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Bactericidal paper trays doped with silver nanoparticles for egg storing applications

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Abstract. **In this study, a cost-effective way to deposit the silver nanoparticles (AgNPs) on paper egg trays was developed, which proved suitable for prolonged storage of table eggs for house-hold use without deterioration of egg quality. Silver nanoparticles were synthesized based on chemical reduction approach and mixed with gelatin–chitosan mixer used as a colloidal stabilizer as well as fixing agent. AgNPs-doped paper egg trays were characterized by TEM, SEM, FTIR, EDX and XRD. AgNPs containing egg trays were tested for its bactericidal effect against commonly found bacteria on egg shells, *E. coli*, *S. aureus*, *Streptococcus* spp and *Salmonella* spp. Storing of eggs in the AgNPs-deposited paper egg trays improved the shelf-life of the eggs by more than 14 days compared to controls (eggs stored in conventional trays). In conclusion, the developed paper trays possessed strong antimicrobial activity and it could be an effective storage material for eggs.**

Keywords. **Nanoparticles; gelatin; chitosan; silver nanoparticle; egg storage.**

1. Introduction

Development of new compounds or modifications of the existing ones for improved antimicrobial activity is a high priority endeavour, wherein nanotechnology provides a promising means with its wide range of antimicrobial formulations. Combating this by using nanostructured coats possessing antimicrobial properties have been attracting attention in recent years [1–5]. Silver nanoparticles (AgNPs), by virtue of their antimicrobial properties, are used as a germicidal compound in commercial products such as catheters, clothing, toys, cosmetics and plastics. AgNPs release free radicals; induced reactive oxygen species and cause oxidative injury inside bacterial cells. The sol–gel method is a widely used technique for coating AgNPs on the surfaces because the particles are synthesized through wet chemical process. In the sol–gel method, two different approaches such as dip coating and spin coating were commonly employed [6–13].

Microbial contamination is considered as a serious issue in pharmaceutical and food packaging industries. The surface-centered microbial infections play an important role in food spoilage, spread of food-borne infections and bio-fouling of materials [14–16]. The worldwide trend in the fast growing poultry industry, especially in developing nations, is towards the development of alternative strategies to manage infectious diseases.

Eggs are a wholesome nutritious diet. Its affordability and ease of availability has made it a protein-rich food source to all sections of the society. Consumption of good quality eggs is thus of paramount importance. In recent years, disease outbreaks associated with bacterial contamination of egg and egg products were reported worldwide [17]. Several studies have reported a high incidence of bacterial contamination in eggs stored in retail outlets on reusable egg trays without proper hygiene. It is also suggested that reduction of bacterial contamination will help to increase the shelf-life of the eggs [18–20].

In this work, we developed a new method to prepare antimicrobial paper egg trays by using AgNPs. For the surface coating, initially, the silver-doped colloidal solution was prepared using chitosan, gelatin with AgNPs and it was doped on paper egg trays by using airless spray method. The gelatin and chitosan act as adhesives as well as colloidal stabilizers to fix the AgNPs. The prepared egg trays were characterized based on SEM, TEM, EDX, XRD and FTIR. To study the storage applications, fresh eggs were stored in silver-doped egg trays and their internal and external qualities were checked at different day intervals and the results were compared with control samples.

2. Experimental

2.1 *Preparation of AgNPs paper egg trays*

For AgNPs synthesis, 10 ml of 0.1 M cetyltrimethylammonium bromide (CTAB), 0.5 ml of 0.1 M ascorbic acid, 5 ml of 2.5×10^{-4} M tri-sodium citrate solution, 10 ml of

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2.5×10^{-4} M of $AgNO_3$ were taken in a conical flask and finally 1.5 ml of 0.1 M of NaOH solution was added and stirred for 2 h. Then, the nanoparticles were collected by centrifugation at 8500 rpm for 15 min. For the hybrid solution preparation, 2 g of gelatin and 2 g of chitosan were taken separately, the gelatin was dissolved in hot water and the chitosan was mixed with 1 ml of 2% acetic acid solution and 100 ml of distilled water. The gelatin and chitosan solutions were mixed together and stirred for 30 min. Then, 2 g of AgNPs were added and stirred for another 30 min. For the silver nanoparticles deposition, the commercially available double-layered paper egg trays (30 cm × 30 cm, thickness 5 mm, weight 110 g per tray) were sprayed with AgNPs contained colloidal solution using conventional airless spray method and dried at room temperature for 1 h.

2.2 *Characterization*

To quantify the amount of silver in the AgNPs-doped paper egg trays, we performed an acid digestion of the paper trays and analysed the amount of dissolved silver with inductively-coupled plasma atomic emission spectrometer (ICP-AES). Briefly, approximately 100 mg of dried paper was reacted with 5 ml of 70% nitric acid in 5 ml of water and then boiled until the paper disintegrated. After a few minutes of cooling, ~5 ml of hydrogen peroxide (30%) was added and the suspension was reboiled. After cooling, the suspension was filtered through a glass paper filter and the effluent was diluted to 100 ml with water. The silver content of the effluent was measured with ICP-AES (Iris, Thermo Jarrell Ash).

