



WATER SORPTION PROPERTIES AND ANTIMICROBIAL ACTION OF ZINC OXIDE NANO PARTICLES LOADED SAGO STARCH FILM

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ABSTRACT

In this work, sago starch based films have been loaded with ZnO nanoparticles prepared insitu via using an unique equilibration-cum-hydrothermal approach. The films have been characterized by XRD, DSC, SPR, FTIR and SEM analysis. The moisture absorption behavior of plain and ZnO nanoparticles loaded films have been studied at 23, 31 and 37° C. The equilibrium moisture uptake data was found to fit well on GAB isotherm model and the monolayer sorption capacity M_0 for the plain and ZnO nanoparticles loaded films was 0.089, 0.039, 0.021 g/g and 0.042, 0.012, 0.007 g/g at 23, 31 and 37 °C respectively. Moreover, the water vapor transmission rates (WVTR) for plain and ZnO nanoparticles loaded films at 23, 31, 37 °C were 11.19×10^{-4} , 48.9×10^{-4} , 62.1×10^{-4} and 3.73×10^{-4} , 6.21×10^{-4} , 24.8×10^{-4} respectively. These films have shown excellent antibacterial action against model bacteria *E.coli* when investigated qualitatively by zone inhibition method. Films exhibit great potential to be used as packaging films to protect food stuff against microbial contaminants.

Key words: Antimicrobial activity, packaging film, sorption isotherms, permeability

INTRODUCTION

Antimicrobial packaging is a fast developing technology that can be employed to control the microbiological decay of perishable food products (Conte et al., 2007_b). Different organic and inorganic active antimicrobial agents can be incorporated into the packaging film, matrices to prevent undesirable microbial spoilage occurring during storage of packaged fresh food (Ahn et al., 2004). Although, most films, used to preserve food stuff, have been produced from synthetic polymers; nevertheless, for environmental reasons, attention has been focused lately on natural biopolymers such as polysaccharides (Vargas et al., 2009; The et al., 2009; Bourtoom et al., 2008; Bertuzzi et al., 2007), proteins (Perez-Mateas et al., 2009; Sivarooban et al., 2008; Del Nobile et al., 2008) and lipids (Suppakul et al., 2003), or the combination of these components for the preparation of food packaging films. These films are usually loaded with antimicrobial agents which, on coming in contact with food stuff, act upon food-borne microorganisms and inhibit their growth. These agents belong to a wide spectrum of organic/inorganic compounds (Eswaranandam et al., 2004), essential oils (Chaibi et al., 1997), bacteria-originated antibacterial protein (bacteriocins) (Kim et al., 2006), enzymes (Guchilmez et al., 2007), fruit extracts (Conte et al., 2007_a), etc. Although, these antibacterial agents have shown great potential in inhibiting microbial growth in food stuff, the development of new strains of bacteria that are resistant to current antibiotics (Singh et al., 2008) has become a serious problem in public health.

Therefore, the current research has been focused on the search for new bactericides that can effectively reduce the harmful effects of microorganisms. With the emergence of nanotechnology, the search for effective biocidal agents has been concentrated on the development of nanostructures of coinage metals like silver, copper, zinc and gold (Sondi and Salopek, 2004). Recently, there have been several reports regarding the antimicrobial activity of ZnO nanoparticles (Yadav et al., 2006). It has been reported by Jones et al., 2007, on the basis of preliminary growth analysis, that ZnO nanoparticles have higher antibacterial effects on microorganism like *S. aureus* than other metal oxide nanoparticles. Similarly, Tam et al., 2008 have reported antibacterial activity of ZnO nanorods prepared by hydrothermal method. ZnO exhibited fair activity against *E. coli* and *B. atrophaeus*, but it was considerably more effective in the later case (at 15 mM vs. 5mM concentration respectively, showing zero viable cell/count). For both organisms, damage of cell wall was observed. Recently Padmawathy and Vijayaraghavan, 2008 have prepared ZnO nanoparticles of different sizes and characterized them by SEM, TEM and XRD analysis. It was observed that nano ZnO showed enhanced antibacterial activity as compared to bulk ZnO which is attributed to the generation of reactive

oxygen species (ROS) on the surface of ZnO nanoparticles. ROS are species such as superoxide, hydroxyl radicals etc which are actively involved in damage of bacterial cells. In order to facilitate homogeneous distribution of ZnO nanoparticles within the film matrix, we have developed a unique approach that involves preparation of film from aqueous solution of starch and Zn(II) ions by solvent evaporation method, followed by in-situ precipitation of Zn(II) as Zn(OH)₂ within the film matrix, which on hydrothermal treatment yields ZnO nanoparticles loaded starch film. Zinc is a good choice in food contact applications for the following reasons: It is an essential micronutrient and serves an important and critical role in our growth and development. Zinc is available in different forms for supplementation and for fortification (Shi et al., 2008). Zinc oxide has been used in food stuff. It decomposes into Zn (II) ions after entering the body (Yu et al., 2007). It is also used in the food industry (Hotz et al., 2004).

