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Sulfur nanoparticles: Synthesis, characterizations and their applications

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Abstract
Sulfur Nanoparticles were prepared by different methods with different size and shapes, when the sulfur present as nanoparticles they have many practical applications in our life. This research work discuss with sulfur nanoparticles synthesis, characterization and applications. With dandruff being a common everyday problem and the market loaded with antidandruff shampoos and such skin care products, it is obvious to assume resourceful research into this area would be both objective to present scenario and lucrative potentially. Nanoparticles are frequently in use in some very powerful antimicrobial, antifungal cosmetics nowadays especially silver. However most metallic nanoparticles are harsh and often toxic, and concern remains on their ill effects. Sulfur, certified as biocompatible to eukaryotes and as soil nutrient it is often used in medicine. Though Sulfur nanoparticles are not much worked with it still is a strong antifungal agent and have been used in macro amount in shampoos. A surfactant has some natural antimicrobial property and therefore such combination could be a potential antidandruff hair washing formulation. To check its antidandruff activity, experiments have been conducted on *Malassezia furfur*; the causal organism for seborrheaic dermatitis or dandruff, which have been cultured for such study in our lab. Spectroscopy based microbial growth kinetics and colony inhibition studies have been performed to show that nanoparticles of sulfur can reduce the proliferation of *Malassezia* yeast colonies abundantly, and cause cellular damage which inhibits its growth and viability considerably.

Keywords: CTAB surfactant, SEM, sulfur nanoparticles (S-NPs), XRD

1. Introduction
"The smallest of things might have the deadliest of impacts", is a hard learned truth mankind has learned through long years of suffering and surviving. Entities only a few microns of size wiped out entire populations, in form of small pox, and plague and other diseases in one fatal blow. Ironical as it might be, Europe had its population reduced to one third by the 'Black Death', in the Middle Ages, a casualty far more subtle and bigger than what it suffered in the world wars. Though antibiotics and other therapies have been very useful in fighting them, much like H.G. Wells classic idea in 'War of the Worlds', the inevitable weakness of our defences still stands farce in face of disease. After the 1950s, with the boom in genetics and biotechnology, and a general impetus to hygiene consciousness, life expectancy suddenly crossed 100 years on an average and most known pathogenic diseases were deemed curable. However new virus-influenced physiological syndromes and non-pathogenic disorders are now became statistically significant and a focus of medical science. Today, 'Cancer' and 'AIDS' evoke the same dreaded feeling as 'plague' and 'leprosy' would elicit in the middle ages. To combat 'micro', we have 'Nano', a new and increasingly broadening concept which is bringing anew technological and medical revolution. 'Nano', means small enough to be about 10^{-9}meters dimensionally. Fungal diseases are very common but mostly not as fatal and fearsome as bacterial or viral one, so often getting unattended to. In fact, fungal studies lead to the discovery of the First antibiotic, Penicillin, derived From *Penicillium notatum*. But in plants, fungal diseases are quite important. A variety of diseases like 'blights', 'rusts', 'smuts', 'mildews', 'scabs' are fungal diseases. Hence indirectly fungal infections are very important to us, from perspective of agriculture and horticulture, also in fishery sciences and poultry. In human or higher animals, fungus diseases are mostly mentioned in relation to infections of superficial and subcutaneous organs especially skin and other peripheral organs.

2. Materials and Methods
2.1 Materials
The surfactant CTAB (Cetyltrimethylammonium bromide), was gifted from Sigma Aldrich Pvt. Ltd., Germany. All the chemicals were used as received without any further purification. Ultrapure water of pH 6.4–6.5 (Sartorius, Germany) was double distilled again and used for all the experiments, the reagents were filtered with 0.2 nylon 6, 6 membrane filter paper from Pall Life science, USA. Sodium thiosulphate (Pentahydrate) and oxalic acid were used from Rankem (India). Pure slant culture of *Malassezia furfur* (strain 1344) was obtained from Institute of Microbial Technology, Chandigarh, India, and maintained in Emmons modified medium. Emmons modified media was obtained from Himedia Laboratories Pvt. Ltd. Spectrophotometric analysis was done using, UV-1800, Shimadzu, Japan and optical microscope from Hund, Germany was used. Glass wares and plastic wares like petri dish and culture tube were obtained from Tarsons, India.