The chemical composition of nanoparticles and the silver-doped paper egg trays and their crystalline structures were examined by using TEM (S-3400 N model, Hitachi), SEM-EDX (Quanta 200 FEG), FTIR (Perkin Elmer Spectrum1 FT-IR instrument), Raman spectra (Bruker RFS 27: Stand-alone FT-Raman Spectrometer), XRD (Bruker) at the sophisticated analytical instrumentation facility (SAIF), Indian Institute of Technology, Chennai, using established methods. The AgNPs-doped egg trays were dried and the ash was used for the analysis.

2.3 *Determination of the antimicrobial property of AgNPs*

For antimicrobial studies, four different bacterial isolates, one each belonging to *Escherichia coli*, *Staphylococcus* spp, *Streptococcus* spp and *Salmonella* spp, isolated from egg shell surfaces and identified, were used. The bacterial isolates were spread on Muller Hinton agar, and the silver-doped egg tray cartons were cut and used as discs to determine the antibacterial effect similar to that of Kirby-Bauer method for antibiotic sensitivity. The zone of inhibition for the silver-doped egg tray paper disc was measured and compared with standard antibiotic disc.

2.4 *Determination of external quality of the egg*

To study the egg storing applications, the external quality parameters such as (i) egg shape index (SI), (ii) specific gravity (SG) and (iii) depth of air cell were examined at regular time intervals.

(i) *Determination of SI*: Egg shape was determined by using Vernier callipers and from the measurements, the average width and average length of the shape index was calculated from the formula, SI = average width/average length × 100.

(ii) *Determination of SG*: SG of egg was determined by Archimedes' method. The eggs were weighed in air on an electronic balance (Sartorius). The weight of the water displaced by the eggs was determined by submerging the eggs in a beaker of room temperature water on the same tarred scale. Specific gravity of the egg was then determined using the equation, SG = egg weight in air/(displaced water weight × temperature correction).

(iii) *Determination of air cell depth*: Depth of the air cell is the distance from its top to its bottom, when the egg is held air cell upwards.

2.5 *Determination of internal quality of the eggs*

To study the internal quality, different parameters were examined at regular time intervals and they are as follows:

(i) *Albumin index (mm) determinations*: Firmness of egg white is correlated with the albumin quality. The measurements of the albumin height were done on the thick albumin, not touching the yolk and avoiding the chalazae. After breaking, open the egg, the height of the thick albumin is measured using a tripod stand micrometer or a spherometer, while the width and the diameter of the thick albumin is measured by using the Vernier callipers. Albumin index = height of albumin (mm)/average width of albumin (mm).

(ii) *Yolk index (mm) measurement*: Yolk index was determined by measuring the height and diameter of the yolk with a Vernier callipers. The yolk index equation is: yolk index = height of the yolk (mm)/average diameter of yolk (mm).

(iii) *Haugh unit measurement*: The standard tripod micrometer was used to calculate the Haugh unit. This device measured the albumin height in millimetres. The albumin height (H) along with egg weight (W) measured previously was used in the following HU equation:

$$\mathrm{HU} = 100 \log(H + 7.57 - 1.7W^{0.37}),$$

where W = weight of egg (g) and H = height of thick albumin (mm).

2.6 *Determination of silver in egg samples*

After 30 days, the egg samples were analysed for silver content by graphite furnace atomic absorption spectrometry (GF-AA, Perkin-Elmer Analyst 100). The white albumin and yellow layers were collected separately for silver analysis. The white and yellow layers were digested by

heating in 2 ml of nitric acid (70%) at 40–50°C for 30 min, being allowed to cool, and heating with an additional 1 ml of hydrogen peroxide (30%) at 30°C for 30 min. The solution from the egg sample digestion was diluted with water and analysed by GF-AA.

The silver loss from the AgNPs doped egg tray was determined from the GF-AA values. This value was expressed as a percentage of the total silver mass contained in the papers, as determined by ICP-AES measurements.

