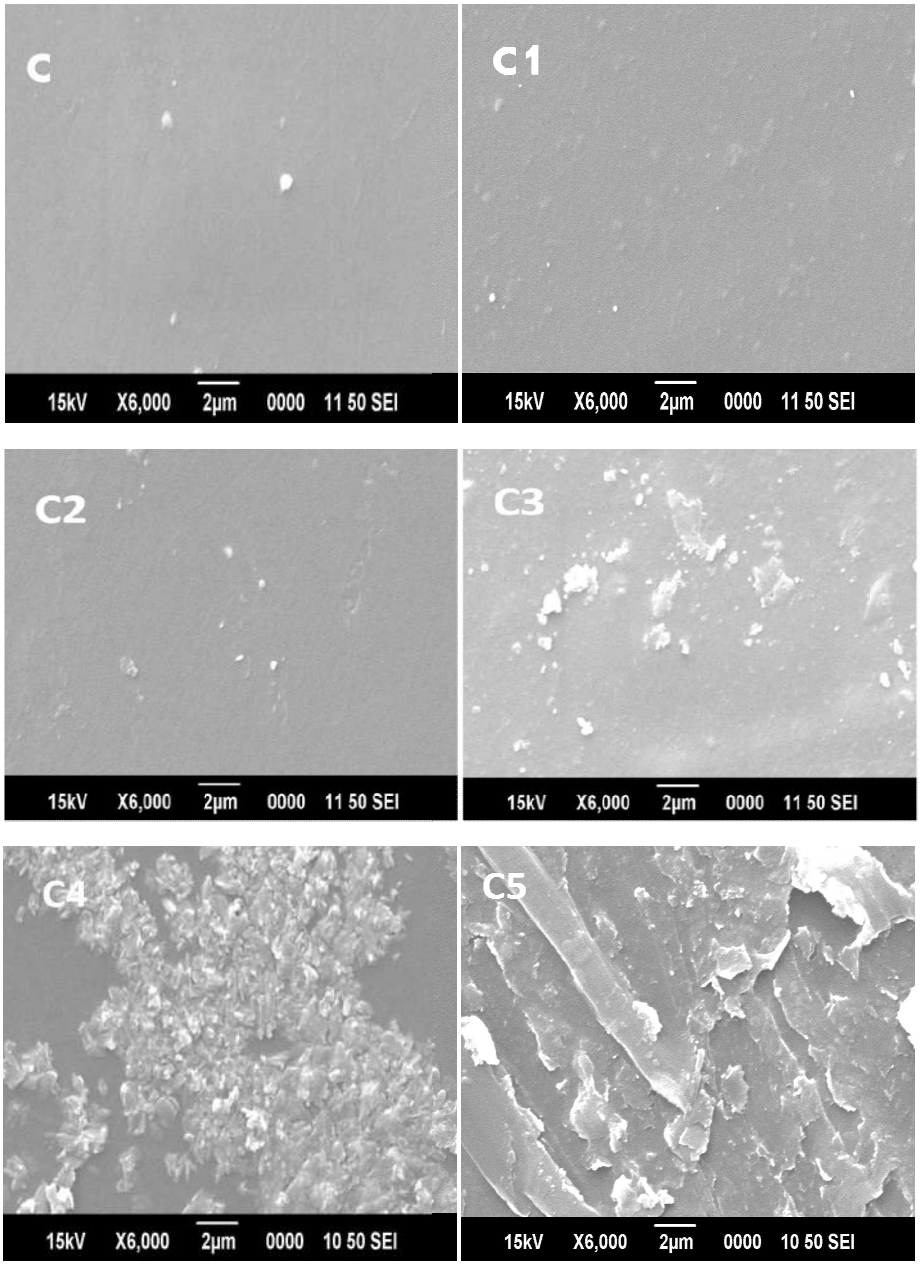


Fig-9. SEM images of films

3.8 Physical properties of films

The contact angle is the measure of wettability of any film with pure water and it is directly linked to hydrophilicity of the films. It is well known that Chitosan films show high degree of wettability due to the presence of a large number of hydrophilic groups [29]. The hydrophilicity of Chitosan can be tuned by various surface modification techniques. The addition of polar substances increases the surface wettability where as nonpolar groups decreases wettability [30,31]. The contact angle values of all films were evaluated (Table-3) to recognize the change in hydrophilicity of Chitosan when GTE powder was incorporated. As shown in Table-3, hydrophilicity decreases initially and then set to increase. A similar trend is observed during water content analysis of composite films. The decrease in wettability/ hydrophilicity can be ascribed to the unavailability of hydrophilic groups of (amino and hydroxyl groups) Chitosan for water molecules when GTE powder was incorporated [32]. There exists a critical concentration of GTE, up to which the hydrophilicity of composite decreases and above that concentration reversal of the said phenomena occurs. This may be due to accumulation of excess GTE powder on the film surface, which can attract water molecules using its own polar groups [33]

Table-3. Physical properties- contact angle, moisture content and solubility.

Film	Contact angle (Θ)	Moisture content (%)	Solubility (%) P^H -7.0	Solubility (%) P^H -5.5
C	80±1.7[a]	19.09±1.7[a]	5 .79± 0.049[a]	12.77± 0.76[e]
C1	94.4±1.61[b]	15.98±0.20[b]	8.3± 0.28[b]	11.25± 0.51[b]
C2	104.8±2.8[c]	15.05±0.48[c]	9.49± 0.24[c]	11.41± 0.86[c]
C3	91.16±1.01[d]	14.19± 1.6 [d]	9.35± 0.61[d]	14.63± 0.86[d]
C4	89.36±2.83[e]	14.89±0.58[e]	14.63±0.10[e]	15.66±0.82[e]
C5	85.43±2.1[f]	17.14±0.57[f]	15.12±0.63[f]	19.88±0.60[f]

All values are average three replicate experiments with standard deviation

Superscript letters (a–f) in each column indicate significant differences between mean values ($P < 0.05$).

