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Biosynthesis of Silver Nanoparticles using Milk Whey and its Applications

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Abstract:
A Green, low-cost, and reproducible biosynthesis of silver nanoparticles using milk whey is reported. The silver nanoparticles were synthesized using milk whey proteins as the reducing agent. It is a novel method of synthesis of silver nanoparticles using most common kitchen waste (milk whey) in order to suggest that it as an eco-amenable and economically viable approach. Silver nitrate (0.25M) solution was used as the source of silver nanoparticles. The optimized condition for the synthesis of silver was found pH-9. This novel green synthesis mechanism was changed their material properties to enhance the utility of that material. The green synthesis was performed in the laboratory ambience. The formation of metallic silver nanoparticles (n-Ag) was confirmed by X-ray diffractogram pattern analysis. The dimensions of synthesized Individual nanoparticles (n-Ag) are found in the range of 10-25 nm. These nanoparticles were characterized by using techniques UV-visible Spectrophotometer. Silver nanoparticles showed antimicrobial activity against S aureus and E. coli. The purpose of the study is to synthesize and characterize the whey protein mediated silver nanoparticles using milk whey.

Key Word: *Biosynthesis; Green approach; Milk Whey; Nanobiotechnology; Nanomaterial; Silver nanoparticle; Antimicrobial activity.*

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I. Introduction

Today, Nanotechnology becomes the quickest thriving area of manufacturing in the world and it provides unrestrained search for new nanomaterials and methods to make them. It is well known to living cells that execute as machines at nanometric scale for many of functions like generation of energy, extraction of targeted materials, etc. efficiently [1]. It had been shown that bacteria able to make an exciting category of microorganisms functioning as “mini” nanofactories. It has natural bestow property of the reduction/oxidation of metal ions into metallic/oxide nanoparticles [2,3].

Nanotechnology finds extensive applications in nanomedicine and provides an emerging new field which results unification of nanotechnology and medicine. The Medicine performs privileged work for the materials and devices designed at nanometric scale for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and is also useful in the permanence and health improvement [4]. The integration of biotechnology and nanotechnology has been emerged to rise Bio- nanotechnology for developing biosynthetic and environmentally friendly technology for synthesis of nanomaterials. From centuries, silver has been known for their effective bactericidal properties. Nanomaterials are much smaller than most of the biological molecules and hence these can be useful for the application in biomedical research in both in vivo and in vitro media [5].

Recently, it has been researched that the interaction between inorganic entities and biological systems has accentuate the portentous applications for the production of nanomaterials having interesting technologic properties [2-3, 6-14]. However, nanomaterials differ from proteins due to their chemical composition, shape, size, density, and aggregation, type of surface and distinct physicochemical (e.g., magnetic, optical and electrochemical) properties.

1. Milk Whey Proteins

The dairy specialists from all over the world have major concern about the Utilization and/or disposal of whey because it contains valuable constituents that should not be wasted. Further, if milk whey gets wasted and/or will become an important environmental pollutant [15]. However, whey protein products are treated as fantabulous foodingredients due to its unique functional features [16].

Whey can be separated from the casein faction of milk during their coagulation of the casein such as rennet or acid coagulated cheese [17]. It is found that the heterogeneity of casein is large among the whey proteins and they have few characteristics such as they are all soluble at pH 4.6 and caseins are precipitated at a temperature of 20 °C [18]. The four major whey proteins represent 90 % of all whey proteins. These are β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La), Bovine Blood Serum Albumin (BSA) and Immunoglobulins (Ig's) as shown in table 1[19-20, 30]. Whey has been considered as a waste product due to its low concentration of milk constituents (6-7% dry matter), [20]. Many cheese factories were built near the waterways and most of them such waste whey was diverted or dumped to these streams or rivers. The problem of pollution from the dumping of whey into waterways and even into municipal sewage systems prohibited by consequent regulations because their conventional treatments are not effective to sufficient reduction of load of whey pollution [21-22]. Nanotechnology has significant scope to utilize the whey waste in the manufacture of nanoparticles. These nanoparticles are very small particles with enhanced properties of catalytic reactivity, thermal conductivity, non-linear optical performance and chemical reactivity due to its large surface area to volume ratio [23].

