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Synthesis of Nano-sized Sulfur Nanoparticles and their Antibacterial Activities

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Abstract
Sulfur nanoparticles (S-NPs) were prepared in this work by precipitation method using sodium thiosulphate and tetraoctylammonium bromide surfactants in conc. hydrochloric acid media. The sizes and shapes of S-NPs were confirmed by scanning electron microscope (SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques. Broth micro-dilution method was applied to determine antibacterial activity of S-NPs against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* reference strains. S-NPs exhibited antimicrobial activity (MIC = 5.47μg/ml) against *Staphylococcus aureus* strain (Gram-positive bacterium). On the other hand, no antimicrobial activity was detected against Gram-negative bacteria isolates (*Escherichia coli* and *Pseudomonas aeruginosa*) at 0.68 to 800 μg/ml.

Keywords*:* Sulfur Nanoparticles (S-NPs), TEM, SEM, XRD, Antimicrobial activities.

1. Introduction
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 [1].

The application of Sulfur Nanoparticles (S–NPs) in nowadays as modification of metal and carbon nanotubes, and synthesis of nanocomposites for lithium batteries [2], agrochemical industries [1] fungicides in agriculture fields [3], anti-cancer agent [4-5], antibacterial agent [6], synthesis of sulfur nanowires with carbon to form hybrid materials with useful properties for gas sensor and catalytic applications [7] and as adsorbent for the extraction of metal ions [8].

Various methods we established to prepare nanoparticles [9]. 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 a uniform sizes NPs. While, the smallest size S-NPs was obtained by using CTAB as stabilizer [10].

Microemulsions system is an attractive and simple method, for example monoclinic S-NPs have been prepared via the chemical reaction between sodium polysulfide and hydrochloric acid, with the oline, butanol and a mixture of Span 80 and Tween 80 (weight ratio 8:1) as the oil phase, surfactant and co-surfactant, respectively. Transparent microemulsions were carried out by mixing the oil phase; a surfactant, co-surfactant, and the aqueous phase in appropriate proportion at room temperature. The S-NPs prepared via this method were found to have an average diameter of ~ 20 nm, with high purity, a narrow size distribution and uniform spherical shape [11].

S-NPs were synthesized from H_2S gas using novel biodegradable iron chelates in water/organic microemulsion system [9]. Ferric malic acid chelate was studied in water/organic micro-emulsion containing n-hexanol, cyclohexane and Triton X-100 as oil phase, a surfactant, co-surfactant, respectively, for the catalytic oxidation of H_2S gas at ambient conditions of temperature, pressure, and neutral pH. The S-NPs was nearly uniform in size (average particle size 10 nm) with narrow particle size distribution (in range of 5–15 nm) as compared to that in aqueous surfactant systems [12].

An electrochemical method is used to prepare the sulfur nanoparticles from thiosulfate ion. The size of S-NPs obtained ~ 35 to 65 nm by adjusting the operation parameters including the initial concentration of sodium thiosulfate. It was found that, the use of cold water and hot alcohol as solvent/non-solvent system along with 100 mL/min flow rate for co-mixing of non-solvent resulted in the formation of S-NPs in a typical size of 250 nm that are fairly homogeneous in shape and have a narrow particle size distribution [13].

In this study, S8-orthorhombic structure s-NPs have been synthesized by quick precipitation method in the presence of tetraoctylammonium bromide (TOAB) that was used as a stabilizer to control the nanoparticles size.

2. Materials and methods

2.1. Chemicals

The chemicals used for this study were taken from the following companies: sodium thiosulphate ($Na_2S_2O_3.5H_2O$) were purchased from Frutarom Co., hydrochloric acid (HCl) 32% concentration was purchased from Merck Co., tetraoctylammonium bromide (TOAB) 98% concentration was purchased from Sigma Co.