Moisture content of the prepared films follows the trend of hydrophilicity order; decreases initially, reaches the optimum value (C3), and then set to increase with the amount of GTE powder. This trend recognizes and justifies the tenets of hyrophilicity of films. The water molecules present in films were held by hydrogen bonds either with polar groups of Chitosan or polyphenol molecules. The presence of water molecules in composite films was vindicated by TGA analysis, where each film gives a characteristic slope for dehydration of water molecules.

Solubility measurement of active packaging material sheds light on the ability of films to withstand moisture condition as well as the skill of packaging films to transfer active molecules from its matrix to raw meat surface during the shelf life keeping [34]. A smart packaging film should be insoluble but at the same time it must release active molecules during its shelf keeping to prevent spoilage of the packed food. In the present case, the pure Chitosan film showed lowest solubility in neutral and acidic P^H, whereas solubility of composite films is linearly proportional to the amount GTE powder (Table-3). It is concluded that addition of tea extract has improved solubility of composite films and similar trend was reported in the case of green and black tea extract powder incorporated composite films [35]. In lower P^H all films have higher solubility due to higher dissolution of Chitosan in acidic condition. As it is well known, pure Chitosan has a lower solubility at neutral P^H due to its semicrystalline nature and strong intermolecular hydrogen bonds. In lower P^H conditions, amino groups of Chitosan can be protonated leading to repulsion between positively charged polymer chains and thereby diffusion of solvent molecules and subsequent dissolution of Chitosan [36]. In contrast to pure Chitosan films, the solubility of composite films depends on various factors such as hydrophilicity, water diffusion ability, the number and ionizability of polar groups, type of fillers, strength of hydrogen bonds and polymer chain relaxation ability[37].Higher solubility of Chitosan GTE powder composite films are not due to enhanced dissolution of Chitosan molecules, but due to the release of GTE powder from the polymer matrix in aqueous media. The weight loss appeared due to the release of GTE has been identified as extended solubility. To confirm

this hypothesis, we carried out solubility experiment with large sized films (6 X 6cm) and kept it in same amount water for 7 days. The slow coloration of the supernatant liquid confirmed the release of GTE powder from the polymer matrix. The intensity of colour is linearly proportional to the amount of GTE powder (Fig-10). Such release of GTE powder in aqueous media has a significant role in deciding antimicrobial and antioxidant properties and it was proved by further studies.

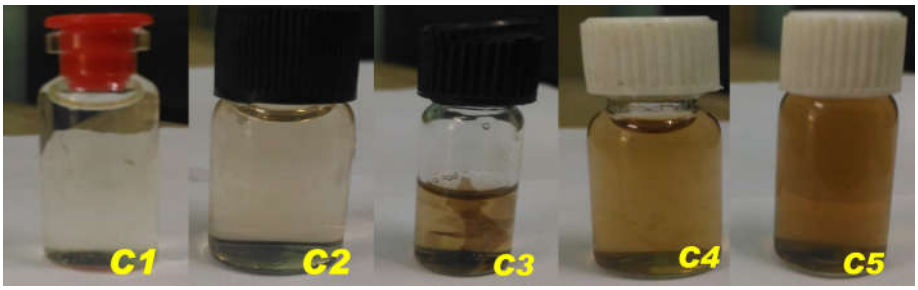


Fig-10. Image of supernatant solution of composite films

3.9 Water Vapour Trasmission Rate (WVTR)

Determination of water barrier ability of packaging film is crucial in developing smart packaging films for raw meat. Excess water molecules inside the pack will lead to undesirable changes in foods, whereas moderate amount of water molecules is tolerable to transfer active molecules from the packaging films to the food surface. Hence, the specific water barrier requirements of a packaging film depend upon product characteristics and mechanisms involved in enhancement of shelf life and quality of food. The values of WVTR are given in the (Table -4). As shown in the result, all composite films exhibite higher

WVTR values compared to the pure Chitosan film except C1. The WVTR is a measure of the easiness with which the water molecules infuse and cross through the material and it is chiefly related to two factors, namely, hydrophilic-hydrophobic ratio of the composite films and its crystallinity. WVTR is inversely related to hydrophobicity and crystallinity of films, whereas it is directly related to the hydrophilicity of the film [7,38,39].The obtained result suggests that the inter play of hydrophilicity-hydrophobicity ratio and crystallinity has paved the way for enhanced WVTR of composite films. It was concluded earlier for composite films based on investigations of physical properties that the hydrophobicity of composite film shows rise and fall like trend whereas crystallinity decreases linearly with the amount of GTE powder. The lowest WVTR value of C1 is attributed to its comparatively higher hydrophobicity (Table-3, contact angle) and moderate crystllinity (as per XRD analysis). Even though C5 film has lowest crysallinity and reasonably higher hydrophilicity, it didn't show any exceptionally higher WVTR property. This anomaly is related to its higher solubility in aqueous media (Table-3). As explained earlier, higher solubility is due to the excess release of GTE powder from the matrix, hence water molecules interact with the component present in the matrix and losing more time to cross the film[40].

Table-4. WVTR values of films

Film	WVTR ($gh^{-1}m^{-2}$)
C	38.19 ± 0.93675^{a}
C1	36.45 ± 0.75^{b}
C2	38.68 ± 0.71^{c}
C3	39.09 ± 1.14^{d}
C4	40.28 ± 0.72^{e}
C5	42.11 ± 0.87^{f}

All values are average of three sets of experiments with standard deviation

Superscript letters (a–f) in the column indicate significant differences between mean values ($P < 0.05$).