MATERIAL AND METHODS

Material

Granules of Sago starch were obtained from local merchant and used as received, Zinc chloride, sodium hydroxide, and glycerol were purchased from Hi Media Laboratories, Mumbai, India and used as received. Nutrient agar, agar-agar type I were received from S. D. Fine Chemicals, Mumbai, India. Different salts, used to prepare saturated solutions to provide desired relative humidity (RH), were purchased from High Media Laboratories Mumbai, India. The Milli-pore water (conductivity 0.06–0.10 μ S/cm and bacterial count < 10 CFU/ml) was used throughout the investigations.

Preparation of Zinc Oxide nanoparticles loaded sago starch (ZONLSS) Films.

PSS (plain sago starch) film was prepared by microwave induced gelatinization of sago starch followed by solvent evaporation. In brief, 1 g of sago starch was added into 20 ml of distilled water at 70° C followed by addition of 0.25 ml of glycerol. The total volume of colloidal dispersion, so obtained, was made up to 25 ml by addition of appropriate quantity of water. The colloidal dispersion was put in microwave oven (LG, model MS-1947C) and irradiated at 640 Watt for 30 seconds, which yielded an almost transparent solution. The solution was poured into Petri dish and put in an electric oven (Tempstar, India) at 50°C for a period of 24 hrs. Finally the film was peeled off and kept in a dessicator for further use. The ZnO nanoparticles loaded sago starch film was prepared by *in situ* formation of zinc oxide within the sago starch film using the

hydrothermal approach. In brief, to 25 ml of the above colloidal dispersion of sago starch in water, a precalculated amount of $ZnCl_2$ was added and the resulting solution was transferred into teflon coated Petri dishes and kept in an electric oven (Tempstar, India) at $80^\circ C$ for a period of 12 h. The film, thus formed, was peeled off and put in a 0.02M solution of sodium hydroxide. After 4 h, the film was taken out and kept in an electric oven at $70^\circ C$ for complete conversion of $Zn(OH)_2$ into ZnO. Finally, the film was washed with distilled water and dried in a dust free chamber at ambient temperature until it was completely dry. The films were designated as ZONLSS(X) where the number X in parenthesis denotes the concentration of Zn(II) ions (in percent W/V) in starch solution.

Characterization of film

FTIR – spectral analysis

The FTIR Spectra of glycerol plasticized plain sago starch film and ZnO nanoparticles loaded sago starch film were recorded on Shimadzu 8400S Fourier Transformation Infrared spectrophotometer using KBr.

XRD analysis

XRD analysis was performed with a Miniflex II desktop X-ray Diffractometer (Japan).

DSC analysis

DSC analysis was performed with a Mettler DSC-30 thermal analyzer with glycerol plasticized sago starch films and ZnO nanoparticles loaded sago starch film. Film of known weight was taken in a sealed aluminium pan and the sample was heated from 40 to $240^\circ C$ at the heating rate of $10^\circ C$ per minute under constant flow of argon gas.

SEM analysis

The morphological features of plain sago starch film and ZnO nanoparticles loaded sago starch film were observed using a JOEL JSM-6390A (Japan) Analytical Scanning Electron Microscope.

UV-Visible spectrum analysis / SPR

The UV-Visible spectrum of the nano ZnO dispersed in distilled water was recorded in a UV-Visible spectrophotometer (Shimadzu 6300) in the range of 300–550 nm. The zinc oxide (ZnO) nanoparticles were prepared by the wet chemical method using zinc nitrate and sodium hydroxide as precursors. Zinc nitrate, 14.874 g (0.1 mol), was dissolved in 500 ml of distilled water under vigorous stirring to ensure complete dissolution. After complete dissolution, 0.2 mol of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 h after complete addition of sodium hydroxide. The solution was then allowed to settle overnight and supernatant was discarded carefully. The remaining solution was centrifuged at 200 rpm for 10 min and the supernatant was discarded. The residual mass was dried at 80 °C for overnight. During drying, complete conversion of Zn(OH)₂ into ZnO took place. A 0.2% (w/v) solution was used to record UV-Vis spectrum.

Equilibrium moisture sorption studies

The moisture sorption isotherms were determined gravimetrically using the static method as described by Alhamadan et al (Alhamadan *et al.*, 1999). Constant relative humidity (RH) atmospheres were obtained with saturated salt solutions (CH₃COOK, K₂CO₃, NaBr, NaCl, KCl, BaCl₂) covering a water activity range from 0.23 to 0.90 at desired temperature. Triplicate preweighed dried samples were placed inside each of the eight desiccators containing the saturated salt solutions. The samples were weighed periodically (every day for a period of seven days) until the percentage of sample mass, changed between two successive measurements, was less than 1%. The moisture adsorbed by samples was calculated using the following expressions:

$$\text{Equilibrium Moisture Content (EMC)} = (W_e - W_o) / W_o \text{ (g/g film)}$$

Where W_o and W_e are weights of the film in the initial and equilibrated state respectively.

