Anti-Microbial Studies Using Sulphur Nano Particles on Dandruff Causing Malassezi Yeasts

Sudarsan Baskar, Pooja Pragati and Karthika Chandrababu

Abstract— Dandruff is a scalp disorder affecting more than half the population and often causes itching. Dandruff may be the result of seborrheic dermatitis, eczema, psoriasis or very commonly, an overgrowth of yeast like fungus called malassezia. Dandruff might also lead to Eye problems, acne, hair loss, allergies, swollen glands, rashes and research in this area would be both objective to the present scenario and lucrative potentially. Nanoparticles are frequently in use in some very powerful antimicrobial and antifungal cosmetics. However, most metallic nanoparticles are harsh and often toxic, and concern remains on their ill effects. Sulphur, certified as biocompatible to eukaryotes and as soil nutrient is often used in medicine. Sulphur nanoparticles is a strong antifungal agent and have been used in macro amount in shampoos. This paper concentrates on synthesizing sulphur nanoparticles in a surfactant system, insitu, such that it could possibly be used in curbing dandruff naturally The objective is to Synthesize and optimize sulphur nanoparticles in surfactant CTAB, GrowM.furfur in culture and optimize its growth and to study antimicrobial activity of sulphur nanoparticles on this strain. 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. Toxicity of nanoparticles is of a grave concern and a lot of effort is given to study and counter its toxic effects, the toxic nature itself serves a good purpose, when and as long as the victim is a microbial pathogen. Sulphur drugs also find applications as antifungal ointments to fight against skin infections.

Keywords—Corneocytes,Hyperprolification,Malassezi yeast, Seborrheaic dermatitis.

I. INTRODUCTION

NANOPARTICLES usually ranging in dimension from 1-100 nanometers (nm) have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change. The nanoparticles possess unique physico-chemical, optical and biological properties, which can be manipulated suitably for desired applications [1-4]. The nanoparticles are finding important applications in the field of medicine for the fact that, biological processes also occur at the Nano scale and due to their amenability to biological fictionalization. The metallic nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials [5-8].

Nanoparticles with antimicrobial activity when embedded and coated on to surfaces can find immense applications. About 95% of human hair is mainly based on keratins, characterized by significant amount of sulphur proteins [9]. The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high [10-14]. Therefore, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections. Sulfur compounds are known to exhibit widespread antimicrobial activity. Antimicrobial activity of fullerenes was observed on various bacteria, like E. coli, Salmonella, and Streptococcus species, so for zinc oxide particles [15-18]. Besides, these materials in their bulk phases, are also slightly, antimicrobial, due to either their metallic nature or in case of sulphur, its interaction with bacterial and fungal cell wall. Here sulphur particles are being widely investigated, as agricultural fungicidal agents, because sulphur being a soil nutrient, removes the chance of residual toxicity. Also on the other hand, sulphur drugs find applications as antifungal ointments to fight against skin infections [19-23]. The sulfur is widely used in different industrial applications such as: production of sulfuric acid, nitrogenous fertilizers, enamels, antimicrobial agents, gun powders, phosphatic fertilizers, plastics, petroleum refining, pulp and paper industries, other petrochemicals, ore leaching processes and different other agrochemical industries [24].

A. Synthesis of sulphur nanoparticles

Nanoparticles can be synthesized in two ways, either in-situ or synthesized separately from final formulation [25]. In case of non-metallic nanoparticles, insitu production would require a precipitation reaction. Here the nanoparticles were of a single element either metallic or non-metallic, 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 a function of the nature and concentration of the surfactant and the amount of nanoparticles formed as

Manuscript received April 03, 2015; revised April 16, 2015.
Sudarsan Baskar is a student with the SRM University, Chennai-603 203 (Phone: +91-8754561964; e-mail: sudarsanbaskar93@gmail.com).
Pooja Pragati is a student with the SRM University, Chennai-603 203 (Phone: +91-7639125999; e-mail: oxford_pooja@yahoo.com).
Karthika chandrababu is an assistant professor with the SRM University, Chennai-603203 (e-mail: karthim.technano@gmail.com).