Table 1: *Major milk whey proteins and their main characteristics*

Whey proteins	Molecular mass (kDa)	Amino acid residues	Approx. amount in milk (g/L)	% of protein
β-Lactoglobulin (β-Lg)	18.277	162	3.2	9.8
α-Lactalbumin (α-La),	14.175	123	1.2	3.7
Bovine Blood Serum Albumin (BSA)	66.463	582	0.4	1.2
Immunoglobulins (Ig's).	103-150	110*	0.7	2.1

2. Nanosilver particles

Nanosilver particles have antimicrobial, antibacterial, and antifungal properties. It has blossoming field of research and could be commercialized. Due to its antibacterial activity, clothing manufacturers have incorporated Ag-NPs into fabrics for socks to neutralize the odor-forming bacteria [24-25]. To preserve the food longer time by inhibiting the growth of microorganism into various food contact materials, such as plastics containers, refrigerator surfaces, storage bags, chopping used to fabricate food containers, refrigerator surfaces, storage bags and chopping boards, etc [26].

II. Material And Methods

The chemicals and reagents of analytical reagent (AR) grade were used. Freshly prepared reagents were used for all the analytical purpose. Whey was prepared from milk (Sudha Shakti milk) obtained from the dairy farm [COMFEED, Deshratana Dr. Rajendra Prasad Dugdh Utpadak Sahkari Sangh Ltd. Barauni Dairy, Barauni (Bihar)]. A 500 ml Sudha Shakti milk was taken in 1000 ml beaker and lemon juice was added to boiling milk. Thereafter, milk whey was taken out by the filtration using clean cotton cloth.

1. Biosynthesis of Nanosilver particles (Ag-NPs)

A 25 ml of milk whey was taken in the conical flask of 500 ml volume. And then milk whey was doubled in volume by mixing equal volume of sterile distilled water. Further, it was heated on steam bath about 10 minutes. Thereafter, the pH was taken and found to be in the range of 5-6 of the milk whey solution. To adjust the pH of the milk whey solution, a small quantity of $NaHCO_3$ was added until it attains pH 7. Now, a 10 ml of $AgNO_3$ solution of 0.25M strength was added to the milk whey solution. Here, the coffee colour solution was found. The milk whey solution was heated on the steam bath up to 80^0C for 5 to 10 minutes. Further, a small quantity of $NaOH$ was added until it attains pH 9. Heating of the solution were continued on the steam bath up to 80^0C for 5 to 10 minutes. At this stage, Ag^+ ion was reduced to Ag-Nps. Greyish colour Ag-Nps was found while supernatant was transparent.

The chemical reactions involved in the solution as follows scheme [1]:

$NaHCO_3 \Leftrightarrow Na^+ + HCO_3^-$

$HCO_3^- \Leftrightarrow OH^- + CO_2$

$AgNO_3 + OH^- \rightarrow Ag_2O + H_2O + NO_3^-$

$Ag_2O + H_2O \Leftrightarrow Ag_2(OH)_2$

$Ag_2(OH)_2 \rightarrow Ag \downarrow + H_2O$

Scheme 1: *Probable Reaction Mechanism for the synthesis of Ag-NPs.*

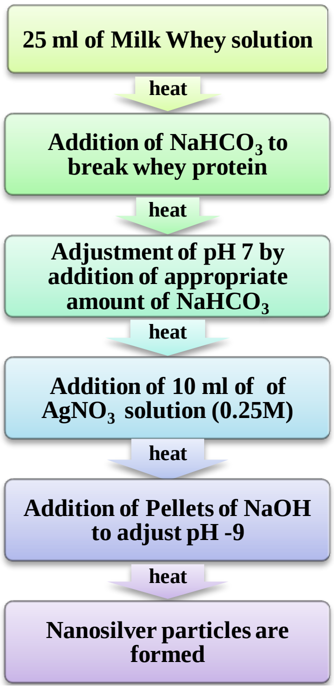

Figure 1:Flow Chart of the Synthesis of nanosilver particles

2. Characterization

The formation of silver Nanoparticles and crystalline nature has been confirmedby the XRD. Absorption and emission of light has been checked with uv-visible spectroscopy. The effect of synthesized silver Nanoparticles on representative Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) has been evaluated by examining the growth of bacterial cells in liquid medium amended with silver nanoparticles. Standard agar dilution method has been used to screen the antimicrobial activity of the synthesized silver nanoparticles. The organism has been introduced on the plates of Muller-Hinton agar and spread uniformly. The wells have been made on the agar plates with the help of a sterile polystyrene tip (4 mm) and antimicrobial effects has been performed with control, 6.25, 12.50, 25 and 50 µg/ml concentrations for the silver nanoparticles. The antimicrobial effect has been determined byscaling the diameter of zone of inhibition around the well.

