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Investigation of water vapour permeation and antibacterial properties of nano silver loaded cellulose acetate film

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Abstract: The study describes water vapour permeation and antibacterial properties of silver nanoparticles (SN) loaded cellulose acetate films. The water vapour permeability of plain cellulose acetate film was found to increase with temperature and activation energy for permeation (Ep) was found to be 46.28 kJ mol-1. In addition, increase in relative humidity also enhanced the permeability parameters of the plain cellulose acetate film. Similarly, use of poly ethyleneglycol (PEG) as plasticizer enhanced the water vapour transmission properties of films but it also produces adverse effect on mechanical properties of the films. The silver nanoparticles loaded films have been characterized by surface plasma resonance (SPR), X-ray diffraction, transmission scanning electron microscopy (TEM) and differential scanning calorimetry (DSC). The average size of the silver nanoparticles, as determined using Scherre's equation, was found to be nearly 84nm. The film showed fair antibacterial activities against model bacteria E. coli. The moisture barrier action of cellulose acetate film was also tested, using grapes pulp as model substract. The grape pulp when entrapped within the cellulose acetate film, exhibited a slower moisture loss as compared to the pulp exposed to open air.

Keywords: Cellulose acetate, silver nanoparticles, edible film, and antibacterial activity

Introduction

The antimicrobial packaging is a fast developing technology that can be employed to control the microbiological decay of perishable food products (Conte *et al.,* 2007). Different organic and inorganic active antimicrobial agents can be incorporated into the film matrix to prevent undesirable microbial spoilage occurring during storage of packaged fresh food (Ahn *et al*., 2004). Most of the films, used to preserve food stuff, are made from synthetic polymers whose accumulation in the nature has caused severe environmental problems, particularly in last two decades. These problems are consequences of extensive degradation period of the synthetic polymers, which can be stretched up to 200 years and of the technological difficulties in recycling of these materials (Cagri *et al*., 2004). An alternative, particularly in the area of food packaging, is the development of environment friendly green polymers as potential naturally occurring biodegradable packaging materials. Therefore, recent past has witnessed tremendous research work on fabrication of edible films composed of different varieties of biopolymers such as zein (Del Nobile *et al*., 2008), soy protein (Sivarooban *et al*., 2008), whey protein (Ozdemir *et al*., 2008), cod gelatin (Perez-Mateos *et al*., 2009), rice starch (Bourtoom *et al*., 2008), cassava starch/gelatin blend (Zhong *et al*., 20080), chitosan (Srinivasa *et al*., 2004), (Ziani *et al*., 2008), (Vargas *et al*., 2009), (Bangyekan *et al*., 2006), (Suyatma *et al.,* 2005) etc. In fact, polysaccharides like chitosan, starch has frequently been exploited for the development of food packaging films. Most recently, we have also reported ZnO nanoparticles loaded chitosan film for the purpose of food packaging (Bajpai *et al*., in press). However, apart from having a number of excellent features such as biocompatibility, biodegradable nature, fair film forming capacity, antimicrobial behaviour etc, it also suffers from some

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serious drawbacks such as its great sensitivity towards moisture and its poor water vapour barrier property (Wiles *et al*., 2000). Moreover, use of solvent such as acetic acid, dilute hydrochloric acid, citric acid for dissolution of chitosan affects mechanical properties of the film in adverse manner, and also reduces its biocompatibility. In addition, acid medium favours degradation reactions, particularly when film is to be used for packaging of food stuff (Suyatma *et al*., 2004, Hosseini *et al*., 2008). Therefore, with the major objectives of minimizing such drawbacks as mentioned above, we hereby propose cellulose acetate based edible film loaded with silver nanoparticles to inhibit any possible growth of microorganism in food stuff. Cellulose acetate is water insoluble and hence has minimum affinity for water vapours. The antimicrobial action of silver has been well known since ancient times to fight infections and control spoilage (Pal *et al*., 2007). Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow-target antibiotics, because silver attacks a broad range of targets in the organisms, which means that they would have to develop a host of mutations simultaneously to protect themselves (Hamouda *et al*., 1999). In addition, silver is also used in trace amount in a 'Ayurvedic' food supplement 'Chyavanprash' which is frequently used in India (en. wikipedia website).

Materials and Methods

Materials

Cellulose acetate (CA), silver nitrate and dimethyl formamide (DMF) were obtained from HiMedia, Mumbai, India and used as received. Nutrient agar, agar – agar type I and nutrient broth were received from S. D. Fine chemicals, Mumbai, India. Different salts used to prepare saturated solutions to provide a wide range of relative humidity (RH) were received from E-Merk, Mumbai, India. Millipore water was used throughout the investigations.

Preparation of silver nanoparticles loaded cellulose acetate (SNLCA) film

The SNLCA film was prepared by reduction of Ag (I) ions by DMF in the presence of dissolved CA. In brief, to a 0.2 percent (w/v) solution of cellulose acetate in DMF, pre-calculated quantity of $AgNO_3$ was added and the solution was transferred into Teflon coated petridishes and kept in an electric oven (Tempstar, India) for a period of 24 h at 60^0C. The dry film was removed by pouring water to the petridish. The films so obtained were washed in distilled water and allowed to dry in an oven at ambient temperature. Similarly, plain cellulose acetate (PCA) films were prepared in which silver was not present.

Film characterization

The films were characterized by different instrumentation techniques. DSC analysis was performed with a Metter DSC-30 thermal analyzer with PC and ZOLC. Film of known weight was taken in a sealed aluminum pan and the sample was heated from 40^0C to 260^0C at a heating rate of 20^0C per min. under the constant flow of argon gas. The X-ray diffraction pattern (XRD) of silver nanoparticles loaded film obtained with a PAN analytical Xpert PRO MPD^R X-ray diffractometer. The UV-Visible spectrum of the silver nanoparticles, was also recorded in UV-Visible spectrophotometer (Shimadzu 6300) in the range of 300-550nm. Tem analysis was carried out in Indian Institute of Technology, Guwahati, India.

Moisture content studies

The moisture sorption isotherms were determined gravimetrically using the static method described by Alhamdan *et al.* (1999). Pre-weighed films were placed in Petri dishes inside glass desiccators containing different saturated salt solutions, providing constant relative humidity environment ranging from 3 to 98 percent as described Oluwamukomi *et al*. (2008). The desiccators were placed inside temperature controlled incubators (Tempstar India), set at desired temperature. The samples were weighed at different time intervals using electronic balance (Denver Germany) with an accuracy of 0.0001g. Equilibrium was considered to have been obtained when three consecutive measurements were found to be the identical. The equilibrium moisture contents were calculated on dry basis from which moisture sorption isotherms were obtained.

Water vapor permeability (WVP) measurement

Water vapor transmission of film was measured using ASTM E 96-93 method (1993) (23). The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the film. A sample was placed in between the cups and the silicon coated ring cover and held with four screws around the cups circumference. The air gap was at approximately 1.5 in between the film surface and desiccant. The water vapour transmission rate (WVTR) of each film was measured at 100% RH and 25 $\pm 1^0$C. After taking initial weight of the test cup, it was placed in glass desiccators containing distilled water to provide100% RH. The cups were taken out at different time intervals and weighed accurately. Three replicates of each sample were measured.

