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Chitosan–silver oxide nanocomposite film: Preparation and antimicrobial activity

SHIPRA TRIPATHI, G K MEHROTRA and P K DUTTA*
Department of Chemistry, Motilal Nehru National Institute of Technology, Allahabad 211 004, India

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Abstract. **The chitosan–silver oxide encapsulated nanocomposite film was prepared by solution casting method. The prepared film was characterized by FTIR, scanning electron microscopy (SEM), thermal studies, and UV-Vis spectroscopy. The elemental composition of the film was studied by energy dispersive X-ray analysis (EDAX). The antibacterial activity of the composite film against pathogenic bacteria viz. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* was measured by agar diffusion method. Our observations suggest that chitosan as biomaterial based nanocomposite film containing silver oxide has an excellent antibacterial ability for food packaging applications.**

Keywords. **Chitosan; silver oxide; nanocomposite; antibacterial; film; food packaging.**

1. Introduction

Antimicrobial enhanced packaging films have great potential for ensuring the safety of foods through controlled release of antimicrobial substances from the carrier film structure to food surface. Recently, antimicrobial packaging has emerged as one of the most reliable and promising tool in the search for the next generation of 'active' packaging (Salleh *et al* 2007). It is well known that silver ions and silver-based compounds are highly toxic to microorganisms (Zhao and Stevens 1998) showing strong biocidal effects on as many as 16 species of bacteria including *E. coli* (Spadaro *et al* 1974). Thus, silver ions, as an antibacterial component, have been used in the formulation of dental resin composites (Yoshida *et al* 1999; Herrera *et al* 2001), ion exchange fibres (Nonaka *et al* 2000) and in coatings of medical devices (Schierholz *et al* 1999). The possible use of silver nanoparticles (AgNPs) as antibacterial agent has, therefore, been investigated as a means of arresting increasing bacterial resistance to conventional bactericides and antibiotics (Gogoi *et al* 2006). Numerous approaches have been used to prepare AgNPs for a rapidly growing list of catalysis, electronics, non-linear optics, and biomaterial applications (Nigam *et al* 2009). For example, laser ablation (Dolgaev *et al* 2002) and inert gas condensation techniques (Turker 2004) have been used to prepare silver nanopowders. A large variety of chemical processes are involved in the preparation of AgNPs with a well-controlled size.

Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes *et al* 2006), magnesium, gold (Gu *et al* 2003), alginate (Ahmad *et al* 2006) and silver have come up but AgNPs have proved to be most effective as they have good antimicrobial efficacy against a wide variety of bacteria, viruses and other eukaryotic micro-organisms (Gong *et al* 2007). AgNPs used as drug disinfectant have some risks as the exposure to silver can cause agyrosis and argyria also; it is toxic to mammalian cells (Gong *et al* 2007).

Chitosan is the second most plentiful natural biopolymer and is relatively cheap (Dutta *et al* 2004). It has attracted considerable interest due to its biological properties, such as antimicrobial activity, antitumor activity, and immune enhancing effect. However, the antibacterial activity of chitosan is influenced by a number of factors, including the species of bacteria, concentration, pH, solvent and molecular mass (Hernández-Lauzardo *et al* 2008). The proposed mechanism for its antimicrobial action is binding to the negatively charged bacterial cell wall, with consequent destabilization of the cell envelope and altered permeability, followed by attachment to DNA with inhibition of its replication (Helander *et al* 2001). Due to excellent antimicrobial property, chitosan film may be used in food packaging (Tripathi *et al* 2008a, 2009a,b; Dutta *et al* 2009). Recently, a chitosan-starch film has been prepared by using microwave treatment which may find potential application in food packaging (Tripathi *et al* 2008b). The recent review on antimicrobial and antioxidative activities of chitosans in food (Friedman and Juneja 2010) also expresses the hopefulness of the different findings of the researchers for further progress to improve microbial food safety and food quality.