a. X-Ray Difffractometer:

The formation of metallic (Ag) nanoparticles was ascertained by X-ray diffraction (XRD) technique using an X-Ray Diffractometer with temperature (lq. N_2-1200^0C variation facility (Make: Bruker, Germany) with Cu Kα radiation $\lambda = 1.5406$ Å over a wide range of Bragg angles ($20° \leq 2\theta \leq 90°$).

The apparent particle size and lattice strain for Ag-nanoparticles were estimated by analyzing the XRD peak broadening, using Williamson–Hall approach:

$$\beta \cos\theta = \frac{K\lambda}{D} + 2\left(\frac{\Delta\xi}{\xi}\right)\sin\theta \qquad \text{... (1)}$$

Where, *D* is the apparent particle size, β is diffraction peak width at half intensity (FWHM), $\Delta\xi/\xi$ is the lattice strain, and *K* is the Scherrer constant (0.89). The term $K\lambda/D$ represents the Scherrer particle size distribution. The values of β were estimated by applying a Lorentzian model. By the utilization of the linear least-square fitting of β cosθ-sinθ data, the apparent particle size and average lattice strain of metal and oxide nanoparticles were estimated.

b. UV-Visible Spectrophotometer

It is a special type of spectrometer which is used to measure the intensity of light. Further, metal and oxide nanoparticles were characterized by using UV-Vis-NIR spectrophotometer with temperature variation facility (Make: Perkin Elmer, UK). This technique refers to absorption spectroscopy in the ultraviolet-visible spectral region. In this technique, it uses light in the visible and adjacent (near-UV and near-infrared) ranges. It determines quantitatively the concentrations of the absorber in the solutions of transition metal ions and highly conjugated organic compounds.

The basic principle of this technique is based on the Beer-Lambert law which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species present in the solution and the path length.

III. Result

a. X-Ray Analysis of Nanosilver (n-Ag) particles:

The investigation of the structure of nanosilver particles has been done by the analysis of X-ray diffraction (XRD). The XRD patterns of synthesized nanosilver particles are shown in the figure 2.

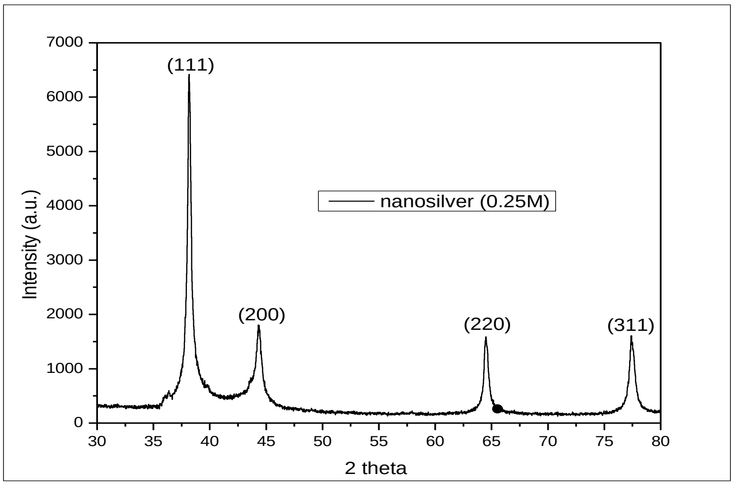

Figure 2: X-ray diffraction patterns of nanosilver particles (Ag-NPs)

The study of the XRD patterns indicates the formation of nanosilver particles. By using Debye-Scherrer formula, the average particle size has been determined from consideration of the XRD-peak at 44.31 degrees.

