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Bio-molecules assisted synthesis of Silver nanoparticles for bio-evaluation

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ABSTRACT:
We describe a bio-molecule assisted method for the facile aqueous phase synthesis of Ag (Silver) nanoparticles, in which Carboxymethyl Cellouse (CMC) is used as a stabilising agent and Ag (Silver) nanoparticles are synthesised. The X–ray diffraction and selected area electron diffraction patterns of the resulting Silver nanoparticles (CMC.AgNp's) indicate that the crystalline structure of the nanoparticles is cubic in nature. In order for CMC.Ag-Np to be stabilised, the hydroxyl (–OH) and carboxylate (–COO–) groups of CMC must come into contact with one another in order for this to happen. The bio-conjugate CMC.AgNp's were produced had a high level of biological activity. The disc diffusion method was used to screen the synthesised CMC.AgNp's antibacterial activity against both gram positive and gram negative bacteria after they had been synthesised. Results showed that CMC.AgNp's improved antibacterial properties as a result of the treatment.

Key Words: Carboxymethyl cellouse (CMC), stabilization, bio-conjugate, antibacterial, enhancement.

I. INTRODUCTION:

The interest in developing metal-based nanoparticles has been growing steadily over the past few years, owing to the potential applications in a variety of research fields, including contact action,[1] sensors,[2] physics, and optics. [3,4] While metal nanoparticles have many advantages, there are some limitations such as particle aggregation, unit cell reactions, and poor solubility that can have an impact on their properties over time. In the literature, it has been demonstrated that self assembly of organic molecules on the surface of metal nanoparticles will improve the solubility and stability of metal nanoparticles. Additionally, the encapsulation of metal nanoparticles with organic compounds will have an effect on the gross properties and will find enhanced applications in asymmetric catalysis,[5] medical specialties, and drug delivery, among other things.[6]

Silver has recently been discovered to have applications in the medical field, [7-10] but the solubility of silver and silver salts (e.g., $AgNO_3$) makes it impractical for use in a number of clinical applications. Because of their ease of synthesis in aqueous/organic phases [11-15] with desired size and shape, nanometer-sized silver particles have piqued the interest of researchers who are interested in a wide range of applications in the medical diagnostic fields, including diagnostic imaging.

Aside from that, Ag-Np's been used in semiconductors with improved performance; the fact that an article of clothing has been modified with a different material is unquestionable. For example, AgNPs have been successfully coated on medical devices that have been used in the prevention of infection during organ transplantation. [16,17]

For the synthesis of AgNPs, biological methods are used, which include microorganisms, plant extracts, and enzymes. [18–19] Because of their availability, non-toxicity, and low cost, plants are considered to be a superior alternative to microorganisms. A large number of plants have been used in the synthesis of nanoparticles [18], and more specifically in the production of AgNPs [18, 20, 21] A cellulose derivative known as carboxymethyl cellulose (CMC) has recently been used to synthesise AgNPs, which is a relatively new development. CMC is water soluble, exhibits chemical stability, and is non-toxic. In addition to its role as a reducing agent, it can also serve as a particle stabiliser in some applications. [22–24] Although temperatures much higher than room temperature or long reaction times are required in order to carry out the Ag+ ion reduction, this is not always possible. [18, 21–23, and 25] In this paper, we describe the process of obtaining CMC.AgNp's. CMC was used as a stabilizer/reducing agent in the continuous reduction of $AgNO_3$ in aqueous solutions at room temperature, with fructose

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serving as the reducing agent and carboxymethyl cellulose serving as the stabilizing/reducing agent. The antibacterial efficacy of CMC.AgNp's also investigated in greater detail.

II. MATERIALS AND METHODS:

Silver nitrate ($AgNO_3$) was 98.5% pure; Sodium carboxymethyl cellulose (CMC, Mw 90.000 from Sigma-Aldrich), were used as received. Deionized and triple-distilled water with conductivity was used.

Preparation of Ag nanoparticles:

The syntheses of the CMC.Ag-Np's were carried out in duplicate using a 250mL jacketed glass reactor. The reactor had an inlet for feeding the fructose aqueous solution. The procedure for the reduction reaction started with loading the 2 mL of 0.01 M silver nitrate aqueous solution (35ml) into the reactor; then, 2 mL of 1% fructose and 98 mL of 0.1 wt% CMC was added to the silver nitrate solution, and the temperature was maintained at room temperature. The reaction mass was allowed to continuous stirring for 6 hours.