3. Results and discussion

In this report, we produced AgNPs using chemical reduction system and it was formed by using CTAB/trisodium citrate/ascorbic acid. For the AgNPs synthesis, initially the molar ratio concentration of the $AgNO_3$/CTAB/trisodium citrate/ascorbic acid was fixed as 1 : 2 : 2 : 2, and the NaOH was added to increase the pH. In the chemical reduction process, CTAB and ascorbic acid induced the formation of AgBr from the $AgNO_3$. The addition of sodium hydroxide simultaneously increases the pH of the solution and it also increases the reducing capacity of the ascorbate. Finally, the silver ions are reduced to form AgNPs. Before adding NaOH, the solution is milk white colour, after adding NaOH the milk white colour changed to golden yellow. The formed AgNPs were confirmed by using spectrophotometry and it showed UV bands at 450 nm. Initially, the silver ions were capped with trisodium citrate to control the particle growth. For the micelles formation, we used the common cationic surfactant CTAB, because previous report indicated that the CTAB helps to form the positively-charged silver particles and it forms a bilayer on the Ag cluster surface. The CTAB micelle concentrates the Ag^+ ions and intermediates the formation of nanoparticles through hydrophobic and van der Waals forces [21]. The formation of bilayer capping of the shell helps the AgNPs to be more stable in aqueous solution. In our method, the role of CTAB was also confirmed by measuring the zeta potential values and it was around +18, which indicate the positive surface nature of the particles. In the micelles, CTAB and ascorbic acid induces the formation of AgBr due to poor reducing property. Finally, the silver ions are reduced to form AgNPs upon addition of NaOH. The addition of NaOH simultaneously increases the pH of the solution and the reducing capacity of the ascorbate. The nanoparticles morphologies were studied by using SEM and TEM. These images confirmed that the synthesized particles are spherical and their sizes were around 40 ± 2 nm (figure 1a). The elemental compositions, crystal structure and the functional group of AgNPs were confirmed by using EDX, XRD and FTIR spectra (figure 1b). The AgNPs showed peaks at 3264 cm^{-1} (strong stretching from $AgNO_3$), 2294 cm^{-1} (OH plane), 1384 cm^{-1} (NO_2) in FTIR. The XRD pattern of results of AgNPs showed the diffraction peaks at $2\theta = 38.08$, 44.49 and 64.20° assigned to the (111), (200) and (220) planes. The diffraction patterns highly matched the database of joint committee on Powder Diffraction Standards (JCPDS) file no. 04-0783. The average grain size of the AgNPs formed in the chemical reduction process was determined using Debye–Scherrer formula, $D = K\lambda/\beta\cos\theta$, where D is particle diameter size, K a constant equal to 0.94, λ wavelength of X-ray source (0.1541 nm), β the full-width half maximum (FWHM) and θ the diffraction angle corresponds to lattice plane (111). For the particle size calculations, $K = 0.94$, $\lambda = 0.154$ nm, β is calculated as 0.0039 radians (β = B1 (observed)−B2 (resolution) × 3.14/180), $2\theta = 64.24$, so $\theta = 32.12$. D was calculated as $D = 0.94 \times 0.154/0.0039 \times \cos 32.12$, so $D = 45.02$. The D value of $2\theta = 38.08$ was calculated as 37.35 nm. The mean size of AgNPs with respect to (111) of Bragg's reflection gave the size as about 37.35 nm. The EDX detector indicated that the prepared nanoparticles contain major amount of Ag and trace amounts of O, Cu and C are present. The carbon and copper peaks correspond to the TEM holding grid. Throughout the scanning range of binding energies, no obvious peak belonging to any impurity is detected. The result indicates that the synthesized product is composed of high purity Ag. The paper egg tray was doped with AgNPs using airless spray approach, and the coating was confirmed by using SEM, EDX, XRD and FTIR. The SEM results indicated that the coating was highly uniform and the thickness was around 5–6 nm (figure 2a). The AgNPs-doped egg trays showed the FTIR peaks at 3524 cm^{-1} (NH symmetric stretching), 2966 cm^{-1} (CH_3 stretching), 2877 cm^{-1} (OH stretching), 1433 cm^{-1} (CH_3 symmetric bonding), 1022 cm^{-1} (N–N stretching), 875 and 830 cm^{-1} (weak bonds of $AgNO_3$ ions), 712 cm^{-1} (C–O–C stretching). Gelatin–chitosan hybrid mixture coated egg tray XRD pattern showed the diffraction peaks at $2\theta = 27.80$, 32.18, 38.01, 46.04 and 64° assigned to the (101), (194), (200), (111) and (36) planes. The EDX analysis of AgNPs–gelatine–chitosan-doped egg tray showed 39.97% of C, 30.68% of O, 2.91% of nitrogen, 10.73% of Ca, 3.89% of Ag and other trace elements (figure 2b). The presence of silver ions in EDX results indicated that the successful doping of silver on paper egg trays. The acid digestion of the AgNPs-doped paper egg tray showed silver content ranging from 20 to 21.4 ± 1.38 mg per 100 g of paper egg tray.