The water transmission rate (WVTR) and other

related parameters were calculated (Bozdemir *et al*., 2003) (24).

$$WVTP = \frac{\Delta W}{\Delta t.A} gs^{-1}m^{-2} \quad (1)$$

$$Permeance = \frac{\Delta W}{\Delta t.A.\Delta P} gs^{-1}m^{-2}Pa^{-1} \quad (2)$$

$$Permeability = \frac{\Delta W.\chi}{\Delta t.A.\Delta P} gs^{-1}m^{-1}Pa^{-1} \quad (3)$$

where $\Delta W/\Delta t$ = the amount of water gain per unit time of transfer,
χ = film thickness,
A = area exposed to water surface cm2, and
ΔP= water vapor pressure difference between both sides of the film,

Antibacterial studies

Antibacterial studies of silver nanoparticles loaded cellulose acetate film was investigated quantitatively and qualitatively by the inhibition zone method (Qin *et al*., 2006) and viable cell count method (Pal *et al*., 2007) respectively, with *E. coli* as model bacteria.

Results and Discussion

Formation of SNLCA film

N,N-dimethylformamide (DMF) is an excellent solvent for a number of polymers while at the same time it is a potent reducing agent for some metal cations (Pastoriza-Santos *et al*., 2000). In the present work, DMF acts as a reducing agent for Ag (I) ions and as a solvent for CA. The overall scheme of formation of SNLCA film may be depicted as below:

When a solution of CA and $AgNO_3$ in DMF is heated at 80^0C, silver ions are first reduced to Ag(0) and later on converted into silver nanoparticles. Here, it is also to be mentioned that reduction of silver salt by DMF can be easily monitored from the color evolution of the solution. Visual observations showed that as the reaction proceeded, the color shifted from light yellow to dark brown through orange and olive green. The oxygen rich species of cellulose acetate molecules act as templates for silver nanoparticles thus resulting in almost homogeneous distribution of silver nanoparticles. When the solution is kept overnight at 80^0C, the silver nanoparticles loaded cellulose acetate film is produced. This scheme has been well shown in Figure 1. In order to confirm the formation of silver nanoparticles, the surface plasmon resonance (SPR) of the film also recorded. The spectrum, shown in the Figure 2 clearly shows a strong peak at nearly 420nm, which is a characteristic surface plasmon resonance of spherical silver nanoparticles (He *et al*., 2002).

Characterization of SNLCA film

XRD analysis

The X-ray diffraction pattern of nano silver loaded cellulose acetate film is shown in Figure 3. The diffractogram exhibits a broad peak at 2θ values of 22.5, which confirms the presence of cellulose acetate network (Valent *et al*., 2005). In addition, four sharp are also observed at 2θ values of 38.2^0, 44.40^0, 64.6^0 and 77.5^0, which can be index to (111), (200), (220) and (311) planes of silver nanoparticles (JCPDS NO. 04-0783). Almost similar values of diffraction peaks for nano silver have been reported by others (Vimala *et al*., 2009). Finally, the Scherre's equation was employed to determine grain size:

$$\mathbf{D = \frac{k\lambda}{(b - b_0)Cos\,\theta}} \quad (4)$$

where cauchy peak type is assumed. Here D is the crystal size, λ is the X-ray wavelength, b is the width of the peak (full width at half maximum), b0 is the instrumental peak broadening and the scherrer constant K =0.89. The crystal size for plane (111) was found to be nearly 86nm.

DSC analysis

The glass transition temperature of a polymer given information about transition of the polymer from glassy to rubbery state. Generally, it is believed that if in a polymer- metal composite there are weak electrostatic interactions between metal and polymer then T_g of the composite is expected to decrease as the incorporated metal compounds act like plasticizers or impurity and increase the mobility of polymer molecules, thus resulting in decrease in T_g (Kwon *et al*., 2005). The DSC thermograms recorded for plain CA and SNLCA film have been shown in the Figure 4. It was observed that glass transition temperature for plain and silver loaded film was 117 and 179^0C respectively, thus indicating an appreciable enhancement in the degree of crystallinity of the polymer due to incorporation of silver nanoparticles. This suggests that silver nanoparticles get fairly embedded within the polymer network and the macromolecular chains are fairly cross linked by silver nanoparticles through interactions with electron rich species present along cellulose acetate chains. Almost similar results have also been reported for

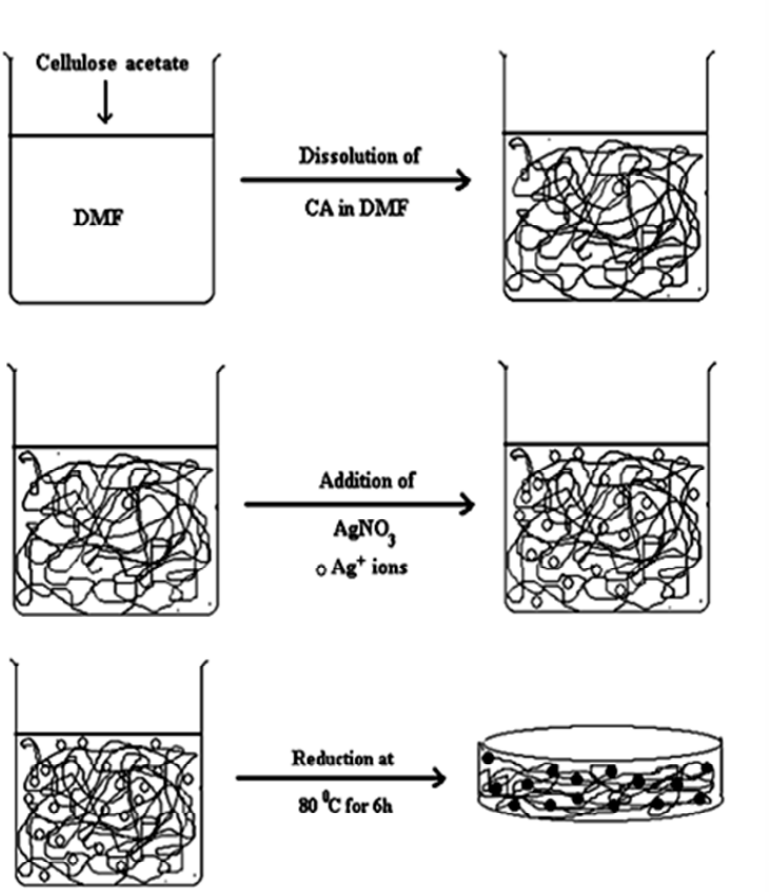


Figure 1. Scheme for formation of nano silver loaded film.