In this communication, we report the synthesis of chitosan–silver oxide nanocomposite film via solution casting method. The ultimate objective was to study the antibacterial activity of the nanocomposite film. The nanocomposite film showed significant antibacterial activity against

*Author for correspondence (pkd_437@yahoo.com)

Escherichia coli, Staphylococcus aureus, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The film was characterized by FTIR, TGA, SEM and EDAX analyses. The prepared method is an easy and processable one and the obtained data may be useful for food packaging applications.

2. Experimental

2.1 *Materials*

Chitosan (79% deacetylated) was obtained from the Central Institute of Fisheries Technology (CIFT, Cochin). Acetic acid and tri-sodium citrate were obtained from CDH. Silver nitrate was obtained from Thomas Baker. The test strains, *Escherichia coli* MTCC 1303, *Staphylococcus aureus*, ATCC 6538, *Bacillus subtilis* ATCC 6633, and *Pseudomonas aeruginosa* MTCC 2453 were procured from IMTECH, Chandigarh.

2.2 *Preparation of chitosan–silver oxide nanocomposite film*

The silver–oxide nanoparticles were prepared by sodium citrate reduction of $AgNO_3$ (Jin and Dong 2003). After the addition of trisodium citrate (1% w/v) into $AgNO_3$ solution, black precipitates were obtained. The precipitates were filtered and rinsed with distilled water. The precipitates were then dispersed in distilled water. The prepared silver oxide sol (1 mL) was added into the chitosan solution (1% w/v in 1% acetic acid) and stirred for 2 h. The mixture solution was cast onto glass plates and dried at room temperature for 48 h to obtain the composite film.

2.3 *Characterizations*

The infrared spectra were recorded on Perkin Elmer RX1 FTIR spectrophotometer model. UV-Vis spectrum of the chitosan–silver oxide nanocomposite film was recorded on 1650PC UV spectrometer by tapping the film in the UV-Vis spectroscopy cell. Thermal degradation processes were investigated using TGA (Perkin Elmer Pyris 6) at a heating rate of 5 °C under Ar atmosphere. The morphology of the chitosan–silver oxide nanocomposite film was examined by a scanning electron microscopy (JEOL, Model JSM-6390LV) after gold coating.

3. Results and discussion

3.1 *UV-vis spectrum*

The UV-vis spectrum of chitosan–Ag_2O nanocomposite film is shown in figure 1. A single peak at 425 nm in the spectrum of the composites arises due to the excitation of surface plasmon vibrations of Ag atoms.

3.2 *Fourier transform infrared spectroscopy (FTIR)*

The infrared spectra of chitosan and chitosan based silver oxide nanocomposite film are shown in figure 2. For chitosan spectrum (figure 2a), the characteristic peaks assignment of chitosan are 3429 cm^{-1}(O–H stretch overlapped with N–H stretch), 2921 and 2867 cm^{-1} (C–H stretch), 1640 cm^{-1} (amide II band, C–O stretch of acetyl group), 1592 cm^{-1} (amide II band, N–H stretch), 1485–1380 cm^{-1}(asymmetric C–H bending of CH_2 group) and 1087 cm^{-1} (skeletal vibration involving the bridge C–O stretch) of glucosamine residue. While for the chitosan based silver oxide nanocomposite film (figure 2b), the spectral band appears at 3418 cm^{-1} (axial O–H group of chitosan), 2918 and