3.1 *Determination of antimicrobial property of silver-doped egg trays*

To compare the antimicrobial activity of AgNPs and the AgNPs mixed solution were tested against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* and *Salmonella* species in MHA plates. The results indicated that the AgNPs and the AgNPs mixed solution showed similar antimicrobial activity against *S. aureus* and *Streptococcus*. But with *Salmonella*, the AgNPs mixed solution showed 42% more antimicrobial activity than AgNPs (figure 3). The mode of bactericidal action in the AgNPs-doped hybrid mixture is unclear. Based on our studies, we found the minimal inhibitory concentration (MIC) needed for AgNPs to

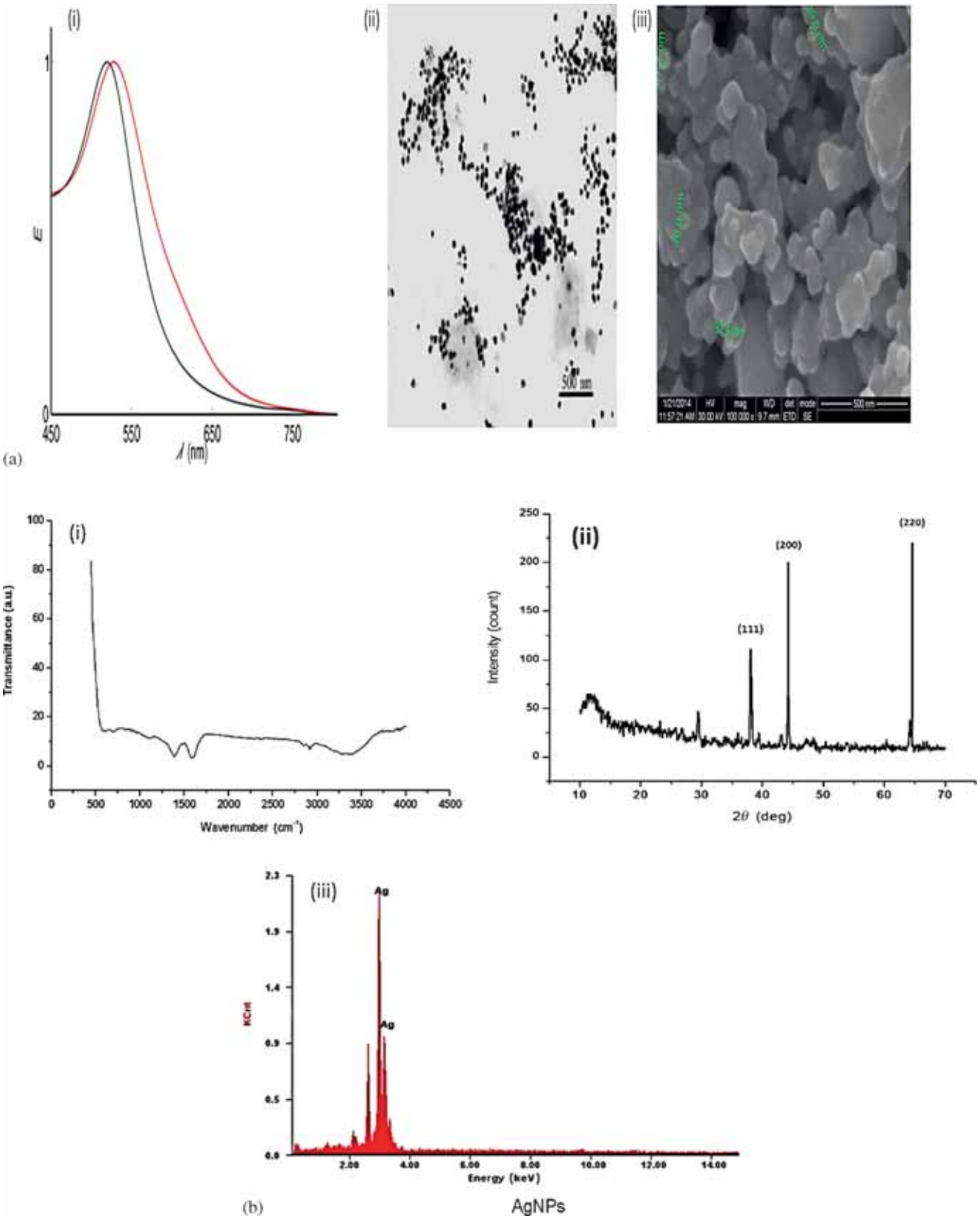

Figure 1. (**a, i**) Results of absorption spectra of silver nanoparticles and the size measurement results of silver nanoparticles. The UV absorption spectrum was recorded using spectrophotometer and the size measurement of silver NPs by (**ii**) transmission electron microscope and (**iii**) scanning electron microscope. (**b**, **i**) Fourier transforms infrared absorbance spectroscopy, (**ii**) X-ray diffraction and (**iii**) energy dispersive X-ray microanalysis results of silver nanoparticles.