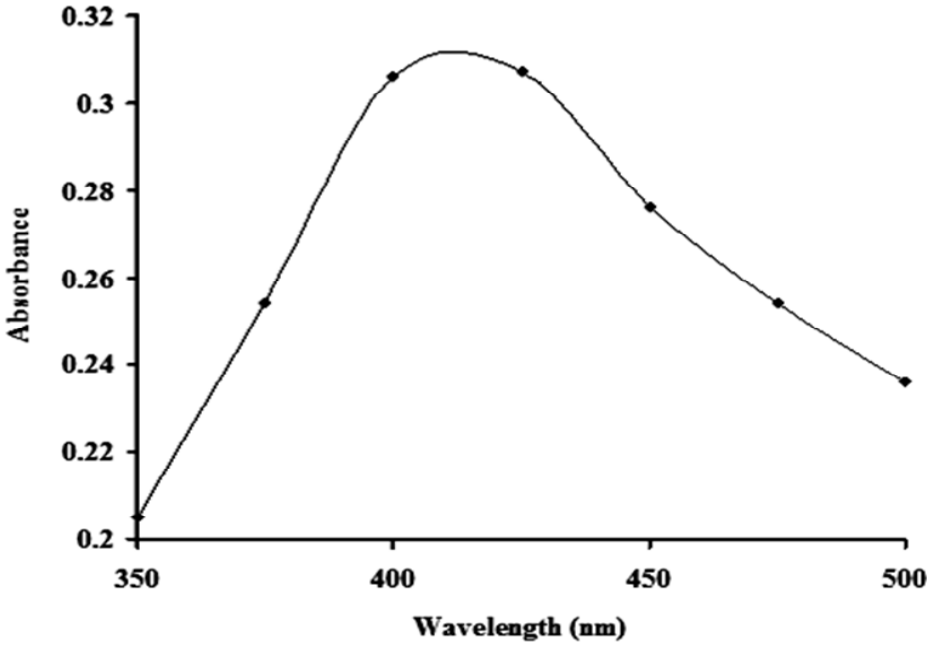


Figure 2. Surface Plasmon spectum for Ag nanoparticles

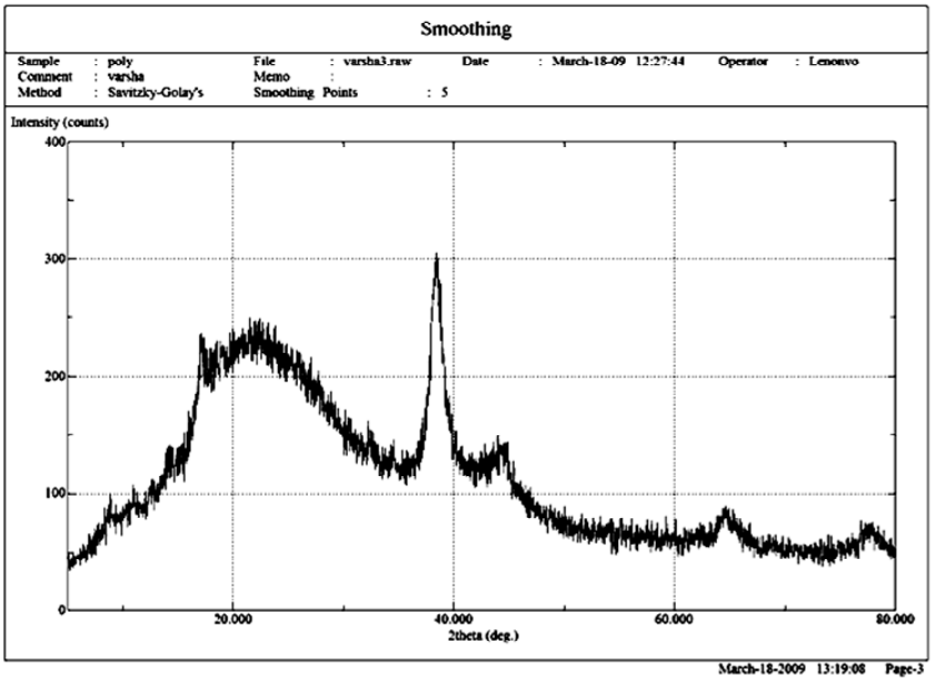


Figure 3. X-Ray Diffraction pattern for Ag nanoparticles loaded cellulose acetate film

