



A study on antifungal properties of biodegradable chitosan/lactic acid based polymer blend films

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Abstract: The objective of this investigation is the study and characterisation of chitosan/lactic acid based polyester blend films which are used as bio-packaging material for potential food applications. The first part of the investigation was related to the synthesis of Poly (hexane diol lactate-co-hexane diol sebacate) polyester, PHLSe by melt polycondensation method. The synthesised polyesters were characterised by FTIR, ¹H NMR, ¹³C NMR and DSC analysis. Biodegradable blend films based on chitosan with synthesised polyester (PHLSe) was prepared using acetic acid-acetone solvent system by solution mixing and film casting method. This blend film was also investigated for its miscibility using FTIR spectra. FTIR spectra showed the changes in the finger print region of the chitosan amide band in the blend, indicating compatibility between hydrophobic chitosan with synthesised polyester. Morphologies of these blend films were viewed using Scanning electron microscopy. The chitosan/polymer blend film was used to study antifungal activity on three fungal strains.

Keywords: Chitosan, lactic acid, Antifungal activity, Bio-packaging.

Introduction

Mycotoxins can be characterized as secondary metabolites of various toxigenic fungi. Mycotoxins occur in a wide variety of foods and feeds and have been implicated in a range of human and animal diseases. The mycotoxins attract attention because of the significant economic losses associated with their impact on human health and animal productivity. Several approaches were adopted towards diminishing the human and animal exposure to mycotoxins. According to Torres et al the use of cultivars less susceptible to toxin production, cultural practices and moreover antifungal compounds has been examined¹.

The development of active materials based on antifungal coatings and films could be one solution to limiting the growth of these phytopathogens. In addition, due to environmental considerations, the elaboration of new edible or biodegradable bioactive packaging constitutes a very interesting option complementary to recycling. Chitosan was thus used as a polymeric matrix to produce films from renewable resources which exhibit potential antifungal properties on mycotoxinogen strains, because of its good film-forming properties and its recognized antimicrobial activity. Chitosan in its free polymer form has been proved to have antifungal activity against *Aspergillus niger*, *Alternaria alternata*, *Rhizopus oryzae*, *Phomopsis asparagi* and *Rhizopus stolonifer*²⁻⁴. From these findings, it could be concluded that antifungal activity of chitosan was influenced by its molecular weight, degree of substitution, concentration, types of fungus and types of functional groups in chitosan derivatives⁵⁻⁸. Basically, the antifungal activity is contributed by the polycationic nature of chitosan.

Chitosan is a multi-purpose material that has found a wide range of applications ranging from dietary regime constituents, food packaging material, drug release components and environmental pollutants⁹.

Unfortunately, the hygroscopic properties of the bio-packaging containing polysaccharides are responsible for their weak moisture barrier and thus have little or no influence on the dehydration/ rehydration phenomena of the foodstuffs, a property crucial for maintaining organoleptic and microbiological food qualities. One strategy to overcome the drawback of the high sensitivity to moisture of chitosan is to associate with more moisture-resistant polymer, while maintaining the overall biodegradability of the product. The association of chitosan and lactic acid based polymer was thus considered in the form of composite (blending) films. Lactic acid is a thermoplastic, high strength, high modulus polymer and is considered biodegradable and compostable¹⁰. Moreover, it is possible to use it for food contact, i.e. it is classified as GRAS (Generally Recognized As Safe, GRAS)¹¹. The hydrophobic nature of this polyester could reduce the hydrophilic nature of chitosan-based films and consequently improve their moisture barrier properties and decrease overall the water / matrix interactions.

With this point of view, we report on the synthesis and characterisation of chitosan/lactic acid based polymer composite for domestic applications.

2. Experimental

2.1 Materials

Lactic acid (Merck AR grade) and Sebacic acid (Lancaster AR grade) were recrystallised from deionised water and used. Chitosan (Aldrich), Hexane diol (Lancaster, AR grade), Titanium tetra isopropoxide, used as catalyst, purchased from Lancaster were used as such. All the other materials and solvents used were of analytical grade.