$$D = \frac{0.89\lambda}{\beta cos\vartheta} \qquad \text{... (2)}$$

Where 'λ' is wave length of X-Ray (0.15406 nm), β is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter (size). The average particle size of nanosilver is calculated to be around 17.24 nm. Silver nanoparticles are obtained by the reduction of Ag^+ ions by the utilization of milk whey. Table 2 shows the Bragg reflections corresponding to the diffraction plane (111), (200), (220) and (311) sets show the face-centered cubic lattice structure of nanosilver particles. The crystalline nature of the nanosilver particles is found to be fcc[27]. Space group of the crystal structure is found to be F m -3 m (225). The cell parameter is $a = b = c = 4.07780\,\mathring{A}$ and $\alpha = \beta = \gamma = 90°$.

Table 2: Two theta (2θ), intensity and diffraction plane of nanosilver particles sample

2θ (degree)	Corresponding Intensity	FWHM	Crystallite Size	Diffraction Plane
38.17	6419	0.4429	19.82	111
44.31	1807	0.7664	11.69	200
64.51	1586	0.4850	20.22	220
77.43	1606	0.6162	17.25	311

b. Analysis of UV-Spectra of Nanosilver (n-Ag) particles:

It is the beauty of UV-Visible spectroscopy that provides a mechanism to monitor the nanoparticles which change over time. The above figure 3 shows the UV- spectra of the nanosilver particles and absorbance is found approximately at 264 nm. While, it was found as 419 nm [28]. The synthesized Ag-NPs were characterized by UV-visible spectroscopy. It has been proved that the UV-visible spectroscopy is a very useful technique for the analysis the nanoparticles. On the analysis of the UV-spectra, it has been found that the surface Plasmon resonance occurs at approximately 264 nm.

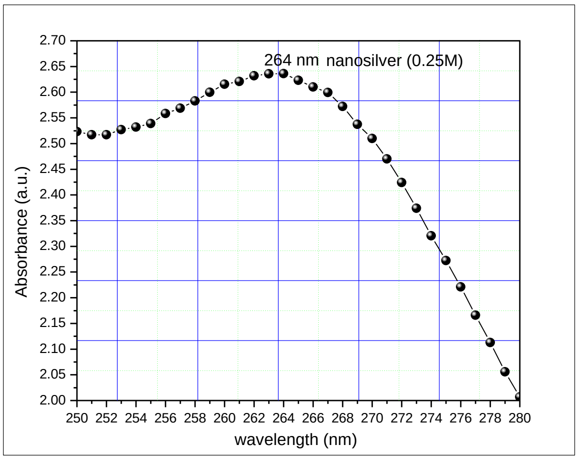

Figure 3:: UV-Spectra of nanosilver particles (Ag-NPs)

The silver metal particles become electronically coupled on the aggregation of silver nanoparticles. This coupled system has a different value of SPR than that of individual particles. On the aggregation of multinanoparticle, the Plasmon resonance will be red shifted to a longer wavelength than the resonance of an individual particle. Thus, aggregation is observed as an intensity increase in the red/infrared region of the spectrum.

The absorbance of biogenic Ag-NPs synthesized by milk whey proteins was found at about 264nm. The intensity peak was found to be for the absorbance 264nm. The higher peak intensity of the spectra showed the presence of Ag-NPs produced by milk whey through the reduction of silver ions to elemental silver. It reveals that the potential of Ag-NPs synthesis by milk whey and also overcome the problems of environmental pollution of milk whey.

c. Assessment of Antimicrobial Activity

Figure 4 illustrates the antimicrobial activity of silver nanoparticles synthesized using Milk Whey on *S. aureus* and *E. coli*. Five wells (diameter ~ 4 mm) were made on the agar plates with the help of a sterile polystyrene tip. Control, 6.25, 12.5, 25 and 50 µg/ml concentrations of silver nanoparticles had been prepared and used in the assays. Control wells were assigned at centre of the both plates.

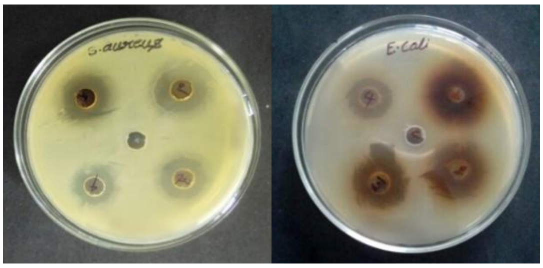

Figure 4:Zone of inhibition of silver nanoparticles(a) S. aureus(b) E. coli.