Water vapor permeation studies

Water vapor permeability (WVP) of the ZONLSS films was determined gravimetrically using a modified ASTM E96-00 (2000) procedure. The permeation cell (acrylic cups) had an internal diameter (id) of 4.4 cm and an external diameter (ed) of 8.4 cm (exposed area: 15.205

cm²). They were 3.5 cm deep and contained CaCl₂ (0% RH; 0 Pa water vapor partial pressure). Film was placed between the cell and its acrylic ring shaped cover (4.4 cm id and 8.4 cm ed) which was adjusted to the cup with four screws located describing a cross. A 7 mm air gap was left between the films and the CaCl₂ layer. The preweighed covered cell was put in a temperature and RH controlled chamber, maintaining desired RH and temperature. Mass measurements of cups were done at regular time intervals using an electronic balance (Denver, Germany) with the accuracy of 0.0001g. All tests were conducted in triplicate and WVP and other related parameter were calculated using following expressions.

$$\text{Water Vapor transmission rate(WVTR)} = \Delta W / \Delta t \cdot 1/A \text{ gs}^{-1} \text{ m}^{-2} \dots\dots (1)$$

$$\text{Permeance(P)} = \Delta W / \Delta t \cdot 1/A \Delta P \text{ gs}^{-1} \text{ m}^{-2} \text{ Pa}^{-1} \dots\dots\dots (2)$$

$$\text{Water Vapor Permeability(WVP)} = \Delta W / \Delta t \cdot \chi / A \Delta P \text{ gs}^{-1} \text{ m}^{-1} \text{ Pa}^{-1} \dots(3)$$

where $\Delta W / \Delta t$ is the amount of water gain per unit time of transfer, χ is the film thickness (m), A is the area exposed to the water transfer (m²) and ΔP is the water vapor difference between both sides of the film. All the experiments were done in triplicate and average values have been reported in the data.

Antimicrobial studies

The biocidal action of ZnO nanoparticles loaded sago starch films (ZONLSS) was investigated in qualitative manner, by the zone inhibition method (Qin et al., 2006) respectively, with *E. coli* as the model bacteria. One hundred micro liters of the inoculums solution was added to 20 ml of the appropriate soft agar, which was over laid on to Petri dishes. Square shaped films were cut from the test films and placed on the bacterial lawns. The Petri dishes were incubated for 48 hrs at 37° C in the aerobic incubation chamber. The Petri dishes were examined visually for zones of inhibition around the square shaped films , and the size of the zone diameter was measured at two cross sectional points and the average was taken as the inhibition zone. The same procedure was followed with plain sago starch film.

RESULTS AND DISCUSSION

Formation of ZONLSS film

The overall scheme for formation of ZONLSS film may be given as follows: When zinc chloride and sago starch are dissolved in water, the Zn (II) ions bind with electron rich species, like oxygen atoms, present in the sago starch molecules in the dissolved state. Later on, when the

film is allowed to be dried at 80°C, the film matrix has almost uniformly distributed Zn (II) ions bound to oxygen atoms. Finally, when the film is put in aqueous solution of sodium hydroxide, the OH⁻ ions enter into the network and cause precipitation of Zn(OH)₂ which, on thermal curing, yields ZnO nanoparticles. Here, it is also worth mentioning that since thermal dehydration of Zn(OH)₂ precipitate yields ZnO particles, there is the chance of formation of relatively larger sized particles, as was also indicated by SEM analysis.

Characterization of ZONLSS film

FTIR analysis

Figure 1.(a) and (b) represent the FTIR spectra of Plain sago starch film and ZnO nanoparticles loaded sago starch film. FT-IR analysis shows that the absorbance near 2960 cm⁻¹ and 1485 cm⁻¹ due to C-H stretching and bending vibration, and absorbance band between 1200 cm⁻¹ and 1000 cm⁻¹ due to C-O stretching. in Glycerol plasticized sago starch film get shifted to lower frequency region due the pesence of ZnO nano particles in the ZnO nanoparticles loaded sago starch film, this difference in IR spectrum of plain sago starch film and ZnO nanoparticles loaded film can be explained on the basis of restricted modes of vibration in the sago starch polymer due to presence of ZnO nano particles in the film.