2.2. Synthesis

The copolyester PHLSe was synthesised by a two step melt polycondensation method. The polycondensation flask was a three neck flask equipped with a nitrogen inlet, a condenser and a thermometer. A magnetic stirrer was used to stir the reaction mixture. The reaction mixture is 0.1mol Lactic acid, 0.1 mol Sebacic acid and 0.2mol 1,6 Hexane diol. The reaction mixture is purged with nitrogen and heated in an oil bath. The temperature of the reaction mixture is raised to 150°C in 20min. Then, the temperature is gradually raised in 10°C steps every minute to the fixed reaction temperature of 210°C to remove water being the esterification by product. When water ceased to be generated, a predetermined amount of titanium tetra isopropoxide, (TTiPO) (0.1mmol) catalyst is added to the reaction mixture. Subsequently, the pressure of the reaction system was gradually decreased and polycondensation is continued at 210°C under a final reduced pressure lower than 0.5mmHg. The reaction was terminated when the rotation of the mechanical stirrer is stopped. The resulting crude copolymer was dissolved in chloroform and then poured into excess of dry cold methanol to purify the polyester. The precipitated copolyester was dried in a dessicator for further characterisation.

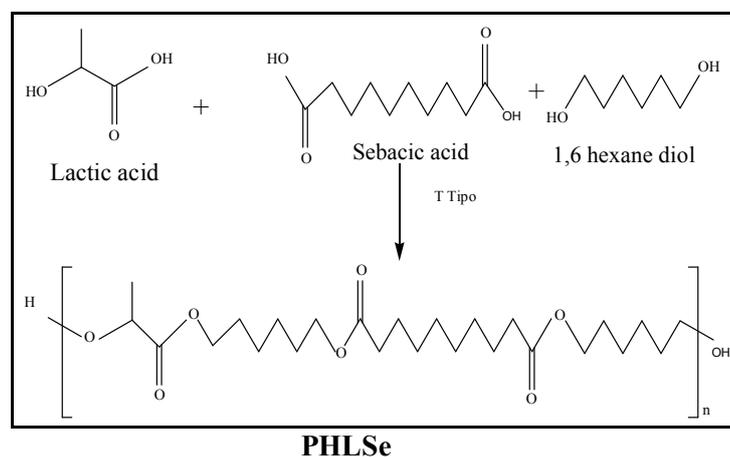


Figure 1. Summary of the synthesis reaction of copolyester, PHLSe

2.3 Preparation of chitosan/polyester blend film

Chitosan solution was prepared by dissolving chitosan in 1.0% (v/v) acetic acid aqueous solution with a 1.0 wt % chitosan concentration. In a typical experiment for preparing with PHLSe /chitosan thin film, 0.5 g of chitosan was first dissolved into 50 mL of 1.0% (v/v) acetic acid aqueous solution and then, a certain amount of acetone was slowly added with vigorous stirring for 2 h, followed by adding 2% (w/v) PHLSe solution in acetone drop by drop with vigorous stirring for additional 2 h. Subsequently, this newly obtained gelatinous mixture obtained was degassed under reduced pressure and cast into Teflon petri dish. The film with dish was then immersed into NaOH-CH₃CH₂OH aqueous solution, finally we collect polymer blend film and is characterized by various analytical techniques.

2.4 Characterisation:

2.4.1. Inherent Viscosity

The inherent viscosity of polymer solutions in chloroform was measured at room temperature using Ubbelohde Viscometer.

2.4.2. Solubility test

Solubility of random copolyester PHLSe was determined using various solvents qualitatively. Polyester maintains a good solubility in acetone, CHCl₃, THF, DMF and DMSO. The copolyester is insoluble in water, methanol and ethanol.

2.4.3. Fourier- Transform Infrared (FTIR) Spectroscopy

IR Spectra of the copolyesters were recorded using a perkin Elmer IR spectrometer in the range of 3800cm⁻¹ to 480cm⁻¹. The samples were embedded in KBr pellets.