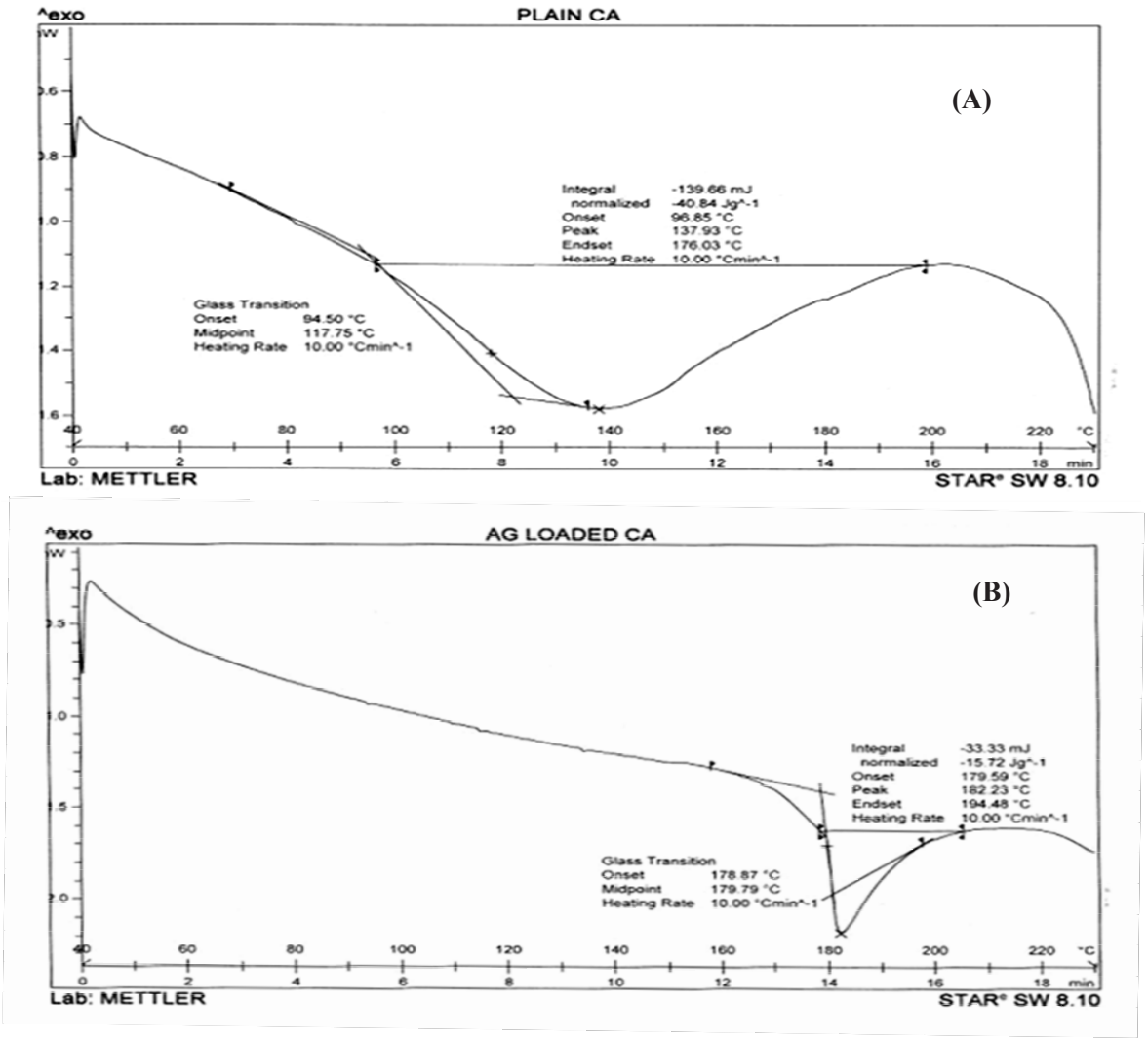


Figure 4. DSC thermogram for (A) PCA and (B) SNLCA film

nano ZnO loaded chitosan film (Napierala *et al*., 2006). The sharpness of the endotherm observed for SNLCA film also indicates enhanced degree of crystallinity.

TEM

Figure 5(A) shows TEM image of the zinc oxide nanoparticles present within the cellulose acetate film. It is quite evident that there is almost uniform particle size distribution of nanoparticles within the matrix. The particle size distribution, as displayed in Fig.5 (B) indicating that the particles formed fall within the narrow range of 10 to 130nm with nearly 51% particles having average diameter of 70nm. However, the value obtained is not in close agreement with that determined using Scherre's equation.

TGA analysis

Figures 6(A) and (B) show the thermograms obtained for PCA and SNLCA film in the overall temperature range of 30 to 800^{0}C respectively. The results obtained reveal that both of the films show marginal weight loss in the initial stage, which might be due to evaporation of moisture present on the films. This moisture loss is quite marginal and occurs in the temperature range of 30 to 70^{0}C. This early moisture loss also indicates hydrophobic nature of the base material cellulose acetate, which has negligible affinity for water vapours. The first initial decomposition temperatures, T_{id} for PCA and SNLCA films were observed to be 260 and 288^{0}C respectively. A higher value of T_{id} for nanosilver-loaded film is due to presence of silver nanoparticles within the film. The overall percent weight loss obtained in the temperature range of 30 to 600^{0}C were found to be nearly 94.2 and 74.6 respectively, thus again establishing the higher thermal stability of silver nanoparticles loaded film as compared to plain CA film.

Water vapor permeation studies

Since one of the main functions of edible films is to decrease the moisture transfer between the food and the surrounding atmospheres, water vapour permeability is a significant physical parameter. High water vapour permeability of a film considerably restricts its use as potential packaging material (Morillon *et al*., 2000). Usually, over small temperature ranges, the temperature dependence of permeability can be represented by an Arrhenius type equation and may be given as (Rogers *et al.,* CRC press).

$$WVP = WVP_0 \exp\left(-\frac{Ep}{RT}\right) \qquad (5)$$

where Ep is apparent activation energy for permeation phenomena (kJ mol-1), R is gas constant (8.314 J mol-1K-1), and T is the absolute temperature (K).The effect of temperature on amount of water vapours permeated through the plain CA film in the environment with relative humidity (RH) of 100% has been shown in the Figure 7. It can be see that the quantity of water vapours permeated through the film increases with temperatures. This may be due to the enhanced motion of polymer segments and also because of increased kinetic energy of permeating water vapour molecules. The dynamic uptake data was analyzed to determine the water vapour transmission rate (WVTR), permeance (P) and water vapour permeability (WVP) of films at three temperatures using equation (1), (2) and (3) respectively. The various parameters have been depicted in the Table 1.

In order to determine the activation energy for permeation (i.e. E_p), logarithmic form of equation (5) was employed. The value of E_p, as determined from the slope of the linear plot between lnWVP and 1/T (see Figure 8) was found to be 46.23 kJmol^{-1}. An appreciably higher value of activation energy may be attributed to the hydrophobic nature of the cellulose acetate, which offers higher energy barrier to the permeating water vapours. The value of E_p obtained in this study is quite comparable to those reported in the case of edible films obtained from other hydrophobic materials such as polyvinylidene chloride (61.9 kJmol^{-1}), polypropylene (42.2-65.3 kJ mol^{-1}), polyethylene (33.4-61.7 kJ mol^{-1}), and waxed laminated cellulose (59.4 kJmol^{-1}) (Kester *et al*.,1989; Forssell *et al*., 2002). Thus it may be concluded that WVP increases with temperature and the hydrophobic nature of CA puts strong barrier against permeation of water vapours. We also observed that due to hydrophobic nature of the cellulose acetate film, it exhibited nearly 10.6 percent moisture content in the environment with RH of 100%, while under similar condition chitosan film demonstrate nearly 72.9 percent moisture uptake.

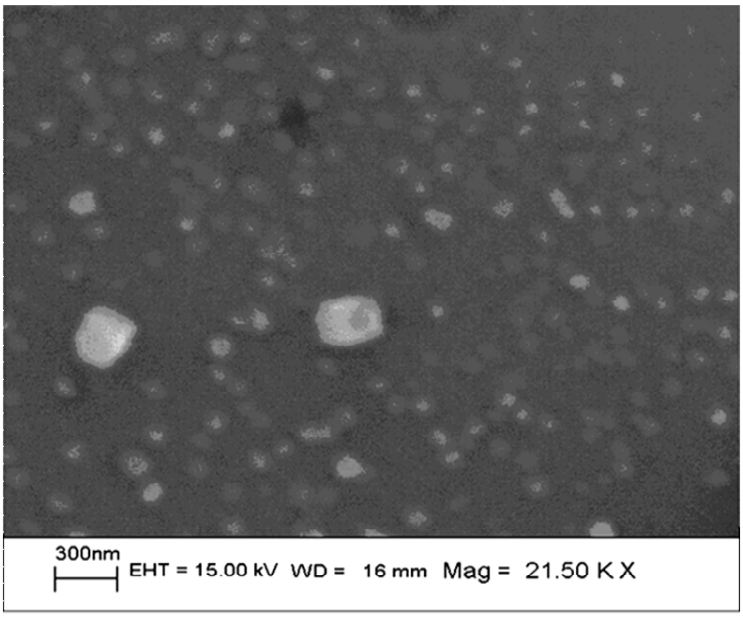
Effect of plasticizer on film properties

Plasticizers are able to increase the free volume between polymeric chains. In doing so, the ease of movement of polymeric chains with respect to each other is dramatically enhanced. The addition of plasticizers to edible film is required to overcome film brittleness caused by extensive intermolecular