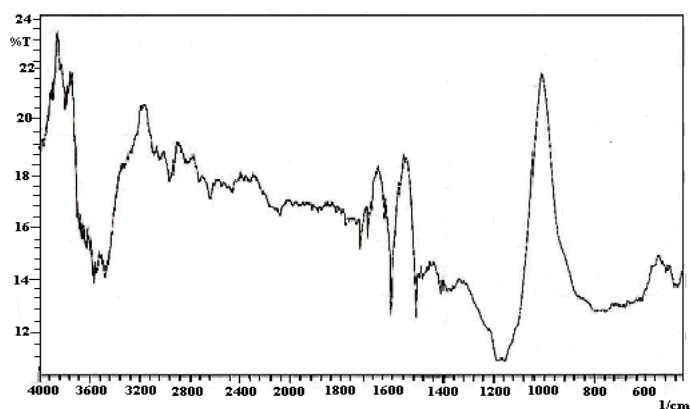


Figure 1 a) FTIR of Plain sago starch film

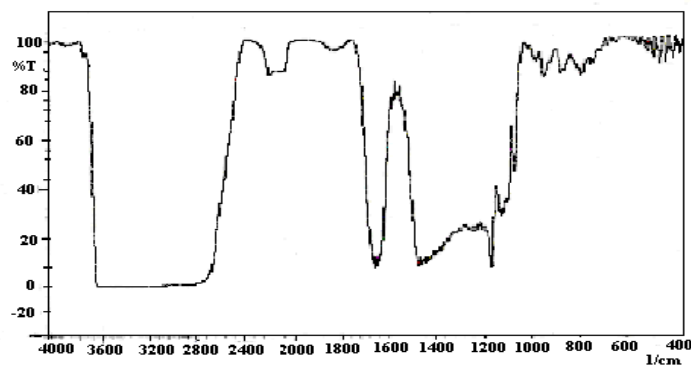


Figure 1 b) FTIR of ZnO nano particles loaded sago starch film

UV –Visible / spectral analysis

The surface plasmon resonance (SPR) is a characteristic of metal nanoparticles. The room temperature UV-Vis absorbance spectrum for the ZnO particles has been shown in Figure 2. The sharp absorbance peak, located at about 362nm, corresponds to the band gap of 3.42 eV. This is almost in accordance with the value of bulk ZnO (Yang *et al.*, 2002), thus suggesting excellent crystal quality of the ZnO nanoparticles. Therefore, no blue shift was observed in UV-Vis spectrum, revealing that nanoscale ZnO particles obtained are not small enough to show quantum confinement related effects. In Surface Plasmon Spectrum for ZnO nanoparticles an asymmetric tail can also be found on higher wavelength of the peak, induced by light scattering.

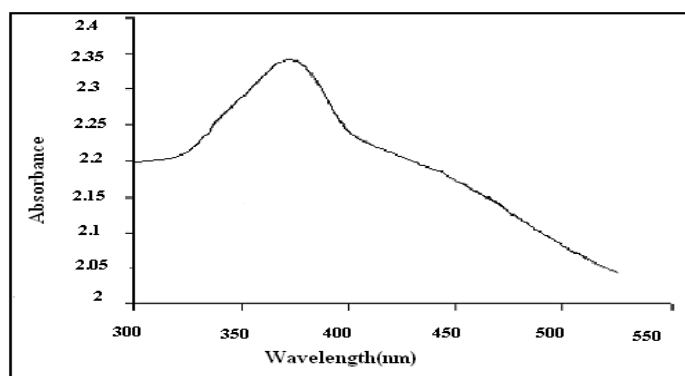


Figure 2 Surface Plasmon Spectrum for ZnO nanoparticles

XRD analysis

Figure 3 shows the X-ray diffraction pattern of ZnO nanoparticles loaded sago starch film. The peaks, observed at 2θ values of 31.7° , 34.4° , 36.2° , 47.5° , 56.6° , 62.8° and 69.1° , correspond to the reflections at (100), (002), (101), (102), (110), (103), (112) planes respectively [JCPDS76-0704]. Almost similar value have also been reported by **Yadav et al., 2006**. The above XRD pattern also consists of some additional sharp peaks showing presence of crystalline starch matrix. The diffraction peaks observed at 5.6° , 17° and 23° indicate that crystalline type of sago starch is intermediate to that of Type A and Type B starches (**Ahmad et al., 1999**).

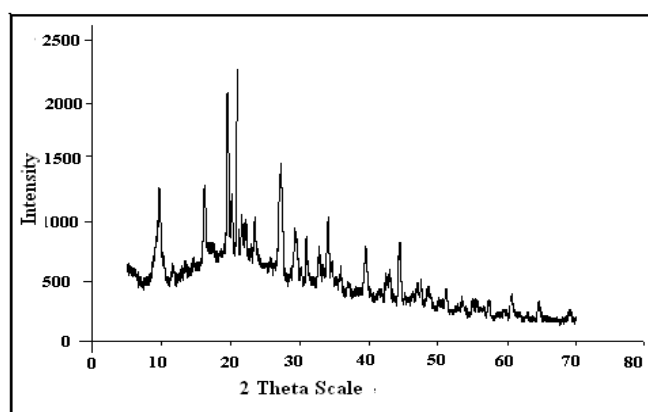


Figure 3 XRD of ZnO nanoparticles loaded sago starch film

SEM analysis

Scanning electron microscopy (SEM) is an effective tool to study the surface morphology of materials. Figure 4 (a) and (b) give a comparative depiction of the SEM images of plain sago starch film and zinc oxide nano particles loaded sago starch film (ZONLSS) respectively. It is quite evident from Figure 4(a) that PSS film exhibits smooth surface while the surface of ZONLSS film, as shown in Figure 4(b), demonstrates crystalline structure of ZnO nanoparticles in an almost uniformly distributed manner. The average size of the ZnO nanoparticles was found to be approximately 977 nm which, in fact, lies far away from the prescribed size of nanoparticles (**Bajpai et al., 2007**).

