Antimicrobial nanopackaging for food products: Prospects and limitations

Jasim Ahmed, Ph.D.
Environment & Life Sciences Research Center
Kuwait Institute for Scientific Research
KUWAIT

*The School of Packaging
Michigan State University, USA

Layout of the presentation

- Importance of Antimicrobial Packaging
- Nanoparticles as Antimicrobials
- Nanopackaging
- Essential oil as Antimicrobials
- Combinations of NPs and EOs
- Applications and limitations

Our da

Company	Product #	Death	Fatal injury	Loss
Maple Leaf Canada-2008 Listerosis	220	13	38	$20 Million
E. Coli –outbreak *2011* Germany	Vegetables	22	2400	Huge
US in 2019 Turkey 2019	Beef/basil/ tahini…. Spinach	- ??	-	-

Food borne disease

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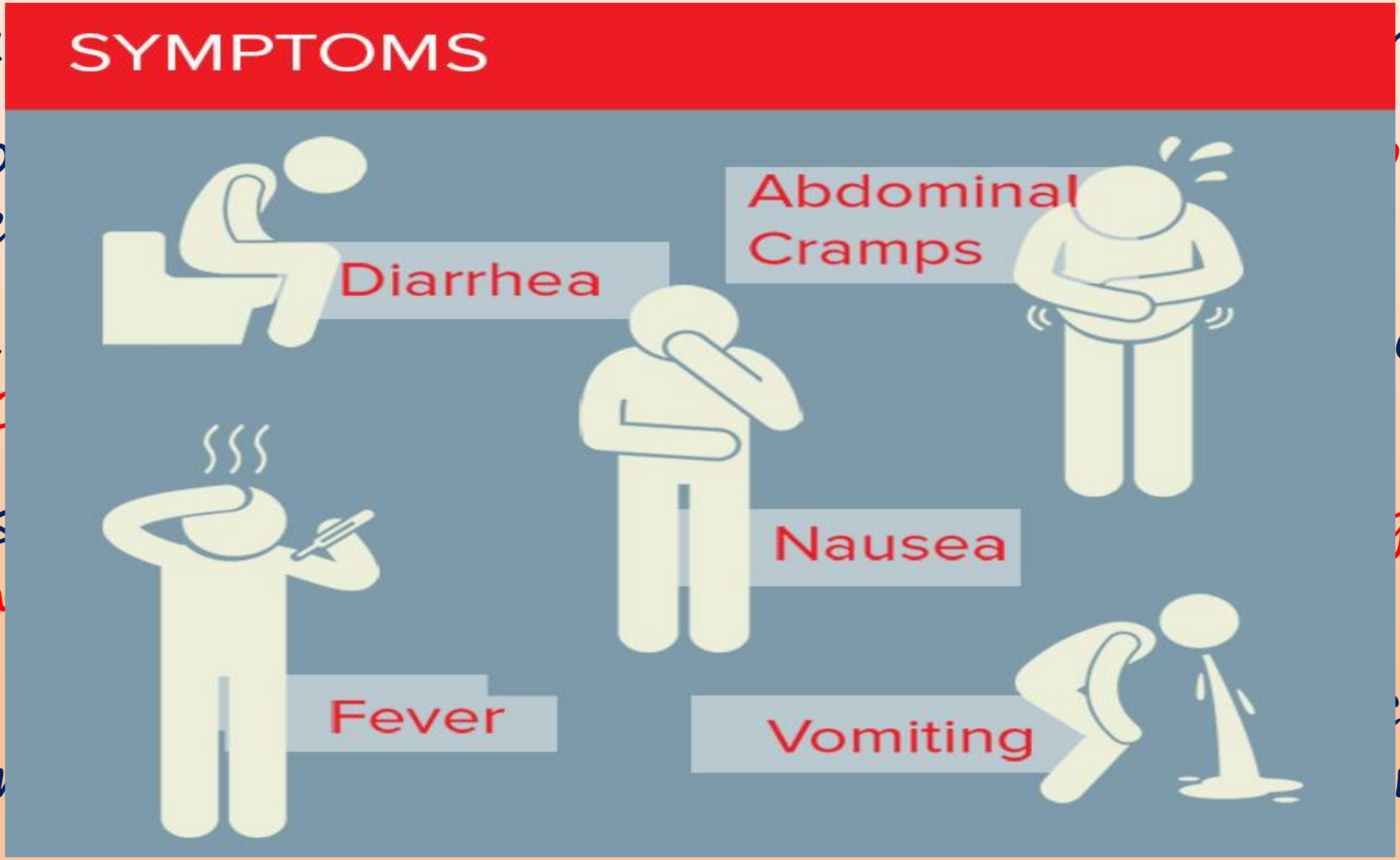

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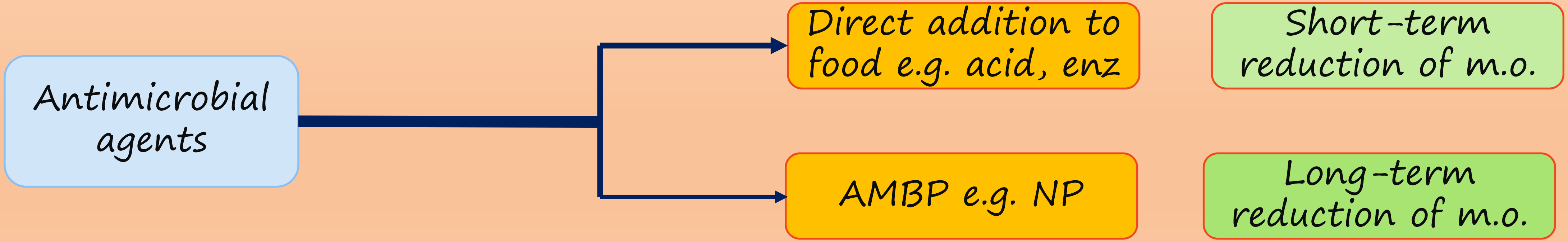
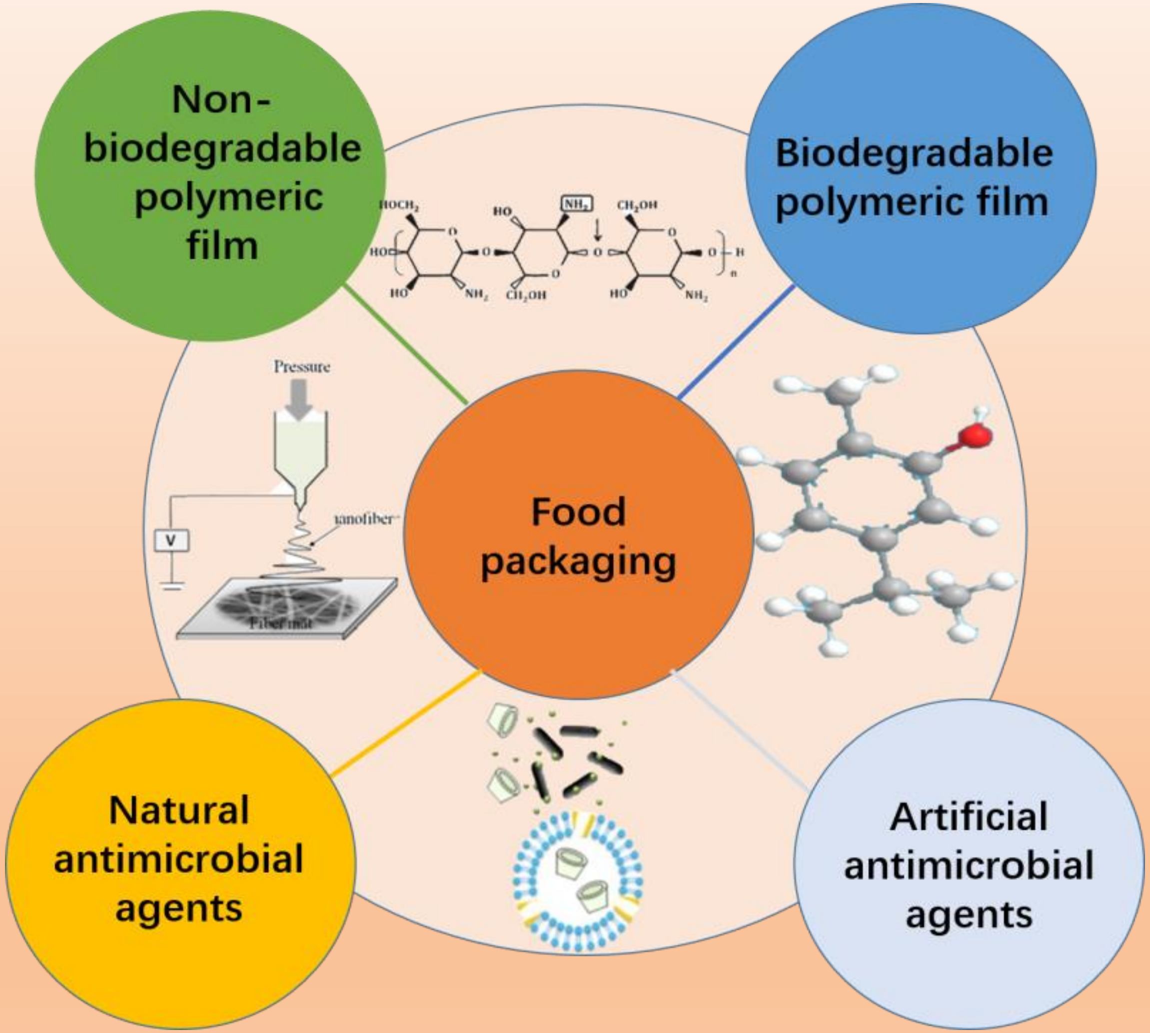


Food Packaging and Antimicrobial agents



Ref: Huang, T.; Qian, Y.; Wei, J.; Zhou, C. Polymeric Antimicrobial Food Packaging and Its Applications. *Polymers* **2019**, *11*, 560.

Why do we need Biodegradable Packaging?

World

A tsunami of plastic waste is about to be unleashed on the world, because China will no longer be the West's rubbish dump

China's decision to stop importing about half the world's scrap looks set to collapse the recyclables market and have profound implications for household recycling schemes around the globe

The Washington Post
Published: 12:11pm, 21 Jun, 2018

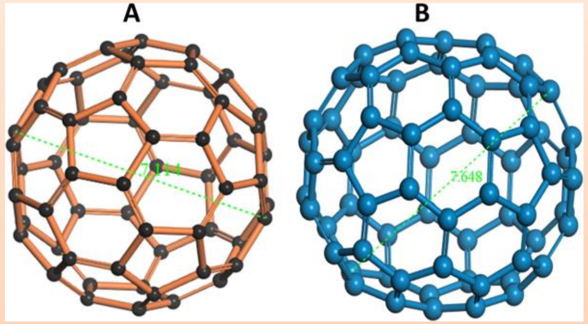
Packaging Nanotechnology

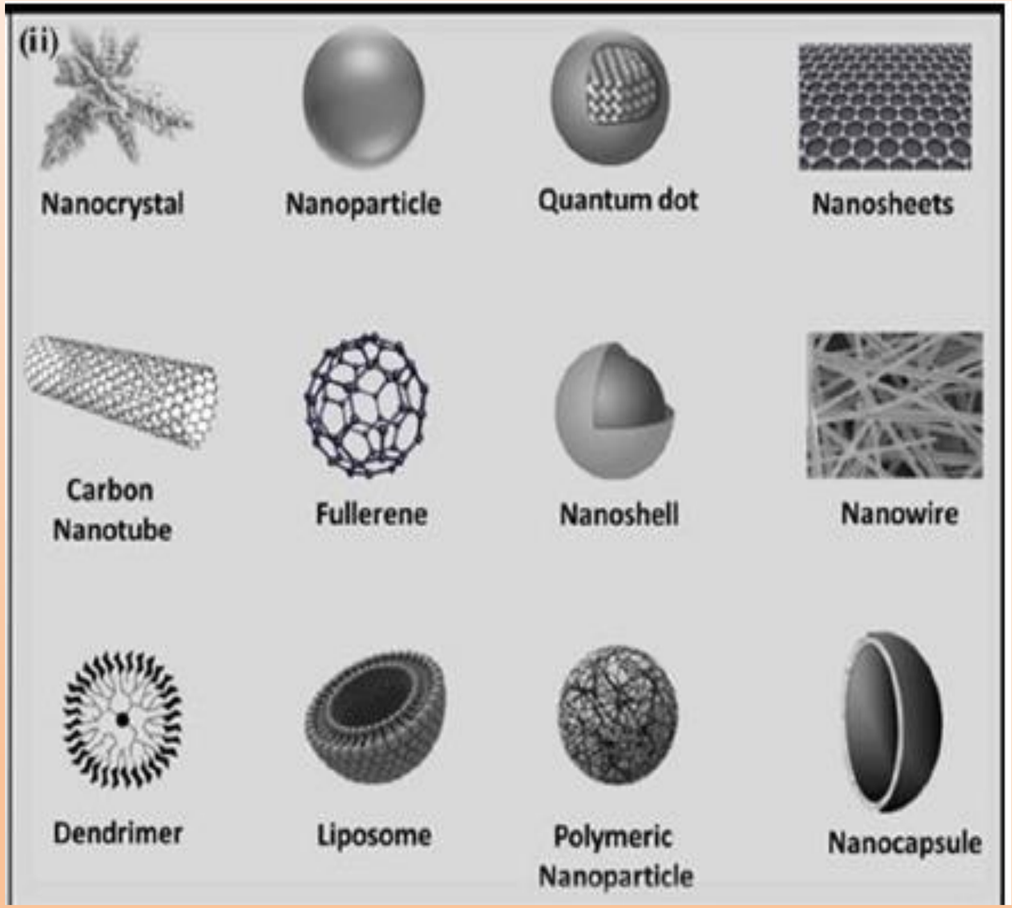
- Packaging Nanotechnology is an interdisciplinary area of research, development & industrial activity which involves the design, manufacture, processing and application of packaging materials filled with particles with nanometer dimensions.

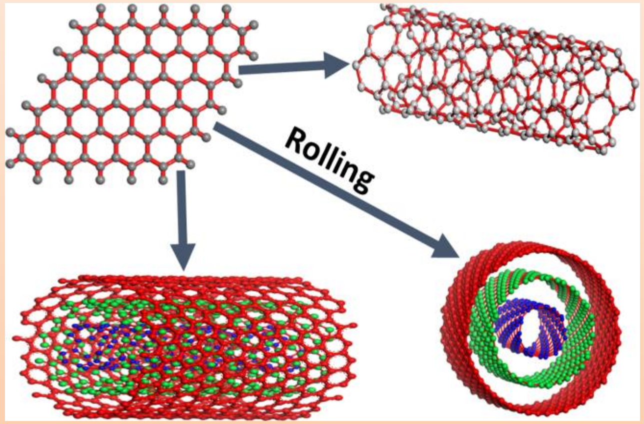
  –Improved packaging: NPs mix into the polymer matrix to improve the gas barrier properties e.g., polymer/clay nanocomposites.

  –Active packaging: NPs interact directly with the food or the environment to allow a better protection of the food, such as Ag NP as potent antimicrobial agents.