3.10 Measurement of tensile strength (TS) of films

Tensile strength is measured by dividing maximum load (N) with specimen's cross sectional area (mm^2) in N/ mm^2 or map and it is the assessment of the ability of a film to withstand the external stress. The tensile strength (TS) values of films was compared and are given in (Table-5). The TS values of composite films have significantly ($P < 0.05$) improved when extract powder was incorporated, but the trend is not in a linear fashion. TS values of composite films have initially increased with the gradual addition of extract powder, reaching an optimum value and then it is set to decrease. Pure Chitosan film has a TS value 41.82 ±0.79 MPa. The increase of TS values of composite films can be justified by the structural changes promoted by the

addition of GTE powder. As it is well recognized, TS values are linearly related to the strength of inter molecular attractions present in the film [41]. When GTE powder was incorporated, the polyphenol molecules can form new inter molecular hydrogen bonds with the polar groups of Chitosan. This bond can build up a cross linking effect leading to the enhancement of TS of composite films [42,43]. For a fixed amount of Chitosan precursor, there will be an optimum level of fillers for maximum cross linking effect. Hence, in the case of incorporation of GTE powder more than the optimum level, the excess amount will be accumulated in the matrix and causes surface discontinuity leading to the decrease of TS value. The surface discontinuity or heterogeneity originated due to the accumulation of GTE powder was vindicated by SEM images.

Table-5. Tensile strength values of films

Film	**Tensile streangth (Mpa)**
C	41.82 ± 0.79^{a}
C1	55.16 ± 1.6^{b}
C2	59.36 ± 0.89^{c}
C3	62.38 ± 1.4^{d}
C4	60.65 ± 0.42^{e}
C5	59.08 ± 1.12^{f}

All values are average of three sets of experiments with slandered deviation

Superscript letters (a–f) in the column indicate significant differences between mean values ($P < 0.05$).

3.11 Antioxidant properties of films

DPPH is one of the stable organic radical and widely used for the determination of antioxidant property of active packaging films [44]. The free radical scavenging ability of prepared films was calibrated at different time intervals in aqueous media (solvent present in the raw meat) and compared (Table-6). As shown in the result, the scavenging ability of the bare Chitosan film was lowest and it exhibits 31.9% of scavenging activity after 25 hrs immersion in water. The maximum activity was shown by C5 film sample in all time intervals and its efficiency has reached at 95.13% after 25hours long immersion.

The limited antioxidant activity of Chitosan was justified as the reaction of radical species with hydroxyl group at the C-6 position and amino group at C-2 position. These functional groups can transfer hydrogen to the unstable radical species and will form macromolecular radicals, but the reaction is relatively slow and futile, hence there is always a search for alternative methods to improve its antioxidant property [45]. Compared to pure Chitosan, GTE powder incorporated composite films showed increased scavenging activity. The fifteen fold increase in antioxidant activity of C5 film (after 1h immersion) justifies the direct involvement of polyphenol molecules in antioxidant activities. The faster and enhanced radical scavenging activities of composite films are directly linked to the release of GTE powder from the polymeric matrix [35]. The ability of water molecules to replace

GTE powder from matrix has been proved by solubility test (Fig -10). Hence, the aqueous media in contact with Chitosan – GTE composite film will contain enormous tea poly phenol molecules and these molecules react with DPPH radicals, decreases its concentration. The speed and efficiency of scavenging activity of the sample are proportional to the amount of tea polyphenol molecules presents. The unusual antioxidant activity of GTE powder is due to special structural features of each catechin molecules present in the extract. Catechins and epicatechins having three hydroxyl groups in the B ring are called gallocatechins, and their esterified form to Gallic acid (3,4,5 trihydroxy benzoic acid) at these three hydroxyl groups are named as catechin gallates. It was reported that ortho-trihydroxyl groups in the B ring of gallocatechins are responsible for its anti oxidant activities and in the case of catechine gallates, carboxylic acid and hydroxyl groups together plays a major role in deciding antioxidant activity. Due to the high reactivity of these groups, they can donate hydrogen and an electron to DPPH radical.

$$F\text{-}OH + R^{*} \rightarrow F\text{-}O^{*} + R\text{-}H \quad \text{..... (3)}$$

The radical scavenging ability of different components of GTE powder follows the order epicatechin gallate = epigallocatechin gallate > epigallo catechin> Gallic acid> epicatechin = catechin. Anyhow, Chitosan film containing GTE powder is an excellent antioxidant packaging material for raw meat. The water molecules present in meat content will trigger the periodic release of GTE powder from matrix towards the meat surface; the presence of these active molecules will prevent the undesirable lipid oxidation of packed meat.

Table-6. Percentage of scavenging activity of films

Time (h)	Scavenging activity (%)					
	C	C1	C2	C3	C4	C5
1	5.76± 1.32[a]	16.07± 1.54[a]	20.29±1.45[a]	34.11±1.43[a]	80.17±2.92[a]	84.23±2.39[a]
2	9.62± 1.7[b]	24.13±1.98[b]	30.81±1.67[b]	42.29±1.98[b]	84.53±3.01[b]	85.91±2.98[b]
3	16.75±1.87[c]	30.43± 1.66[c]	40.82±2.65[c]	49.45±2.45[c]	89.79±2.56[c]	91.33±1.97[c]
24	21.11±2.01[d]	66.87±2.43[d]	70.12±2.31[d]	84.22±2.98[d]	92.33±2.13[d]	91.93±2.34[d]
25	31.93±2.31[e]	71.87±2.86[e]	75.48±2.67[e]	84.33±2.56[e]	93.55±3.43[e]	95.13±1.90[e]

All values are average of three sets of calibrations with standard deviation

Superscript letters (a–e) in each column indicate significant differences between mean values ($P < 0.05$).