2.4.4. Nuclear Magnetic Resonance(NMR)

¹H NMR spectra were obtained with a Jeol Model GS X 300 MHz NMR Spectrometer by using CDCl₃ as solvent. The measurements were carried out at room temperature.

2.4.5. Differential Scanning Calorimetry (DSC)

The DSC scans were recorded at a heating rate of 10° C/min using a Perkin-Elmer Pyris I analyser. Indium was used as the calibration standard.

2.4.6. X-ray Diffraction Analysis

A Siemens D 500 diffractometer with CuK α filtered radiations was used for assessing the crystallinity of the polymers. The samples were scanned over the range of 2 θ angle, from 5 ° to 50 °

2.4.7. Scanning Electron Microscopy (SEM)

The surface morphology of the composites were determined by scanning electron microscopy (SEM).

2.4.8. Mechanical Properties

The tensile and mechanical properties were studied on Hounsfield densitometer equipped with a 50 KN load cell at room temperature. The Polymer and Composites thin films samples were prepared according to the ASTM standard (45x5x0.2mm, length x width x thickness) and pulled at a strained rate of 10 mm min⁻¹. Using the measurements, stress Vs strain plot was drawn. Young's modulus was calculated from the initial slope of the curve of the tensile stress versus strain.

3. Results and Discussion:

3.1. Inherent Viscosity and solubility studies

The inherent viscosity of the copolyesters was measured in chloroform using ubbellhode viscometer.

Table – 1 Inherent Viscosity of Copolyester PHLSe

Polymer	Inherent viscosity η_{inh} (dL/g)
PHLSe	0.97

The synthesised copolyester is soluble in CHCl_3 , acetone, DMSO and insoluble in alcohols and water.

Table – 2 Solubility of Copolyester PHLSe

S.No	Polymer	Acetone	CHCl_3	DMSO	CH_3OH	Ethanol	THF	DMF	Water
1	PHLSe	+++	+++	++	--	--	++	++	--

+++ - Freely Soluble, ++ - Soluble, -- Insoluble

The inherent viscosity and solubility of the copolyester is presented in table 1 and 2 respectively.

3.2. Fourier- Transform Infrared (FTIR) Spectroscopy

FTIR spectra of copolymer for PHLSe polyester is shown in the Figure 2. The pronounced peaks around 1734 cm^{-1} suggest the presence of carbonyl ($\text{C}=\text{O}$) groups from the ester. The bands centered at around 2931 cm^{-1} was assigned to methylene($-\text{CH}_2-$) groups for diacids/diols and observed in all the spectra of the polyester. The C-H symmetric stretching of aliphatic- CH_2- group observed at 2931 cm^{-1} . Strong vibrational mode observed at 1217 cm^{-1} is associated with C–O–C asymmetric stretching mode aliphatic ester.