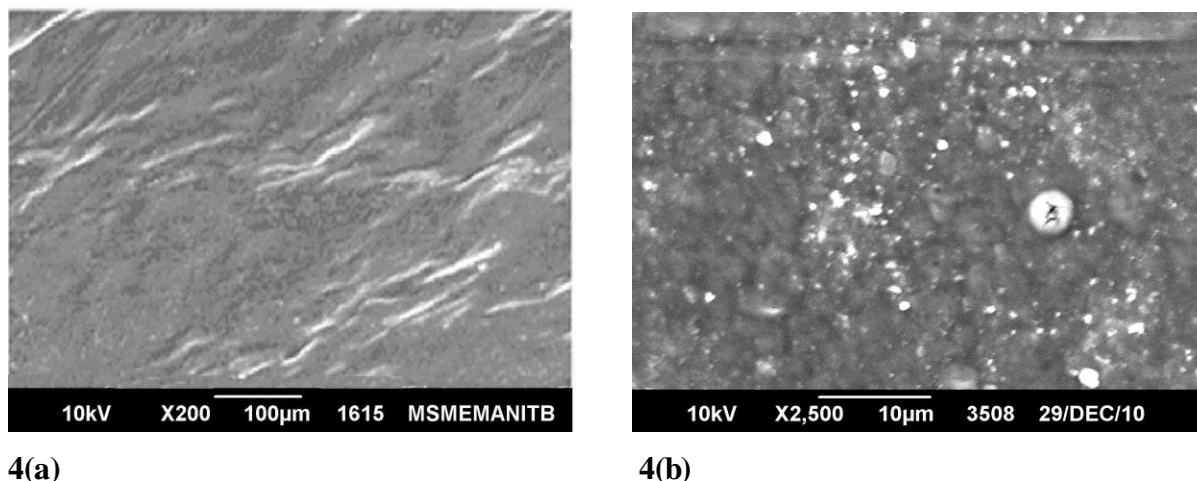


Figure 4 (a) SEM image of Plain sago starch film, (b) SEM image of ZnO nano particles loaded sago starch film

DSC analysis

In order to investigate the effect of presence of ZnO nanoparticles on the structure of starch film, DSC analysis of plain sago starch film (PSS) and ZnO nano particles loaded sago starch film (ZONLSS) was carried out. The results, as shown in Figure 5(a) and (b) respectively, clearly indicate sharp endothermic peaks in thermograms of both plain and nano ZnO loaded films. The melting temperature (T_m) of PSS and ZONLSS films were found to be 142.3 and 104.7 °C respectively. The observed decrease in T_m due to incorporation of ZnO nanoparticles may be attributed to the fact that presence of ZnO in the starch matrix decreases the intermolecular forces between polar groups of starch molecules, thus partly decreasing the crystallinity of the film matrix. This finally results in lowering of crystalline melting temperature. Almost similar type of results have been reported by **Chen et al., 2008**. It is also interesting to see that the enthalpy of fusion, ΔH_m for PSS and ZONLSS film were found to be -121.38 J g⁻¹ and -121.77 Jg⁻¹ respectively, showing almost no appreciable change in enthalpy value due to incorporation of ZnO nanoparticles. This indicates that there are no specific interactions between ZnO nanoparticles and starch molecules which could suppress the starch retrogradation as was observed in the work reported by **Chen et al., 2008**. A close look at the two thermograms reveals one more interesting fact. The glass transition temperature, T_g of plain sago starch film is found to be nearly 118.24° C while the thermogram of ZONLSS film does not display occurrence of glass transition phenomenon. This indicates that presence of ZnO nanoparticles reduces the intramolecular H-Bonding interactions to such a great extent that not much thermal energy is required for transformation from glassy to rubbery state. In other words at ambient temperature the ZONLSS film exists in nearly rubbery state.