2.2 Media composition and culture conditions
Media for growth and maintenance contains:

Dextrose 30 g/L
Peptone 15 g/L
Agar 16 g/L
Corn oil 2 ml/L).

Growth conditions: Temperature: 290K
pH: 6.7 optimum, however pH of 5-6 was maintained in our experiments
Incubation time: 9 days
Aerobic growth necessary.

2.3 Media preparation and culture maintenance

The growth media with the given composition was prepared in conical flasks and autoclaved at 15l psi for 15 min (394 K). Initially cultures were grown in liquid media and when substantial growth was observed, sub culturing was done onto agar plates using glass petridish (10mm diameter). Growth conditions were maintained as required and a couple of cultures were stored at freezing temperature using glycerol slants for long term storage. Active cultures were refrigerated. However, to observe the lipid dependence of growth for the organism sub culturing was done using media where corn oil was replaced by almond oil, coconut oil and butter and observed. The same proportions were used for both liquid and solid cultures.

2.4 Synthesis of Sulfur nanoparticles

The important aspect of study involves, synthesis and application of nanoparticles. Nanoparticles or single crystal material can be synthesized in two ways, either in-situ or synthesized separately from final formulation. In case of non-metallic nanoparticles, production would require a precipitation reaction [Ghosh, Choudhuri and Paria, 2012] [20, 21]. Generally, microemulsion and bulk aqueous system is commonly used technique in synthesizing nanoparticles in such cases, where particle size control is most important [Deshpande *et al.*, 2008, Xie *et al.*, 2009] [14]. But microemulsion process involves several reagents with specific compositions, so being very cumbersome. Also other technical difficulties involved are process scaleup, separation and purification of the particles from the microemulsion, and consumption of huge amounts of surfactants.

The other quite simple method developed recently by Paria and co-workers is using surfactants as capping agents to control the size of particles formed. Here the nanoparticles were of a single element either metallic or nonmetallic, and the reaction was a simple reduction or disproportionate reaction respectively. The function of the surfactant was as a capping agent to control and keep the size of the particles to nanometer range. The size of the particles is function of the nature and concentration of the surfactant. The amount of nanoparticles formed as product depend on the reactants [Choudhuri and Paria, 2010, Ghosh, Choudhuri and Paria 2012] [8, 20, 21]. For Sulfur nanoparticles synthesis a simple disproportionate reaction, using thiosulphate and different acids, both organic and inorganic, were used. Stock sodium thiosulphate was prepared by dissolving solid thiosulphate in double distilled water and the same was done for oxalic acid. In an acidic solution, sodium thiosulphate undergoes through a disproportionate reaction to sulfur and sulfonic acid according to

$$Na2S2O3 + 2H+ \rightarrow 2Na+ + SO2 + S \downarrow + H2O$$
$$SO2+ H2O \rightarrow H2SO3$$

After mixing the reactants, 40 mins equilibrium time was given for the completion of reaction organic acids. After equilibration, the sample was sonicated for 2 minits and particle size was measured by DLS method immediately. CMC of CTAB was measured by Wilhelmy plate, technique using surface tensiometer (DCAT-11EC, Data Physics, Germany). A constant temperature of28˚C± 0.5 ˚C was maintained throughout the experiments. The Sulfur formed as a product of this reaction has the same molarity as thiosulphate added according to the stoichiometry of the reaction (since complete reaction is taking place) and hence for future experiments, concentration of nanoparticles of Sulfur has been calculated on that basis.