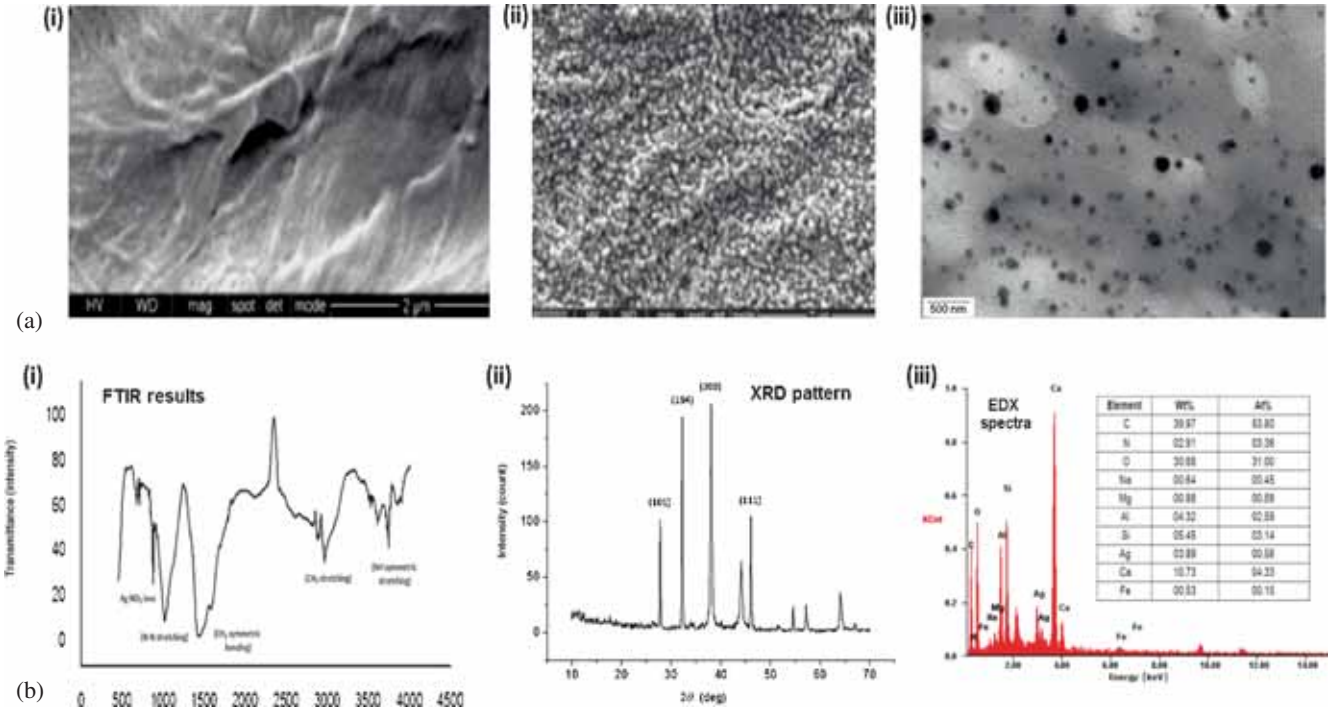

Figure 2. (**a**) SEM images showing (**i**) uncoated paper, (**ii**) silver-doped paper egg trays corresponding to 2 μm and (**iii**) silver nanoparticles-doped egg trays (doping corresponds to 500 nm). (**b**) FTIR, XRD and EDX analyses results of hybrid mixture-coated paper egg tray.

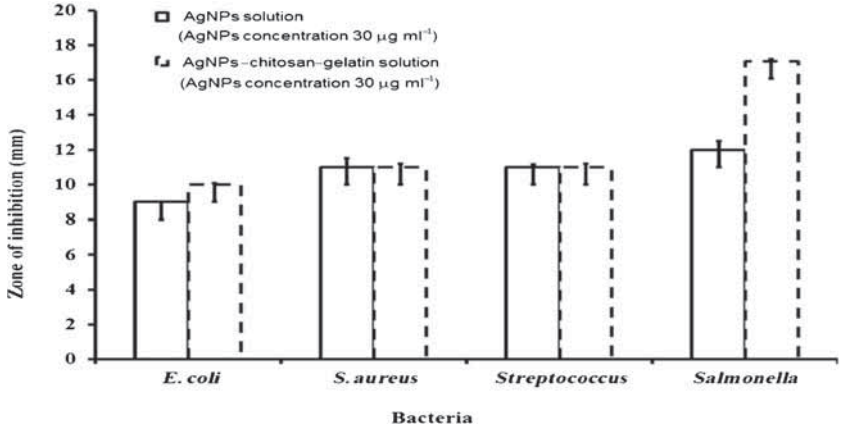

Figure 3. Comparitive antimicrobial study results of silver nanoparticles and silver nanoparticles-doped colloidal stabilizers.

inactivate bacteria ranges from 30 to 50 μg ml^{-1} and the value was varied based on bacterial species.