3.12 Antimicrobial activity

Optical density (OD) values of composite films at four different time intervals are given in (Table 7and 8). The growth of microorganism in the media is directly linked to its turbidity. Higher the turbidity, higher the OD value and lower the antimicrobial efficacy of the film [46,47]. The percentage of inhibition was determined by comparing the OD value of control media (without film sample) in each time interval.

$$\% \text{ of inhibition} = [(OD)_c - (OD)_S / (OD)_c] \times 100$$

$(OD)_c$ is the optical density values of control media and $(OD)_s$ is the optical density values of film sample containing media.

All composite films have shown higher antimicrobial efficacy compared to pure Chitosan films, C4 and C5 films have big leap in

antimicrobial efficacy. The enhancement of antimicrobial property of composite films rationalizes the tenets of controlled release of GTE powder from the polymer matrix in aqueous media. The exceptionally higher antimicrobial activity of C4 and C5 films are related to the enhanced release of GTE powder from the polymer matrix. The biocidal activity of GTE was well recognized by various systematic studies. There was a report that an army surgeon recommended the use of tea in soldiers' water bottles as a prophylactic against typhoid. It was generally agreed that the antimicrobial activity of GTE powder is due to the presence of catechins [48]. Among the different catechins, gallocatachins and corresponding gallates have a chief role in antimicrobial activity [49]. These molecules specifically bind to the peptide glycan present in the cell wall of microorganism leading to the precipitation. There are a few other suggestions that gallocatechins and their gallates damages lipsosame or attack and destroy lipid bilayer of bacterial cell wall or generate H_2O_2 for biocidel activity. Anyhow, further research is required to elucidate the specific involvement of each type of tea catechin molecules in their antimicrobial activity [50].

Table -7. OD values and Percentage of inhibition of films against Staphylococcus aureus.

Time (h)	**% of inhibition**					
	C	**C1**	**C2**	**C3**	**C4**	**C5**
5	36.11	30.56	8.33	33.33	19.44	38.89
20	22.80	28.94	41.22	35.96	73.68	78.94
25	20.47	27.55	29.92	16.53	80.31	80.31
30	23.91	34.23	35.32	39.67	82.06	84.78

Table-8. OD values and Percentage of inhibition of films against E.coli.

Time (h)	% of inhibition					
	C	C1	C2	C3	C4	C5
5	12.9	18.1	2.78	2.6	22.22	13.89
20	13.17	21.70	2.8	19.44	6.4	6.8
25	14.18	23.40	23.25	26.35	55.03	60.46
30	38.89	47.22	24.11	31.91	92.19	97.87

4.1 Packaging application

Since raw meat becomes rapidly unfit for use due to microbial contamination, the efficiency of composite pouches (Fig-11) for extending the shelf life of raw meat was evaluated by plate count procedure. It is well known that a packaging film inhibit growth of microorganisms can extend the shelf life of packed food. The comparison of total plate count of microorganisms (Table -9) of meat samples packed in Chitosan-GTE composite pouches and LDPE pouches obviously emphasize the ability of composite pouches to enhance the shelf life of raw meat. As shown in the result bacterial count of meat samples stored in composite pouches are significantly ($P < 0.05$) lower than polyethylene pouches. There was no growth detected in a meat sample stored in composite pouches for one day and the rate of growth of microorganisms in meat samples stored in composite pouches are relatively low. Whereas meat samples stored in polyethylene pouch has exhibited moderately higher bacterial count from the first day of storage and there was an exponential growth of

microorganisms with a number of storage days. After six days of storage the total plate count of microorganisms in polythene bag was 40 times larger than microorganisms found in composite pouches (Fig-12). This result has undoubtedly underlined the ability of composite pouches to inhibit the growth of microorganisms in raw meat and enhance its shelf life. The inhibition efficiency of composite pouch is attributed to the periodical diffusion of active molecules from the packaging material onto the meat surface. The water content in the meat sample may facilitate the diffusion of active molecules. The slow growth of microorganisms in composite packed meat samples with number of packaging days implies the declining of diffusion and decrease of efficiency with storage days.

Fig-11. Image of flexible pouch

Table-9. Total count of bacteria (Log cfu/g) present in meat sample during storage at 4^0 C

Storage time (h)	Meat sample	Total count	
		Exp-1(3-dilutions)	Exp-1(4-dilutions)
24h(1day)	LDPE pouch	4.066 ± 0.05^a	4.43 ± 0.04^a
	Composite pouch	N.G.D	N.G.D
48h (2 days)	LDPE pouch	4.42 ± 0.27^b	5.1 ± 0.44^b
	Composite pouch	1.96 ± 1.7^a	1.33 ± 2.3^a
144h (6days)	LDPE pouch	5.59 ± 0.017^c	5.99 ± 0.23^c
	Composite pouch	4.15 ± 0.07^d	4.15 ± 0.15^d

N.G.D -no growth detected

[a-d] Means within columns having the same superscript do not differ significantly ($p > 0.05$).