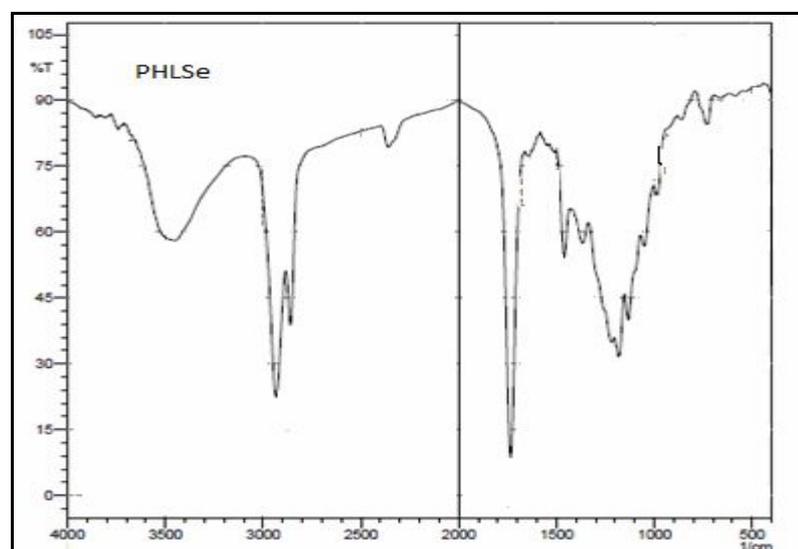


Fig.2. IR spectra of PHLSe

3.3. ^1H NMR spectroscopy

The chemical shift values obtained from ^1H NMR spectra of the copolyester is follows. Methylene protons of diols ($-\text{CO}-\text{O}-\text{CH}_2-$) at 4.03ppm (multiplet), methyl protons of LA at 1.59ppm and methine protons of LA at 5.1ppm.

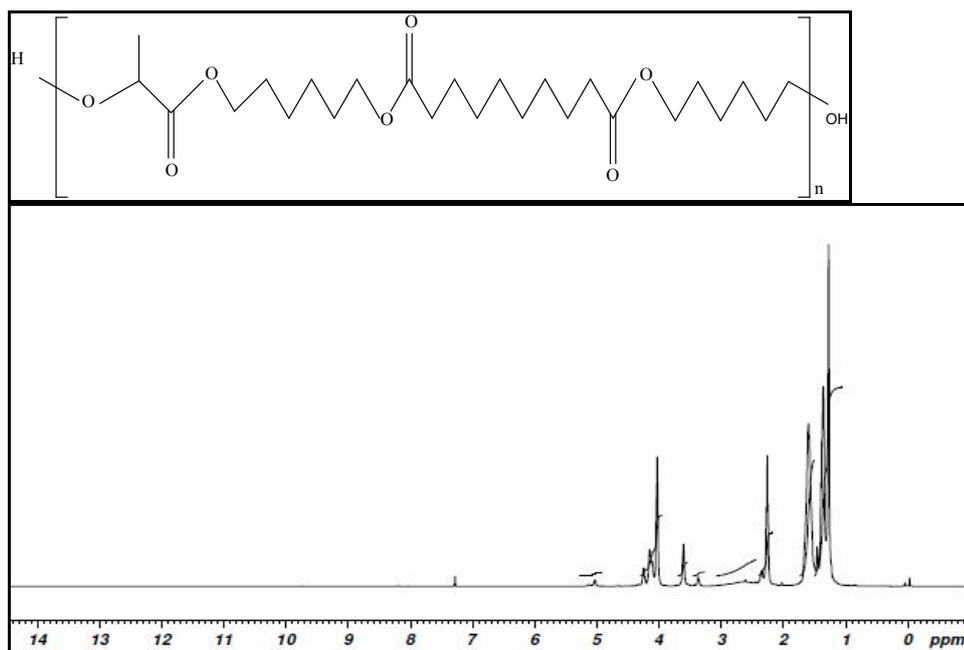


Fig. 3. ¹H NMR spectra of copolymer PHLSe

3.4. ¹³C NMR Spectroscopy

The chemical shift values obtained from ¹³C NMR Spectra of the copolyester is follows. Methylene carbons of diols (-CO-O-CH₂) at 63.60ppm(multiplet), methyl group of LA at 20.22ppm(multiplet) and methine group of LA at 64ppm(multiplet).