ISBN: 978-988-14047-0-1
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

product depended on the reactants [Choudhuri and Paria, 2010, GhoshChoudhuri and Paria, 2012]. For sulphur nanoparticles synthesis a simple disproportionate reaction, using thiosulphate and different acids, both organic and inorganic, were various methods we established to prepare nanoparticles. Among them, use of wet chemical precipitation method by dissolving the sodium thiosulfate in double distilled water and different acidic solutions, using different surfactants (CTAB, TX-100, SDBS, and SDS) as stabilizer to control the particle size. The anionic stabilizer SDBS was found to be highly effective for obtaining uniform sizes NPs. While, the smallest size S-NPs was obtained by using CTAB as stabilizer. The antibacterial activity of sulphur nanoparticles was determined using broth micro dilution method.

B. Antidandruff agent

As the pathogenesis of dandruff involves hyper proliferation of keratinocytes, followed by deregulation of keratinization, followed by clumping together of corneocytes, manifesting itself as large flakes of loose skin, therefore, keratolytic agents such as salicylic acid and sulphur that loosen the attachments between the corneocytes and allow them to get swiped off, is a possible treatment of removing dandruff. Hence notably, sulphur is already a conventional antidandruff agent. Compound selenium sulphide is already used to treat dandruff, as it is known to have anti-Malassezia effects. Other compounds include imidazole [Shuster, 1984] and hydroxypyridones [Milani et al., 2003]. Zinc pyrithione is known to inhibit growth of yeast cells of Malassezia, through inhibition of iron-sulphur proteins [Reeder et al., 2011] and is widely used in common antidandruff shampoos [Cardin et al., 1990]. Silver nanoparticles have been, tested to be used in shampoos, firstly to increase wettability and secondly to act as fungicidal agent. However potential toxic effect of silver nanoparticles in shampoos and soaps, have created quite a concern. While, a large number of in vitro studies indicate that Ag NPs are toxic to the mammalian cells derived from skin, liver, lung, brain, vascular system and reproductive organs and has the potential to induce genes associated with cell cycle progression, DNA damage and apoptosis in human cells at non-cytotoxic doses sulphur nanoparticles in eco-friendly and clinical trials.show no alarming toxic effects on eukaryotes [Ahamed et al., 2010]. A milder concoction of surfactant formulated with nanoparticles, less toxic and yet effective like sulphur, would give good wettability and be an efficient cleanser, not toxic to the skin therefore would be a lucrative direction for research.

C. Effect of surfactants:

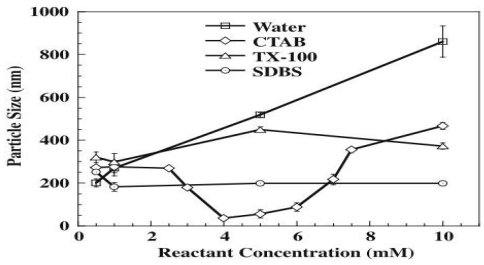


Fig 1: Variation of particle size with the thiosulphate concentration in the absence and presence of surfactants by oxalic acid catalyzed reaction [Choudhuri and Paria, 2010].

II. SULPHUR AND ITS BIOLOGICAL INTERACTIONS

A simple in-situ synthesis protocol includes using an

organic acid that reacted with a thiosulphate compound in presence of several surfactants, in concentrations above critical micellar concentration (CMC) which led to slow control of the size of the sulphur found. The lowest size (30 nm) particles were obtained in a certain reactant concentration range using CTAB surfactant and oxalic acid.Lime sulphur has shown to restrict growth of *M. canis* spores and other related species. While, a large number of in vitro studies indicate that Ag NPs are toxic and has the potential to induce genes associated with cell cycle progression, DNA damage and apoptosis in human cells,while at non-cytotoxic doses sulphur nanoparticles is eco-friendly and clinical trials show no alarming toxic effects on eukaryotes [Ahamed et al., 2010,].Also sulphur is a soil nutrient, and therefore causes no chance of soil or water pollution, if waste amounts accumulate in the environment [Palmiter and Smock, 1957, Ellis et al., 1998]. Thus it can probably be good as antidandruff, shampooing component.