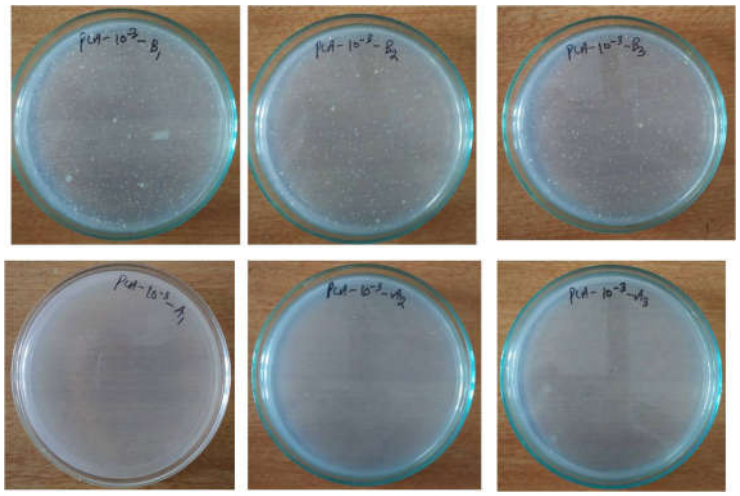


Fig-12. Image of total plate count of microorganisms of meat samples in LDPE pouch (above) and composite pouch (below).

5.1Conclusions

The aim of this investigation was the fabrication of an Eco friendly, smart packaging film for the extension of raw chicken meat. To optimize a suitable candidate for packaging applications five different Chitosan-GTE powder composite films were synthesized by a simple one pot procedure. All films were well characterized by FT-IR (ATR), XRD and SEM. Physical, thermal and mechanical features of composite films were evaluated and compared with pure Chitosan films. It was observed that incorporation of GTE powder has improved all properties, but not in linear fashion. The smartness of composite films towards antimicrobial and antioxidant properties was calibrated by in-vitro analysis. DPPH scavenging activity studies have undoubtedly proven enhanced antioxidant ability of composite films. Similarly, antimicrobial studies also confirmed the ability composite films to inhibit the growth of Gram-positive and Gram-negative bacteria. Finally, the usefulness of composite films to substitute current polyethylene bags to store meat and related items were analyzed. The C4 composite film was crafted to pouch like packaging material for investigating the potential of film to enhance the shelf life of raw meat. The result was highly encouraging that the rate of bacterial growth in meat samples stored in composite pouches was significantly lower than that of polyethylene pouch.

6.1 References

[1] M.P. Almajano, R. Carbó, J.A.L. Jiménez, M.H. Gordon, Antioxidant and antimicrobial activities of tea infusions, Food Chem. 108 (2008) 55–63. doi:10.1016/j.foodchem.2007.10.040.

[2] H. Bozkurt, Utilization of natural antioxidants: Green tea extract and Thymbra spicata oil in Turkish dry-fermented sausage, Meat Sci. 73 (2006) 442–450. doi:10.1016/j.meatsci.2006.01.005.

[3] H. Zhang, J. Wu, X. Guo, Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality, Food Sci. Hum. Wellness. 5 (2016) 1–10. doi:10.1016/j.fshw.2015.11.003.

[4] A. Pinotti, M.A. Garca, M.N. Martino, N.E. Zaritzky, Study on microstructure and physical properties of composite films based on chitosan and methylcellulose, Food Hydrocoll. 21 (2007) 66–72. doi:10.1016/j.foodhyd.2006.02.001.

[5] M. Cheng, J. Deng, F. Yang, Y. Gong, N. Zhao, X. Zhang, Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions, Biomaterials. 24 (2003) 2871–2880. doi:10.1016/S0142-9612(03)00117-0.

[6] S. Fakhreddin Hosseini, M. Rezaei, M. Zandi, F.F. Ghavi, Preparation and functional properties of fish gelatin-chitosan blend edible films, Food Chem. 136 (2013) 1490–1495. doi:10.1016/j.foodchem.2012.09.081.

[7] S.R. Kanatt, M.S. Rao, S.P. Chawla, A. Sharma, Active chitosan-polyvinyl alcohol films with natural extracts, Food Hydrocoll. 29 (2012) 290–297. doi:10.1016/j.foodhyd.2012.03.005.

[8] Y. Zhong, Y. Li, Effects of surfactants on the functional and structural properties of kudzu (Pueraria lobata) starch/ascorbic acid films, Carbohydr. Polym. 85 (2011) 622–628. doi:10.1016/j.carbpol.2011.03.031.

[9] P. Mayachiew, S. Devahastin, Effects of drying methods and conditions on release characteristics of edible chitosan films

enriched with Indian gooseberry extract, Food Chem. 118 (2010) 594–601. doi:10.1016/j.foodchem.2009.05.027.

[10] A. Kaushik, R. Khan, P.R. Solanki, P. Pandey, J. Alam, S. Ahmad, et al., Iron oxide nanoparticles-chitosan composite based glucose biosensor, Biosens. Bioelectron. 24 (2008) 676–683. doi:10.1016/j.bios.2008.06.032.

[11] J. Brugnerotto, J. Lizardi, F.M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, An infrared investigation in relation with chitin and chitosan characterization, Polymer (Guildf). 42 (2001) 3569–3580. doi:10.1016/S0032-3861(00)00713-8.

[12] Pawlak, M. Mucha, Thermogravimetric and FTIR studies of chitosan blends, Thermochim. Acta. 396 (2003) 153–166. doi:10.1016/S0040-6031(02)00523-3.

[13] V.A. Fassel, M. Margoshes, The Infrared Spectra of Aromatic Compounds. 1 1 . Evidence Concerning the Interaction of r-Electrons and a-Bond Orbitals in C-H Out-of-plane Bending Vibrations', 78 (1952) 8–11.

[14] U. Siripatrawan, B.R. Harte, Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract, Food Hydrocoll. 24 (2010) 770–775. doi:10.1016/j.foodhyd.2010.04.003.

[15] L. Wang, Y. Dong, H. Men, J. Tong, J. Zhou, Preparation and characterization of active films based on chitosan incorporated tea polyphenols, Food Hydrocoll. 32 (2013) 35–41. doi:10.1016/j.foodhyd.2012.11.034.