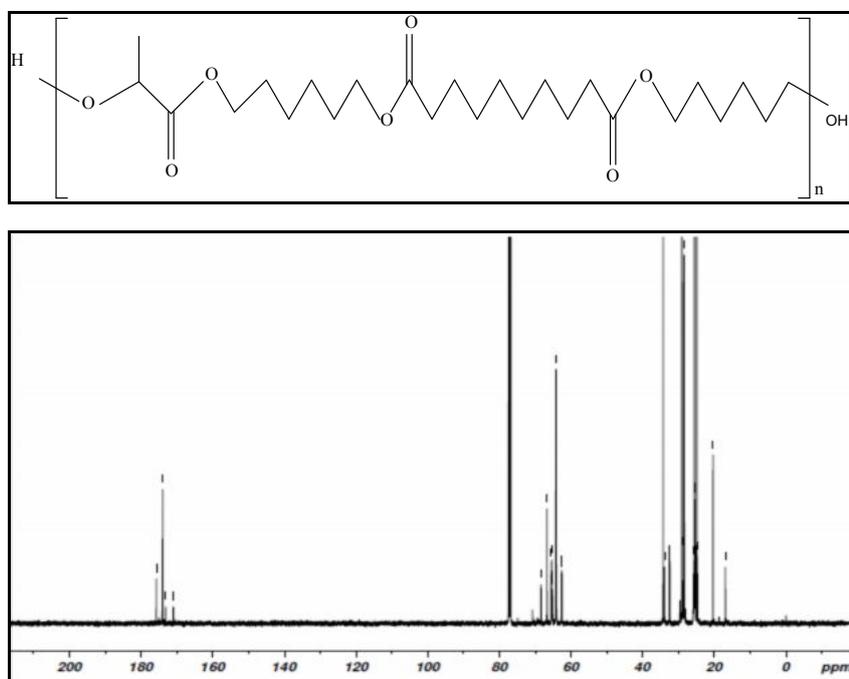


Fig. 4. ¹³C NMR spectra of copolymer PHLSe

3.5. Differential Scanning Microscopy

Differential ScanningCalorimetry (DSC)Analysis. DSC is a technique for determining the quantity of heat either absorbed or released when a substance undergoes a physical or chemical change. Several parameters

could be estimated by performing a DSC scan, such as the glass transition temperature (T_g), melting temperature, crystalline level and oxidation¹².

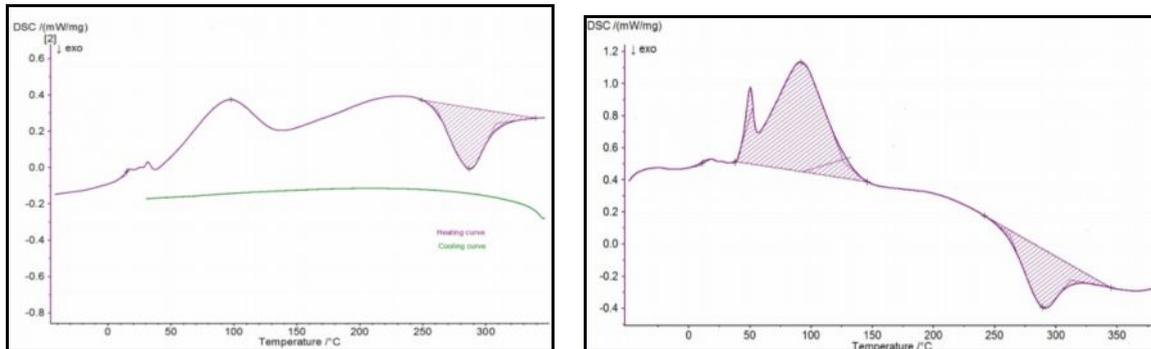


Fig. 5. DSC thermograms of copolymer PHLSe and chitosan/PHLSe blend

3.6. X-ray Diffraction (XRD)

X-ray diffractogram of the polyester, chitosan/polyester blend and chitosan are presented in Figure 6. Since both chitosan and PHLSe are semicrystalline polymers, their crystalline property will certainly affect the structure and property of blended thin films. The X-ray pattern of the pure chitosan membrane shows two characteristic peaks located at 2θ about 10.2° and 20.2° . It is known that chitosan always contains bound-water (5%) even when it has been extremely dried. The incorporation of bound-water molecules into the crystal lattice, commonly termed hydrated crystals, generally gives rise to a more dominated polymorph which can be normally detected by a broad crystalline peak in the corresponding X-ray pattern, and therefore, the crystalline peak centered at around 10.2° is attributed to the hydrated crystalline structure of chitosan¹³. Another peak registered near 20.2° is reported to be the indication of the relatively regular crystal lattice (110, 040) of chitosan^{14,15}.