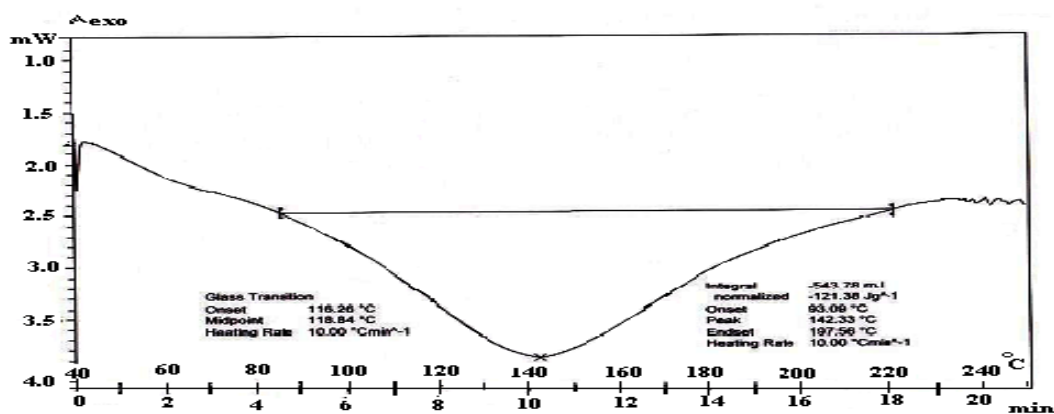


Figure 5a)

Figure 5 (a) DSC of Plain sago starch film

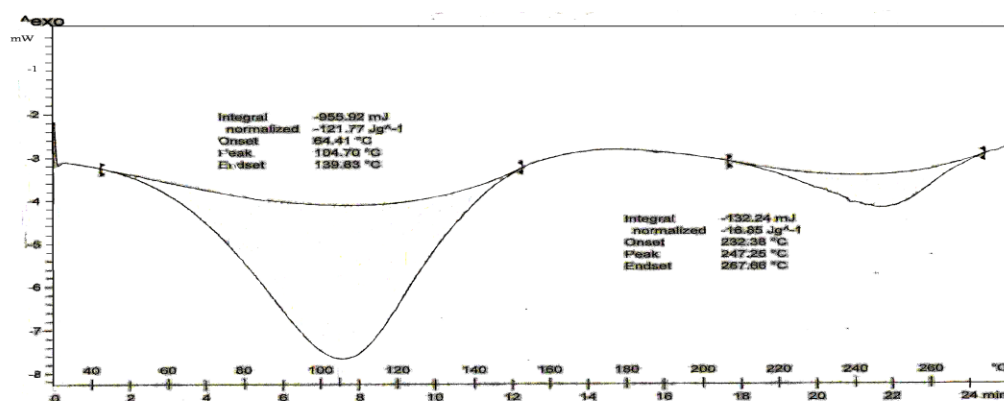


Figure 5 (b) DSC of ZnO nanoparticles loaded sago starch film

Moisture sorption isotherms

Water vapor pressure and temperature are the most significant factors, determining the moisture uptake properties of a food packaging film. In fact, higher moisture content considerably restricts its use as potential packaging material. The equilibrium moisture contents of plain sago starch film (PSS) and zinc oxide nanoparticles loaded sago starch (ZONLSS) films were investigated in the environment of varying relative humidity (RH) at 23, 31, and 37° C. The sorption isotherms for PSS and ZONLSS films are displayed in Figure 6 a) and 6 b). These isotherms show typical sigmoidal shape, thus confirming class II classification in which the polymer absorbs relatively smaller quantity of water at lower water activities and larger amount at higher relative humidity (Al-Mutaseb, 2004). In fact, sigmoidal Type II curves are typical of most of the biopolymer materials like cellulose (Ayranci, 1996), casein (Fabra et al., 2010) and

soy-protein (Cho et al, 2002). The explanation for the nature of the isotherms may be that, at low water activities, physical sorption on active sites of film occurs only on the surface.