The antimicrobial activity of the hybrid mixture-coated egg tray layers were also tested against *E. coli*, *S. aureus, Streptococcus* and *Salmonella* species on MHA plates. For this study, initially, the egg tray was cut into circular bits (radius = 2 cm and the Ag concentration was 30 μg per 0.5 cm). The results revealed that the hybrid mixture-coated egg tray disc showed microbial growth inhibition effects against all bacteria and the results were comparable with standard antibiotic discs (table 1). The zone of inhibition value for *E. coli* was estimated at 10.0 ± 0.07 mm, for *S. aureus*, it was 11.0 ± 0.20 mm, for *Streptococcus* spp, 11.0 ± 0.19 mm and against *Salmonella* showed the values around 17.0 ± 0.09 mm.

3.2 *External and internal characteristics of eggs stored in antimicrobial egg tray*

The external and internal characterizations of the egg were assessed based on Bureau of Indian Standards (BIS grades) specification. The qualities were analysed over an interval

Table 1. Comparative antimicrobial study results of antimicrobial egg trays and the standard antibiotic disc against four different bacterial isolates.

Name of the samples	Zone of inhibition values (mm)			
	E. coli	*S. aureus*	*Streptococcus*	*Salmonella*
Antimicrobial egg tray (Ag nanoparticles quantity in egg tray (30 μg per 0.5 cm)	10 ± 0.07	11 ± 0.20	11 ± 0.19	17.1 ± 0.09
Standard antibiotic disc				
Amikacin (30 mcg)	1.1 ± 0.05	0	0	15.6 ± 0.42
Ampicillin (30 mcg)	2.2 ± 0.06	0	30.4 ± 0.48	3.3 ± 0.49
Cefotaxime (30 mcg)	8.1 ± 0.15	27.36 ± 0.42	11.4 ± 0.42	15.1 ± 0.05
Chloramphenicol (30 mcg)	7.13 ± 0.15	9.36 ± 0.4	25.4 ± 0.51	17.2 ± 0.19
Ciprofloxacin (30 mcg)	2 ± 0.12	16 ± 0.16	27.4 ± 0.31	17.3 ± 0.49
Ceftazidine (30 mcg)	0	0	0	10.4 ± 0.45
Erythromycin (30 mcg)	5.2 ± 0.10	0	0	9.2 ± 0.19
Kanamycin (30 mcg)	10.1 ± 0.12	0	0	11.3 ± 0.49
Penicillin-G	10.3 ± 0.04	0	0	10.1 ± 0.12
Tetracycline (30 mcg)	4.2 ± 0.10	0	0	13.2 ± 0.19
Oxytetracycline (30 mcg)	0	20.2 ± 0.48	10.3 ± 0.49	5.3 ± 0.49