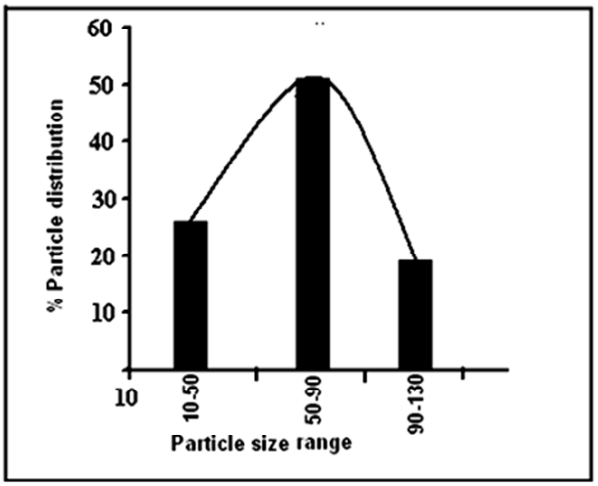
Table 1. Kinetics of permeability of plain chitosan film and cellulose acetate film at room temperature

Parameters	Chitosan*	Cellulose Acetate
WVTR (gs-1m-2)	17.5 X 10^{-2}	1.25 X 10^{-2}
Permeance (gs-1m-2Pa-1)	2.79 x 10^{-5}	1.95x 10^{-5}
Permeability (gs-1m-1Pa-1)	5.59 x 10^{-10}	7.82 x 10^{-10}

*Data reported from our previous work (Bajpai et al., in press)



(A)



(B)

Figure 5(A). TEM image of (A) silver nanoparticles loaded cellulose acetate film and (B) Particle size distribution

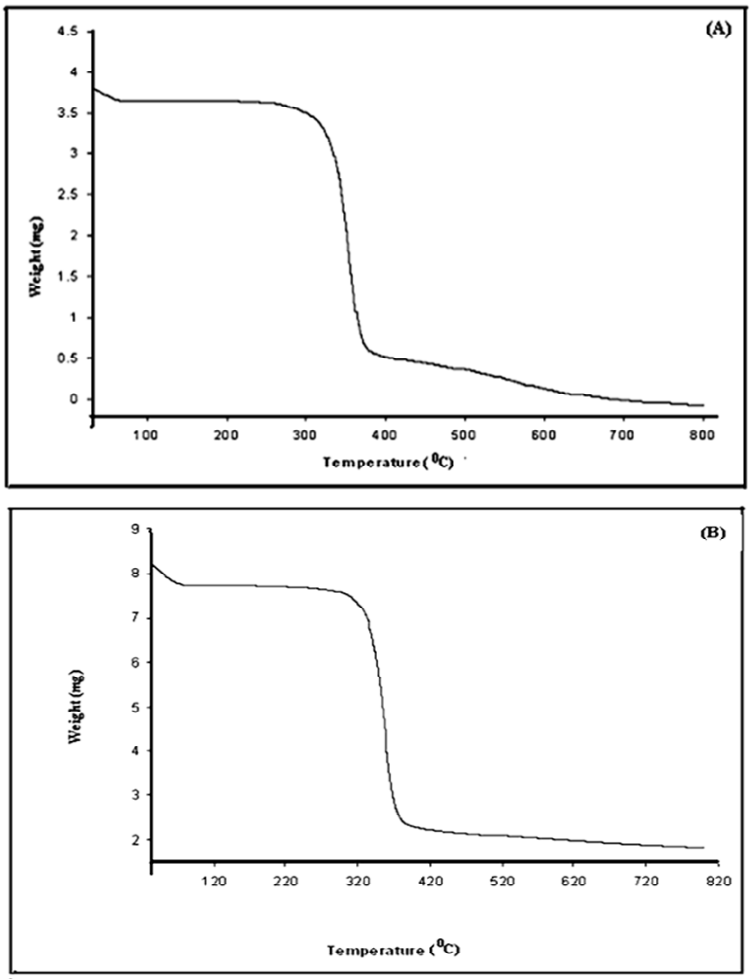


Figure 6. Thermograms for (A) Plain cellulose acetate and (B) nanosilver loaded film

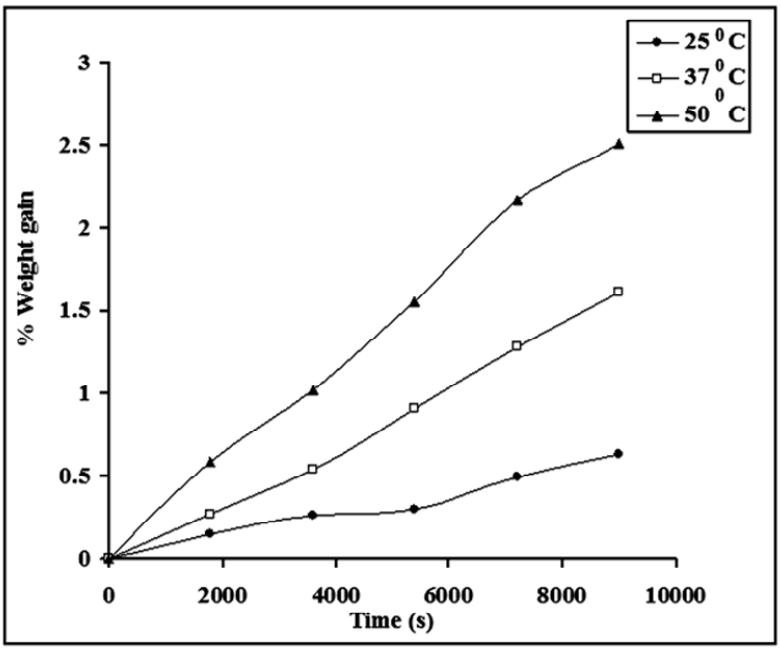


Figure 7. Kinetics of water vapor permeation through plain cellulose acetate film at different temperatures