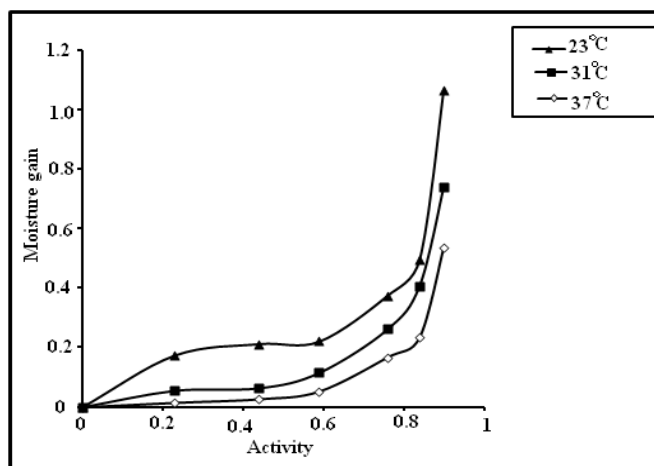


Figure 6 (a) Moisture sorption isotherm of Plain sago starch film

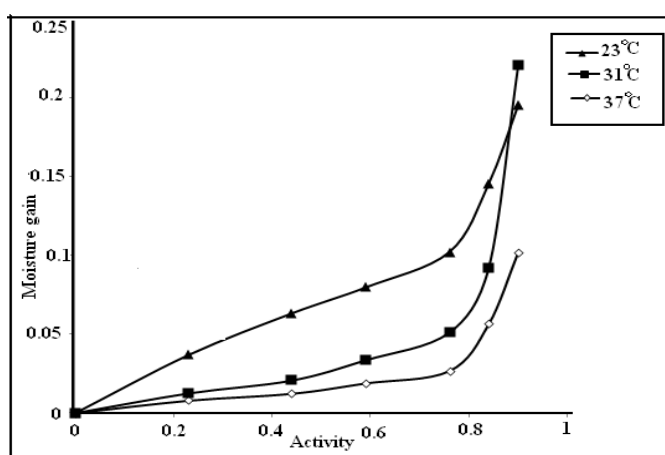


Figure 6 (b) Moisture sorption isotherm of Zinc oxide nano particles loaded sago starch film

However, in the intermediate water activity range, sorption takes place at less active sites also. It is also clear from Figure 6 a) and b) that the moisture content of films decreases with increasing temperature at any given RH. This may simply be attributed to the fact that as the temperature increases, water vapor molecules possess more kinetic energy and hence, show less tendency to get sorbed onto films, thus causing a decrease in moisture uptake. Similar type of behavior has also been reported earlier by other workers (Goula et al., 2008). Hence, moisture content uptake may be regarded as an exothermic process. A close look at the isotherms, displayed in Fig 6(a) and 6(b) reveals that for a given temperature, PSS films demonstrate higher moisture uptake as compared to the ZONLSS film. This may probably be attributed to the fact that in plain sago starch film the polar $-OH$ groups act as binding sites for incoming water vapor

molecules and so cause fairly high moisture uptake. However in ZnO nanoparticles loaded films the electrostatic binding between oxygen atoms of Zinc oxide and hydrogen atom of –OH groups of sago starch may partially reduce the polarity of these hydroxyl groups and thus less number of moisture (water) molecules are attached to them. The equilibrium moisture sorption data, obtained at three temperatures, was applied on a well known GAB isotherm model (Venden Berg et al., 1981), given as:

$$M_c = M_o CK_{aw} / [(1 - K_{aw})(1 - K_{aw} + CK_{aw})] \dots\dots\dots(4)$$

Where M_c is the moisture content of the material on a dry basis (g/g dry basis), M_o is the moisture content sorbed as monolayer, C and K are sorption constants .

The values of various GAB parameters ,namely M_o, C, K ,obtained for both PSS and ZONLSS films at 23,31 and 37° C are depicted in Table 1. The monolayer moisture capacity M_o is higher for the PSS films as compared to ZONLSS films thus supporting our arrangement that hydroxyls of plain starch are better active sites. Moreover, for a given film , M_o decreases with increase in temperature which is a simple outcome of the fact that at high temperature the enhanced kinetic energy of water vapor molecules disfavors their accumulation on starch molecules.. The monolayer moisture content M_o is recognized as the moisture content affording the longest time period with minimum quality loss at a given temperature. Therefore, at a given temperature, the safest water activity level is that corresponding to M_o or lower. The another parameter C measures the adsorbent –adsorbate interactions which are exothermic in nature and are favored at lowered temperature. Therefore C is expected to decrease with increase in temperature. This parameter ,therefore, shows negative temperature dependence which is also clear from the following well known relationship between C and T

Table 1 GAB Parameters for PSS and ZONLSS film.

GAB parameters	23 ° C		31 ° C		37 ° C	
	PSS	ZONLSS	PSS	ZONLSS	PSS	ZONLSS
K	0.995	0.854	1.07	1.031	1.09	1.025
C	8.73	10.69	162	9.19	2.8	19.36
M_o	0.089	0.042	0.039	0.012	0.021	0.007

$$C = C_G \cdot \exp(\Delta H_c / RT) \dots\dots\dots(5)$$

Where ΔH_c is function of heat of sorption of water; $\Delta H_c = H_m - H_n$ here H_m and H_n are heat of sorption of the monolayer and multilayer of water respectively. The C values for the plain starch film, as given in Table 1, also show same pattern i.e. negative temperature dependence. However, in the case of ZONLSS film values of C are deserved to increase with temperature. Iglesias and Chirife (Iglesias et al., 1982) studied more than 30 different foods and found that in around 74% of them C did not increase with decrease in temperature. In the case of ZONLSS film there may be some irreversible change in starch due to hydrothermal approach followed to form in situ ZnO particles. However, in all the cases the values of C , obtained are ≥ 2 , which is indicative of occurrence of sigmoidal Type II isotherm (Farahnaky et al., 2009).

The value of K provides a measure of the interactions between the molecules in the multilayer with the adsorbent, and tends to fall between the energy values of the molecules in the monolayer and that of liquid water. If K is equal to 1, the multilayer have properties of liquid water (Gabas et al, 2007). The values obtained in the present work are in the range of 0.854 to 1.09.