Some distinct changes in diffractogram of PHLSe/chitosan film are also observed. As shown in Figure 6, the original peak of chitosan component at 10.2° almost completely vanishes in X-ray pattern of the blend film. Although two crystalline peaks are recorded for the film, a narrow peak presumably matched with PHLSe has been shifted to around 18.12° , and another broad peak registered at about 21.35° differs from the original peaks of the PHLSe or chitosan component in both shape and position. The obtained results signify that two components, chitosan and PHLSe, have interacted with each other in a certain manner so that the original crystalline structures of each component have been disturbed or partially damaged to a different extent¹⁶.

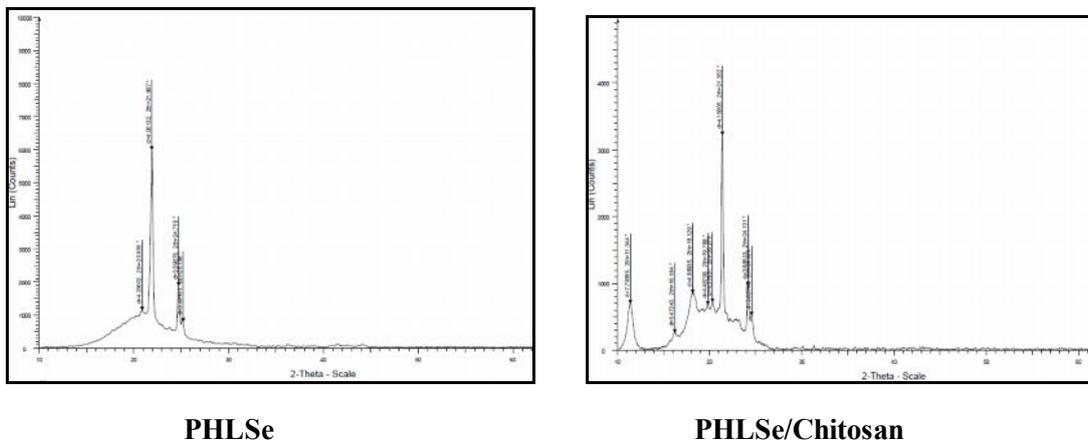
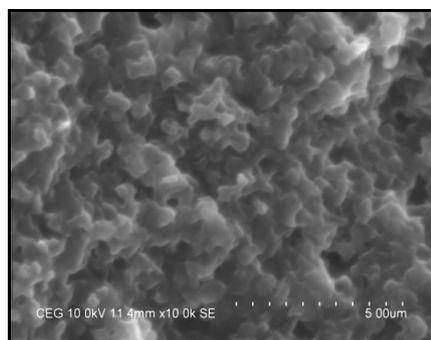


Fig. 6 X-ray Diffraction Patterns of PBLSA, Chitosan and Chitosan/PBLSA Blend.

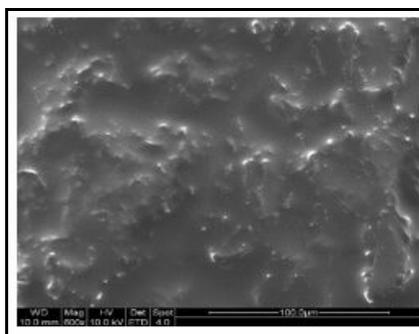
3.7. Scanning Electron Microscopy

The morphology of the films of polyester and chitosan/polyester blend are examined through scanning electron microscopy (SEM). The images of the polyester and chitosan/polyester blend are presented in the figure 7. The image shows the phase separation in the blend films at microscopic level.

