Biopolymeric Synthesis of Ag-Glycogen Nano-composite in Inert Medium: Characterization and Antibacterial Activity

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Abstract— In the Synthesis of Metal–Oxied Based Nanocomposite, glycogen biopolymer is a good stabilization agent. We are proposing a synthesis of Ag-glycogen based nanocomposite in inert atmosphere. The samples with different contents of silver were prepared by two diverse procedures that include two inert atmosphere, synthesis in the presence of Neon gas and in the presence of Argon gas. The outcome also revealed that the optical properties of the obtained nanocomposite samples sturdily depend on the process of preparation. The TEM images of the two nanocomposites demonstrate the existence of nanoparticles with average diameter of 9.5 and 10.1 nm, correspondingly. Antimicrobial activity tests were conceded against Escherichia coli and we found that and microbial growth was steadily reduced as the concentration of Ag vary.

Keywords: Nanocomposite, Nanoparticle, Nanocomposite, Biopolymer

I. INTRODUCTION

Due to large number of –OH groups, polysaccharide chains like Glycogen complexate fit with metallic ions in solution. Hybrid system of inorganic nanoparticles and polysaccharide biopolymers is observing a remarkable activity in bio-nanoscience. These biopolymers make them excellent controlled environments for development of metallic and semiconductor nanocrystal. In previous starch were using a good stabilizing agent in the formation of metallic crystals, but now days Glycogen is also getting attention in various biocompatible metal encapsulate formation. Various studies shown in which polysaccharide biopolymers of animal foundation used for the synthesis of inorganic nanoparticles. glycogen highly branched (1→4)(1→6)-linked α-d-glucoses And it primarily produced by the liver and the muscles. Glycogen is higher in molecular weight and molecules are packed into spherical granules. In this study we formed silver nanoparticle in glycogen in the presence of two different inert environment of Neon and Argon and tried to determine the difference. Various studies shown that silver nanoparticles exhibit antibacterial activity .the antibacterial activity vary by factor likes concentration, size and capping agents. Ag+ formation causing antibacterial activity in most of studies. glycogen is a biocompatible polymer of animal origin and we are using it as a stabilizing agent with an inert atmosphere for the formation of silver nanoparticles. We measured that it would be fascinating from a fundamental point of view to explore the antimicrobial effects of this material prepared by two different inert atmosphere, previous studies shown that this combination of Ag-Glycogen exhibit antibacterial properties but we are exploring this formation by imposing two different inert atmosphere and calculating the difference in the outcome.

II. EXPERIMENTAL

A. Synthesis of Nanocomposite

1) Material

Liver based glycogen ammonium hydroxide (NH_4OH), silver nitrate (AgNO3) and D-glucose were purchased from Thomas Baker India. Doubly distilled water was used during the experiment.

For the preparation of Ag-glycogen nanocomposite we used modified tollens method. by reducing $[Ag(NH3)2]+$ ions in the presence of aqueous D-glucose with micro-wave irradiation we prepared silver nanoparticles .and for study of inert atmosphere in the preparation of nanocomposite , we used two small size glass chamber(flask type). For the preparation of Ag based nanocomposite both glass chamber filled with, a 1mL of 0.1M solution of AgNO3 and 3mL of 0.1M solution of d-glucose were added to 50mL of a 0.7% aqueous solution of glycogen andthan both chamber filled with Argon and Neon gases at relatively high pressure and sealed the chambers. The mixtures were stirred for 3hr at room temperature and after that NH4OH was added in both the chambers drop wise to maintain the pH around 7-8. at the end conventional heating was applied and after evaporation of water we got the nanocomposite film

III. METHODS

A. Characterization of the Nanocomposites

To study morphology of Encapsulated Ag nanoparticles in Glycogen matrix, transmission electron microscopy (Philips CM 200 instrument) at a 80 kV operating voltage was performed. A water dispersal of the nanocomposite was deposited on carbon coated (Argon Sample) and formvar coated (Neon sample) copper grids with a fine pipette. The samples were left to dry for some time before transferred to the TEM chamber. Particle sizes was determined corresponding circular area of 134 (Argon) or 162 (Neon sample).

The surface morphology of nanocomposite was investigated by scanning electron microscopy (SEM) using a JEOL JSM 6460LV instrument, sample were coated with carbon prior to SEM investigation. The X-ray diffraction (XRD) of Argon and Neon sample were performed on a Philips PW3710 Xray diffractometer (Cu radiation, _ = 0.150 nm).

Antibacterial study were performed on Gram-negative bacteria E. i ATCC 25923. Agar was used for investigation of growth of microorganism, supplemented with 0.5% (v/v) yeast extract. quantitative testing of the microbicidal activity was performed at pH 7. For inoculum, the microorganisms were cultivated in yeast extract at 37 ◦C

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and left overnight. The obtained numbers of E. coli and in inoculums was, 2.4×107 CFUmL−1

B. Antimicrobial Activity Testing

To determine antimicrobial activity of Ag-glycogen nanocomposite, two study were performed, agar-well diffusion method and the quantitative testing. For study of cells reduction activity we performed agar-well diffusion method. In this method 65_L of fresh overnight cultures of indicator strains were added in 7mL of soft yeast (0.5% of agar–agar in yeast). The soft agar was robustly mixed and than poured over Petri plates with previously dried up yeast on the surface of which sterile ceramic tubes (5mm diameter) were placed. After solidifying the soft Agar, the tube removed and than obtained wells were filled with 60_L of Ag–glycogen aqueous solutions. All the plates were incubated at 37 ◦C for 24 h. The width of inhibition zones measured in millimeters. For the inhibitory activity of nanocomposite the samples were taken from the clean zones with a loop, surface-plated onto yeast and incubated for up to 48 h.