Water vapor permeation studies

Since the main function of an packaging or edible film or coating is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product, water vapor permeability (WVP) should be minimum. The nano zinc oxide loaded sago starch film (ZONLSS) were placed under relative humidity of 100% at three different temperatures, namely 23, 31 and 37^o C, and were investigated for moisture permeation through films. The results, as shown in Figure (7a) and (b) clearly show that the amount of water vapor permeated through films increases with temperature. This may simply be attributed to the enhanced movements of polymeric segments of film and also due to increased kinetic energy of the permeating water vapor molecules.

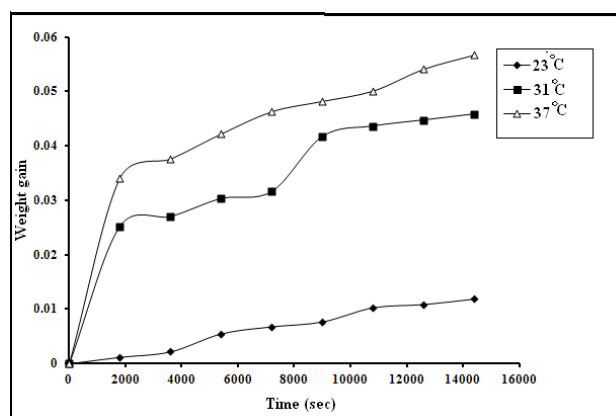


Fig 7a) Kinetics of water vapor transmission through PSS film.

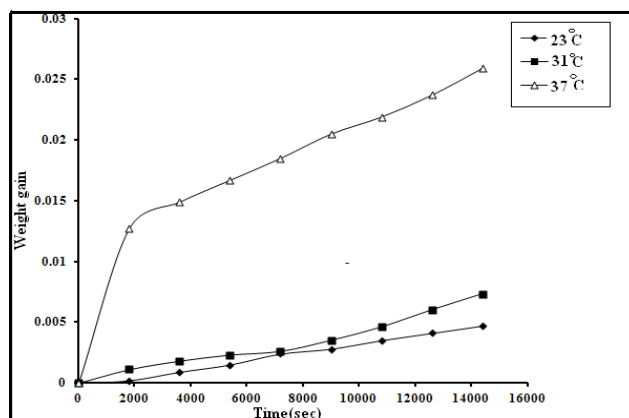


Figure7b) Kinetics of water vapor transmission through ZONLSS film.

The dynamic uptake data was also used to determine various kinetic parameters (Equation 1, 2 & 3) which are given in Table 2. It is noticeable that values of WVTR, at a given temperature, are higher for plain sago starch film as compared to Zinc oxide nanoparticles loaded sago starch film. This may be attributed to the fact that binding of ZnO particles to hydroxyl groups of starch segments may produce additional crosslinks, thus imparting a complexities or compactness to the film matrix. This finally lowers the water vapor permeation.

Table 2 Various parameters obtained for water vapor permeation through PSS and ZONLSS film at 100 % relative humidity.

Water vapor Permeation parameters	23 ⁰ C		31 ⁰ C		37 ⁰ C	
	PSS	ZONLSS	PSS	ZONLSS	PSS	ZONLSS
WVTR (gs ⁻¹ m ⁻²)	11.19x 10 ⁻⁴	3.73x10 ⁻⁴	48.9 x 10 ⁻⁴	6.21 x 10 ⁻⁴	62.1x10 ⁻⁴	24.8 x10 ⁻⁴
Permeance (gs ⁻¹ m ⁻² Pa ⁻¹)	1.78 x 10 ⁻⁷	0.59 x 10 ⁻⁷	7.79 x 10 ⁻⁷	0.99 x10 ⁻⁷	9.91 x 10 ⁻⁷	3.96 x10 ⁻⁷
Permeability (gs ⁻¹ m ⁻¹ Pa ⁻¹)	4.10x 10 ⁻¹¹	1.36 x 10 ⁻¹¹	17.91x 10 ⁻¹¹	2.27 x 10 ⁻¹¹	22.7x10 ⁻¹¹	9.11 x10 ⁻¹¹

Antibacterial investigations

The antibacterial action of ZONLSS film was tested against *E-Coli* as model bacteria, taking plain Sago starch (PSS) film as control. The results of investigations have been well depicted in Figure 8. It is clear from Figure 8 that there is dense population of bacterial cells in the petriplate supplemented with PSS film while a clear zone of inhibition appears around the piece of ZONLSS film in the petri plate as shown in Figure 8. films. Therefore, it is clear that zinc oxide nanoparticles loaded sago starch film has the potential to inhibit bacterial colonies.



Figure 8 Antimicrobial activity in (A) PSS film (control) (B) ZONLSS film (6% ZnO nanoparticles).

CONCLUSION

From the above study it may be concluded that the water vapor permeation properties of sago starch based films, are greatly influenced by temperature. These films can be used as packaging film to restrict moisture permeation, as well as bacterial growth in food products.

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