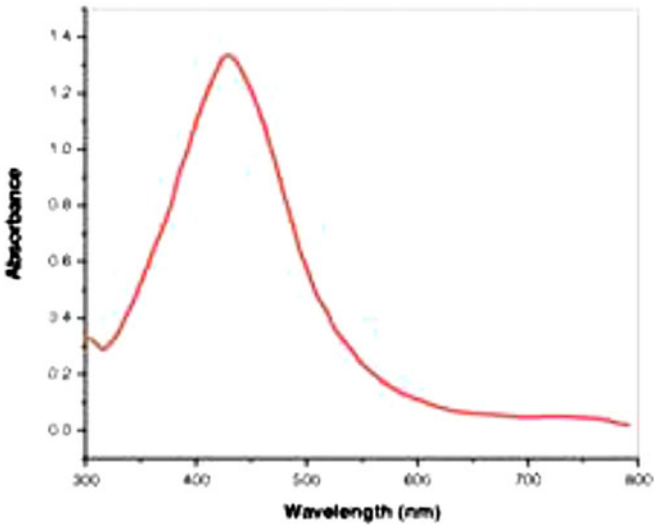

Figure 1. UV–visible spectrum of chitosan–Ag_2O nanocomposite film (in tapping mode).

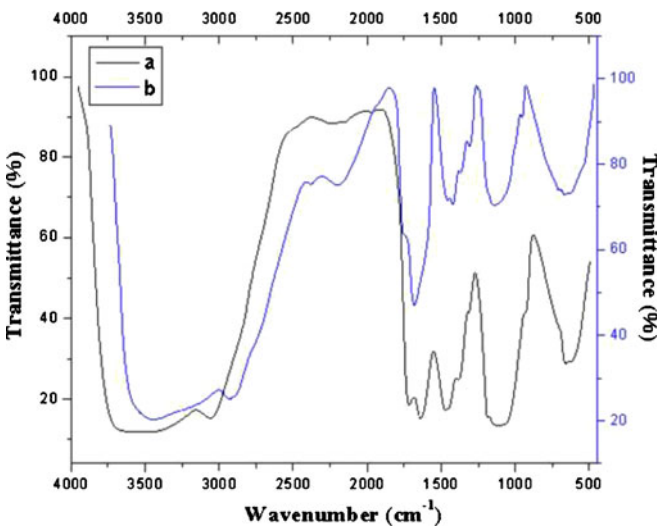

Figure 2. FTIR spectra of (**a**) chitosan, and (**b**) chitosan–Ag_2O nanocomposite film.

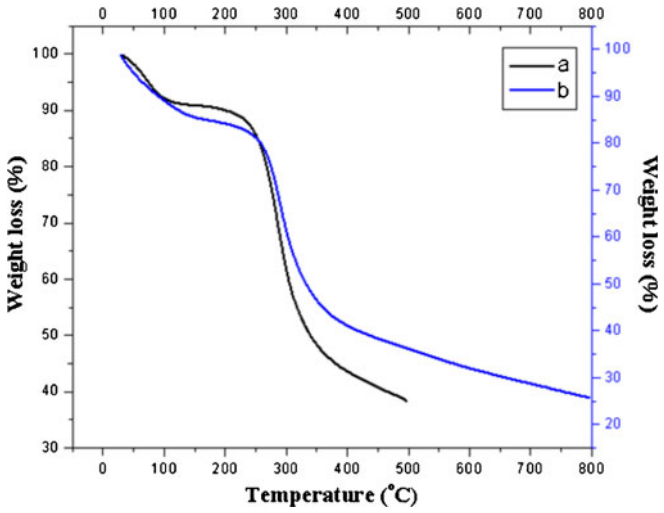

Figure 3. TGA thermograms of (**a**) chitosan, and (**b**) chitosan–Ag_2O nanocomposite film.

2837 (symmetric or asymmetric CH_3 stretching vibration attributed to pyranose ring of chitosan), 2154 cm^{-1} (C–N asymmetric band stretching), 1621 cm^{-1} (C=O carbonyl group vibration), 1381 cm^{-1} (C–C stretching vibration and asymmetric C–H bending of CH_2 group), 1262–1109 cm^{-1} (skeletal vibration involving the bridge C–O stretch) of glucosamine residue whereas the bands around 615 and 437 cm^{-1} are ascribable to intrinsic stretching vibrations of the metal–oxygen bond.

The FTIR analysis suggests that the prepared chitosan silver nanocomposite film consists of an intermediate/or complex of tri-ammonium citrate, chitosan and metal ions.