Shapes of nanoparticles







Types of nanoparticles used in food packaging

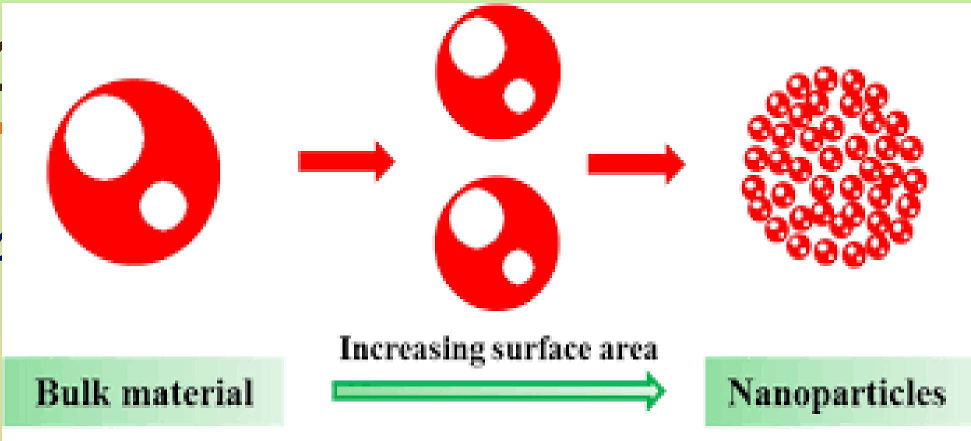
Inorganic

- Metals and metal oxides, clay nanoparticles & many more.
- Ag, Cu, CuO, ZnO, TiO_2, MgO and Fe_3O_4

Organic

- Phenols, halogenated compounds, quaternary ammonium salts, plastic polymers,
- Natural polysaccharide or protein materials such as chitosan, chitin, cellulose and whey protein isolates

Nanoparticles as antimicro



- NPs demonstrated the effective bactericio
- Because of their extremely large surface better contact with the microorganism.
- Close or direct contact with the microorganism is an essential requirement for the mechanisms of action of the antimicrobial agents
- The contact can happen:

i) For a nonvolatile substance: it diffuse and solubilize into the food surface in which the microorganisms are located.

ii) For a volatile substance: the headspace around the surface of the food and in the food itself absorb by indirect contact.

Nanoparticles and microbial inactivation mechanism

- NPs can inhibit microbial growth by:

    a) Influencing the protein structure by denaturation or alteration

    b) Altering the cell membrane proteins or membrane lipids

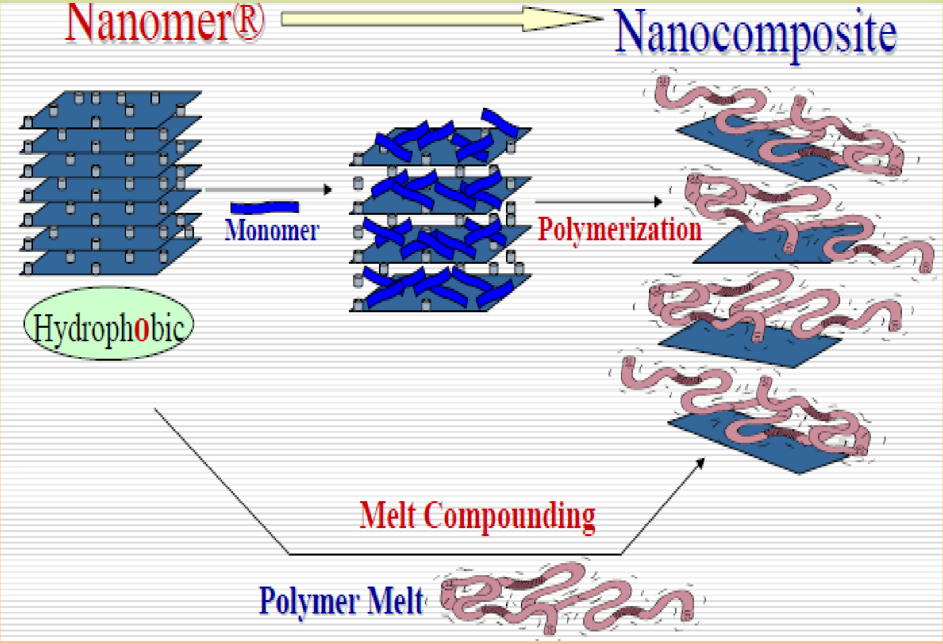
    c) Blocking the synthesis of cell wall components

    d) Preventing replication, transcription, and translation of nucleic acids

    e) Disrupting cellular metabolism

Nanocomposites (NC) and its formation

NC forms by incorporation of particulate fillers (e.g. clay/metal) into neat polymer matrix.



Courtsey: Nanocor

Nanocomposite Preparation techniques:

- Solvent casting

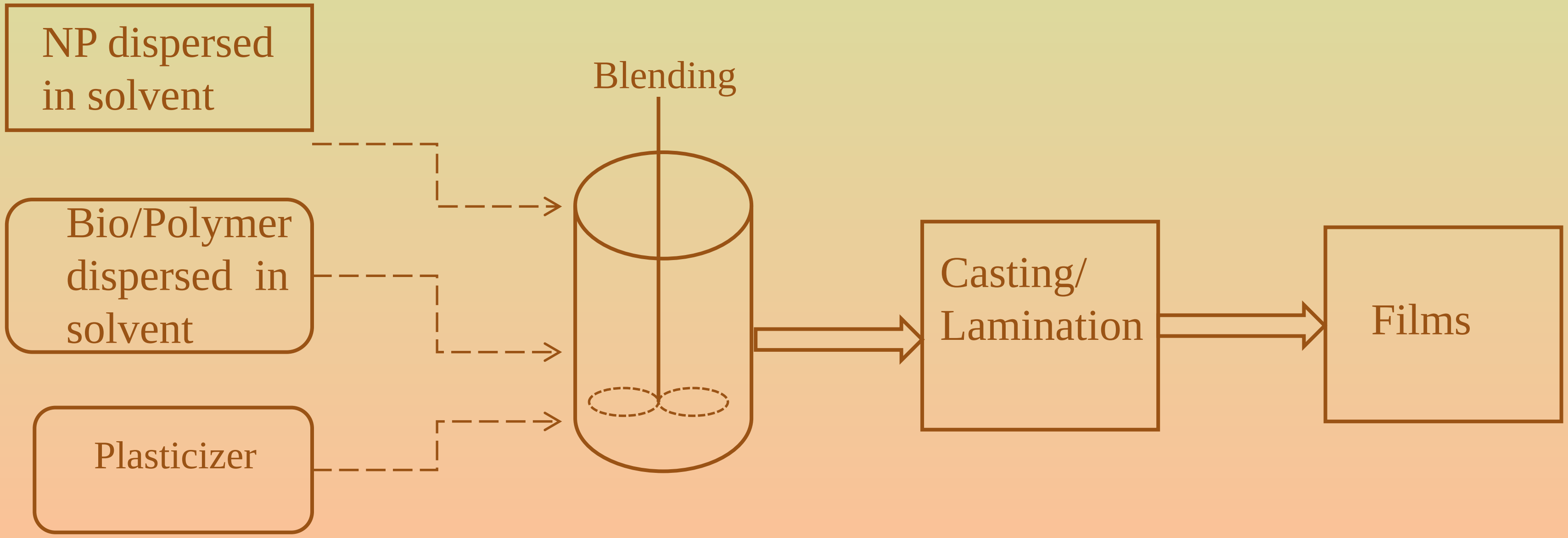
-Simple process

- Melt extrusion

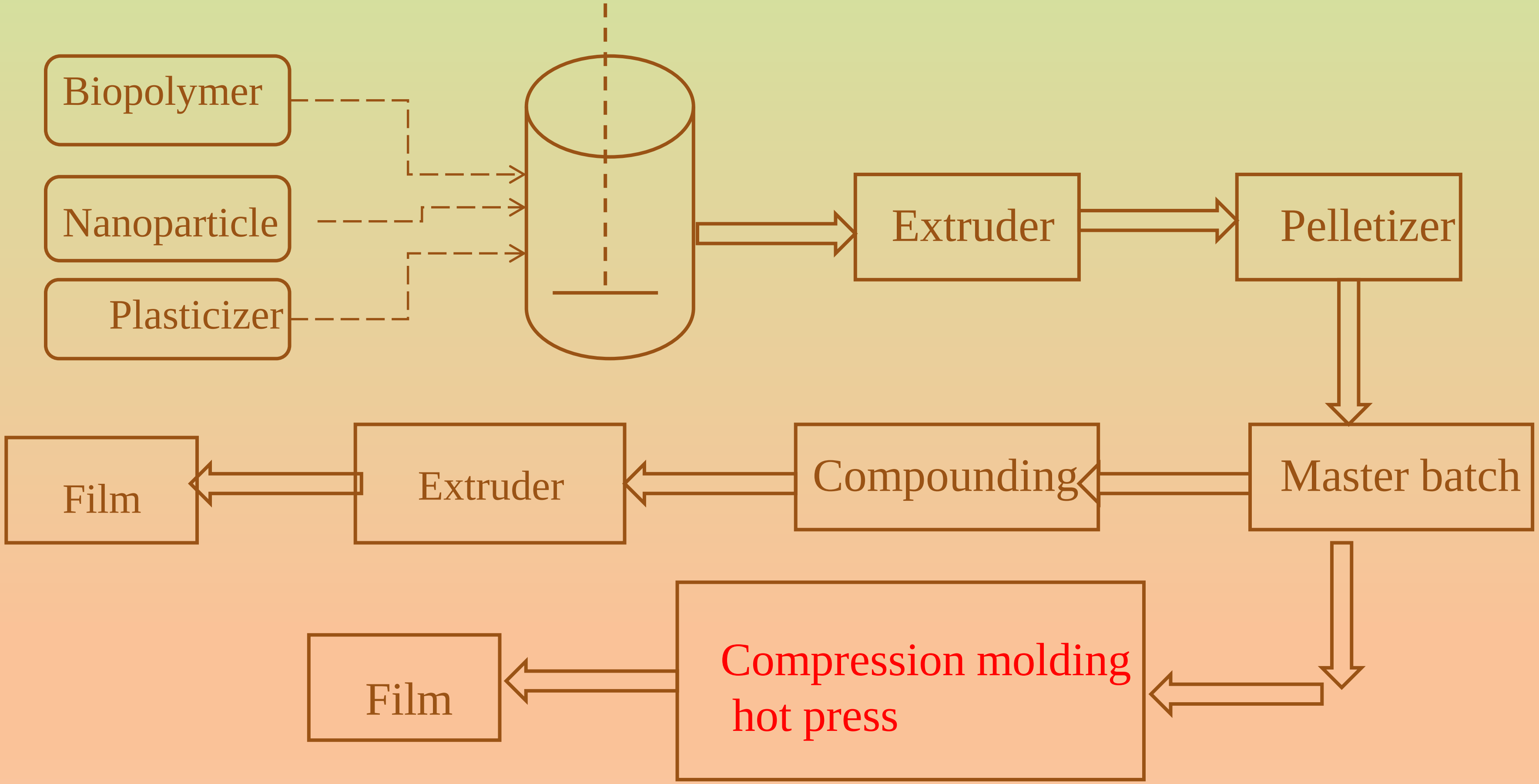
-Heat above melting point and extrude

- In situ polymerization

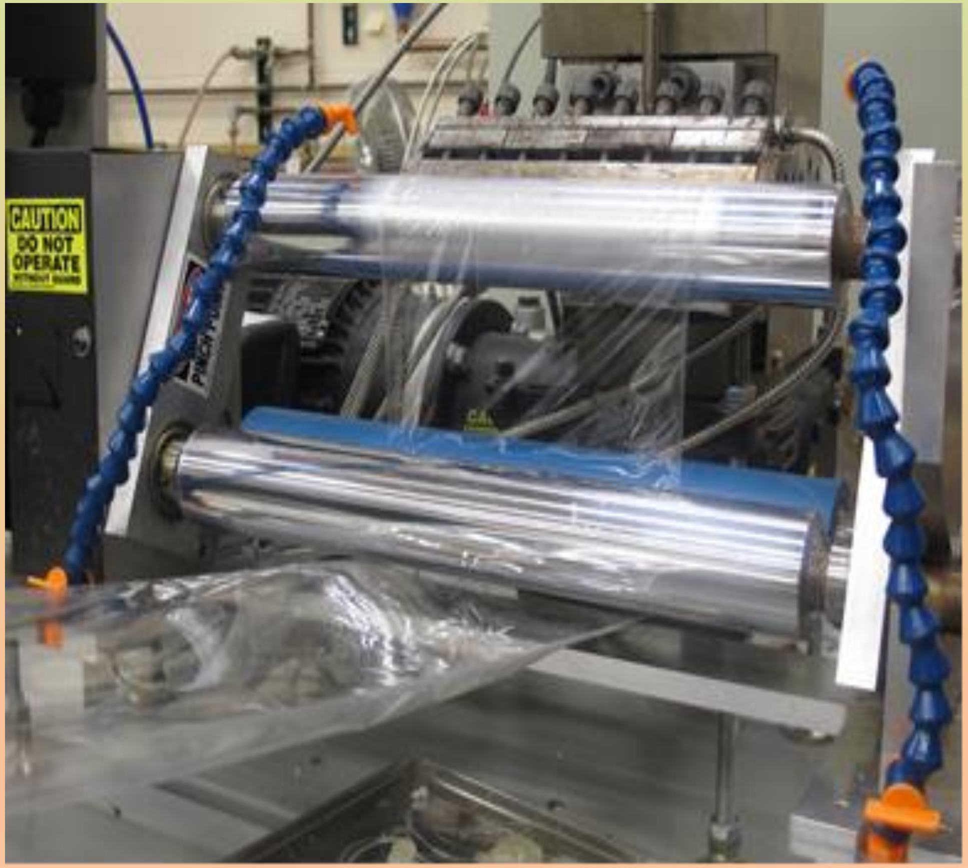
Nanocomposite film preparation by solvent casting



Antimicrobial-nanocomposite preparation by extrusion



Extrusion of PLA-based films & Packaging Development



Biodegradable Antimicrobial Nano-packaging-My lab

Materials

- Polylactides (PLA)
- Agar

Brittle, poor in thermo-mechanical properties
Needs property improvement

Nanoparticles

- Zinc Oxide (ZnO)
- Surface treated ZnO
- Ag/Cu Alloy
- Graphene Oxide (GO)
- Nanoclay

Plasticizers

- Polyethylene glycol (PEG)
- Glycerol

Characterization of composites and films

Thermomechanical and microstructural Properties

- Melt Rheology
- Thermal Analysis (DSC &TGA)
- Tensile Measurement
- Microstructure

-SEM/TEM/AFM

-XRD

-FTIR

Film Barrier Properties

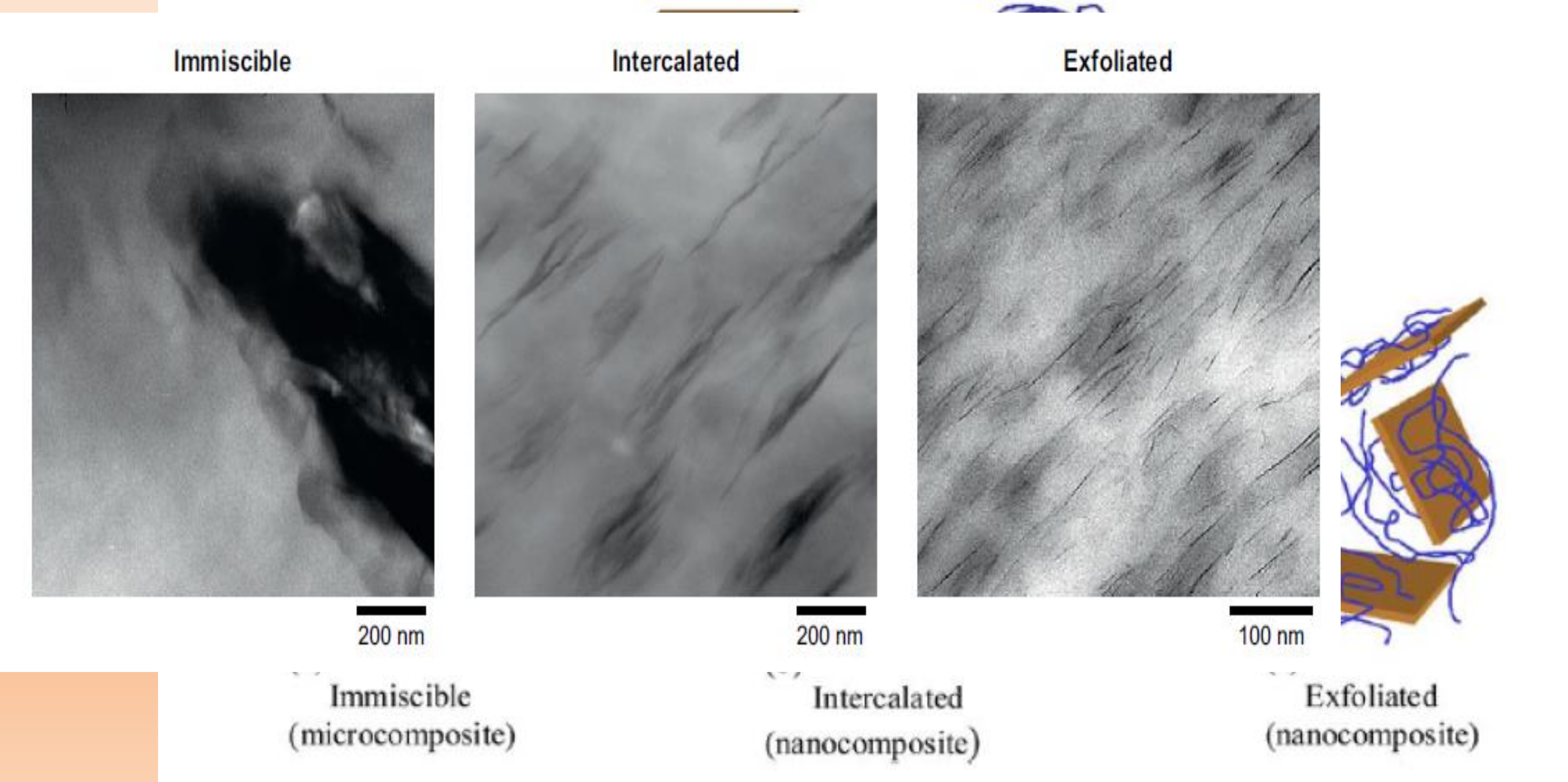
- Water vapor transmission rate (WVTR)
- Oxygen transmission rate (OTR)