3. Results and Observations

3.1 Growth of *M. furfur*

Malassezia furfur grew as white to tan cream in colour and smooth pasty yeast like appearance over the solid medium and pale opaque mass in liquid medium. Growth was show ever immensely dependent on the lipid source provided in media if all other components remain same. In standard corn oil, which is the specified lipid source in literature, the culture grows optimally after 6-7 days. However growth is profuse in coconut oil and butter, the latter taking only 3-4 days of incubation. The fungus shows growth restriction and hibernation in presence of almond oil. This fact was established by inoculating the cells onto S-Agar with almond oil in which the cells showed initial sporadic growth for 2 days and then no growth at all; however they grew again on sub-culturing onto butter showing that they had not lost their viability.

3.2 Characteristic morphology of *M. furfur*

Detailed microscopic analysis of the cell showed bottle shaped cells with one side round and other flattened, over which a bud like structure growth. Constriction is observed at the intersection. Very rare hyphae and no spores have been observed. With aging, cells and show wavy nature; single layered colony formation is observed. This aggregation is better observed with methylene blue staining as shown in figure 1.

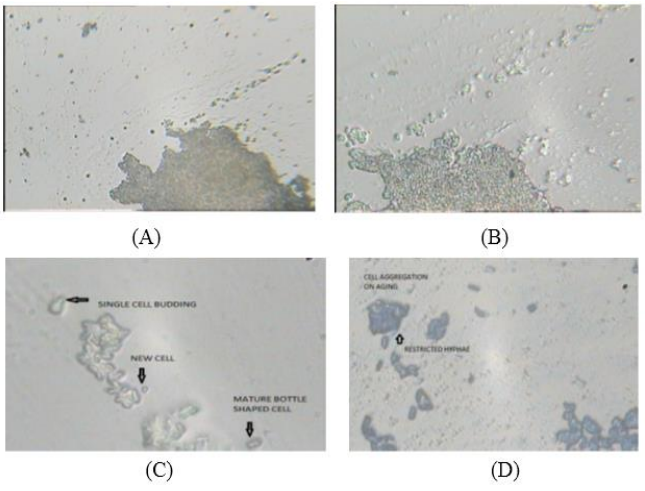


Fig 1: .Microscopic view of *M.furfur* showing detailed cell morphology (40X optical lens): (A) and (B) shows aggregation and colony structure, (C) and (D) shows identifiable distinct structures on staining with methylene blue.

3.3 Sulfur nanoparticle formation in CTAB

Sulfur nanoparticles were synthesized by reacting thiosulphate with oxalic acid in 1:6 stoichiometric proportions in pure CTAB solution, with surfactant concentration fixed at a concentration above its CMC value

(0.93 mM/L). This makes sure that the number of free surfactant monomers in solution is constant throughout the process and system is stable. Bulk Sulfur could be prepared by the same means, only if CTAB is not present in reaction media. The CTAB was later added to keep uniform concentrations. Bulk Sulfur solutions become pale white and turbid (precipitation observed on standing), while nano Sulfur solutions were completely transparent.

3.4 XRD and SEM analysis of nanoparticles

To confirm the structural aspects of the nano Sulfur formed, an XRD analysis was done of the solution sample. The XRD samples are prepared by successive washing by distilled water, to get pure sulfur peaks .The positions and intensities of the XRD diffraction peaks are same as the literature values for orthorhombic sulfur with S 8 structure [JCPDS PDF number 77-0145].