III. RESULTS AND DISCUSSION:

Analysis Using UV-VIS Spectroscopy: The UV-VIS absorption spectra of the CMC.AgNp's shown in Figure 2. A carboxymethyl cellulose aqueous solution was used as a blank in this experiment. The absorption spectra of CMC.Ag-Np show absorption bell-shaped bands with a maximum around the 423nm wavelength range. This band has been linked to the surface plasmon resonance (SPR) of CMC.AgNp's has been used to confirm the reduction of Ag+ to Ag0 in the presence of CMC.AgNp's. Moreover, transmission electron microscopy has confirmed that CMC.AgNp's a spherical shape, which is consistent with previous findings (TEM). In one study, it was discovered that a maximum absorption at 423 nm indicates the presence of particles with sizes between 15 and 40 nm.

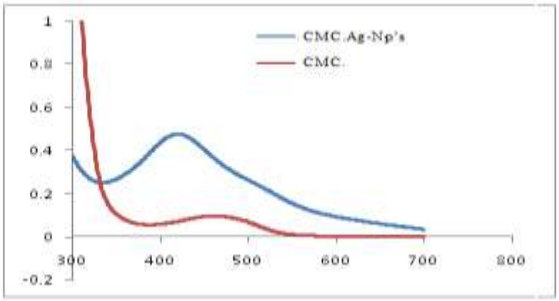

Figure-1: UV-VIS absorption spectra of the CMC.Ag-Np's

TEM Studies:

TEM and selected area electron diffraction pattern (SAED) data were obtained on an FEI Technai G2 S–Twin. For TEM analysis, a few drops of the Ag colloidal samples dispersion onto the carbon coated copper TEM grid and dried overnight.

In order to know the morphology of the prepared CMC.Ag-Np's, we have carried out TEM measurements. The prepared CMC.Ag-Np's TEM image is shown in Figure-2. It can be seen that mono-dispersed spherical shaped particles with an average size of 20 nm.

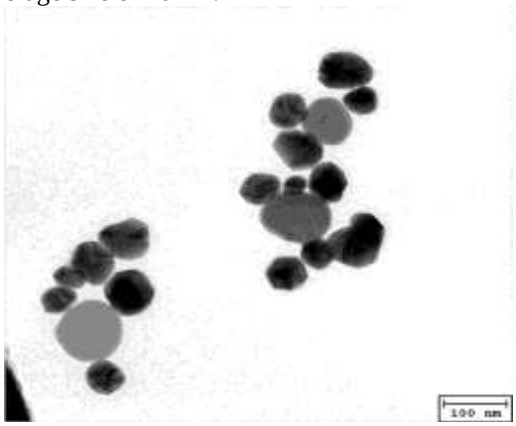

Figure-2: Prepared CMC.Ag-Np's TEM image

The SAED pattern of the prepared CMC.Ag-Np's nanoparticles is shown in Figure 3. It can be observed that the four rings in Figure 3 reflect the (111), (200), (220), and (311) planes, respectively, which represents the fcc structure of Ag nanoparticles obtained using bio-molecules of fructose.

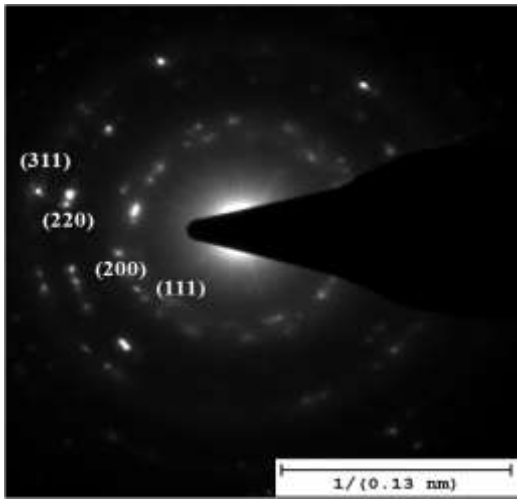

Figure-3: SAED pattern of the prepared CMC.Ag-Np's nanoparticles

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XRD Studies:

To understand the crystalline structure of the synthesized CMC.Ag-Np's nanoparticles, we have carried out XRD measurement. The result is presented in Figure 4. Diffraction peaks at two theta = 38.47, 46.22, 65.05, 82.05 and 87.01° related to the indexed planes (111), (200), (220), (311) and (222) respectively, which are consistent with the fcc structure of the Ag.