Antimicrobial Analysis

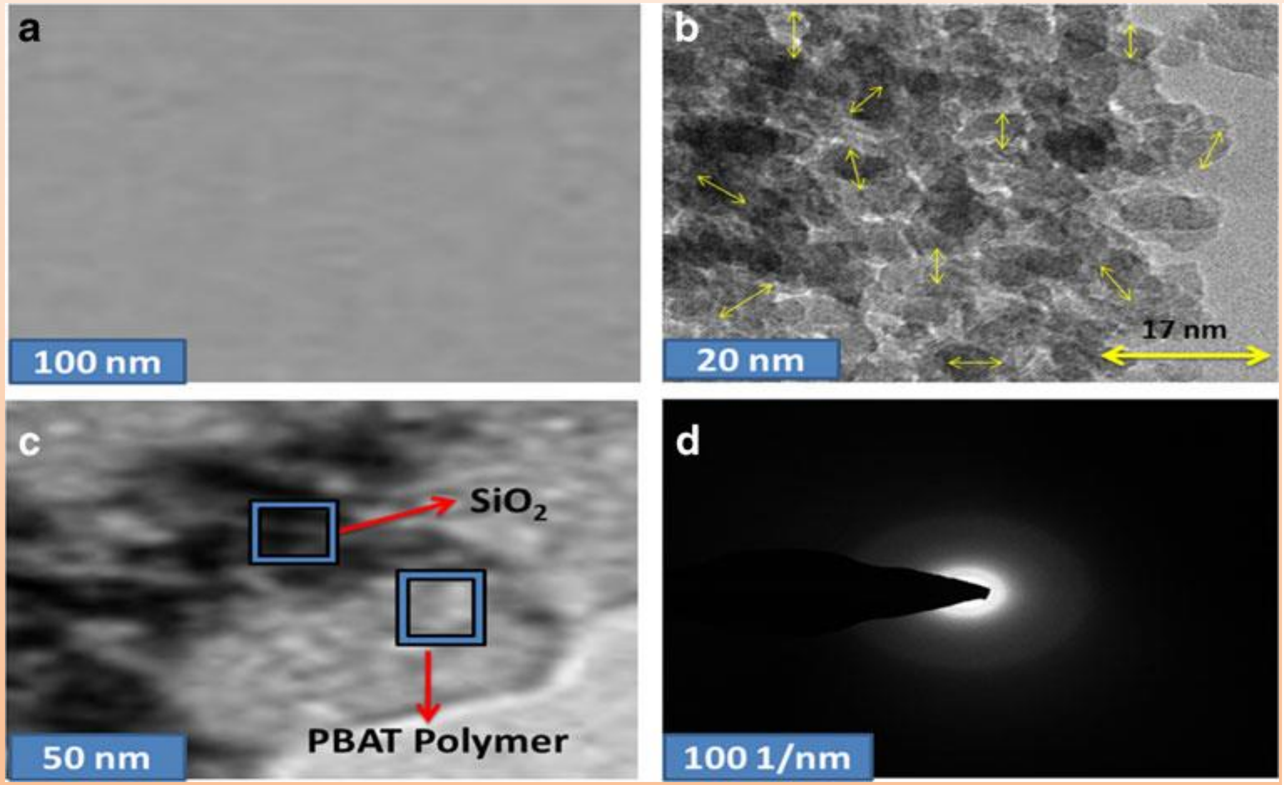
- Against gm +ve test organisms
- Against gm –ve test organisms

Nanoparticles dispersion in polymer system

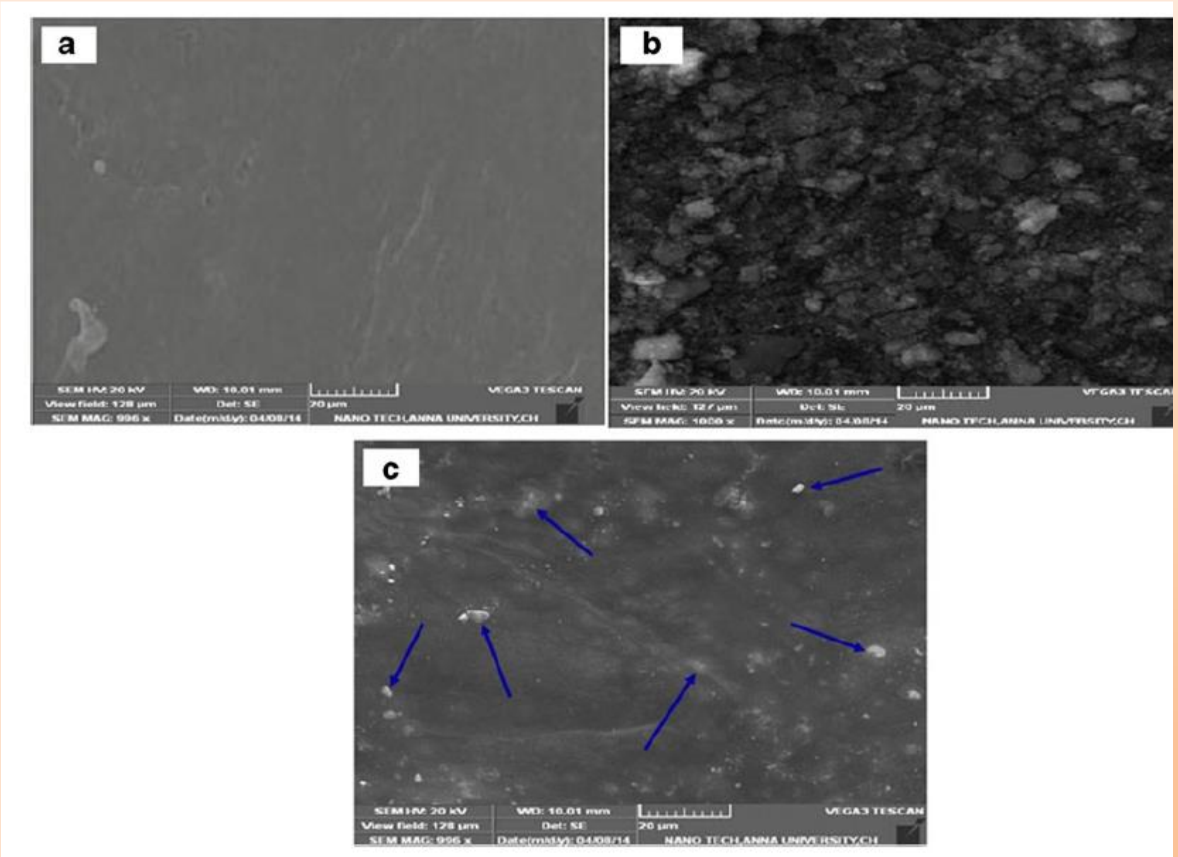
Distribution, property improvement and safety are the main keys for the nanoparticles.



Poly(butylene adipate-co-terephthalate)/SiO2 Films



TEM of **a** PBAT, **b** SiO_2 NPs, **c** PBAT/SiO_2, **d** SAED pattern of PBAT/SiO_2 NC film



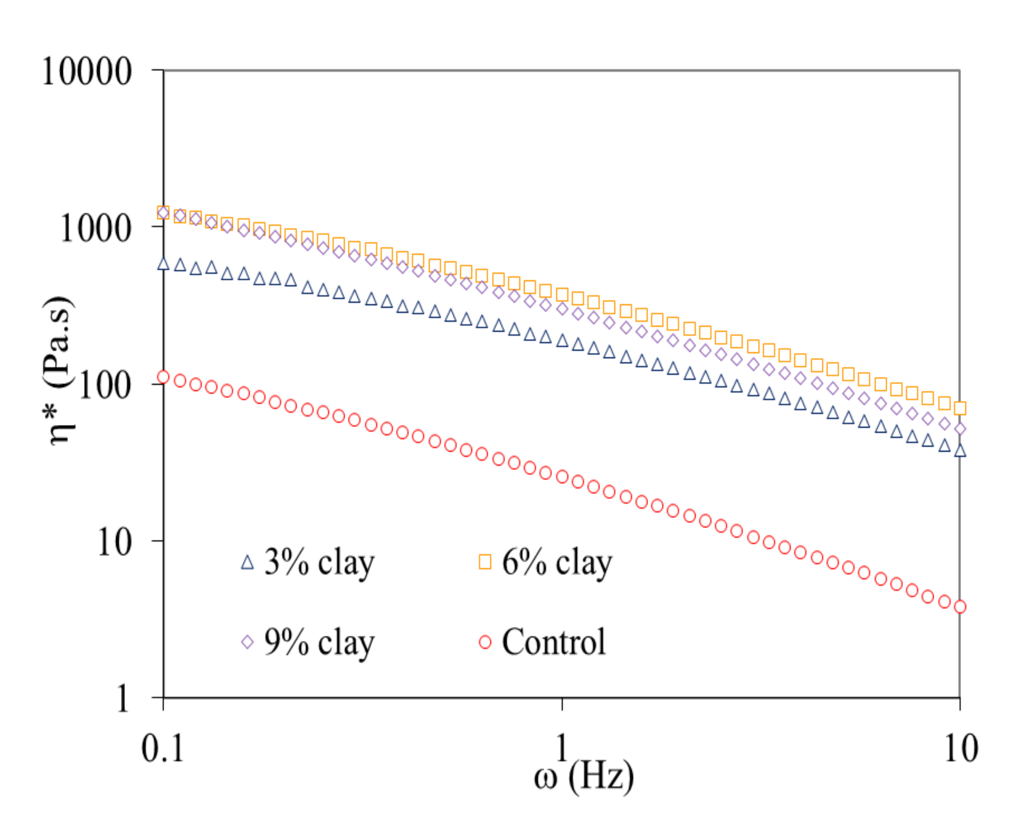
SEM of **a** PBAT, **b** SiO_2 NPs, **c** PBAT/SiO_2 NC film

Ref: Venkatesan & Rajeswari, 2015 Silicon

Melt rheology of Biopolymer Films

- Melt rheology provides a deep scientific and engineering understanding of how thermoplastics behave at melt whilst being processed.
- Study provides information on the processability of polymers or composites and also predict the dispersibility of fillers in the composite.

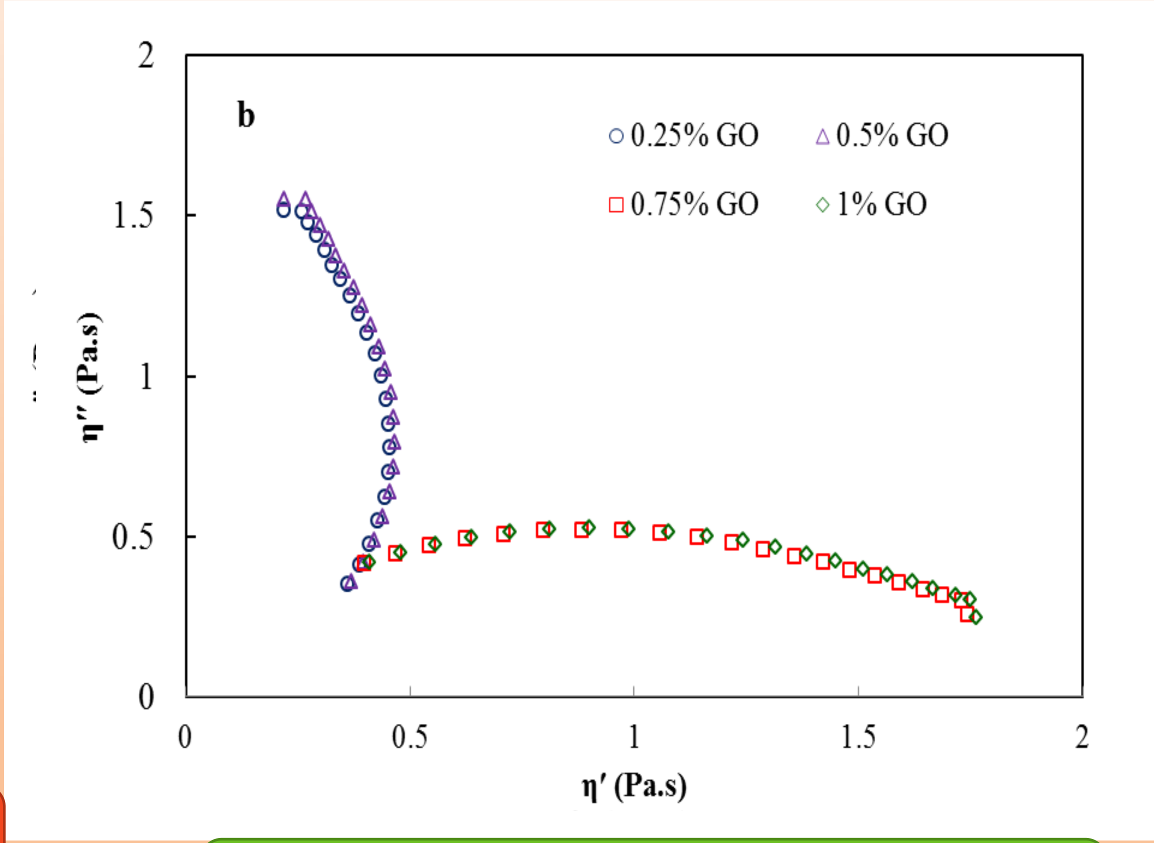
Melt rheology of PLA/NC films at 190 °C



Mechanical property increases with clay concentration

Ref: Ahmed et al. (2010) J Fd Sci

Cole-cole plot (PLA/clay & PCL/GO)