[16] L.-H. Li, J.-C. Deng, H.-R. Deng, Z.-L. Liu, L. Xin, Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes, Carbohydr. Res. 345 (2010) 994–998. doi:10.1016/j.carres.2010.03.019.

[17] S.F. Wang, L. Shen, Y.J. Tong, L. Chen, I.Y. Phang, P.Q. Lim, et al., Biopolymer chitosan/montmorillonite nanocomposites: Preparation and characterization, Polym. Degrad. Stab. 90 (2005) 123–131. doi:10.1016/j.polymdegradstab.2005.03.001.

[18] Y. Xu, X. Ren, M.A. Hanna, Chitosan/clay nanocomposite film

preparation and characterization, J. Appl. Polym. Sci. 99 (2006) 1684–1691. doi:10.1002/app.22664.

[19] I. Yamaguchi, K. Tokuchi, H. Fukuzaki, Y. Koyama, K. Takakuda, H. Monma, et al., Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites, J. Biomed. Mater. Res. 55 (2001) 20–27. doi:10.1002/1097-4636(200104)55:1<20::AID-JBM30>3.0.CO;2-F.

[20] Y.X. Xu, K.M. Kim, M. a. Hanna, D. Nag, Chitosan–starch composite film: preparation and characterization, Ind. Crops Prod. 21 (2005) 185–192. doi:10.1016/j.indcrop.2004.03.002.

[21] S.C.M. Fernandes, C.S.R. Freire, A.J.D. Silvestre, C. Pascoal Neto, A. Gandini, L.A. Berglund, et al., Transparent chitosan films reinforced with a high content of nanofibrillated cellulose, Carbohydr. Polym. 81 (2010) 394–401. doi:10.1016/j.carbpol.2010.02.037.

[22] Z. Pieiro, M. Palma, C.G. Barroso, Determination of catechins by means of extraction with pressurized liquids, J. Chromatogr. A. 1026 (2004) 19–23. doi:10.1016/j.chroma.2003.10.096.

[23] A.K. Atoui, A. Mansouri, G. Boskou, P. Kefalas, Tea and herbal infusions: Their antioxidant activity and phenolic profile, Food Chem. 89 (2005) 27–36. doi:10.1016/j.foodchem.2004.01.075.

[24] M.C. Gómez-Guillén, M. Ihl, V. Bifani, A. Silva, P. Montero, Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (Ugni molinae Turcz), Food Hydrocoll. 21 (2007) 1133–1143. doi:10.1016/j.foodhyd.2006.08.006.

[25] J. Kim, Z. Cai, H.S. Lee, G.S. Choi, D.H. Lee, C. Jo, Preparation and characterization of a Bacterial cellulose/Chitosan composite for potential biomedical application, J. Polym. Res. 18 (2011) 739–744. doi:10.1007/s10965-010-9470-9.

[26] S. Tripathi, G.K. Mehrotra, P.K. Dutta, Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications, Int. J. Biol. Macromol. 45 (2009) 372–376. doi:10.1016/j.ijbiomac.2009.07.006.

[27] J.F. Rubilar, R.M.S. Cruz, H.D. Silva, A.A. Vicente, I. Khmelinskii, M.C. Vieira, Physico-mechanical properties of chitosan films with carvacrol and grape seed extract, J. Food Eng. 115 (2013) 466–474. doi:10.1016/j.jfoodeng.2012.07.009.

[28] C.G.T. Neto, J.A. Giacometti, A.E. Job, F.C. Ferreira, J.L.C. Fonseca, M.R. Pereira, Thermal analysis of chitosan based networks, Carbohydr. Polym. 62 (2005) 97–103. doi:10.1016/j.carbpol.2005.02.022.

[29] S.B. Rao, C.P. Sharma, Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential, J. Biomed. Mater. Res. 34 (1997) 21–28. doi:10.1002/(SICI)1097-4636(199701)34:1<21::AID-JBM4>3.0.CO;2-P.

[30] V. Tangpasuthadol, N. Pongchaisirikul, V.P. Hoven, Surface modification of chitosan films. Effects of hydrophobicity on protein adsorption, Carbohydr. Res. 338 (2003) 937–942. doi:10.1016/S0008-6215(03)00038-7.

[31] M. Moradi, H. Tajik, S.M. Razavi Rohani, A.R. Oromiehie, H. Malekinejad, J. Aliakbarlu, et al., Characterization of antioxidant chitosan film incorporated with Zataria multiflora Boiss essential oil and grape seed extract, LWT - Food Sci. Technol. 46 (2012) 477–484. doi:10.1016/j.lwt.2011.11.020.

[32] S.M. Ojagh, M. Rezaei, S.H. Razavi, S.M.H. Hosseini, Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water, Food Chem. 122 (2010) 161–166. doi:10.1016/j.foodchem.2010.02.033.

[33] A.S. Ferreira, C. Nunes, A. Castro, P. Ferreira, M.A. Coimbra, Influence of grape pomace extract incorporation on chitosan films properties, Carbohydr. Polym. 113 (2014) 490–499. doi:10.1016/j.carbpol.2014.07.032.

[34] M. Abdollahi, M. Rezaei, G. Farzi, Improvement of active chitosan film properties with rosemary essential oil for food packaging, Int. J. Food Sci. Technol. 47 (2012) 847–853. doi:10.1111/j.1365-2621.2011.02917.x.

[35] Y. Peng, Y. Wu, Y. Li, Development of tea extracts and

chitosan composite films for active packaging materials, Int. J. Biol. Macromol. 59 (2013) 282–289. doi:10.1016/j.ijbiomac.2013.04.019.