3.3 *Thermogravimetric analysis*

Thermal degradation of the chitosan and chitosan–Ag_2O nanocomposite film is shown in figure 3. Two weight losses are observed in the chitosan TGA curve (figure 3a). The weight loss at 50–150 °C is due to the moisture vaporization. The other weight loss at 200–300 °C is due to the degradation of chitosan molecule. In the TGA curve of the chitosan–Ag_2O nanocomposite film too two weight losses are noticeable (figure 3b). The weight loss at 70–150 °C is due to the moisture vaporization. The other weight loss at 200–310 °C is due to the degradation of chitosan and silver oxide molecule. This result shows the slight change in thermal stability of the nanocomposite film as compared to the chitosan alone due to presence of silver in the composite film.

3.4 *Morphological characterization*

Scanning electron microscopy (SEM) was used to investigate the surface morphology of chitosan–Ag_2O nanocomposite film with reference to chitosan film and silver oxide nanoparticles. The SEM pictures of chitosan, silver oxide and chitosan–Ag_2O nanocomposite film are shown in figure 4. The chitosan–Ag_2O nanocomposite film has aggregated particle structures (figure 4c), however, the micrographs of chitosan, and silver oxide (figures 4a and b) are uniform. The particles in nanocomposite film were found with almost spherical morphology. However, some of the agglomeration of nanoparticles (may be due to presence of the capping agent) were also found (figure 4c), and the surface was somewhat rough. It is noteworthy that the particles are non-uniformly mixed in a chitosan matrix.

The elemental composition of chitosan–Ag_2O nanocomposite film was studied by energy dispersive analysis of X-rays (EDAX). Figure 5 depicts the EDAX analysis from a selected area. The EDAX analysis confirmed that the nanocomposite structure contained about 4 wt % Ag, 31 wt % carbon, 7 wt % nitrogen, 28 wt % oxygen, 0·40 wt % sodium, and 27 wt % Au (table 1). The presence of trace of Au was, however, also identified by EDAX analysis. We feel that this could be due to gold coating of nanocomposite film during the SEM analysis. The result corroborates the formation of chitosan–Ag_2O nanocomposite film.

3.5 *Antimicrobial activity*

Various methods are employed to prepare antimicrobial chitosan films and coatings for food packaging applications. Solution casting method is one of the popular methods. As a general practice, chitosan films are prepared cross-linked by agylcone geniposidic acid (Mi *et al* 2006), ternary chitosan-glucomann-nisin (Li *et al* 2006), blending of ferulic acid incorporated starch-chitosan (Mathew and Abraham 2008), incorporation of garlic oil, potassium sorbate and nisin (Pranoto *et al* 2005). The derivatized chitosan such as O-carboxymethylated chitosan blended with cellulose from LiCl/N,N-dimethylacetamide solution (Li *et al* 2002) has also been reported for antibacterial study. Most recently, Tripathi *et al* (2008a, 2009a,b, 2010) have synthesized chitosan based antimicrobial films for food applications employing supercritical carbon dioxide and microwave technique. The novelty of this method lies in achieving the film formation without addition of any cross-linker or plasticizer. The aim of the present work is to evaluate antibacterial activity of the nanocomposite film, so the antibacterial activity of the film and its solutions (1 and 2) against *E. coli, B. subtilis*, *P. aeruginosa* and *S. aureus* was measured by agar diffusion method. After 24 h incubation at 37 °C, the chitosan–silver oxide nanocomposite film showed effective antibacterial effect on Gram-positive *B. subtilis, S. aureus* and Gram-negative *E. coli* and *P. aeruginosa*.

