Supporting information

Fabrication of Antibacterial Silver Nanoparticle – Sodium Alginate-Chitosan Composite Films

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MATERIALS AND METHODS

Chemicals and Growth media

Chitosan (high MW, >75% deacetylated) was obtained from Sigma Aldrich Chemical Pvt. Ltd., Kolkata, India and alginate from Loba Chemie Pvt. Ltd., Mumbai, India. Silver nitrate, acetic acid (glacial, 99–100%) and acetone were purchased from Merck India Ltd, Mumbai, India. Luria-Bertani Broth (LB), Nutrient broth (NB) and brain-heart infusion (BHI) growth media were purchased from HiMedia, Mumbai, India. For preparing solid media, the nutrient media was supplemented with 1.5 % bacteriological agar (HiMedia Laboratories Ltd., Mumbai) as solidifying agent.

Bactericidal activity of the composite

The antibacterial activity of Alg-Ag NPs was tested against E. coli and B. cereus MTCC 1305 as representative strains of Gram negative and Gram positive bacteria respectively by well diffusion method. The suspensions of bacterial cultures in the range of 10^5-10^6 cfu ml^{-1} were made and streaked over the surface of agar plates to obtain uniform growth. After the plates were dried for
5 min, 4 distinct wells were punctured into the agar plates and filled with Alg-Ag NPs solution and alginate solution as control. The zones of inhibition were observed after incubation at 37°C overnight.

RESULTS

Figure S1. XRD pattern of Alg-Ag NPs showed peaks at 38.2° and 44.3° corresponding to the (111) and (200) planes of the cubic structure of metallic Ag. Absence of peaks at 32.8°, 46.2° and 54.6° corresponding to Ag₂O suggests that Ag₂O does not form during the synthesis.

Ag NPs were prepared at higher Ag NO₃ concentrations (2 × 10⁻³ M and 4 × 10⁻³ M) keeping alginate concentration and temperature as 0.2 % and 90 ºC, respectively. UV-Vis spectra measurements (Figure S2) showed broad peaks at ~409 nm and ~413 nm for NPs prepared using 2 × 10⁻³ M and 4 × 10⁻³ M Ag NO₃ concentration, respectively indicating wide distribution of NPs. This was confirmed by TEM measurements. For NPs prepared using 2 × 10⁻³ M Ag NO₃ concentration, particles of various shapes were formed (Figure S3a). The particle size distribution was calculated by measuring diameters of spherical particles. The average size was calculated to be 5.65 ± 2.4 nm (Figure S3c). In case of NPs synthesized using 4 × 10⁻³ M Ag
NO$_3$, TEM images showed that the particles of various irregular morphologies were formed (Figure S3b). Most of the particles were found to be aggregated.

**Figure S2.** UV-vis spectra of Ag NPs prepared using 5 times and 10 times initial Ag NO$_3$ concentrations (i.e. $2 \times 10^{-3}$ M and $4 \times 10^{-3}$ M respectively) and 0.2 % alginate at 90 °C.

**Figure S3.** TEM images of NPs synthesized using (a) $2 \times 10^{-3}$ M and (b) $4 \times 10^{-3}$ M of Ag NO$_3$, (c) Particle size distribution of NPs prepared using $2 \times 10^{-3}$ M Ag NO$_3$. 
**Figure S4.** Photographs of antimicrobial test results of (i) alginate and (ii-iv) Alg-Ag NPs against (a) *E. coli* and (b) *B. cereus* MTCC 1305 strains.

**Figure S5.** Photograph of different (C) chitosan, (1) 1:1, (2) 2:1 and (3) 4:1 films
Figure S6. Optical micrographs of (a) chitosan film (b) 1:1, (c) 2:1 and (d) 4:1 blended films.

REFERENCES