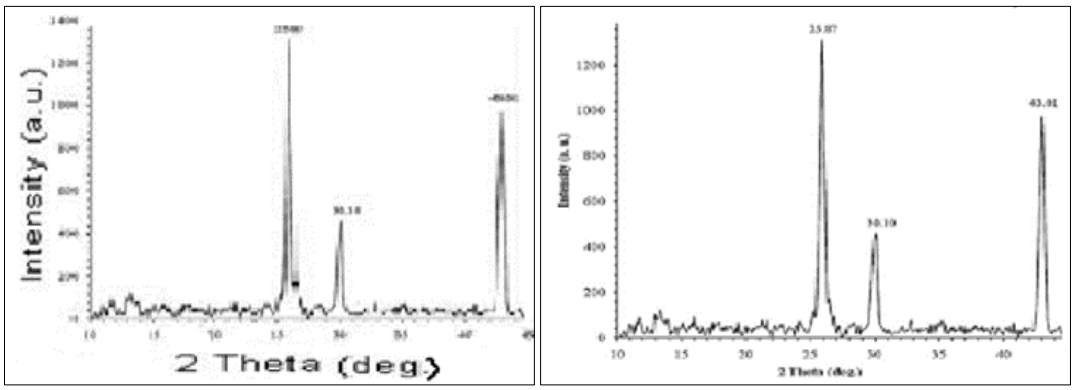
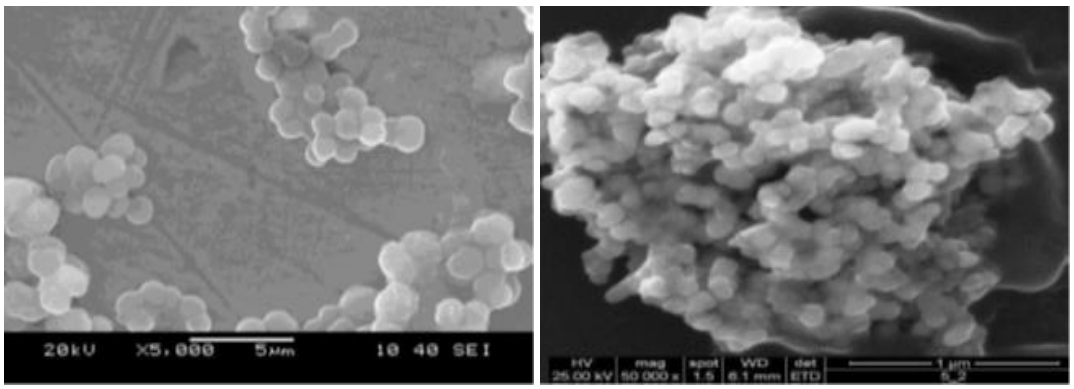


Fig 2: positions and intensity of the XRD diffraction peaks of Sulfur nanoparticles



(A) **(B)**

Fig 3: SEM images of nano Sulfur obtained using thiosulphate, oxalic acid and CTAB, at different magnifications, (A) 5000X, (B) 50000X

3.5 SEM analysis of *M. Furfur*

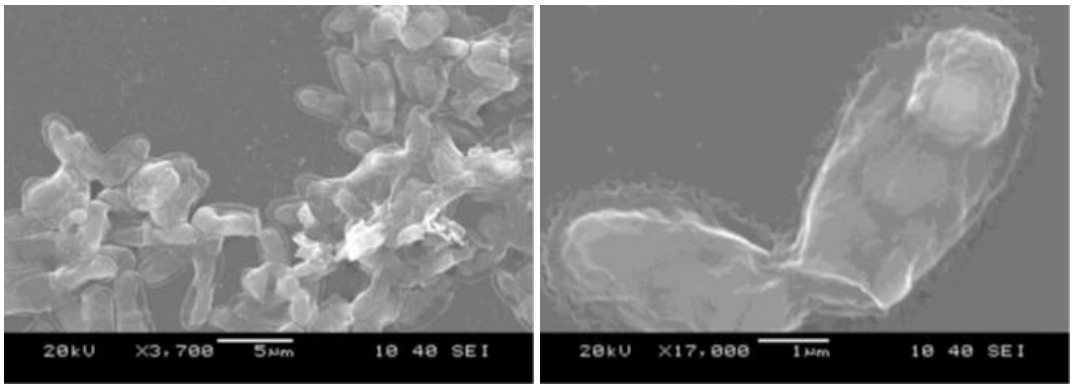


Fig 4: SEM figure of normal *M. furfur* cells, at different magnifications

The figure 4 shows the electron microscopic view of *M. furfur* cells, before and after treatment with sulfur nanoparticles. Besides the morphological structures already seen through optical microscopy like, no spore or hyphae and bowling pin like structures, other notable features include:

- Transparent lipid sheath covering around the cell
- Elongated cells with 'phialide fitted with collarette' structure of cell
- Size of single mature cell is around 1µmX10µm.