The ability of the film and its solution (1 and 2) to inhibit growth of the tested strains are listed in table 2. The inhibitory activity was measured based on the diameter of the clear inhibition zone. If there was no clear zone surrounding, it was assumed that there was no inhibitory zone. Contact area was used to evaluate growth inhibition underneath

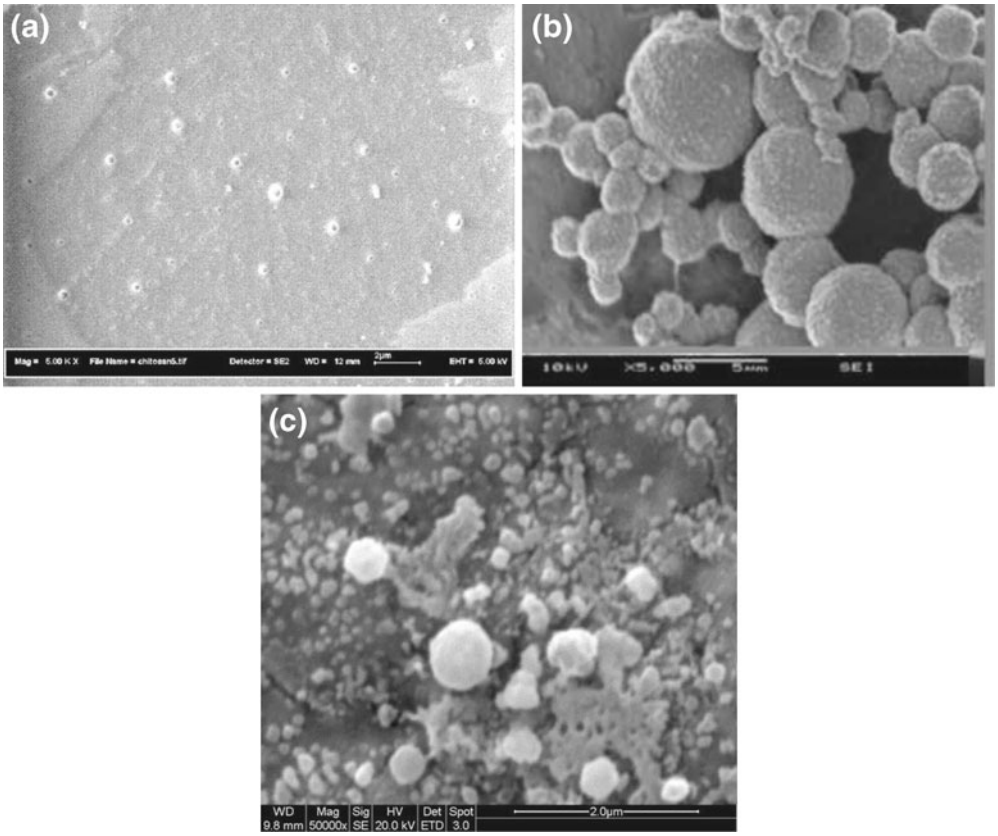

Figure 4. Scanning electron micrographs of chitosan, silver oxide and chitosan–Ag_2O nanocomposite films (**a**), (**b**) and (**c**), respectively.

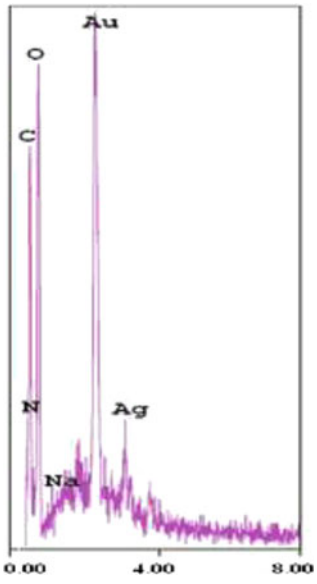

Figure 5. EDAX profile of chitosan silver oxide nanocomposite film.

film discs in direct contact with target microorganisms in agar. In terms of surrounding clearing zone, the control chitosan film did not show inhibitory effect against all tested microorganisms.