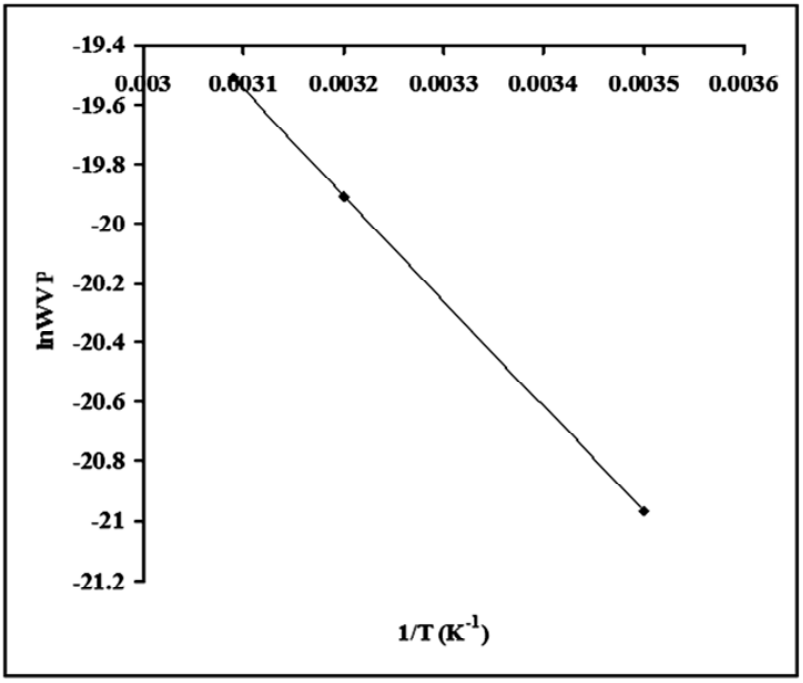


Figure 8. Plot between lnWVP and 1/T for evaluation of activation energy

forces. Plasticizers reduce these forces, thereby improving flexibility and extensibility of the film (Zhong *et al*., 2008). This, in turn, may affect various significant properties of edible films e.g. water vapour transmission, tensile strength and percent elongation at break etc (Aulton *et al*., 1981). In order to investigate this we prepared PEG loaded films, namely PCA (0) and PCA (10) where number in parenthesis denotes the weight percent of the plasticizer polyethylene glycol (PEG 400) present within the film. The kinetics of permeation of water vapour through the films at RH of 100 percent has been shown in the Figure 9. It is quite clear that permeation of water vapours through the film PCA (10) is more as compared to the plain cellulose acetate film i.e. PCA (0). This may simply be explained on the basis of the fact that added plasticizer molecules increase the free volume between the polymer chains thus resulting in enhancement in the ease of movement of macromolecular chains with respect to each other. The reduction in molecular interactions between polymer chains causes an increase in the amount of water vapour permeating through film. It was found that water vapour transmission rate increased from $12.20X10^{-2}$ to $15.71X10^{-2}$ $kgs^{-1}m^{-2}$while permeability was increased from $78.2 X 10^{-11}$ to $100 X 10^{-11}$ $kgs^{-1}m^{-1}Pa^{-1}$for plain and PEG loaded films respectively. The observed findings were further supported by the fact that addition of plasticizer caused an increase in the percent elongation at break from 1.22 to 1.73 while tensile strength decreased from 21.17 to 17.90 MPa respectively. In other words, addition of PEG 400 has resulted in enhancement in water vapour permeation as well as in flexibility of films.

Although, polyethylene glycol is a well-known plasticizer but its antiplasticization action, when used in higher concentrations cannot be ignored. In order to investigate the effect of higher concentration of PEG 400 on the plasticizing property of film, a number of PEG loaded films, namely PCA (20), PCA (40), PCA (60), PCA (80) and PCA (100) were prepared and their DSC thermograms were recorded (see Figure 10). Finally the on-set glass transition temperatures T_g were plotted against weight percent of PEG 400 in the films. The results, as depicted in Figure 11 reveal some interesting facts. Initially, there is a slight decrease in T_g when amount of plasticizer is increased from 20 to 40 wt%, With further increase in PEG content, glass transition temperature tends to increase, then attains an optimum value of 167.05^0C and then it again begins to decrease with further increase in PEG 400 content. The observed findings may be explained as follows; In the initial stage, the observed slight decrease may be attributed to the plasticizing action of PEG 400 as discussed earlier. However, when PEG content is further increased, the large number of oxygen atoms present in polyethylene glycol molecules may form H-bonding interaction with hydrogen atoms of cellulose acetate chains, thus producing rigidity within the film matrix. This causes T_g to increase. When PEG content reaches 80 wt percent, maximum T_g is attained. However, on further

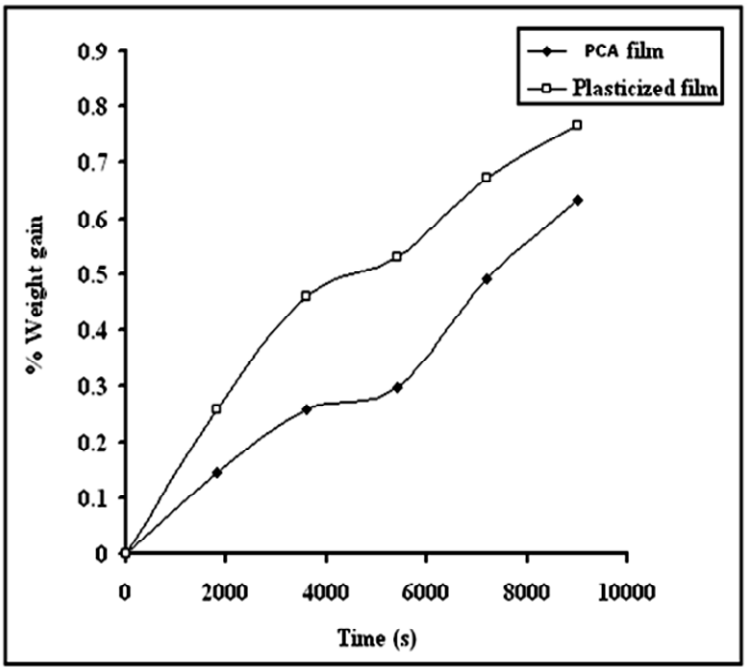


Figure 9. Kinetics of water vapor permeation through plain cellulose acetate film PCA(O) and plasticizer added film PCA(10) at 25^0C