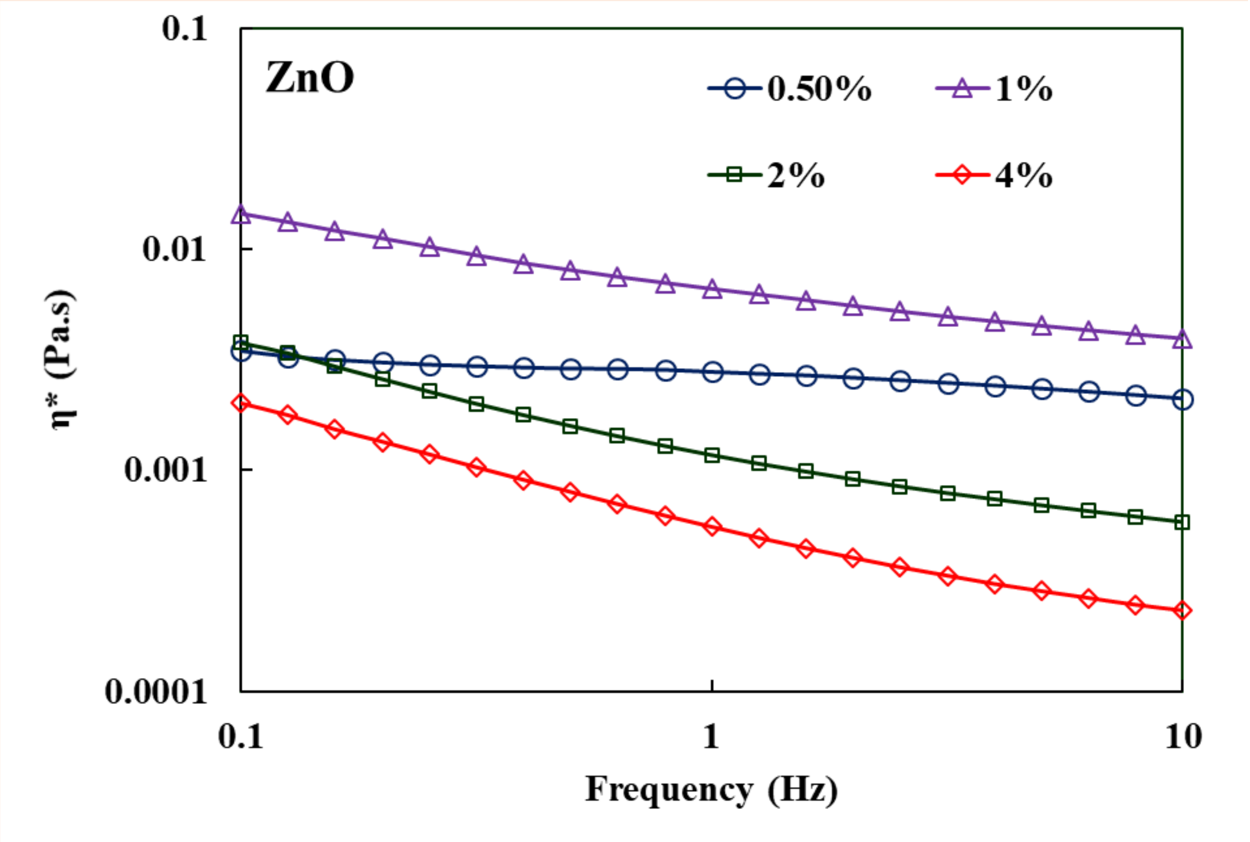


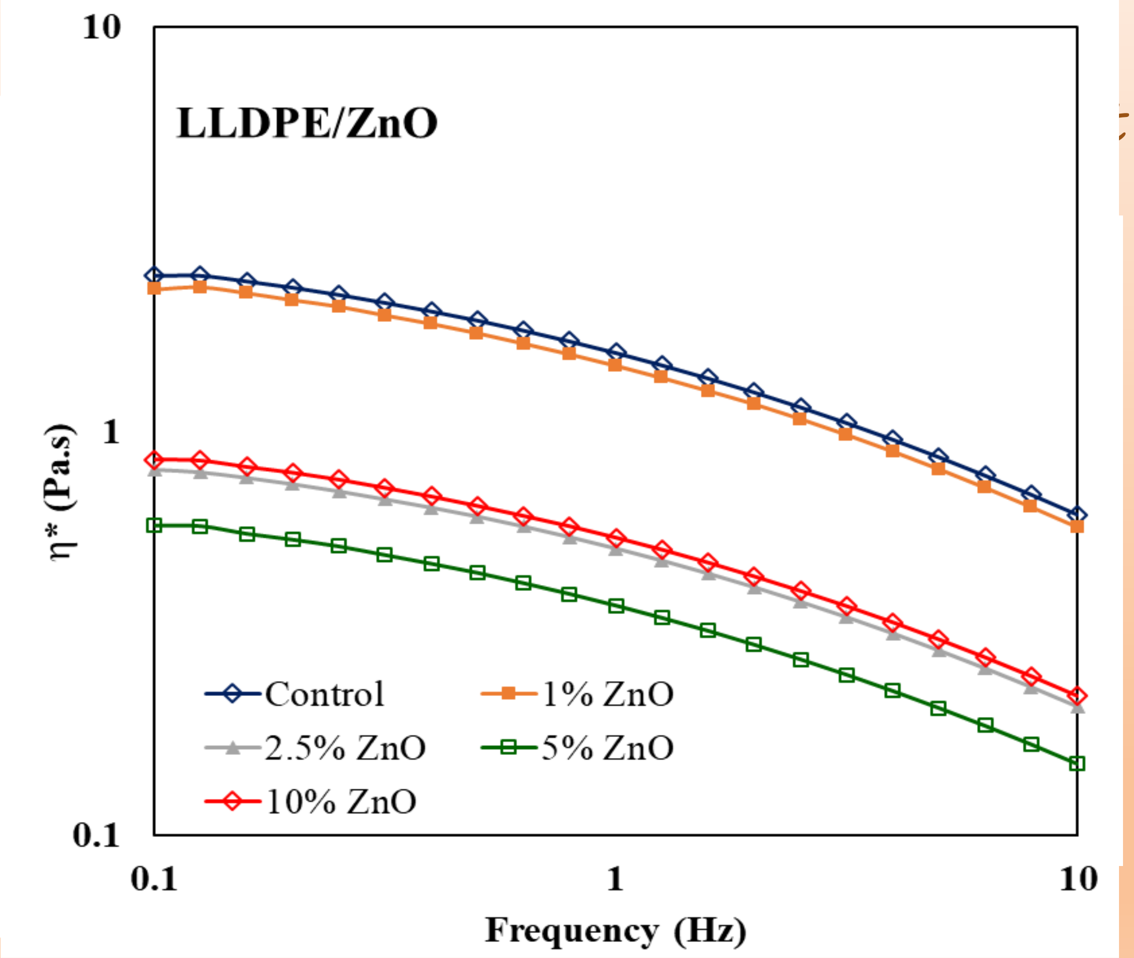
Semi-circular curve indicates uniform distribution of NP

J. Ahmed et al. *Thermochimica Acta 659 (2018) 96–104*

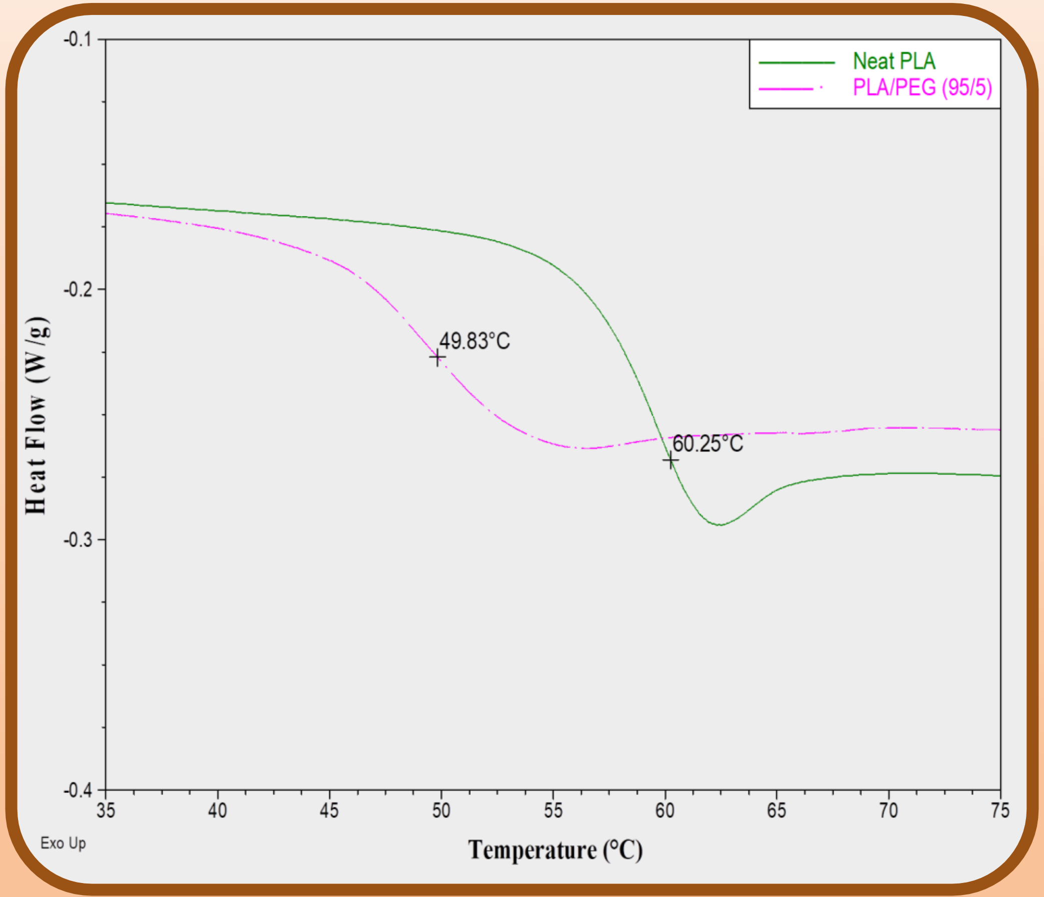
PLA/PEG/ZnO melt rheology

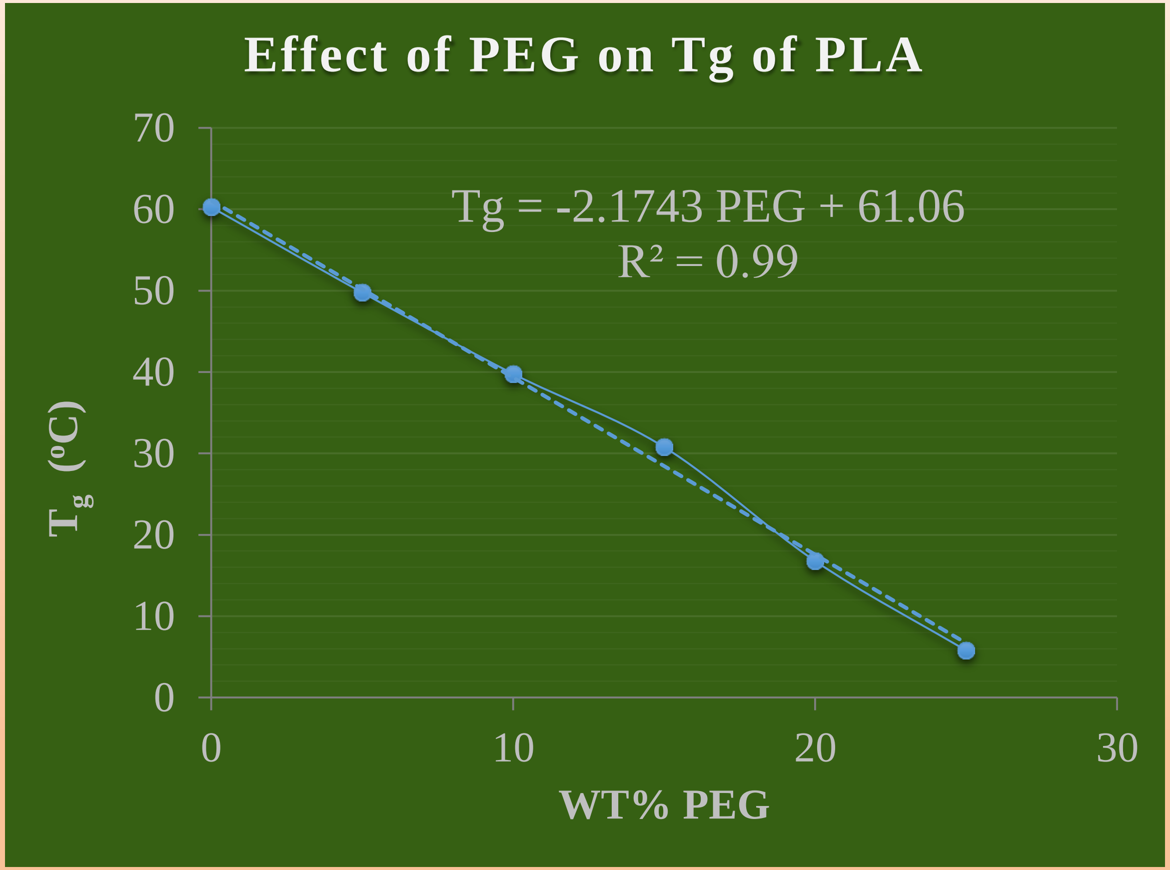
Melt rheology at 170 °C



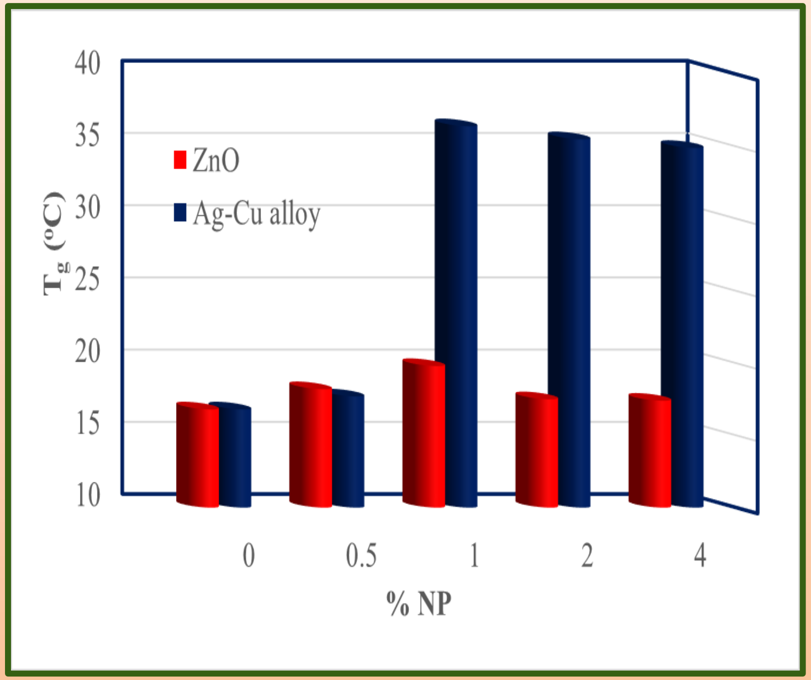


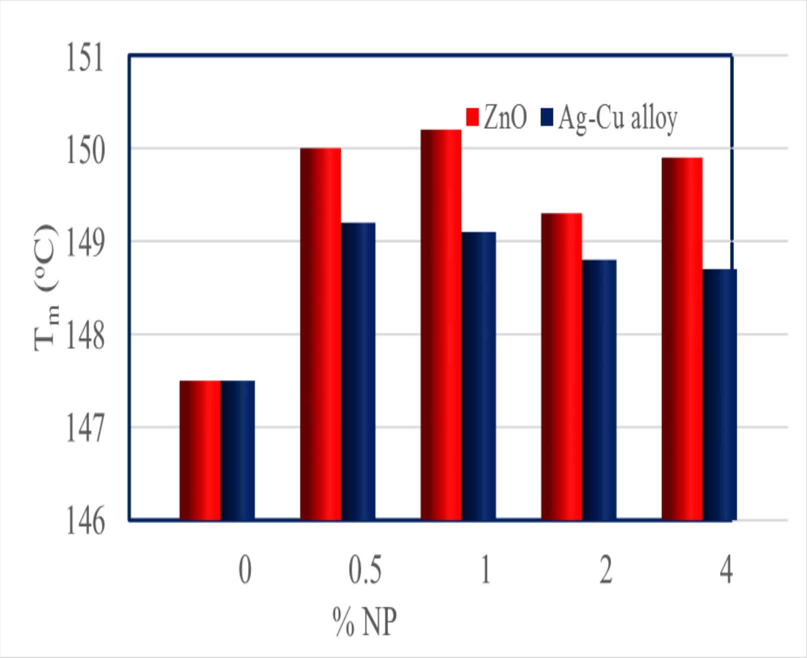
Plasticization of Polyethylene glycol to PLA and the Tg