The inhibitory zone of chitosan–silver oxide nanocomposite (CS–Ag_2O) film, its solutions 1 (diluted to 50 % in aqueous 0·1 % acetic acid), and 2 (as such) are shown in figure 6. In terms of surrounding clearing zone, chitosan–silver oxide nanocomposite film, its solutions (1 and 2) showed a very clear inhibitory effect against Gram positive

Table 1. Elemental composition of chitosan–silver oxide nanoparticles film.

Element	Wt%
CK	31·59
NK	07·03
OK	28·93
NaK	00·40
AuM	27·43
AgL	04·62
Total	100·00

and Gram negative bacteria. Our results have revealed that solutions 1 and 2 show very clear and greater inhibitory effect against *P. aeruginosa*, *S. aureus, E. coli* and *B. subtilis* in comparison to CS–Ag_2O nanocomposite film. One of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi *et al* 1999). In the Gram-positive bacteria, the major constituent of its cell wall is peptidoglycan and there is very little protein. The cell wall of Gram-negative bacteria on the other hand is thinner but more complex and contains various polysaccharides, proteins and lipids beside peptidoglycan. The cell wall of Gram-negative bacteria also has an outer membrane, which constitutes the outer surface of the wall (Black 1996). Elemental silver has been believed to function antimicrobially either as a release system for silver ions or as a contact-active material (Chan *et al* 2004). In the present study, the chitosan nanocomposite films seem to be contact-active.

In general, chitosan film itself showed some antimicrobial effect even though it did not reveal inhibitory zone in any microorganisms tested. This is reasonable as chitosan has the innate characteristic of antimicrobial activity itself (Darmadji and Izumimoto 1994). According to Brody *et al* (2001), the antimicrobial effect of chitosan occurred without migration

Table 2. Antibacterial activities of film and solutions.

Sample CS–Ag_2O	Diameter (mm) of inhibitory zone against bacteria			
	E. coli	*B. subtilis*	*P. aeruginosa*	*S. aureus*
Solution 1	14	19	22	22
Solution 2	16	20	24	23
Film disc (*d*)	10	10	13	14

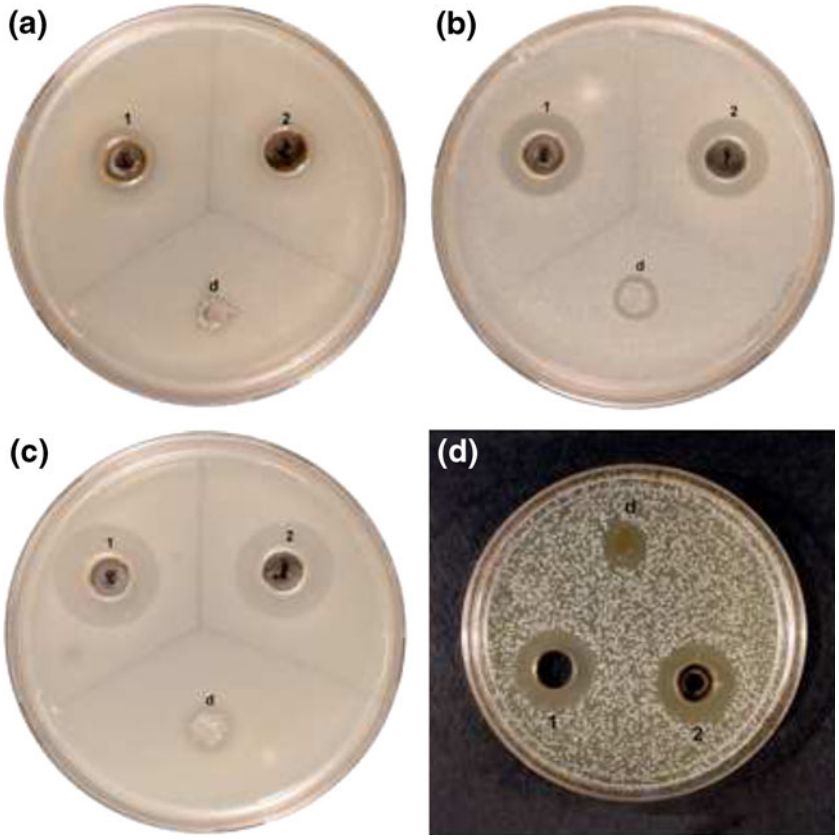

Figure 6. Inhibitory effect of chitosan–silver oxide nanocomposite film and solutions (1 and 2) against (**a**) *E. coli*, (**b**) *B. subtilis*, (**c**) *P. aeruginosa*, and (**d**) *S. aureus*.