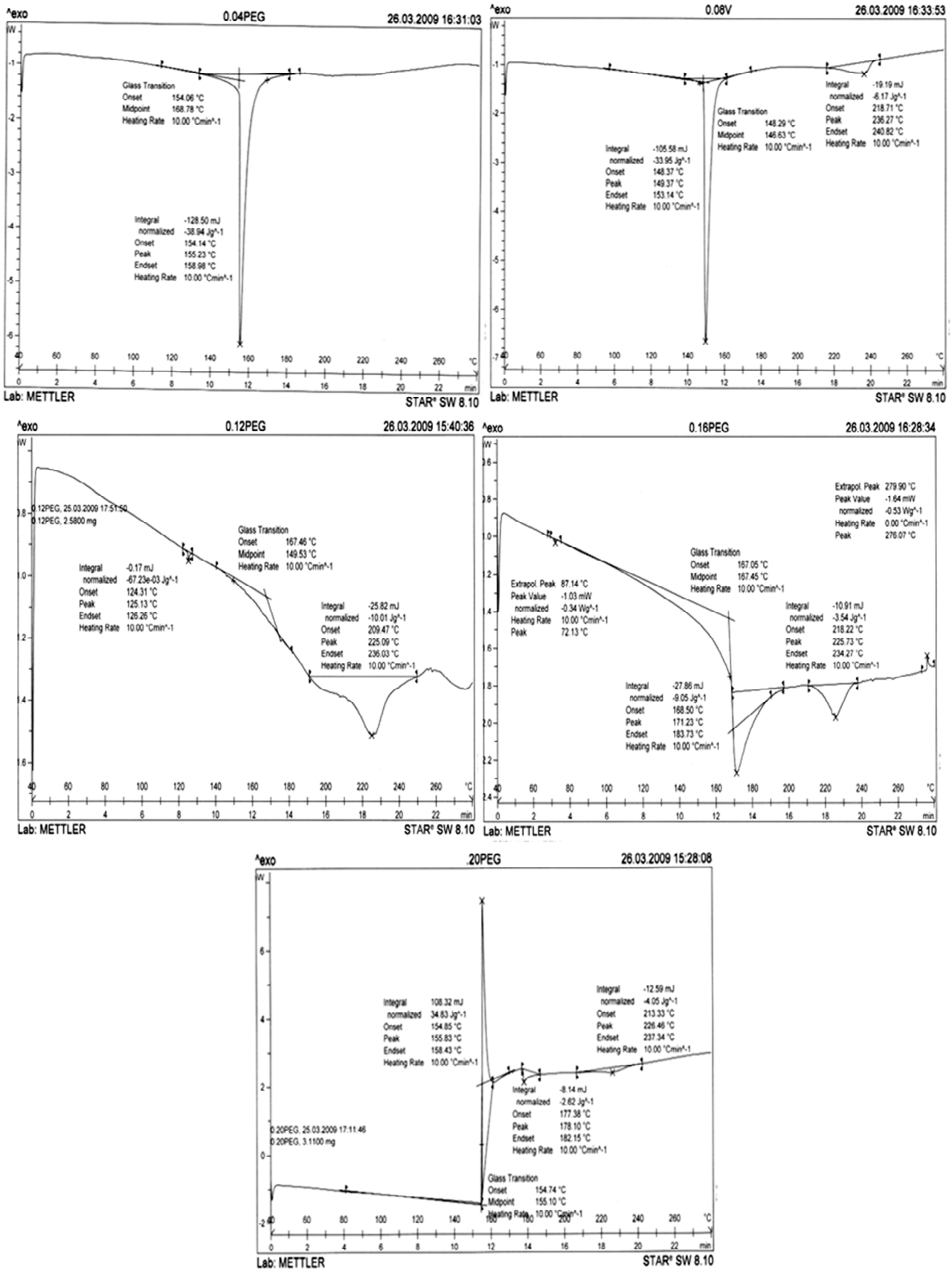


Figure 10. DSC thermograms of plain cellulose acetate films, containing varying amounts of the plasticizer PEG 400

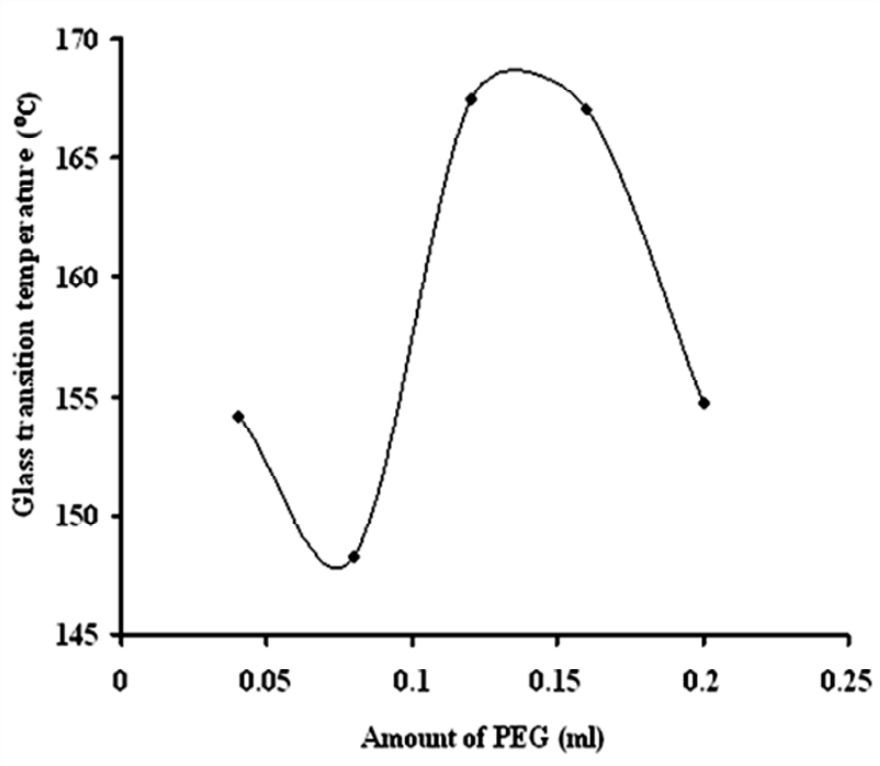


Figure 11. Effect of plasticizer content on glass transition temperature, T_g of the fil

increase in the PEG content, the glass transition temperature again decreases which might be due to phase separation as such a high concentration of plasticizer may cross the limit of compatibility with cellulose acetate chains causing phase segregation and physical exclusion of the plasticizer.

Antibacterial action of SNLCA films

The biocidal action of nano silver loaded films was studied quantitatively and qualitatively against model bacteria *E-Coli* using nanosilver loaded films SNLCA(20), and SNLCA(50) where the number in parenthesis denotes the amount of silver in mg, used to prepare the film. The results of zone-inhibition test, as shown in Fig.12, indicate that Petri plate, supplemented with plain cellulose acetate film (i.e. control set) shows a dense population of bacterial colonies around the circular films. However, the Petri plates, containing circular pieces of nano silver loaded films demonstrate clear zones of inhibition surrounding the circular films. The average diameters of the zones measured longitudinally and transversally, were found to be nearly 2.0 cm and 2.5 cm respectively for the plates supplemented with pieces of SNLCA (20) and SNLCA (50) films respectively. It is quite clear that diameter of circular inhibition zone around SNLC (50) film is greater than that around the film SNLCA (20). This may be attributed to higher silver content in SNLCA (50) film. Finally Figure 13 shows a comparative depiction of time-dependent growth of bacterial colonies in nutrient broth (NB) supplemented with pieces of plain as well as nanosilver loaded films. The results reveal that growth rate of bacterial colonies is suppressed to a greater extent in the NB which contains pieces of nanosilver loaded film. On the other hand, there is sharp increase in the number of bacterial cells in NB containing pieces of plain cellulose acetate film. Therefore, it may be concluded that silver nanoparticles loaded cellulose acetate film has great potential to be used for inhibition of microbial growth in foodstuff.