[36] I.A. Sogias, V. V. Khutoryanskiy, A.C. Williams, Exploring the factors affecting the solubility of chitosan in water, Macromol. Chem. Phys. 211 (2010) 426–433. doi:10.1002/macp.200900385.

[37] A. Biro, Water politics and the construction of scale, Stud. Polit. Econ. (2007) 9–30. doi:10.1002/bip.

[38] M.P. Souza, A.F.M. Vaz, H.D. Silva, M.A. Cerqueira, A.A. Vicente, M.G. Carneiro-da-Cunha, Development and Characterization of an Active Chitosan-Based Film Containing Quercetin, Food Bioprocess Technol. 8 (2015) 2183–2191. doi:10.1007/s11947-015-1580-2.

[39] J.T. Martins, M.A. Cerqueira, A.A. Vicente, Influence of ??-tocopherol on physicochemical properties of chitosan-based films, Food Hydrocoll. 27 (2012) 220–227. doi:10.1016/j.foodhyd.2011.06.011.

[40] L. Wang, Q. Wang, J. Tong, J. Zhou, Physicochemical Properties of Chitosan Films Incorporated with Honeysuckle Flower Extract for Active Food Packaging, J. Food Process Eng. (2015) 1–8. doi:10.1111/jfpe.12305.

[41] S. Il Park, Y. Zhao, Incorporation of a High Concentration of Mineral or Vitamin into Chitosan-Based Films, J. Agric. Food Chem. 52 (2004) 1933–1939. doi:10.1021/jf034612p.

[42] C. Pastor, L. Sánchez-González, A. Chiralt, M. Cháfer, C. González-Martínez, Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol, Food Hydrocoll. 30 (2013) 272–280. doi:10.1016/j.foodhyd.2012.05.026.

[43] D. Altiok, E. Altiok, F. Tihminlioglu, Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications, J. Mater. Sci. Mater. Med. 21 (2010) 2227–2236. doi:10.1007/s10856-010-4065-x.

[44] L. Sanchez-Gonzalez, C. Pastor, M. Vargas, A. Chiralt, C. Gonzalez-Martinez, M. Chafer, Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes, Postharvest Biol. Technol. 60 (2011) 57–63. doi:10.1016/j.postharvbio.2010.11.004.

[45] S. Woranuch, R. Yoksan, Preparation, characterization and antioxidant property of water-soluble ferulic acid grafted chitosan, Carbohydr. Polym. 96 (2013) 495–502. doi:10.1016/j.carbpol.2013.04.006.

[46] X.F. Liu, Y.L. Guan, D.Z. Yang, Z. Li, K.D. Yao, Antibacterial Action of Chitosan and Carboxymethlyated Chitosan, J. Appl. Polym. Sci. 79 (2000) 1324–1335. doi:10.1002/1097-4628(20010214)79:7<1324::AID-APP210>3.0.CO;2-L.

[47] Z. Li, X.P. Zhuang, X.F. Liu, Y.L. Guan, K. De Yao, Study on antibacterial O-carboxymethylated chitosan / cellulose blend from LiCl / N , N-dimethylacetamide solution, Polym. Commun. 43 (2002) 1541–1547.

[48] J.M. Hamilton-Miller, Antimicrobial properties of tea (Camellia sinensis L.)., Antimicrob. Agents Chemother. 39 (1995) 2375–7. doi:10.1128/aac.39.11.2375.

[49] T.S. Yam, S. Shah, J.M.T. Hamilton-Miller, Microbiological activity of whole and fractionated crude extracts of tea (Camellia sinensis), and of tea components, FEMS Microbiol. Lett. 152 (1997) 169–174. doi:10.1016/S0378-1097(97)00197-3.

[50] T. Shimamura, W. Zhao, Z. Hu, Mechanism of Action and Potential for Use of Tea Catechin as an Anti- infective Agent, Science (80-.). 6 (2007) 57–62. doi:10.2174/187152107779314124.

CHAPTER 8

SUMMARY AND FUTURE SCOPE OF WORK

In the backdrop of rapidly depleting petroleum resources and increased environmental awareness, motivation for developing new eco-friendly Green composite materials has been at the helm of current research. Today Green composites have grabbed greatest attention of material Chemist, which are non toxic and biodegradable. They have inherent capacity to replace synthetic plastic materials in large scale. Recent advances in genetic engineering and composite sciences open up new avenues of research leading to the development of biodegradable packaging materials. Direct exploitation of bare biopolymers for packaging applications are prevented by many of its intrinsic shortcomings. Low barrier properties, brittleness etc are the major hurdles preventing them from wide application for packaging purposes. Cost effectiveness, easiness of fabrication also challenges for the utilization of biopolymers for packaging utilization. Still many windows are open and researches are actively involving to address each issue to come up with biopolymer composites as the future packaging materials.

In the present work we have fabricated two different types of Green composites, where polymer was Chitosan and the other materials were nano ZnO particles and Green tea extract. Both materials were prepared aiming to make use of them for 'smart packaging' applications. Raw meat, one kind of rapidly perishable food packed with prepared composite materials and shelf life studies were carried out. The whole work has three parts, the first two parts cover synthesis, characterization and packaging application studies of Chitosan-nano ZnO composite materials and the third part includes synthesis,

characterization and packaging application studies of Chitosan-GTE powder composite films.