III. METHODOLOGY

The surfactants CTAB (cetyltrimethyl ammonium bromide) are primarly used. Ultrapure water of pH 6.4–6.5 was double distilled again and used for all the experiments, the reagents were filtered with 0.2 l nylon 6, 6 membrane filter paper. Sodium thiosulphate and oxalic acid were used.Pure slant culture of Malassezia furfur was obtained and maintained in Emmon's modified mediumUnits

A. 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). Sub culturing was done onto agar plates using glass petri plates when substantial growth was observed. 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.

B. Synthesis of sulphur nanoparticles

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 disproportionation reaction to sulfur and sulfonic acid according to

$$Na_2S_2O_3 + 2H^+ \rightarrow 2Na^+ + SO_2 + S\downarrow +$$

$$H_2O\ SO_2 + H_2O \rightarrow H_2SO_3$$

After mixing the reactants, 40 min equilibrium time was given for the completion of reaction organic acids. After equilibration, the sample was sonicated in a bath for 2 min and particle size was measured by DLS method immediately. CMC of CTAB was measured by Wilhelmy plate technique with a surface tensiometer (DCAT-11EC, Data Physics, Germany). A constant temperature 28 ± 0.5˚C

ISBN: 978-988-14047-0-1
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

was maintained throughout the experiments. The sulphur 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 sulphur has been calculated on that basis.

IV. ANTIFUNGAL EFFECT OF SULPHUR NANO PARTICLES ON MALASSEZI

A. Furfur Culture

(i) Cell density measurement

Number of cells was located by Haemocytometer method of counting cells. Here a small amount of pure, viable colonies were scratched from a petri plate and mixed thoroughly with 2ml of sterile water to make a homogenous suspension. A small amount of the solution was put onto the haemocytometer, and cells were counted under an optical microscope using 10X objective lense. The cell density before inoculation was $150x10^4$ cells/ml. This served as a stock solution, from which diluted inoculums were prepared. About 10000, cells/ml was the final cell density used for experiments.

B. Inhibition of Colonial Growth

This test aims to find a concentration of sulfur nanoparticles of specific size for total inhibition of growth by spread plate method [Zhang and Chen, 2009]. Keeping the size constant, SDA agar plates supplemented with different concentrations from 0.0256 mg/ml to 0.128 mg/ml CTAB stabilized nanoscalesulfur particles (~35-55nm),from a stock of 0.128mg/ml to inspect their fungicidal activity (i.e. 0.2 to 1 fraction dilution of maximum working volume of 500µl, 4mM sulphur solution). Working volume added to the plates was 500µl for both nanoparticles solution and microbial seed solution Active culture was dispersed in sterile water, diluted 40 times (Cell density about 500 cells/ml, though in later experiments, more concentrated cell suspensions were used), and a volume of 0.5 mL from diluted culture used as a seed for the CTAB-Sulfur plate studies, SDA plates without nanoparticles and/or surfactant CTAB were cultured under the same conditions and used as negative control, while all other plates served as positive control. The controlled plates were incubated for 3 days at 30°C, while the plates with sulfur nanoparticles incubated for 5 days (as fungal colonies had delayed growth) and the number of colonies on the plates were counted.

V. RESULT AND OBSERVATION

A. Growth of M. Furfur:

The fungus shows growth restriction and hibernation in presence of almond oil. This fact was established by inoculating the cells onto S-D 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.

Fig 2: (from left) Growth of cells in (a) butter, (b) almond oil, and (c) coconut oil after 6 days incubation