Moisture loss kinetics of grape pulp

In countries like India the average room temperature in summer is nearly 37-38^{0}C and hence process of moisture loss from fruits is very fast .We put wrapped grape pulp in plain cellulose acetate film and put it at 37^{0}C in dust free chamber to follow the moisture loss kinetics, along with open pulp taken as control. The result of eight days experiment, as shown in Figure 14 clearly indicate that the moisture loss is very rapid in first 24h for both open and film wrapped pulp and thereafter the moisture loss becomes quite slow. In addition, the open-to-air pulp shows a greater weight loss as compared to the pulp that was wrapped with plain CA film. It can be seen that on day 1 open

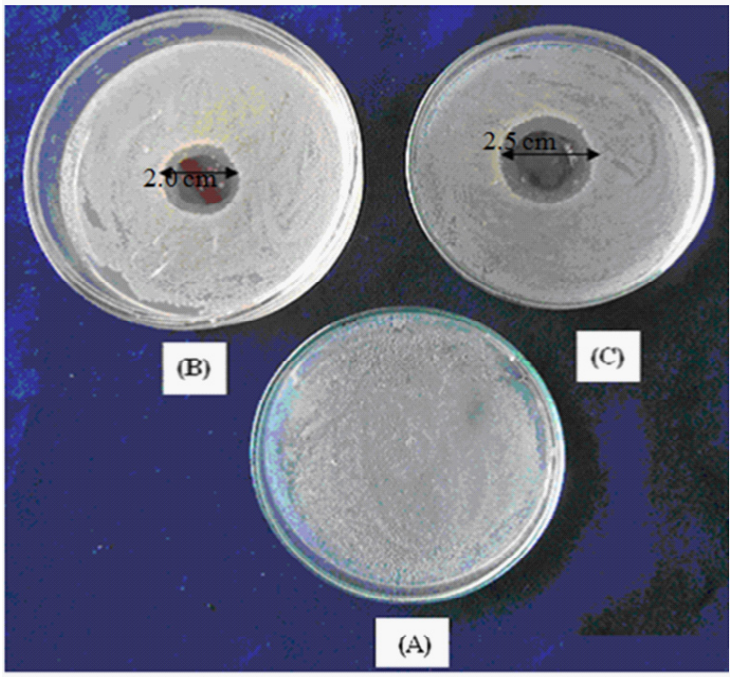


Figure 12. Zone of inhibition in petriplates supplemented with (A) plain cellulose acetate films (B) SNLCA(20) and (C) SNLCA(50) films

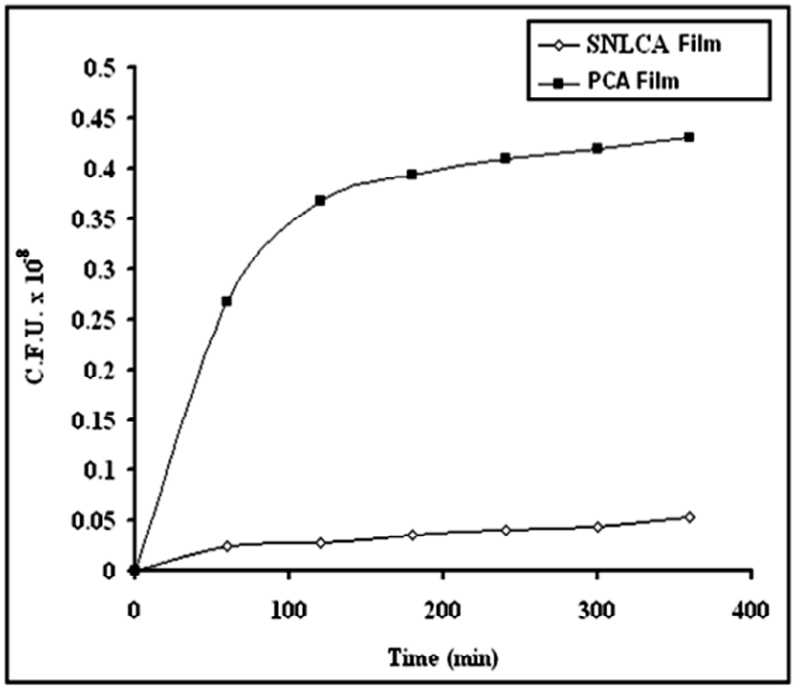


Figure 13. Kinetics of bacterial Growth for plain cellulose acetate film and SNLCA films against *E. coli*

and film wrapped pulps suffer a weight loss of nearly 76 and 60 percent respectively. In order to determine the initial rates of moisture loss, the weight loss for open and film-wrapped pulp was monitored at time interval of two hour, extended over an overall duration of 24h. The results, as depicted in Figure 15 indicate a faster moisture loss in first 2h followed by a relatively slower phase, and the moisture loss is relatively slower for film wrapped pulp.

Conclusion

From the above study it may be concluded that silver nanoparticles loaded silver acetate film has fair antibacterial activity against model bacteria *E. coli*. *As* cellulose acetate has fewer tendencies to permeate water vapors as compared to chitosan, the former can be used as edible film for protection of food stuff against food born microorganisms.

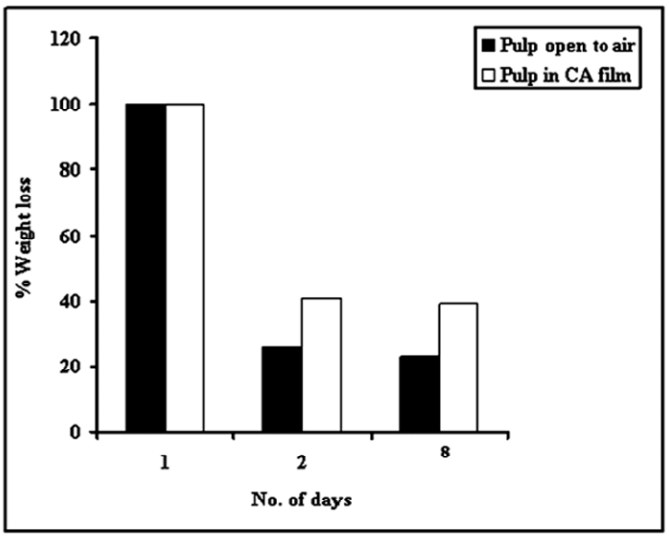


Figure 14. Moisture loss by grapes pulp when placed in open atmosphere and when wrapped in CA film

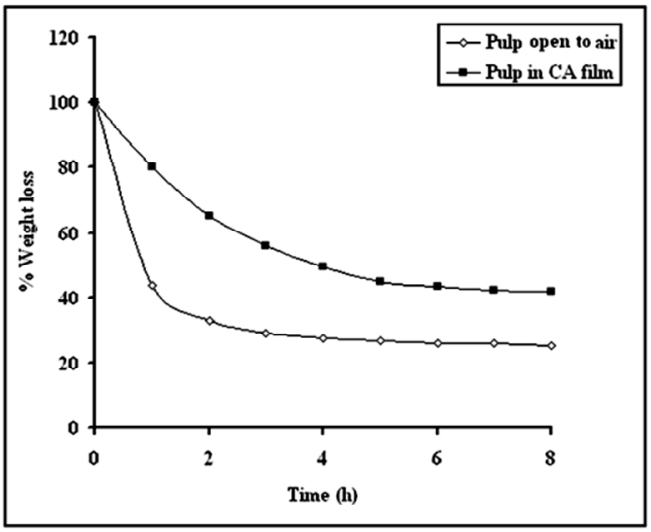


Figure 15. Moisture loss versus time profile for film–wrapped grapes pulp and open to air pulp

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