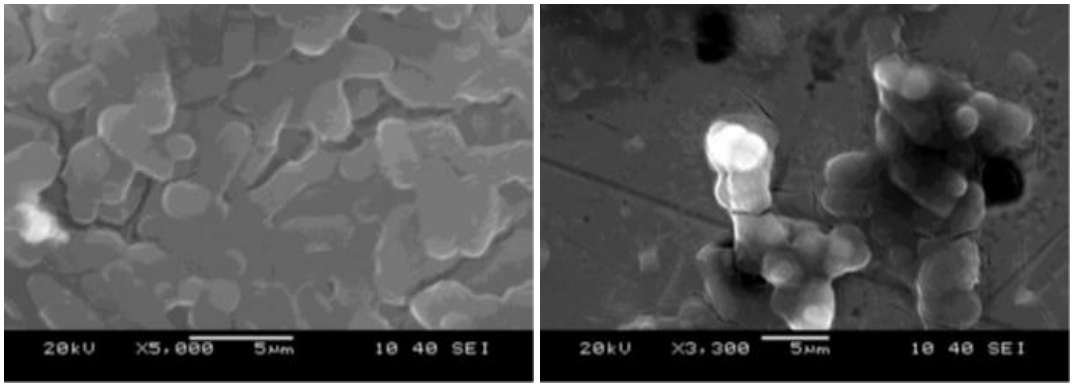


Fig 5: SEM and EDAX of Sulfur nanoparticles treated *M.furfur* cells.

After treatment of *M. furfur* cells, with nano Sulfur solutions, the cells show certain alterations in structure.

- Firstly, the roughness of the cell surface increases.
- Thinner or no lipid sheath covering the cell.
- The cells appear less elongated, more constricted and almost spherical in structure.

The changes observed after treating the yeast with Sulfur nanoparticles under SEM, explains why reduction in colony size and alteration of culture texture from smooth slimy paste to discontinuous flakes have seen while studying agar plates. The constriction of the lipid sheath is a result of surfactant action, while the cell roughness is a due to increased porosity of the cell boundaries in presence of nanoparticles which infiltrate through the sheath. Also since the cell size is substantially getting reduced, the same is seen in agar plates, where the colonies become notably diminished in size, when growing in presence of the nanoparticles-surfactant solution. Thus the yeast inhibition efficiency of the nanoparticles is comparably high and almost equivalent to the values obtained for conventional fungicidal agents.

5. Conclusion

Therefore we conclude that both surfactant CTAB and sulphur nanoparticles are antimicrobial in nature and their inhibitory action increases with their concentration. The fungus *M. furfur* is growing consistently in the media with healthy growth and no inherent effect. Butter has been the best lipid source showing growth in 4 days which is much less than 7 days growth observed earlier. Evidently, nano Sulfur of 40 ± 10nm, has greater efficiency as the fungicide, over bulk Sulfur (1000nm approx.), which is about 20% more in the case of *M. furfur* cells. Concentration of nano Sulfur, corresponding to total inhibition of growth, depends upon initial strength of inoculums. The close microscopic views, give us a clear idea of the yeast cell's interaction with nano Sulfur-CTAB solution. Gross changes in structure of the viable yeast cell which have been studied under electron microscope, shows the initial attempt of the yeast to adapt to the environment, and condense within. With all these experiments done, we could very well say that Sulfur nanoparticles, thus synthesized in surfactant base, is a good potential antidandruff agent, and the concept could develop a formulation of surfactant and nontoxic Sulfur nanoparticles for treatment of dandruff, in future.

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