B. Characteristic Morphology of M.Furfur

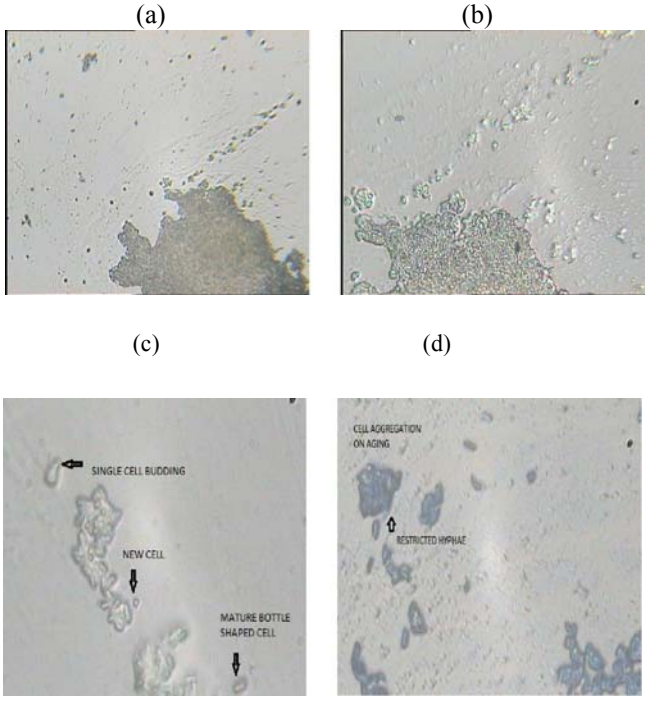


Fig 3: (a) and (b) showed aggregation and colony structure, (c) and (d) shows identifiable distinct structures on staining with methylene blue.

C. Sulphur Nanoparticle Formation in CTAB:

Sulphur 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 sulphur 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 sulphur solutions become pale white and turbid (precipitation observed on standing), while nanosulphur solutions were completely transparent.

ISBN: 978-988-14047-0-1
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

Fig 4: Physical appearance of sulfur suspension, from 4m M thiosulphate solution: (a) Turbid bulk sulphur, (b) Transparent nanosulphur.

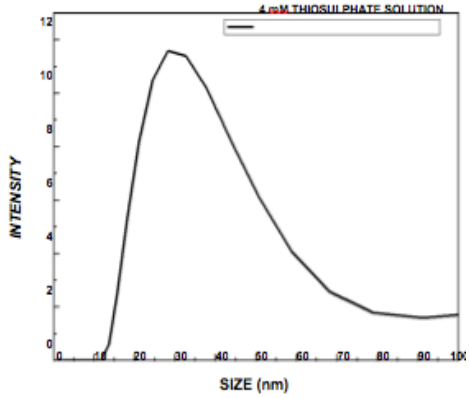
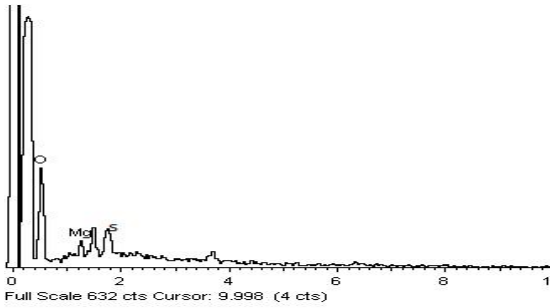


Fig 5: Intensity vs size distribution curve and particle vs size distribution curve of sulfur nanoparticles formed from 4mM thiosulphate concentration in CTAB.

D. XRD AND SEM ANALYSIS OF NANOPARTICLES



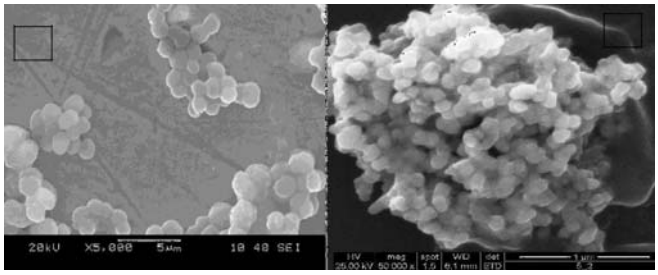


Fig 6: SEM images of nanosulphur obtained using thiosulphate, oxalic acid and CTAB, at different magnifications, (A) 5000X (B) 50000X.Below, EDAX data of the same

VI. CONCLUSION

We therefore conclude from our experiments, that both surfactant CTAB and sulphur nanoparticles are antimicrobial in nature and their inhibititory action increases with their concentration, for a fixed amount of inoculums. The fungus M. furfur is growing consistently in the media with healthy growth and no inherent decaying effect. Butter has been the best lipid source showing growth in 4 days, which is much less than 7 days growth observed earlier. Evidently, nanosulphur of 40±10nm, has greater efficiency as the fungicide, over bulk sulphur (1000nm approx.), which is about 20% more in the case of *Malassezia.* Concentration of nanosulphur, corresponding to total inhibition of growth, depends upon initial strength of inoculums. The close microscopic views, gives us a clear idea of the yeast cell's interaction with nanosulphur-CTAB solution. Gross changes in structure of the viable yeast cells 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 sulphur nanoparticles, thus synthesized in surfactant base, is a good potential antidandruff agent, and the concept could develop a formulation of surfactant and nontoxic sulphur nanoparticles for treatment of dandruff, in future.