The effect of antimicrobial activity has been determined by measuring the diameter of zone of inhibition around the well. Very good inhibition zones could be observed in both of the organisms indicating thereby the antimicrobial activity of silver nanoparticles against *S aureus* and *E. coli*. Therefore, antimicrobial activity against these microorganisms was good at almost every concentration of silver nanoparticles which has been indicated by the diameter of zone of inhibition. Here we would like to emphasize that the similar pattern of result may be expected from other pathogenic and odourogenic microbes.

IV. Discussion

Generally, whey obtained as a by-product from dairy plant or industry gets wasted. This common waste is treated as an environmental pollutant. Its usefulness is a growth media for the production of silver nanoparticles. Proteins-the primary constituents of the living systems are naturally bestowed biological ligands, because they contain amino acids, which act as multidentate chelate ligands, providing an amenable spatial fixation and act as a medium themselves due to defined dielectric properties. The value of Partial Molar Volume (PMV) remains an important thermodynamic quantity harboring pivotal information regarding solvent-solute interactions along with solute behavior/structure in solvated state. It remains to be the most imperative deal while analyzing the effect of pressure on a defined chemical reaction. The biological sources (cultures, extracts or broths) are nothing but mere conglomerates of different metabolites, which are released abreast entity due to heat. Bio-ligands and metal ions make coordination complexes of broadly biological relevance because metal ions are the sites of Lewis acids capable of accepting lone pairs of electrons donated by the ligand, which behaves as Lewis base [29]. A major portion (approx. 30–35%) of constituent amino acid is cysteine and carries soft SH-groups [30]. Figure 5, illustrates thiol (-SH) groups in cysteine involve as nucleophile in many of enzymatic reactions [31].

Due to a favorable chelate effect; metal chelate complexes are quite stable, because there is always a favorable entropic factor accompanying the release of non-chelating ligands. Similarly, pKa values of coordinate ligands also play significant role dependent upon tuning of redox potential. Finally, different biopolymers affect the thermodynamic stoutness of a metal center, since it can control, through its three-dimensional structure such as stereochemistry, available ligands for coordination, local hydrophilicity and or hydrophobicity, steric blockage of coordination sites and hydrogen bonding formation. This enrapturing thermodynamic interaction/ interdependence keep molecules agile and ensure the formation of nanomaterials is dependent upon the modulation of experimental cues.

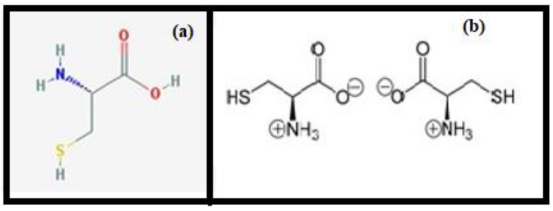

Figure 5:(a) Structure of cysteine having SH-group, and (b) zwitterionic form of cysteine at neutral pH [31].

In the studies of the effect of antimicrobial behavior of silver nanoparticles against S aureus and E. coli., we have been observed a very good inhibition zone around the well. Inhibition zones observed in both of the organisms indicate the antimicrobial behavior of silver nanoparticles.

V. Conclusion

The process of the nanotransformation that involves the synthesis of silver nanoparticles employing the milk whey proteins (common kitchen waste) was found less expensive, more reproducible, least time consuming, and a truly green approach of synthesis. Milk whey proteins contain amino acids and are naturally bestowed biological ligands. These amino acids act as multidentate chelate ligands and providing an amenable spatial fixation and also act as a medium themselves due to defined dielectric properties. It is observed that the milk whey contains chemical constituents having zewitters ions are readily interconvertible in aqueous medium. This release an appreciable amount of free energy (ΔG) which is suitable for the nanotransformation. The biological source like milk whey mere conglomerates of different metabolites, which are released abreast entity due to heat. Bio-ligands and metal ions make coordination complexes of biological relevance. The milk whey proteins act as both stabilizing and reducing agent for the synthesis of silver nanoparticles. The antimicrobial behavior was found in silver nanoparticles.

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