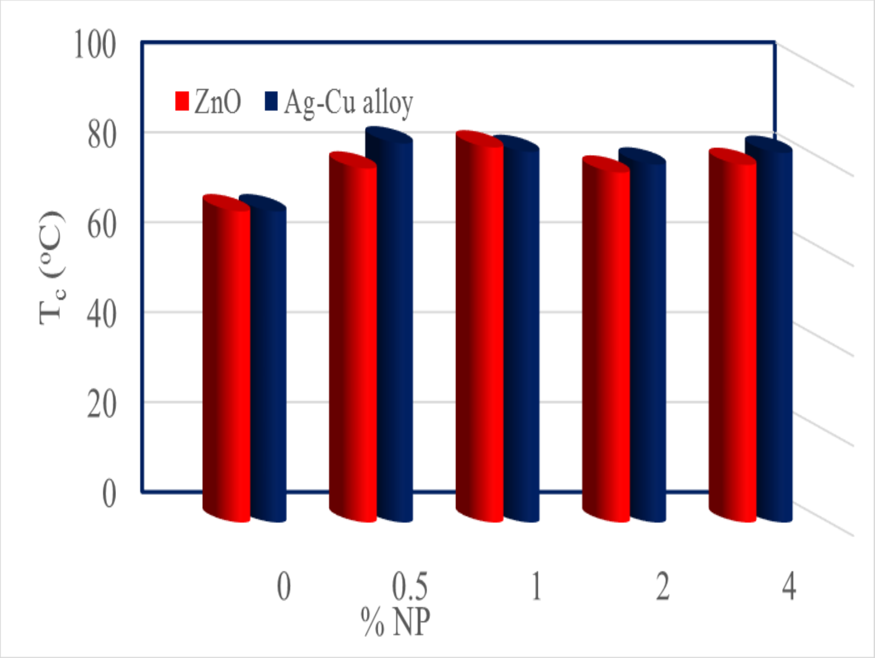




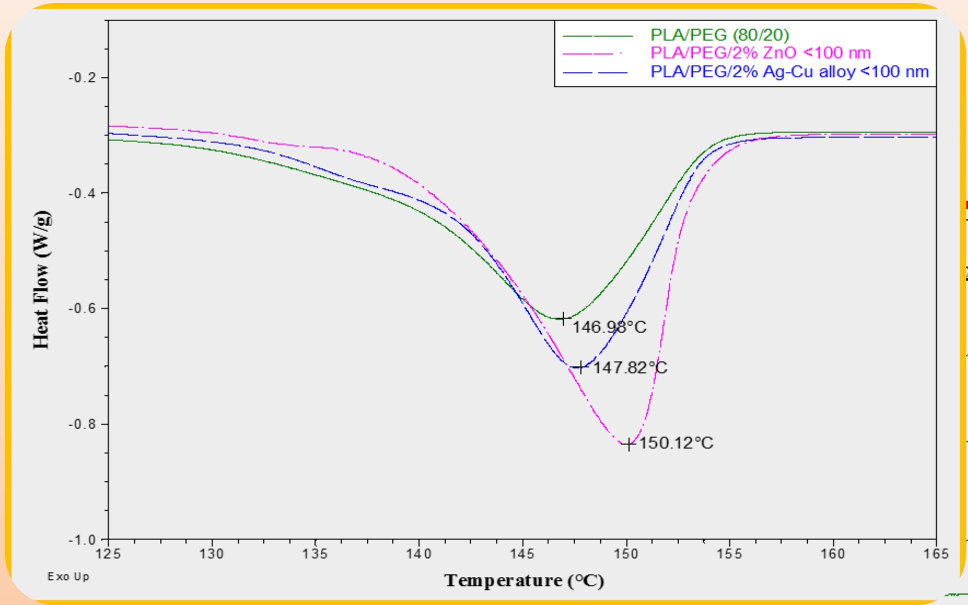
Effect of metallic NP concentration on thermal properties of PLA/PEG NC films

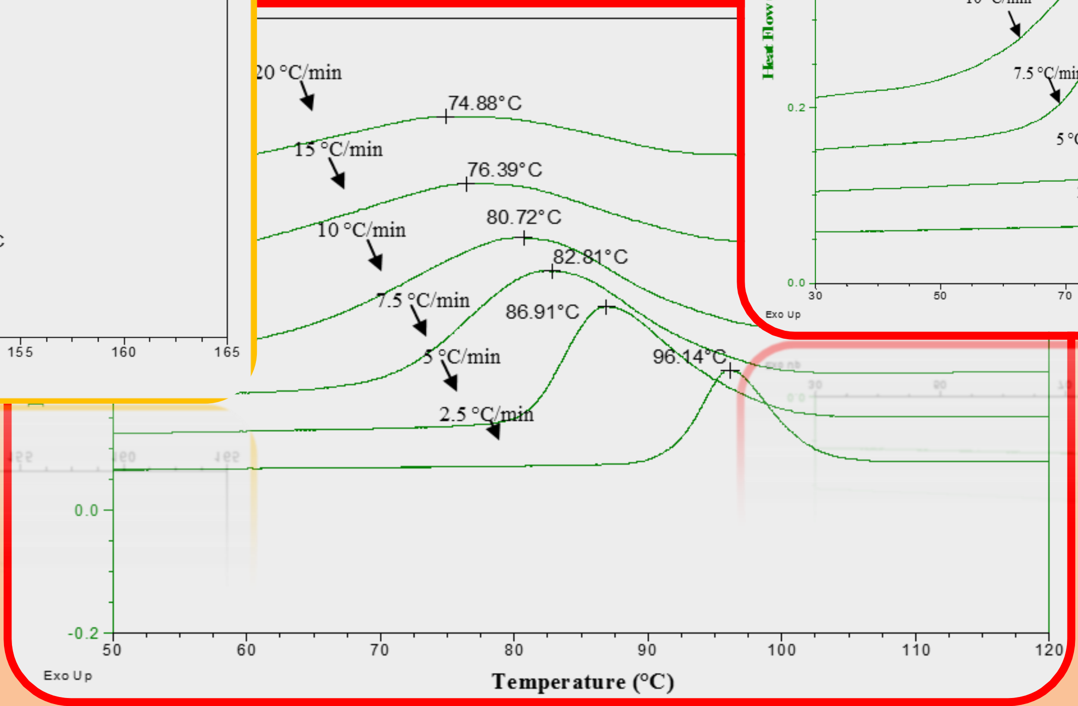




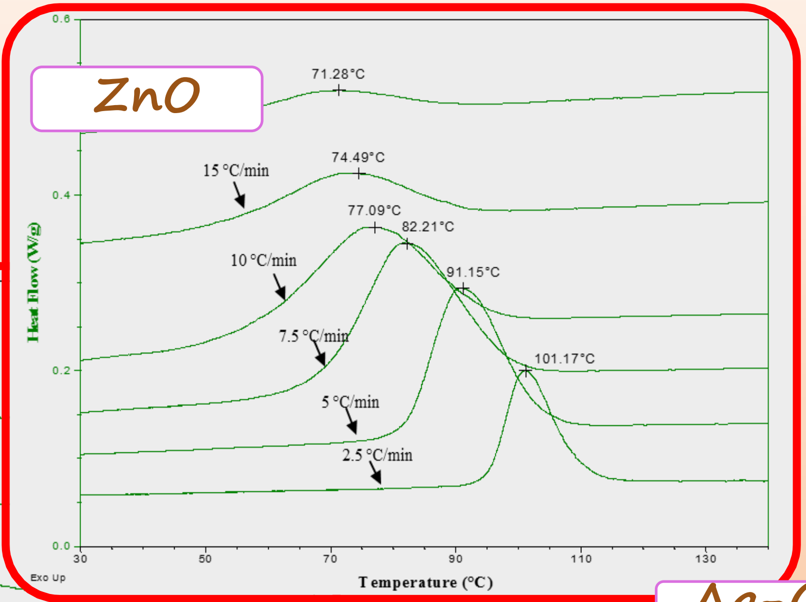


Effect of NP on melting and crystallization of PLA/PEG/NP





ZnO



Ag-Cu alloy

Mechanical properties of biocomposite films

Sample	Thickness (mm)	Tensile strength (MPa)	EAB (%)	Modulus (MPa)
PLA/PEG (80/20)	0.52±0.03	18.91±0.91	12.33±1.28	1312.3±28.68
PLA/PEG/2% ZnO-50nm	0.63±0.02	24.41±1.31	5.46±1	1613.23±31.5
PLA/PEG/2% ZnO-100nm	0.69±0.03	22.63±0.86	7.96±0.55	1541.8±23.69
PLA/PEG/2% Ag-Cu-100nm	0.71±0.05	23.59±1.1	10.93±2.18	1502.86±13.62

Film sample	Thickness (mm)	Tensile strength (MPa)	EAB (%)
Control FSG	0.061±0.002[e]	19.2±1.2[c]	54.2±2.1[a]
FSG/0.5% Ag-Cu NPs	0.069±0.003[d]	20.4±0.8[bc]	50.7±3.8[ab]
FSG/1% Ag-Cu NPs	0.077±0.002[c]	23.9±2.6[b]	45.4±2.4[bc]
FSG/2% Ag-Cu NPs	0.089±0.004[b]	28.6±1.7[a]	40.6±3.9[c]
FSG/4% Ag-Cu NPs	0.098±0.002[a]	19.6±3.1[c]	32.9±2.8[d]

Transparency of agar and fish skin gelatin films with Ag-Cu NPs

Control

1% Ag-Cu

2% Ag-Cu

4% Ag-Cu

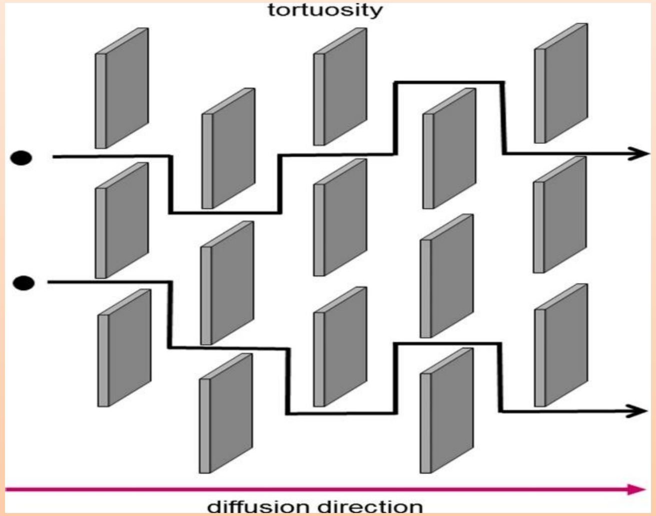
Control

2% Ag-Cu

4% Ag-Cu

Barrier properties of nanocomposite films

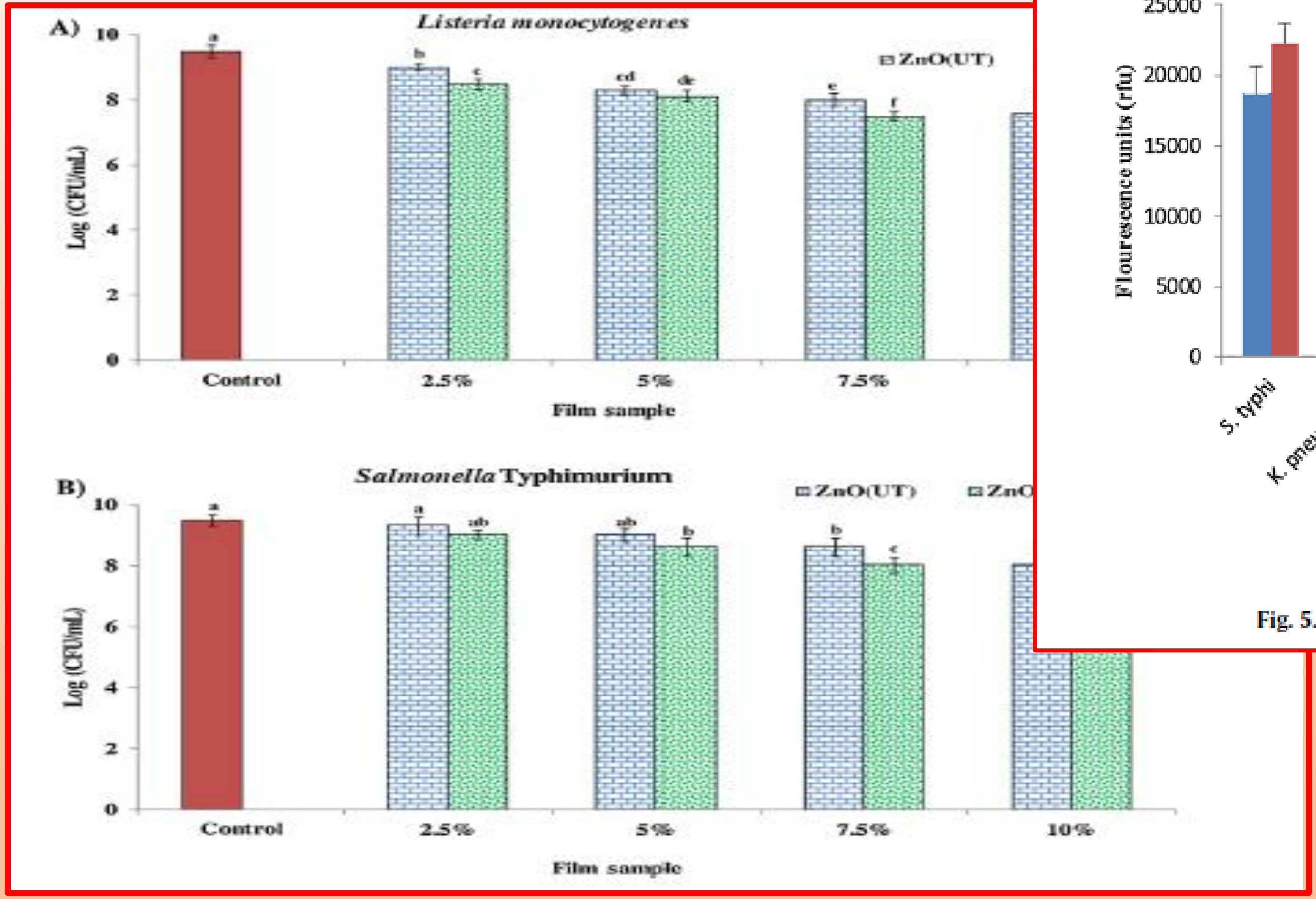
Crab shell chitosan/GO



Sample	WVTR gm/[m² - day]	OP (cc mm/m² d atm)
CS	22.4±1.05[a]	8.60±0.43[a]
CS/0.5% GO	20.1±0.82[b]	7.26±0.56[b]
CS/1% GO	15.7±1.65[c]	5.70±0.36[c]
CS/2% GO	9.8±0.68[d]	2.99±0.42[d]