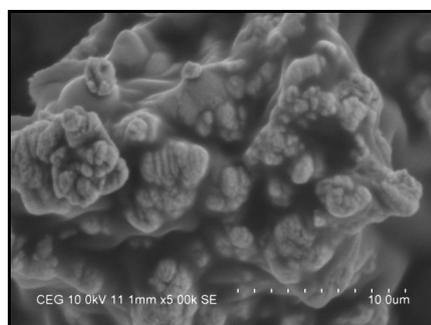
The SEM image shows that the thin film possess a clear two – phase structure, i.e., a great number of dispersed small balls are embedded into a continuous matrix. It indicates that the continuous matrices should be ascribed to the chitosan, and those dispersive balls are composed of PHLSe. An interesting observation is that some small balls with a relatively large diameter are hollow inside.



PHLSe



Chitosan



PHLSe/Chitosan

Fig. 7. SEM Images of PHLSe, Chitosan and chitosan/ PHLSe blend

3.8. Mechanical Properties

The mechanical resistance of film was studied according to three parameters: tensile strength (TS), Young’s modulus (Y) and ultimate elongation at break (UE). Tensile testing is usually used to evaluate the tensile strength and elongation at break of the polymer composite.

Table 3 Mechanical properties of the copolyester PHLSe.

Copolyester	TensileStrength(MPa)	Young’sModulus(MPa)	Elongationatbreak(%)
PHLSe	1.243	0.639	10.23

The mechanical properties of the synthesised polymer /chitosan blend film is tabulated in Table 3. Tensile strength, Young's modulus and elongation at break are calculated from mechanical study graph of the polyester/chitosan blend film .

3.9. Antifungal activity of polymer composite

The antifungal properties of composite film was studied on a solid medium for the three fungi: *Aspergillus flavus*, *Candida albicans* and *Aspergillus niger*. The antifungal activity of the film is presented in the table 4. It was difficult to deposit composite films on the agar medium. The latter retracted after contact with the medium, preventing a ‘coating’ on the whole of the surface of the agar medium. However, as observed for composite films showed an antifungal activity against the three fungal strains, only visible under the points of contact between the agar and the film. The antifungal activity of chitosan based composite film was thus

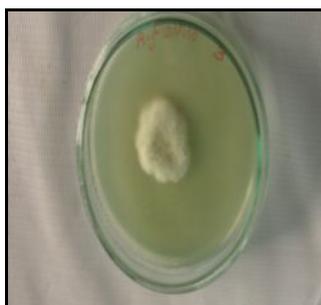
primarily due to the chemical nature of the chitosan and to the protonation of the amino group. These bio-packagings would be thus potentially used to reduce the development of mycotoxinogen fungal strains. They are interesting candidates in improving food safety of some foodstuffs.

Table 3 Antifungal activity of the copolyester PHLSe.

Sample	Zone of Inhibition(mm)		
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Candida albicans</i>
1	Nil	Nil	Nil



Aspergillus niger



Aspergillus flavus



Candida albicans

Fig. 8. Fungal Images of chitosan/ PHLSe blend

4.Conclusion

The random polyester PHLSe was synthesised through an economical method of two step melt polycondensation of lactic acid, sebacic acid and 1,6 hexane diol, in the presence catalyst titanium tetra isopropoxide. The synthesised polyesters were characterised by means of solubility, viscosity measurements. The polyester exhibit good solubility in many common solvents which is an important quality in view of its applications. FTIR, ¹H NMR, ¹³C NMR spectral analysis, XRD, DSC and SEM analysis and mechanical properties were characterised by this polymer and polymer composite. The synthesised polyester is utilised for preparing chitosan/polyester blend film by solution mixing and film casting method. Chitosan is a promising biodegradable polymer for active food packaging. However, because of its sensitivity to moisture, we have blended it with PHLSe, thus obtaining biodegradable film blends that are completely from renewable resources. In spite of their incompatibility, the incorporation of lactic acid based polymer could improve the water vapor barrier and hydrophobicity of chitosan. The synthesised polymer composite tested on antifungal activity and it shows absence of fungus in the polymer surface medium. Hence it is suitable for food packaging.

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