of active agents. As chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan is inhibited. Chitosan is incapable to diffuse through the adjacent agar media (Coma *et al* 2002). The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material. The chemical structure and the crosslinking level of the films also affect this phenomenon (Cagri *et al* 2001). When antimicrobial agents are incorporated, there will be diffusing materials through agar gel, and furthermore, resulting in clearing zone on the bacterial growth. Incorporating antimicrobial agents into chitosan edible film thus improves antimicrobial efficacy of chitosan, as diffused antimicrobial actively would add to non migrated antimicrobial potency of chitosan.

Chitosan has strong affinity towards metal ions because of the presence of numerous amine and hydroxyl groups (Varma *et al* 2004). Under alkaline condition chitosan can reduce Ag^+ ions to AgNPs (Murugadoss and Chattopadhyay 2008).

The outer membrane (OM) of Gram-negative bacteria such as *E. coli* consists of lipopolysaccharides (LPS) containing phosphate and pyrophosphate groups which render the cell surface negatively charged (Prescott *et al* 2002). As chitosan is a cationic polymer, it can attach to the *E. coli* cell wall by electrostatic interaction.

Below pH 6·5 chitosan can interact with the bacterial cell wall to destabilize it and alter cell permeability (Helander *et al* 1998, 2001). This process is probably enhanced by the binding of AgNPs to thiol-containing proteins present in the cell wall, with some of the AgNPs penetrating the cell wall to compromise permeability (Feng *et al* 2000; Gogoi *et al* 2006). This can lead to leakage of proteins and other intracellular constituents, and inactivation of the organisms (Helander *et al* 2001).

It has been proposed that AgNPs could interact with sulfur containing intracellular proteins in bacteria (Feng *et al* 2000). Further, chitosan has been studied in terms of bacteriostatic/bactericidal activity to control the growth of a wide variety of bacteria. Chitosan has several advantages over other types of disinfectants because, according to Kim and Choi (1998), it possesses a higher antibacterial activity and a broader spectrum of activity. The inhibitory activity of chitosan was observed on surface spoilage bacteria (Ouattara *et al* 2000a,b; No *et al* 2002; Savard *et al* 2002; Coma *et al* 2003; Gerasimenko *et al* 2004) and on various pathogen food strains (Siragusa and Dickson 1992; Helander *et al* 2001; Tsai and Su 1999; Tsai *et al* 2000; No *et al* 2002). The present investigation, therefore, indicates that the composite was efficient for inactivating bacteria, possibly due to synergistic effect of both the Ag_2O nanoparticles and chitosan in the composite. The results suggest that the antimicrobial activity of chitosan can be enhanced with incorporation of silver oxide nanoparticles into chitosan film matrix as an easy processable technique for food packaging applications.

4. Conclusions

The chitosan–silver oxide nanocomposite film was prepared via solution casting method. The morphology of nanocomposite film was examined by SEM. The antibacterial effects of nanocomposite films against *E. coli, B. subtilis*, *P. aeruginosa* and *S. aureus* were examined by agar diffusion method. The results reveal a nanoparticle formation within the chitosan matrix. It was proved that the nanosilver containing chitosan film had an excellent antibacterial performance. Typically, the nanocomposite film may be used to wrap foods that are highly susceptible to microbial growth or directly used as a surface coating on perishable fruits and vegetables to enhance microbial safety and extend food shelf life.

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