J. Ahmed et al. / Food Hydrocolloids 71 (2017) 141–148

Antimicrobial activity of ZnO/PLA films



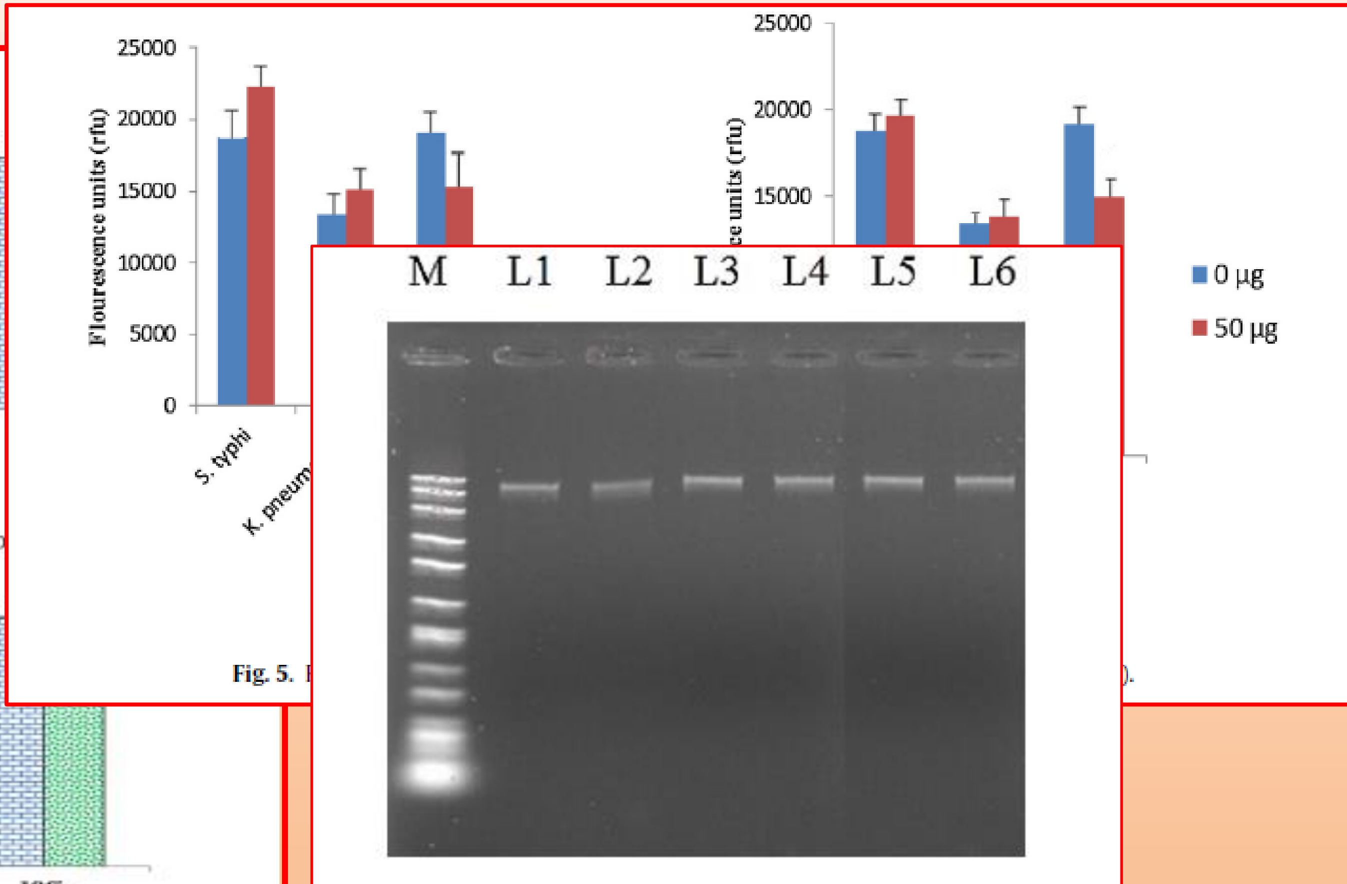


Fig. 5.

g. 6. DNA Fragmentation analysis for ZnO and TiO_2 nanoparticles. ZnO nanopar-les (L1). *Salmonella typhi* (L2) *Klebsiella pneumonia* (L3) *Shigella flexneri*.

Arfat et al. / International Journal of Biological Macromolecules 101 (2017) 1041–1050

Venkatasubbu et al. / Colloids and Surfaces B: Biointerfaces 148 (2016) 600–606

Nanoparticles as AMB agents at a glance

NP	I	esults
ZnO (Sig		log reduction
ZnO (Ev		-3 log reduction
ZnO surf		-4 log reduction
Ag-Cu (S		log reduction
Ag		gnificant reduction
GO (Sign		o significant reduction <1
Clay (So		o significant reduction <1

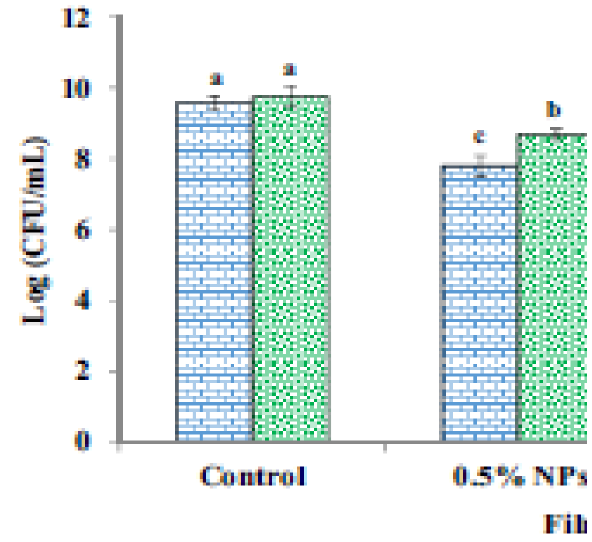


Fig. 5. Antibacterial activities of control (
cytogenes and S. typhimurium. Bars repres
lowercase letters on the bars indicate sig

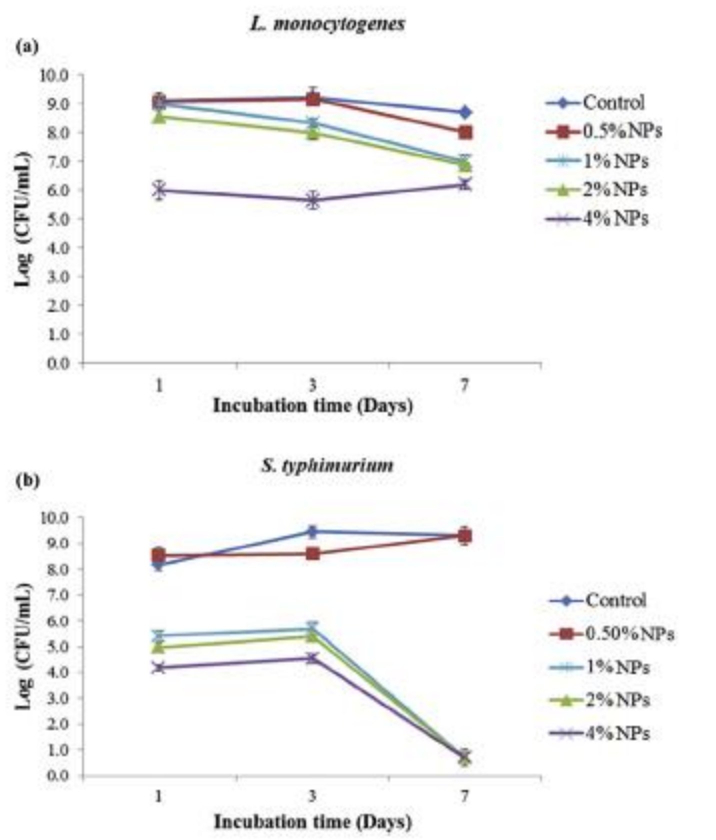


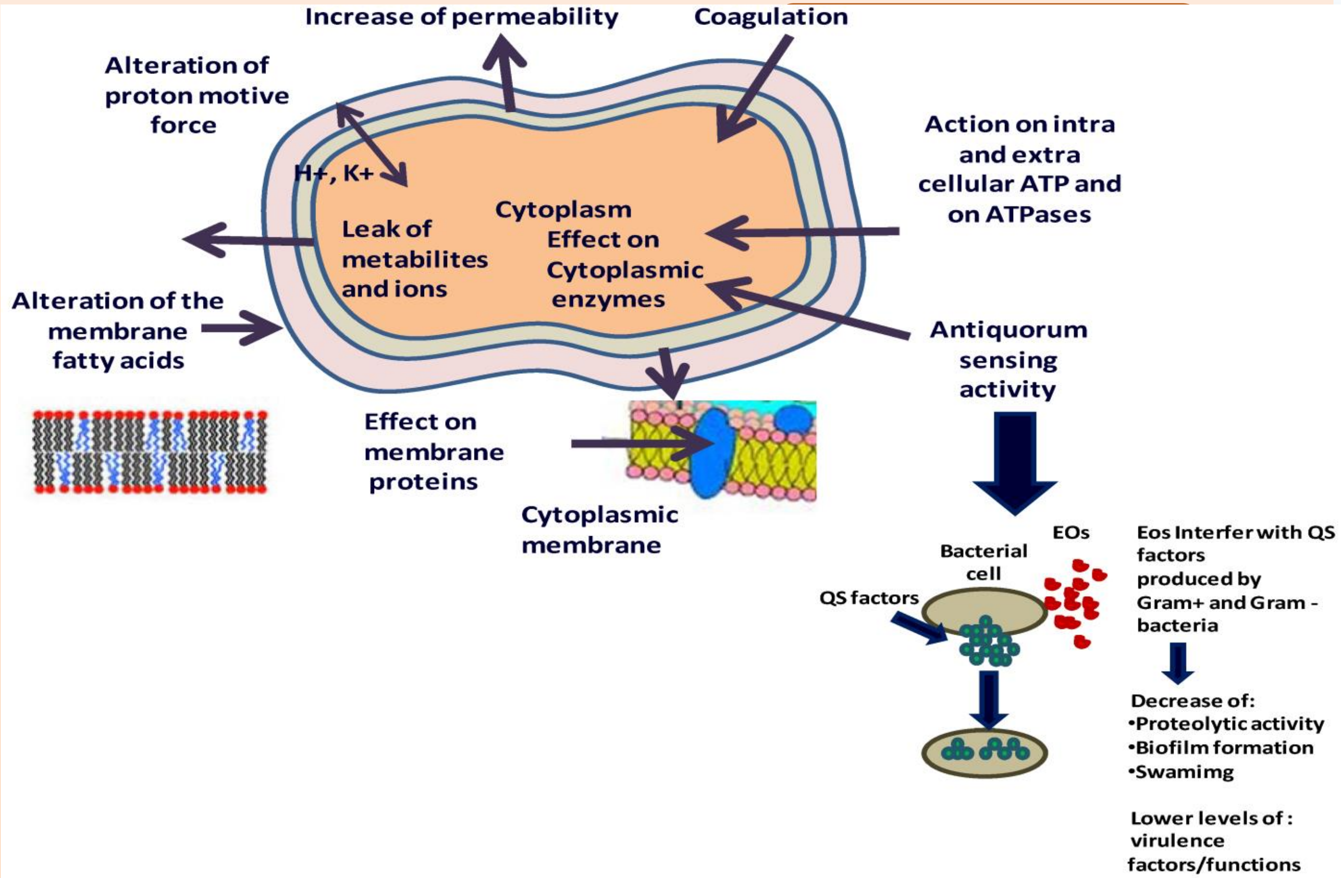
Fig. 8. Antibacterial activities of FSG films incorporated with 0.5–4% Ag-Cu NPs against (a) L. monocytogenes and (b) S. thyphimurium after 1, 3 and 7 days incubation at room temperature.

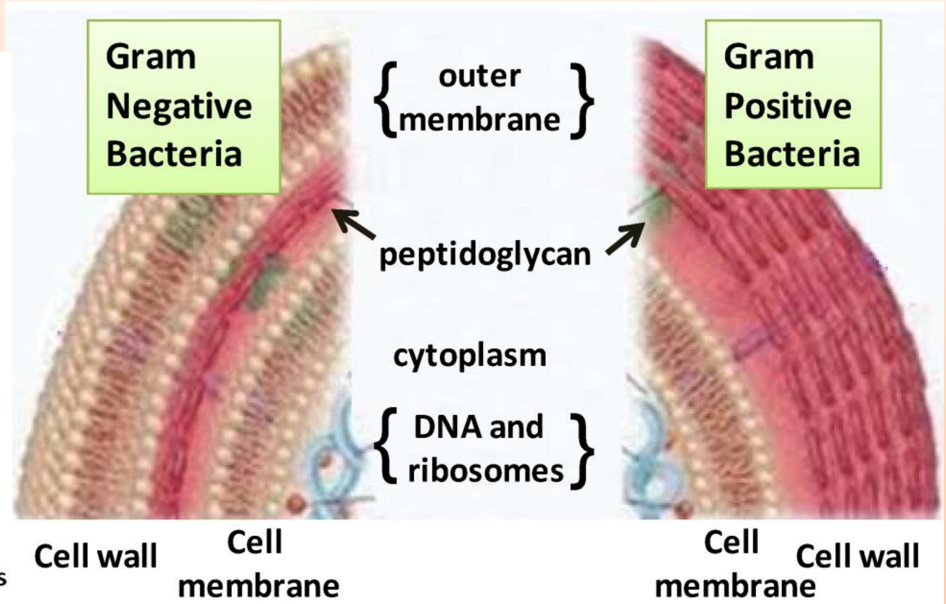
Carbohydrate Polymers 157 (2017) 65–71

Conclusions of nanopackaging

• Property improvement	Excellent
• Antimicrobial properties	Moderate

Essential oils and microbial inactivation





Biodegradable Antimicrobial Nano-packaging

Materials

- Polylactides (PLA)
- Chitosan
- Gelatin
- Hydrocolloids
- Agar

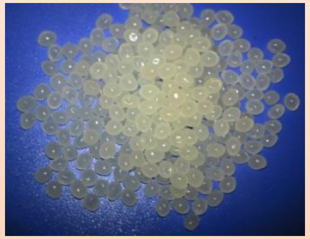
Essential oils

- Clove
- Cinnamon
- Garlic

Plasticizers

- Polyethylene glycol (PEG)
- Glycerol

PLA/PEG/Essential oil based films by solvent casting

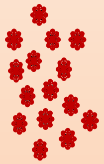


PLA

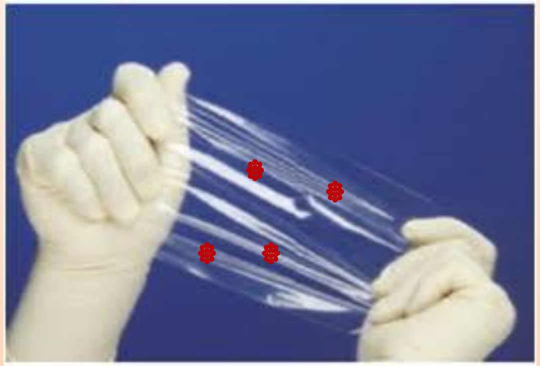
+

PEG

+



Essential oil



Active film

PLA film

PLA/PEG film

Antimicrobial Properties of PLA-based films

In vitro studies

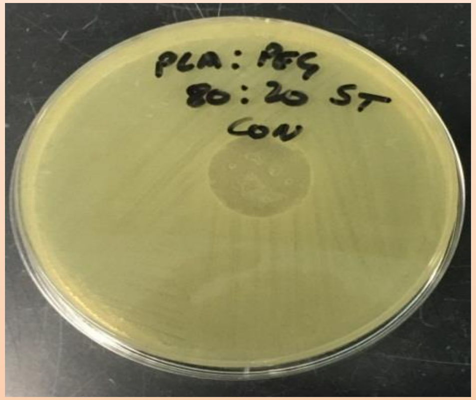
- *Staphylococcus aureus* (gm +ve) and *Campylobacter jejuni* (gm –ve)

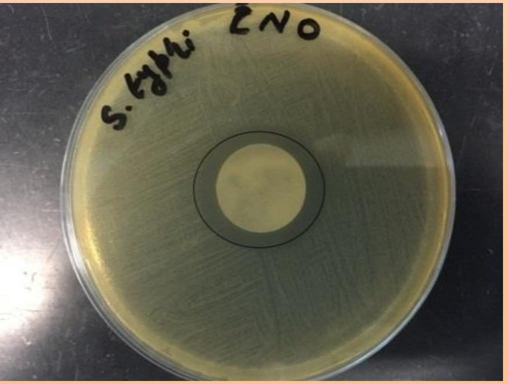
-Agar diffusion method

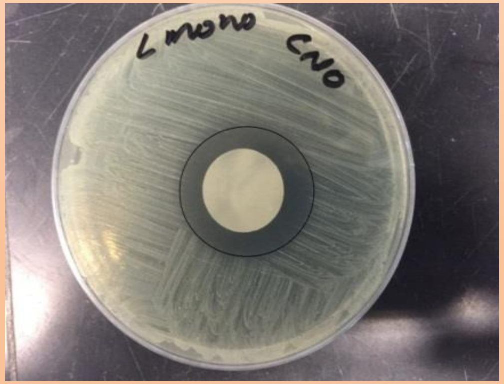
-Liquid incubation method

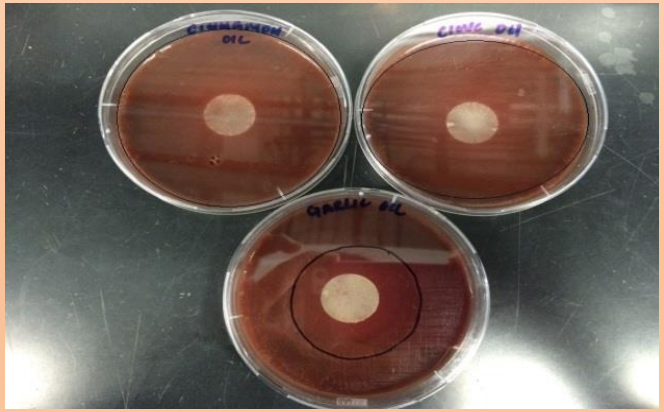
PLA/EO films were tested with:

- **Clove essential oil**
- **Cinnamon essential oil**
- **Garlic oil**









Zone of inhibition indicated by black circle

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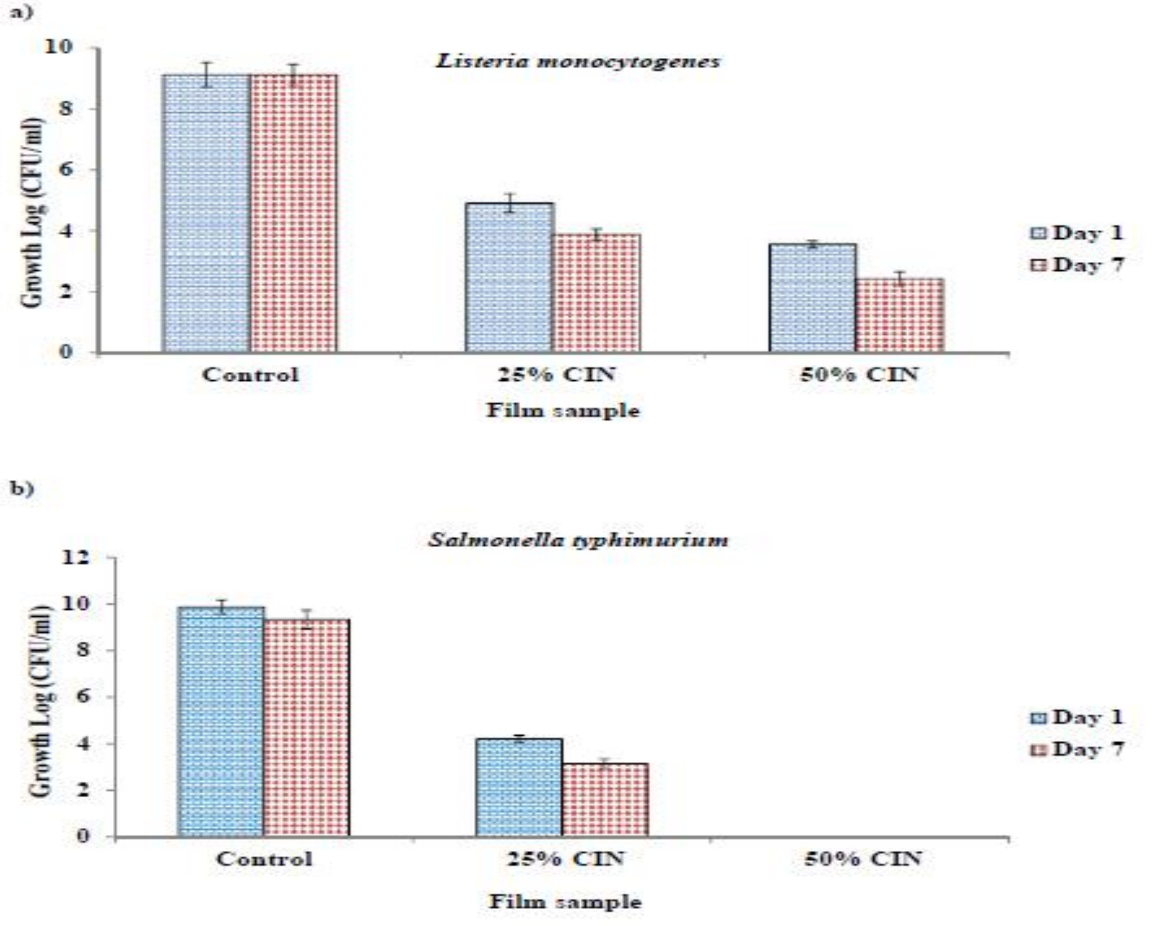
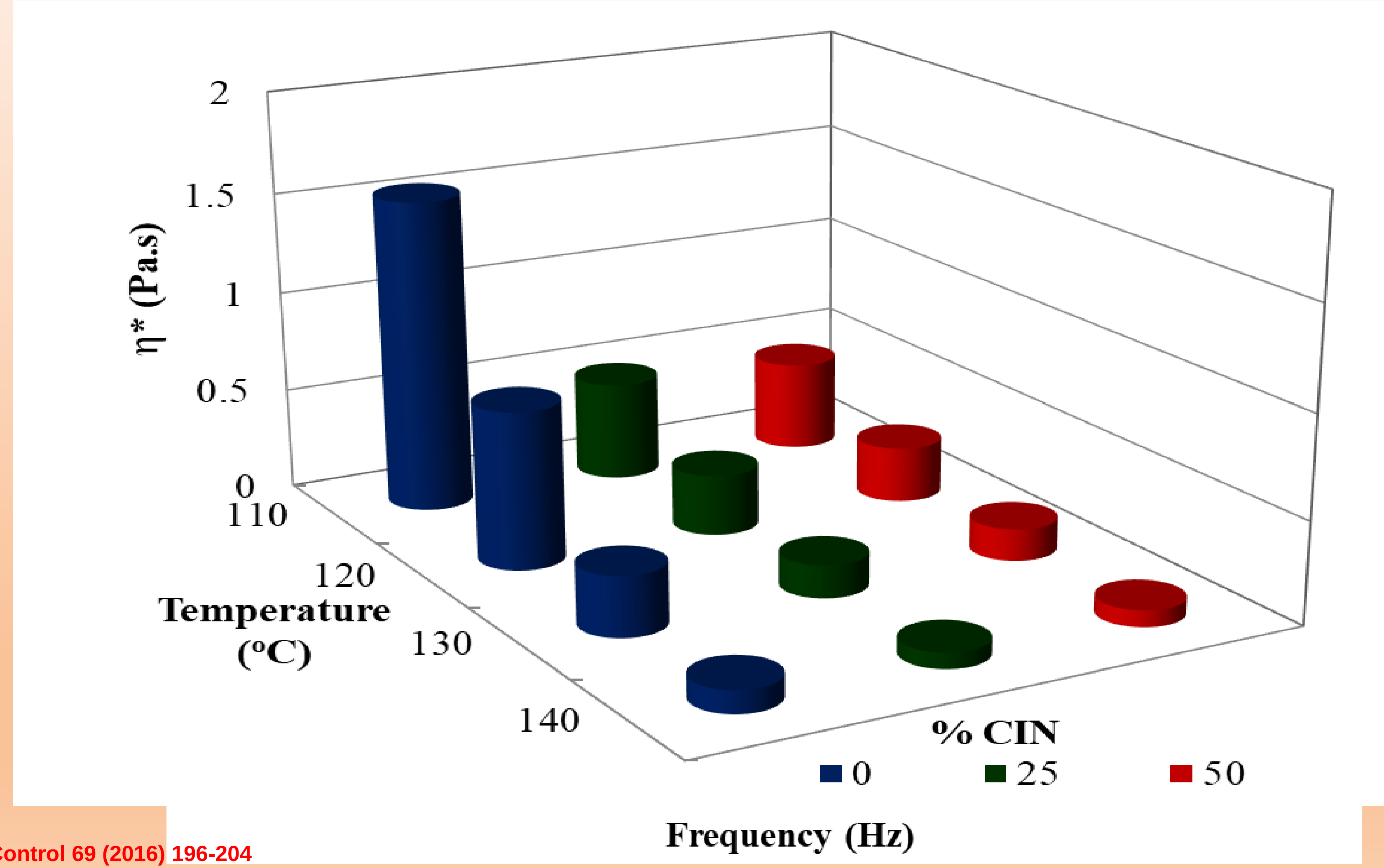


Fig. 5. Antibacterial activities of plasticized PLA films incorporated with 25% and 50% CIN against *L. monocytogenes* (a) and *S. thyphimurium* (b) after incubation at room temperature for 1 and 7 days.

CrossMark

Melt rheology of PLA/PEG/Cinnamon oil



J. Ahmed et al. / Food Control 69 (2016) 196-204

EO-based film properties

Table 1
Thickness and mechanical properties of pla

Sample	Thi	AB (%)	Tensile modulus (MPa)
PLA/PEG (90:10)	0.0	5.37 ± 3.98c	797.90 ± 12.50a
PLA/PEG/25% CEO	0.10	0.60 ± 6.96b	382.97 ± 6.51 b
PLA/PEG/50% CEO	0.1	55.29 ± 8.72a	256.88 ± 1.70c

Values are given as mean ± SD (n = 3).
Different lowercase letters in the same colu

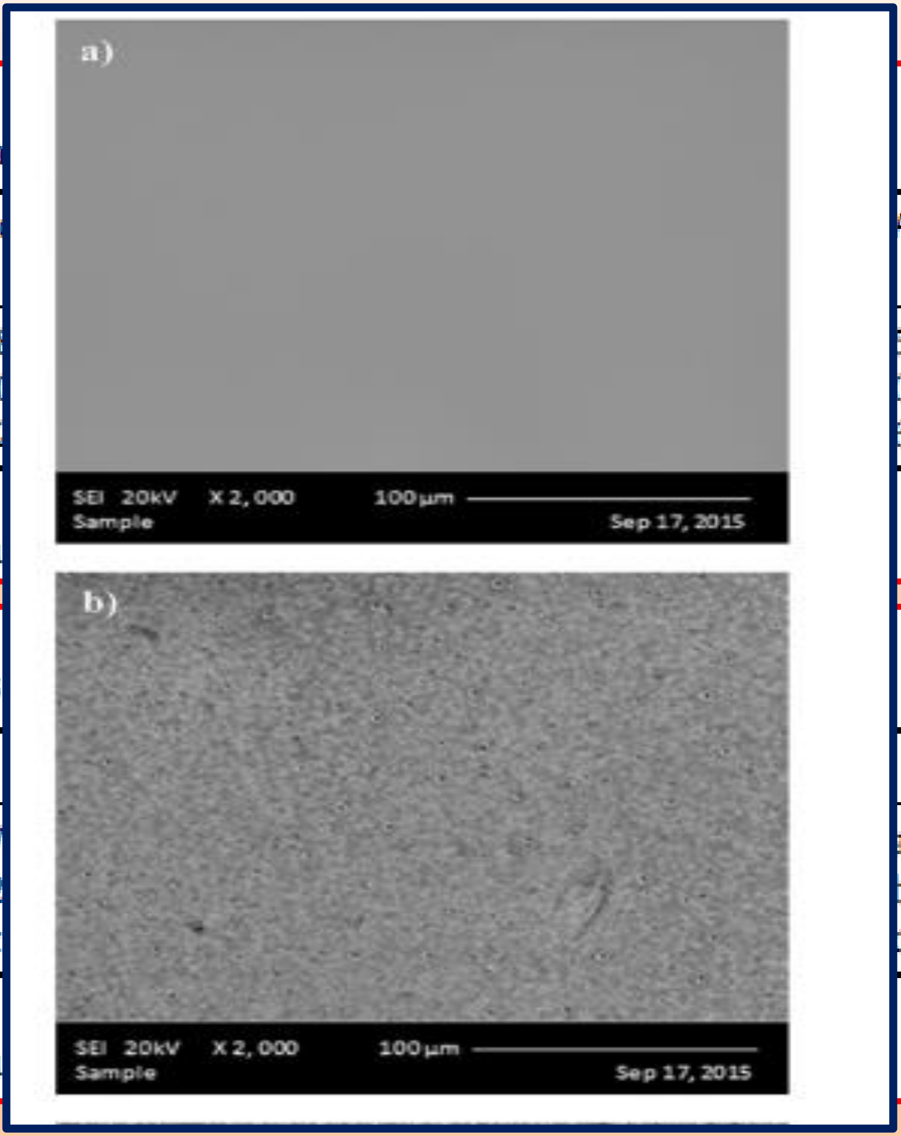


Table 3
Thermal properties of plasticized PLA films

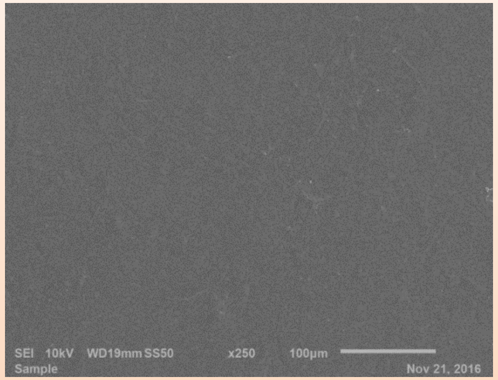
Sample	T_g (°C)		H_c (Jg^{-1})	% X_{cc}
PLA:PEG (90:10)	39.30 ± 0.27	a	22.56 ± 0.42a	32.86 ± 0.64a
PLA/PEG/25% CEO	16.42 ± 0.32	b	18.98 ± 0.14b	27.66 ± 1.26b
PLA/PEG/50% CEO	6.81 ± 0.36c	c	16.16 ± 0.25c	14.41 ± 0.31c

Values are given as mean ± SD (n = 3).
Different lowercase letters in the same colu

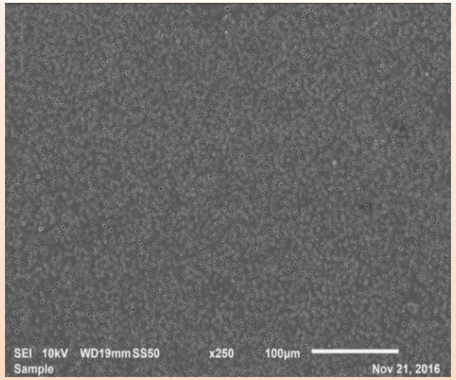
What should be the next?