Chitosan-nano ZnO composite material was synthesised in powder from to explicate all physico-chemical features of the novel composite material. The adopted synthesis method is completely abided by Green chemistry protocols. Cost effective one pot procedure was used to achieve the composite materials. ZnO nano particles were in situ formed in the Chitosan matrix and it was confirmed by FTIR, XRD and SEM analysis. FTIR spectra gave a clear picture of interaction between nano particles and Chitosan matrix. XRD showed characteristic peaks of nano ZnO particles in consistent with JCPDS data base. The thermal stability of the composite was examined by TGA. All composite materials have exhibited superior stability than Chitosan particles. Finally the particle size and the structure of ZnO were determined by XRD analysis. Band gap energy of the composite was determined from UV-Visible spectral analysis. The results show that nano particles still maintain its semiconducting behaviour even they were impregnated in the matrix. Since shelf life of raw meat directly related to number of microorganisms on meat surface, the antibacterial efficacy of composites against E.coli and S.aureus was tested using Zone inhibition method. It was proved that this composite has excellent antibacterial activity and could be used as a natural anti-microbial agent and photo catalyst.

Aiming to fabricate flexible pouches for extending the shelf life of raw meat, above prepared Chitosan-nano ZnO composite material was re synthesised in film form with same kind of chemicals. The synthetic

strategy was changed so as to get composite films in cost effective way without using toxic or hazardous chemicals. Fabrication film material was achieved at room temperature by sole-cast procedure. Four different composite films were prepared by varying the amount precursor Zinc acetate. Characterization of films with various Physico-Chemical methods were carried out and compared with data obtained from Chitosan-nano ZnO powder. Presence of nano ZnO particles in the Chitosan matrix was proved well by FT-IR (ATR), XRD and SEM. Enhanced thermal stability of composite films was established by the TGA and DSC techniques. Sensitive property such as Water sorption ability of films was calibrated and compared with pure Chitosan film. Solubility tests proved that all composite films were less soluble than bare Chitosan film and the solubility of composite films varied inversely with the amount of ZnO particles. The usefulness of composite films to substitute current polythene bags to store meat and related items was analyzed. The C-2 bag came out to be a star candidate by ensuring a total inhibition of bacterial growth. The proposed film exhibited a great potential in the future of packing pouches for meat via promising its impact on extending the shelf-life of raw meat and controlling spoilage through complete inhibition of bacterial growth.

Synthesis of Chitosan-GTE composite material was other aim of present work. Green tea extract is material naturally available in abundant and posses excellent antioxidant and antimicrobial activity. This composite will fall under the category of Green composite with specific properties. The packaging pouches fabricated by this

composite will have smart properties like antimicrobial and antioxidant activities. To optimize a suitable candidate for packaging applications five different Chitosan-GTE powder composite films were synthesized by a simple one pot procedure. All films were well characterized by FT-IR (ATR), XRD and SEM. Physical, thermal and mechanical features of composite films were evaluated and compared with pure Chitosan films. It was observed that incorporation of GTE powder has improved all properties, but not in linear fashion. The smartness of composite films towards antimicrobial and antioxidant properties was calibrated by in-vitro analysis. DPPH scavenging activity studies have undoubtedly proven enhanced antioxidant ability of composite films. Similarly, antimicrobial studies also confirmed the ability composite films to inhibit the growth of Gram-positive and Gram-negative bacteria. Finally, the usefulness of composite films to substitute current polyethylene bags to store meat and related items were analyzed. The C4 composite film was crafted to pouch like packaging material for investigating the potential of film to enhance the shelf life of raw meat. The result was highly encouraging that the rate of bacterial growth in meat samples stored in composite pouches was significantly lower than that of polyethylene pouch.

Two composite materials have significant attributes suitable for packaging of raw meat. Even though both are entirely different materials some sort of comparisons are possible. Both can be fabricated at room temperature and follow green chemistry protocols. Physically Chitosan-nano ZnO composite films seem stronger than Chitosan-GTE composite material. The solubility of Chitosan-GTE

films are far higher than that of Chitosan-nanoZnO composite films.GTE extract composite films are coloured but Chitosan-nanoZnO composite films are colourless. The magnitudes of antimicrobial and antioxidant properties of Chitosan-nanoZnO composite films are lesser than that of Chitosan-GTE composite films. Fabrication of pouches is easier in the case of Chitosan-nanoZnO composite films as compared to Chitosan-GTE composite films. The shelf life of composite Chitosan-GTE composite films is lesser as compared to Chitosan-nanoZnO composite films.

Emerging social concerns and growing environmental awareness throughout the world triggered the search for new materials with similar qualities of synthetic plastics. Each day novel applications and composites are emerging from the biopolymer research to foster the need of end consumers. But instead of much recent advancement, the direct applications of many biopolymer composites are struck with many hurdles. Here, our composite material also requires further intense investigations in diverse fields for future applications. Present study was focused on synthesis and characterization of two novel composite packaging materials for extending the shelf life of raw meat. The adopted procedure of synthesis was simple sole-cast method. Even though adopted procedures are simple, we didn't investigate the possibility melt mixing or other techniques for the preparation of these composite materials. The studies we have undertaken is preliminary, of course more investigations are required before the industrialization of composite materials. Some of the most important areas to be focussed in future are summarized below.

- Generally food packaging materials most cost-effectively fabricated by melt mixing methods. There is a challenge of stabilizing nano particles and Chitosan at high temperatures without compromising its specific properties. Hence future studies must focus on this alternative way of synthesis and applications.

- The release of active molecules from the film surface may surpass restriction limits underscored by current food legislations; hence there is a call for further studies to envisage the kinetics and mechanism of release of functional molecules.

- Instead of mere preservation studies, it is recommended to extend the studies towards the impact of film on qualities of packed food.

- Investigate barrier properties of these composite films and optimise barrier limit for most favourable packaging conditions.

- It would be very interesting if compare the barrier properties of composite films with existing synthetic packaging materials such as polyethylene, polypropylene etc.

- The studies can extend towards various moulding techniques so as to obtain different shaped and sized packaging materials.