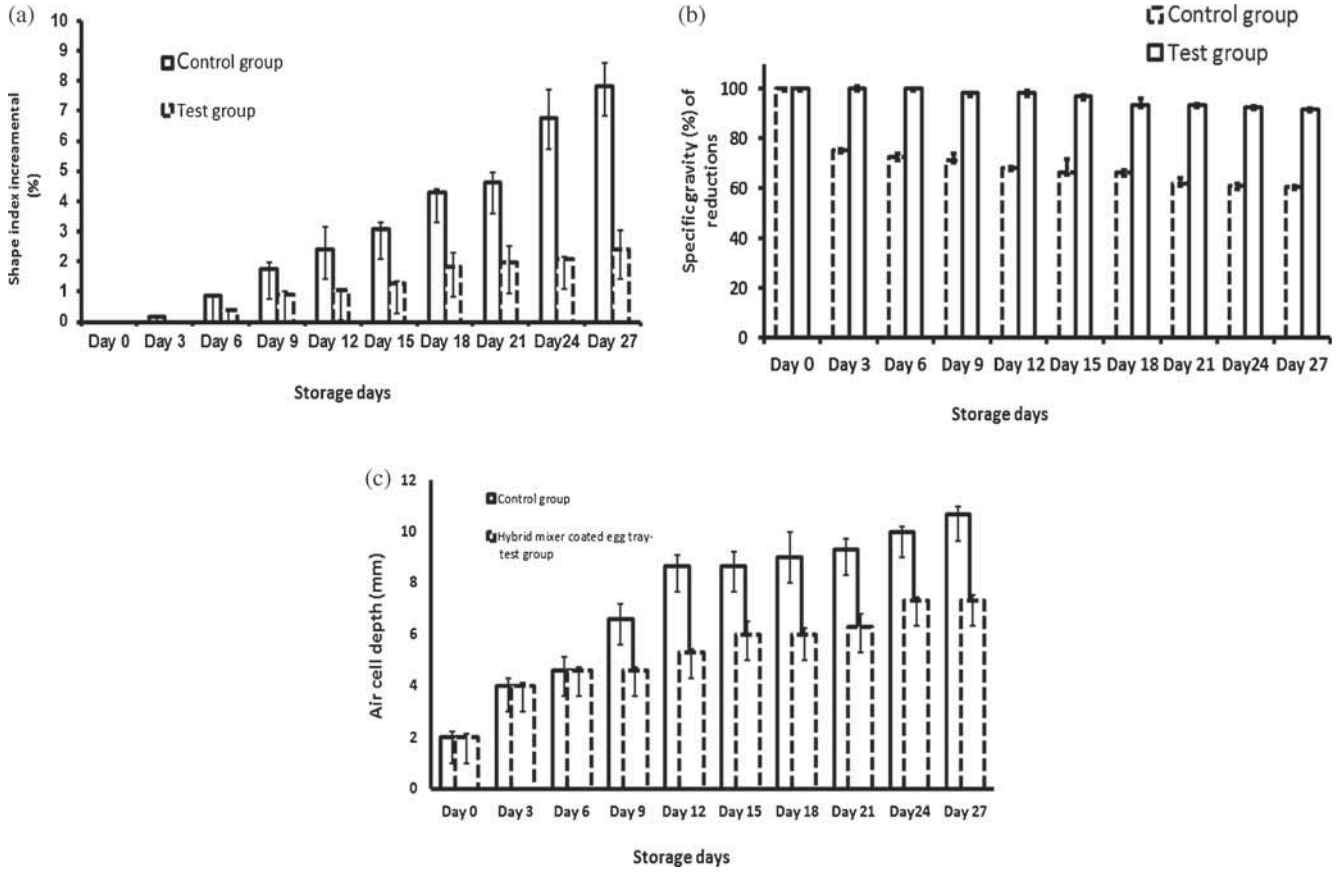

Figure 4. Results of egg external quality parameters. (**a**) Shape index study results, (**b**) specific gravity study results and (**c**) air cell depth study results.

of 3 days up to 1 month and the results of external quality parameters are shown in figure 4. The physical quality factors like egg weight, egg volume, surface area and the colour of the shell were studied up to 30 days. The results indicated that the control and the AgNPs-doped egg trays showed no significant changes with respect to physical quality parameters (weight, volume, surface area and colour) even after 30 days (date not shown).

The change in egg shape index is also main factor due to evaporation of moisture during storage. So, we tested the shape index of the eggs during storage. The results (figure 4a) indicated that the control sample showed up to 8% of the shape index variation from the day 0. But in the test groups, the shape index value was changed only 2% from the day 0.

The specific gravity is also a main factor to assess the egg quality and it reflects the amount of shell, relative to the amount of albumin, yolk and membranes. The specific gravity of the shell is two times higher than other parts of the egg and hence, a good indicator for shell quality. We tested the specific gravity for both control and test eggs (figure 4b), based on the results we found that the specific gravity percentage of the control sample was reduced up to 38% from the day 0 but only up to 8% in treated samples.

The age of the egg is measured based on the air cell depth, which indicates how recently the egg has been laid and an increase in the air cell depth indicates the duration after the lay. The air space is normally visible during candling, and the height of the air cell depth increases in size with the age of the egg. So, we studied the air cell depth for eggs stored in AgNPs-doped egg trays up to 30 days and compared with the control eggs (figure 4c). Results indicated that in the control samples, the air cell depth was increased up to 10.66 mm, while an equivalent increase in the AgNPs-doped egg trays, it was only 7 mm.

The internal quality parameters results for albumin index, yolk index and Haugh unit are shown in figure 5. Albumin index measure the original interior quality of eggs. Albumin is more sensitive to changes than the yolk to external factors. In fresh eggs, the thick albumin is opaque and has a pH of 7.6, and in older eggs the pH ranges from 9.5 to higher indicating lower quality. The two main parameters used for determination of albumin index are viscosity and clarity. Albumin index is a direct measure for calculating the Haugh unit which is a basic parameter in grading the egg consignments. We measured the albumin index up to 27 days based on the cut off values (figure 5a). In the control samples, for the first 2 weeks the albumin index value was reduced up to