REFERENCES

[1] Schmid G., Clusters and Colloids: From Theory to Applications. New York : Wiley& Sons. 1994.
[2] Liu W.T., J. Biosci Bioeng 102 (2006) 7.
[3] Batarseh K., J. Antimicrob. Chemother., 54 (2004) 546
[4] Ober J. A., Materials Flow of Sulfur: US Geological Survey Open File Report 02- 298, 2003.
[5] LaMer V.K., Kenyon A.S., J. Colloid Sci. 2 (1947) 257.
[6] LaMer V.K., Denegar R.H., J. Am. Chem. Soc. 72 (1950) 4847.
[7] LaMer V.K., Ind. Eng. Chem. 44 (1952) 1240.
[8] Chaudhuri R.G., Paria S., J. Colloid Sci. 343 (2010) 439.
[9] Deshpande A.S., Khomane R.B., Vaidya B.K., Joshi R.M., Harle A.S., Kulkarni B.D., Nanoscale Res. Lett. 3 (2008) 221.
[10] Xie X.Y., Zheng W.J., Y. Bai, J. Liu, Mater. Lett. 63 (2009) 1374.
[11] Chaudhuri R. G., S. Paria, J. Sci. 343 (2010) 439.
[12] Guo Y., Zhao J., Yang S., Yu K., Wang Z., Zhang H., Powder Tech. 162 (2006) 83.
[13] Shamsipur M., Pourmortazavi S., Roushani M., Kohsari I., Hajimirsadeghi S., Microchim Acta,173 (2011)445.
[14] Ellis M., Ferree D., Funt R., Madden L., Plant Dis. 82 (1998) 428.
[15] Barkauskas J., Res. Bull. 42 (2007) 1732.
[16] An Y., Nie F., Wang Z., Zhang D., J. Nano Med. 6 (2011) 3187.
[17] Porras I., Appl. Rad. Isotop. 69 (2011) 1838.
[18] Choudhury S., Roy S., Goswami A., Basu S., J. Anti. Chem. 67 (2012) 1134.
[19] Yu X., Xie J., Yang J., Wang K., J. Power Sources 132 (2004) 181.
[20] Zheng W., Y.W. Liu, X.G. Hu, C.F. Zhang, Electrochim. Acta 51 (2006) 1330.
[21] Yong Z., Wei Z., Ping Z., Lizhen W., Tongchi X., Xinguo H., Zhenxing Y., J. Wuhan Univ. Technol. – Mater. Sci. Ed. 22 (2007) 234.
[22] Kobayashi T., Y. Imade, D. Shishihara, K. Homma, M. Nagao, R. Watanabe, T. Yokoi, A. Yamada, R. Kanno, T. Tatsumi, J. Power Sources, 182 (2008) 621.

ISBN: 978-988-14047-0-1
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

[23] Choudhury S., M.Ghosh ,A. Goswami , Curr Microbio. 65 (2012) 91.
[24] Ghanemi K., Y. Nikpour, O.Omidvar, A. Maryamabad , Talanta 85 (2011) 763.
[25] Lan Y., Deng B., Kim C., Thornton E.C., Xu H., Environ. Sci. Technol. 39 (2005) 2087.
[26] Xie X., Li L., Zheng Pu., Zheng W., Bai Y., Cheng T., Liu J., Materials Res. Bull. 47 (2012) 3665.
[27] Li K., Wang B., Su D., Park J., Ahn H., Wang G., J. Powder Sources 202 (2012) 38.
[28] Santiago P., Carvajal E., Mendoza D.M., Rendon L., Microsc. Microanal. 12 (2006) 690.
[29] Ghanemi K., Nikpour Y., Omidvar O., Talanta 85 (2011) 763.

ISBN: 978-988-14047-0-1
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)