- Tailoring of food packaging using a combination of NPs and EOs.
- EO-based films have limitations to deliver the desirable mechanical and barrier properties
- NPs do have the same problem with the flexibility of the film and mostly the antimicrobial property

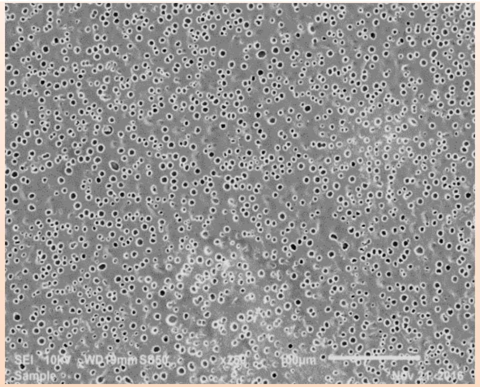
SEM of surface and cross-section of PLA/GO/clove oil films



Control PLA/PEG



PLA/PEG/15% CLO



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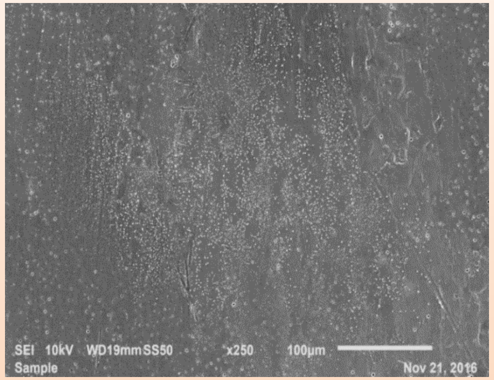

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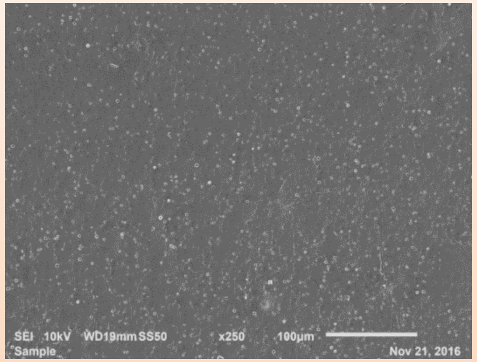

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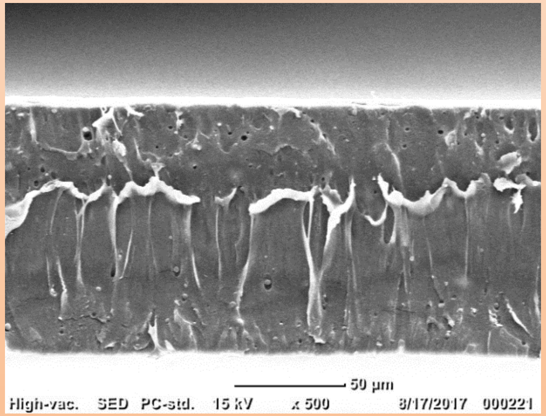
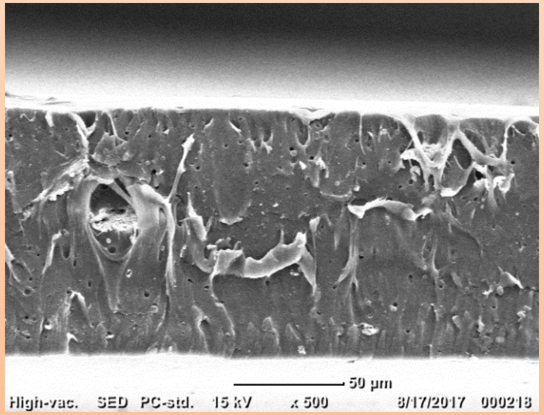


Control PLA/PEG



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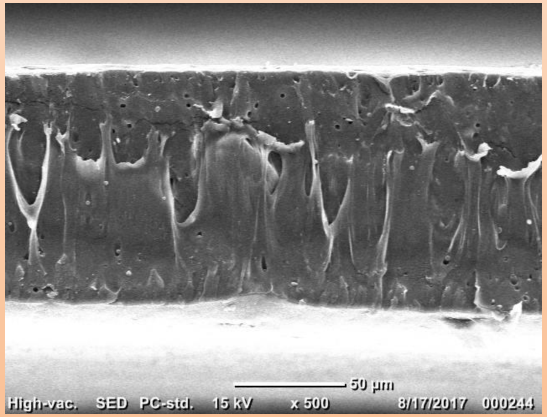

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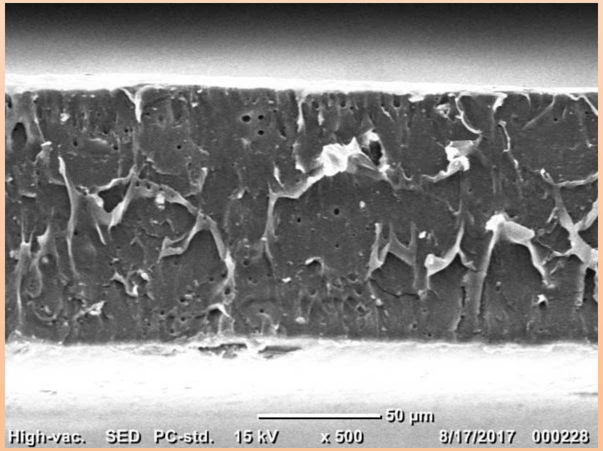

PLA/PEG/1% GO/30% CLO

Arfat et al. / International Journal of Biological Macromolecules 107 (2018) 194–203

Active Chicken Meat Packaging Based on Polylactide Films and Bimetallic Ag–Cu Nanoparticles and Essential Oil

Jasim Ahmed, Yasir Ali Arfat, Anibal Bher, Mehrajfatema Mulla, Harsha Jacob, and Rafael Auras

Food Packaging and Shelf Life 21 (2019) 100355

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Polylactide/poly(ε-caprolactone)/zinc oxide/clove essential oil composite antimicrobial films for scrambled egg packaging

Jasim Ahmed[a,*], Mehrajfatema Mulla[a], Harsha Jacob[a], Giorgio Luciano[b], Bini T.B.[c], Abdulwahab Almusallam[d]

Polyl
films

Yasir A

Compression molded LLDPE films loaded with bimetallic (Ag-Cu) nanoparticles and cinnamon essential oil for chicken meat packaging applications

Jasim Ahmed[a,*], Mehrajfatemah Mulla[a], Yasir Ali Arfat[a], Anibal Bher[b,c,d], Harsha Jacob[a], Rafael Auras[b]

3D-Printing of Biopolymers

Food Hydrocolloids 98 (2020) 105256

Contents lists available at ScienceDirect

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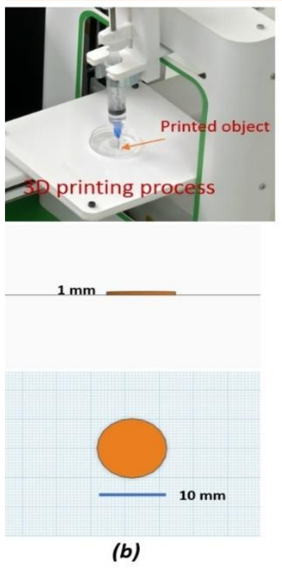
Zinc oxide/clove essential oil incorporated type B gelatin nanocomposite formulations: A proof-of-concept study for 3D printing applications

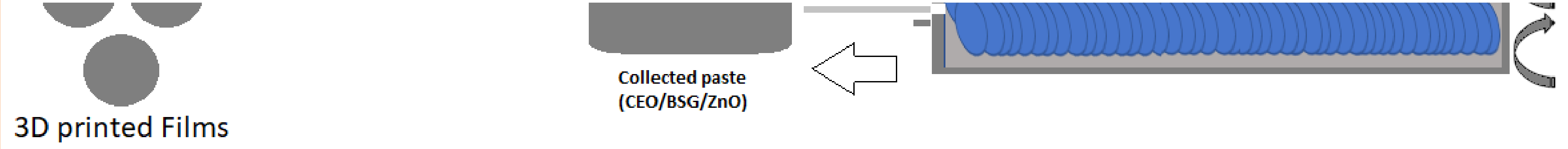
Jasim Ahmed[a,*], Mehrajfatema Mulla[a], Antony Joseph[a], Mohammed Ejaz[a], Mohammed Maniruzzaman[b,c]

[a] Food and Nutrition Program, Environment & Life Sciences Research Center, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, 13109, Kuwait

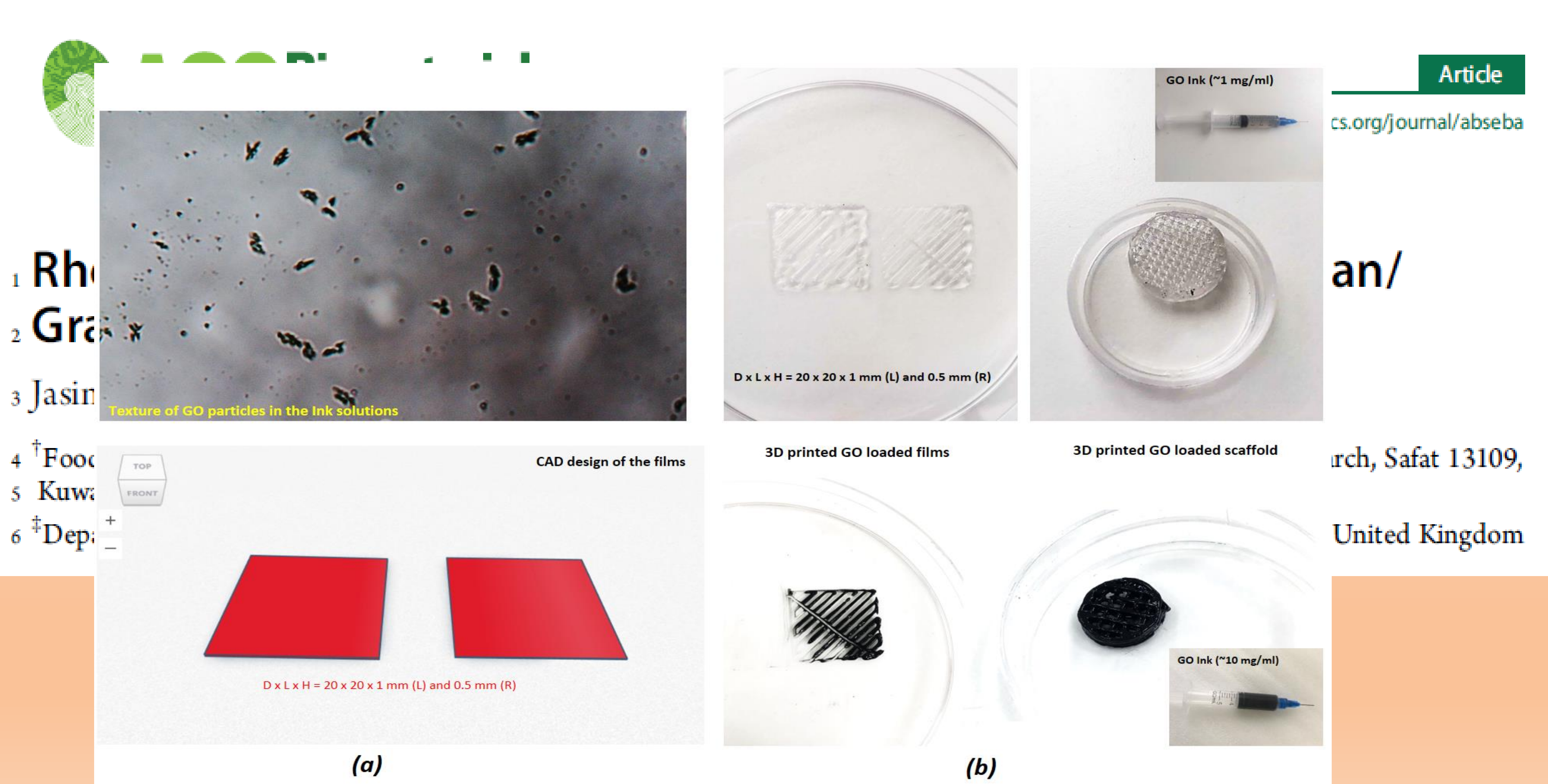
[b] School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ, UK

[c] Division of Molecular Pharmaceutics and Drug Delivery, College of Pharmacy, University of Texas at Austin, 2409 University Avenue, Stop A1920, Austin, TX, 78712, USA



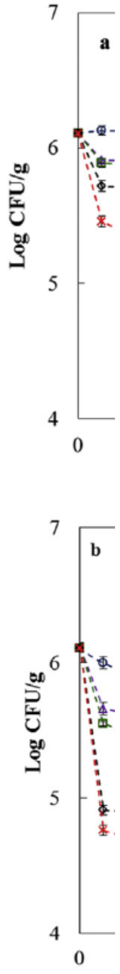


3D-Printing of Biopolymers



Packaging and High-pressure processing

Food Control 78 (2017) 160–168



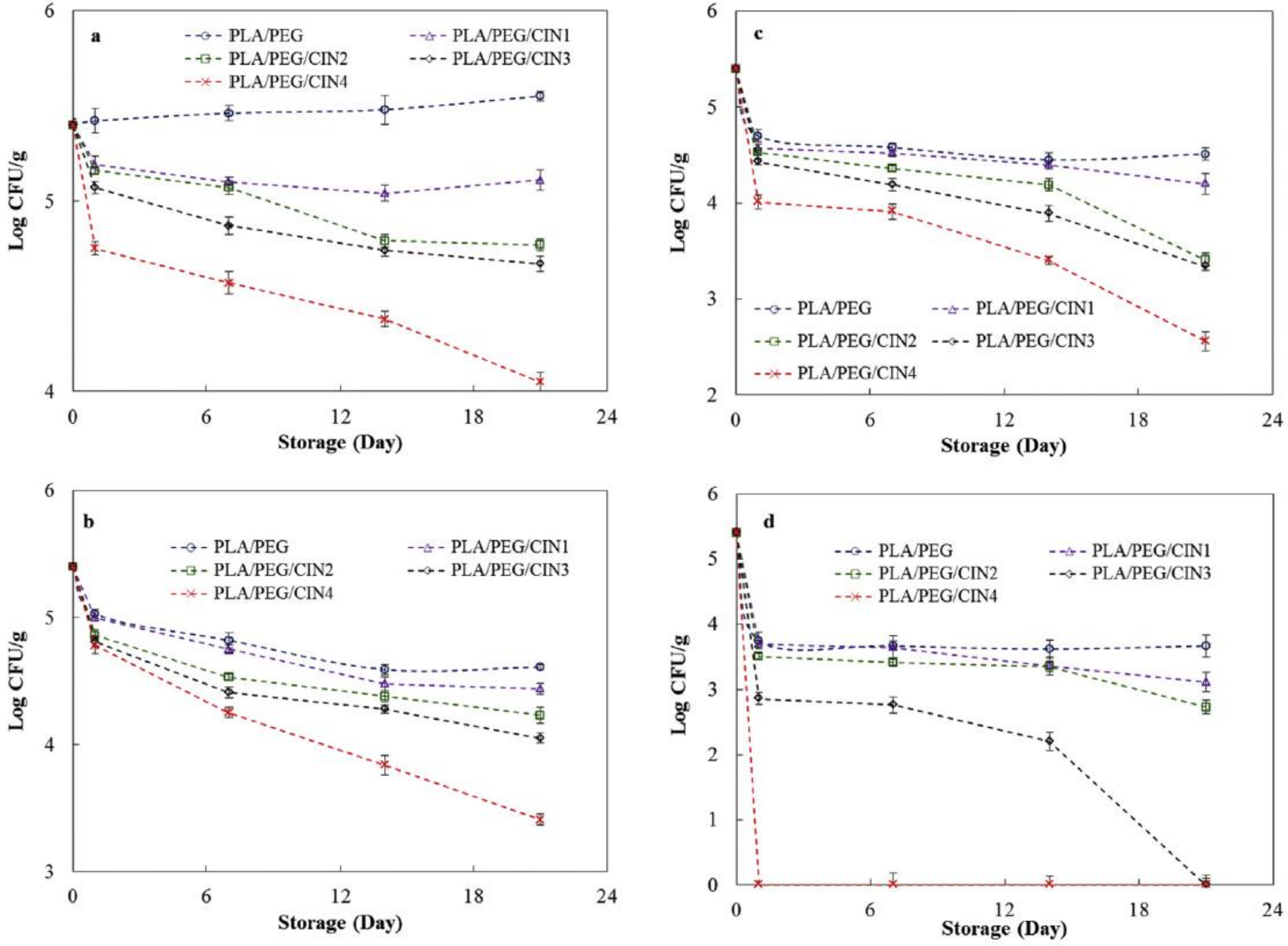


Fig. 1. Growth of L. monocy
200 MPa c) 250 MPa and d)

Fig. 2. Growth of S. Typhimurium in chicken samples packed with PLA/PEG, PLA/PEG/CIN1, PLA/PEG/CIN2, PLA/PEG/CIN3, PLA/PEG/CIN4 films and treated at a) 0.101 MPa b)

CrossMark

CONCERNS: Nanoparticle migration into food

- The migration of NP from different types of NCs into food simulants showed NP migrated into food simulants, but also that the migration was food and heating dependent.
- Acidic food and the classical oven presenting the highest migration level.
- The release of the detached NP from the composites, and dissolution of metal ions upon oxidation.

- The critical parameter driving the migration was the percentage of NP in the NCs more than particle size or storage temperature and time.
- ICPMS data mostly indicated metal migration appears to be below current migration limits established by the EU legislation.

Safety of nanoparticles

- The European Food Safety Authority (EFSA) panel has strong reservation about products for food packaging and food supplements that contain NPs unless authorized.
- Recommendations are not to exceed 0.05 mg/L in water and 0.05 mg/kg in food.
- EFSA recommends in vitro genotoxicity, absorption, distribution, metabolism and excretion tests are required by manufacturers.
- The FDA recommends that manufacturers should study and prepare a toxicological profile for each container with nanomaterials.
- The EPA prohibited the sale of plastic food containers with NPs produced by an American company because their products have not been tested according to USFDA regulations.
- At the moment Canada does not have any regulation on nanomaterials and in many other countries only incomplete food safety regulations are introduced.

At the end……..

- Still it needs time to understand the fate of NPs in the environment and the human body.
- Extensive toxicological studies required for the implementation.
- Bionanoparticles could be used to get the benefits.

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