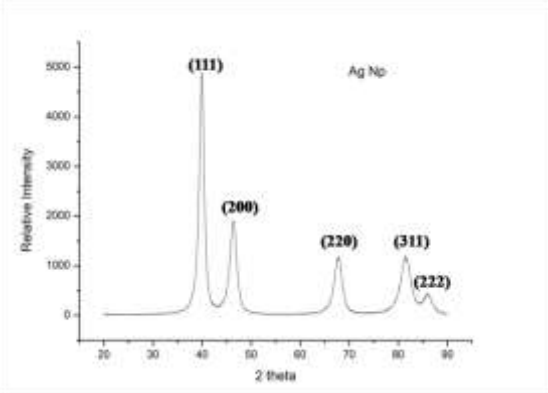

Figure-4: XRD pattern of synthesized CMC.Ag-Np's

FT-IR studies

The functional groups responsible for stabilization of the CMC.Ag-Np's nanoparticles were studied by FT−IR. The FT−IR spectra of the prepared CMC.Ag-Np's nanoparticles are shown in Figure 5. The FT−IR profile of CMC stabilized CMC.Ag-Np's nanoparticles revealed that the functional groups in CMC have played an important role in the stabilization of prepared CMC.Ag-Np's nanoparticles. The FT–IR spectrum of stabilized CMC.Ag-Np's nanoparticles shows the presence of five peaks at 3267, 1593, 1401, 1306 and 1024 cm^{-1}, which are assigned to –OH stretching, –COO– asymmetric stretching, –COO– symmetric stretching, –OH in plane vibration and –COC– stretching, respectively. These observations suggest that the resulting CMC.Ag-Np's nanoparticles are stabilized (anchored) with CMC molecules.

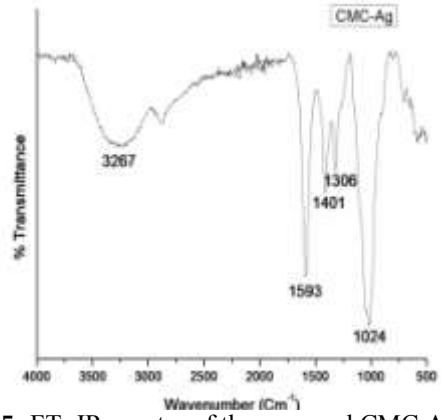

Figure-5: FT−IR spectra of the prepared CMC.Ag-Np's nanoparticles

In vitro bactericidal studies:

The bactericidal studies were carried out using Muller Hilton Broth method. The CMC.Ag-Np's showed excellent action against bacterial strains. The minimum inhibitory concentration (MIC) of CMC.Ag-Np's was calculated with the lowest concentration at which the bacterial growth was inhibited. The results are shown in Table 1. The CMC in conjunction with CMC.Ag-Np's is not an potent inhibitor for gram-negative bacteria.

From the results, it was noticed that there was an increment in the bactericidal activity of CMC-encapsulated CMC.Ag-Np's to the pure CMC. Especially the results obtained for Micrococcus proteus (MP) showed almost equal to that of the ampicillin antibiotic. Whereas, the antibacterial activity on EC & CMC are same and comparatively less than reference antibiotic.

Table-1: The effects of nanoparticles on Gram-negative & Gram-Positive Zone of inhibition in mm (50µl of compound from 1mg/ml)

Sl.no	Compound	EC	PS	KP	BS	ML	MP
01	CMC	18	21	20	22	22	23
02	CMC.Ag-Np's	25	28	26	29	27	26.5
03	Ampicillin	30.1	30.3	27.1	30.5	29	28

Escherichia coli (EC), Pseudomonas syringae (PS), Klebsiella pneumoniae(KP), Bacillus subtilis(BS),Micrococcus luteus (ML), and Micrococcus proteus (MP).

IV. CONCLUSIONS:

Very stable colloidal solutions of silver nanoparticles were obtained, by green method at room temperature in a continuous mode using CMC as a stabilizer/reducing agent and fructose as reducing agent. The characterization of the nanoparticles was carried out by TEM, UVVIS, and

XRD. XRD and EXD confirmed the obtaining of CMC.Ag-Np's. TEM images showed spherical CMC.Ag-Np's with unimodal distribution sizes in the range of 55 to 80 nm. CMC proved to be an effective stabilizer agent. Greater productivities than those reported by other authors were obtained with the advantage of using a lower temperature and minor reaction times. Both bacteria, were inhibited by CMC.Ag-Np's. The higher the CMC.Ag-Np's concentration, the larger the inhibition zone.

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