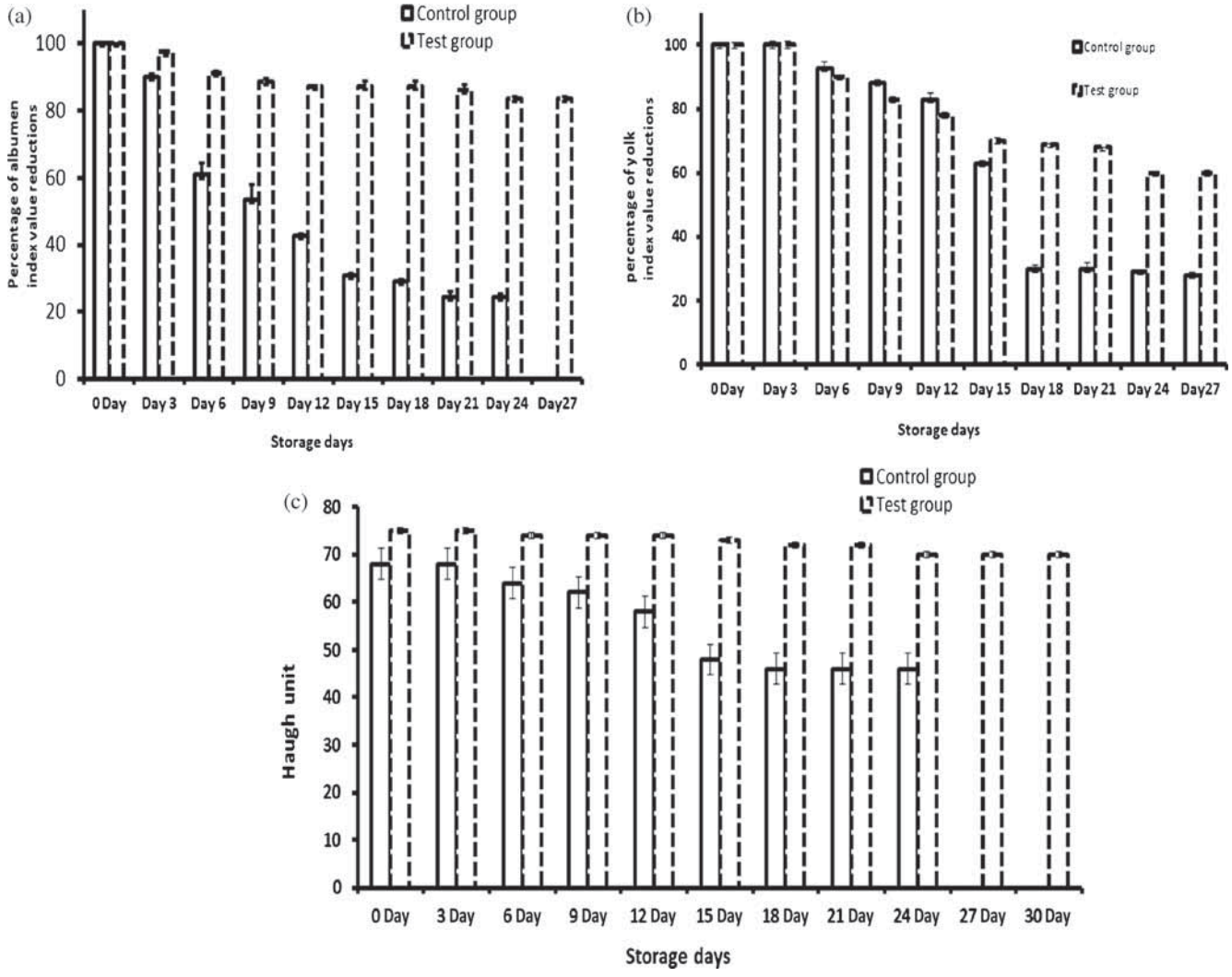

Figure 5. Results of egg internal quality parameters. (**a**) Albumin index study results. (**b**) Yolk index and (**c**) Haugh unit study results.

57.3% from day 0 values, while eggs stored in silver-doped egg trays, it was found to reduce only up to 12.7%.

Yolk index indicates the strength of the vitelline membrane and is therefore an indirect measure of internal egg quality. Yolk index value was calculated up to 27 days (figure 5b), the control sample value was reduced up to 73%, for the first 2 weeks and the yolk index average value was around 37%. After 15 days, the average yolk index value reduced very rapidly. The eggs stored in the AgNPs-doped egg tray showed the yolk index value was reduced around only 40% after 27 days.

Haugh unit also indicated same trend, in the control samples an average Haugh unit of 68.15, the control groups showed a faster rate of decrease in Haugh unit until 15 days of storage and then the values were levelled off at 46 (24 days). After that, the egg yolks were disintegrated in control group, therefore, it was impossible to measure Haugh unit after 24 days of storage. In contrast, the eggs stored in AgNPs-doped egg trays, Haugh unit value was 75.24, and after 30 days, the average Haugh unit value was only slightly reduced to 70.14.

3.3 *Determination of silver in egg samples*

Due to possible human health effects from silver exposure, we analysed the silver content in egg samples using graphite furnace atomic absorption, and the results indicated that the silver content was nil in all 30 samples. The silver loss from the paper egg trays studies indicated that, after 30 days the silver loss in different paper egg tray samples were between 0.04 and 0.1%.

4. Conclusion

In conclusion, we have described a new bactericidal paper egg tray preparation method using AgNPs. The paper egg trays were capable of killing both Gram-positive and Gram-negative bacteria on surface and in solution. The egg storage studies indicated that the egg stored in the silver-doped egg trays recorded high storage days compared with control samples. The method reported here is very easy and highly suitable for storing large-scale commercial eggs. This study opens up a new household material making use of nanotechnology for the future.

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