In second method we used potassium hydrogen phosphate buffer solution of pH 7 to determine the quantitative testing of nanocomposites. The given concentration of pure glycogen solution and the nanocomposites were placed in the glass tubes containing 11mL of hydrogen phosphate buffer solution inoculated with 1.2mL of diluted microbial inoculum. And the resulting mixture with inclusion of samples was vortexed for 15 s and incubated at 37 ◦C in a water bath shaker. 0.2mL aliquots were removed after exposureing the sample for 2h and further diluted with saline solution. After all dilutions 1.2mL aliquots were placed in Petri dishes, overlaid with yeast extract and after 24 h of incubation at 37 ◦C, for percentage of microbial cells reduction was calculated by the following equation.

$$R, \% = \frac{CFU_{cont./Gly} - CFU_{Ag/Gly}}{CFU_{cont./Gly}} \times 100\%,$$

CFUcont./Gly and CFUAg/Gly are the colony numbers forming units per millilitre of glycogen without Ag and the Ag–glycogen nanocomposite. To determine antimicrobial activity 5, 20, 50 and 200_gmL−1 of investigated concentration were added on 10mL of molted yeast extract. 1mL diluted inoculum was added and mixture vortexed for 30 S and placed the mixture in sterile Petri dish. After solidification of the agar, all the plates were incubated for 24 h at 35 ◦C and than appearance of viable cells present in product was examined.

IV. Results and Discussion

A. Morphology and Structure

Fig.1 show image of SEM (A) glycogen as recived (B) Ag-glycogen nanocomposite in Neon and (C) Ag-glycogen nanocomposite in Argon Image(A) shows the glycogen containing α-Particles with range around several hundred nanometers to approximately 1µ m. Image (b) and (C) not showing any particle like structure in morphology. Fig.2 showing TEM image of nanocomposites and it can be seen the well despersion of silver nanoparticles throughout in glycogen matrix. The average sizes of the particles by Neon and Argon methods are dNE = 9.5nm and dAR = 10.1nm (Fig. 2).

Fig.(3) showing XRD spectra of Ag-glycogen nanocomposite. A broad peak at 2Θ ˜20◦ instigate from the glycogen matrix whereas the other two peaks can be assigned to the cubic crystal type structure of silver.

B. FTIR Spectroscopy

FTIR–ATR spectroscopy were performed to study the possible interaction between Ag nanoparticles and glycogen macromolecules. Fig .4 showing the Ftir spectra of glycogen as well as Neon and Argon nanocomposite. By comparing all three spectrum, spectrum of nanocomposite in the region of 1500–1200cm−1, which is characteristic for CH and OH vibrations in alcohols. Generally nanoparticles interact with polymer through –OH groups which create the band at 1417cm−1. particle-macromolecule interaction results in a strong band at 1384cm−1 and the weak modes at 1371 and 1417cm−1 disappears.

C. Antimicrobial Activity

Fig.5 showing the antimicrobial activity of pure glycogen and nanocomposites (Argon sample) againts E. coli. The results for the Neon nanocomposite samples were similar to those obtained for Argon samples and for this reason they will not be reported. Pure glycogen does not exhibit any inhibition zone. but the nanocomposite exhibits distinct effects against E. coli. The form and the size of the clear zone typically depend on the proportion of the rounded area and the size of the inoculum, as well as on the solid medium and the contact area.

For the further clarification of antimicrobial activity of nanocomposite. The indicator strains were exposed for the known concentration of nanocomposites and after 1 and 2 h of exposure the survived cells were measured. Table.1 showing cell count for both methode neon and argon againts E.coli. In between neon and argon based nanocomposite, neon based nanocomposite was more successful as bactericidal agent to E. coli, since it decrease its number by 100.00% after 1 h of exposure. The results shows that Ag-glycogen nanocomposite are efficient antimicrobial agents with fast activity, and proving that the antimicrobial activity generate by Ag nanoparticles. it has also observed that pure glycogen decomposed while nanocomposite is stable for months . fig .6 is showing that concentration of nanoparticles effect the bacterial growth above 50_gmL−1 there was no considerable growth for any microorganisms

V. Conclusions

Results shows that glycogen is a stable agent for the encapsulation of nanoparticles. Preparation of nanocomposite effect its physical properties. the nanoparticles obtain by Neon and argon method are in avg. 9.5nm and 10.1nm respectively .FTIR spectra shows that growth of the Ag nanoparticles takes place via OH groups of glycogen. Antimicrobial test showed that inhibition of microbial increase with increase in Ag content. The number of cells is reduced significantly by nanocomposite and after 2 h a total reduction take place.

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