2.2. Particle synthesis

A mixture of Sodium thiosulfate and TOAB was prepared by combining 50.0 mL of 0.80M $Na_2S_2O_3.5H_2O$ with 20.0 mL of 0.02M TOAB aqueous solutions. The mixture was stirred mechanically at 120 rpm and heated in constant bath at 40 °C. Then 40.0 mL of 1.0 M HCl solution were added to the mixture under continuous stirring. A yellow precipitate was observed immediately, the reaction was stopped after 40 min. The produced yellow precipitate was collected, washed with distilled water and dried.

2.3. Samples characterization

The sulfur nanoparticles were characterized by X-ray diffraction (XRD) using Rigaku Dmax 2500 diffractometer equipped with graphite monochromatized CuKa radiation (k = 1.5406 $\mathring{A}$) employing a scanning rate of 5°/min in the 2h range from 10° to 50°.

The samples for TEM analysis were prepared by deposition of an ultrasonically dispersed suspension of the sulfur product in absolute ethanol on carbon-coated copper grids.

The size and shape of particles were observed under a scanning electron microscope (Inspect F50) and transmission electron microscope (ZEISS EM10CR).

2.4. Bacterial isolates

Antibacterial activity of the sulfur nanoparticles was evaluated against reference bacterial strains obtained from the American Type Culture Collection (ATCC). *Staphylococcus aureus* (ATCC 25923) strain was the Gram-positive bacterial isolate included. In addition, two types of Gram-negative bacteria, which were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa*(ATCC 27853), were examined.

2.5 Antibacterial activity of sulfur nanoparticles

The antibacterial activity of sulfur nanoparticles was determined using broth microdilution method. The applied protocol was similar to that of CLSI [14, 15]. Briefly, nanoparticles, were dissolved in 100% Dimethyl sulfoxide (DMSO) to achieve a concentration of 700 μg/ml. This solution was serially diluted (2-fold) 11 times with nutrient broth (HIMEDIA, India). Well number 11 was considered negative control of bacterial growth, while well number 12 contained nutrient broth only and was the positive control of bacterial growth. The achieved 10 concentrations of sulfur nanoparticles were 0.68, 1.37, 2.73, 5.47, 10.9, 21.88, 43.75, 87.5, 175 and 350μg/ml. To detect any antibacterial activity of DMSO in broth microdilution method conditions, a raw of 10 wells with serial 2-fold dilution of DMSO with nutrient broth was prepared with concentration form 0.098% to 50%. Overnight grown bacterial isolates were applied to all wells except negative control. The final standard bacterial concentration in each well was adjusted to 5×10^5 CFU/ml. After inoculation of bacteria, the plates were covered and incubated at 35 °C for 18 hours. Broth microdilution method was performed in duplicate for each isolate. Minimal inhibitory concentration (MIC) was considered to be lowest concentration that did not show any visible growth in the test media.

3. Results and Discussion

3.1. Synthesis of sulfur nanoparticles

S-NPs were synthesized by redox comproportionation of $Na_2S \cdot 5H_2O$ in concentration hydrochloric acid and using tetraoctylammonium bromide (TOAB) as stabilizer according to equation 1.

$$Na_2S_2O_3.5H_2O \xrightarrow[\text{TOAB in Conc. HCl}]{\text{Stirred at T = 40 C}^o} \text{5.7 nm S-NPs}$$

After synthesis, the particles were centrifuged and washed extensively with water to remove any soluble impurities (such as unreacted sulfite) and then filtered. The S-NPs were collected in good yield; the purity of the product was confirmed to be 99% by HPLC.

3.2. Analysis of X-ray diffraction (XRD)

Fig.1. shows that the XRD analysis of the as prepared sulfur nanoparticles had broad peaks. The position and intensities of the diffraction peaks of all samples were compared with standard a-sulfur particle diffraction pattern [16]. The average crystallite size of the as-prepared sulfur particles is about 5.7 nm, according to the Debye–Scherrer formula:

$$D=0.9\lambda/b \cos\theta$$

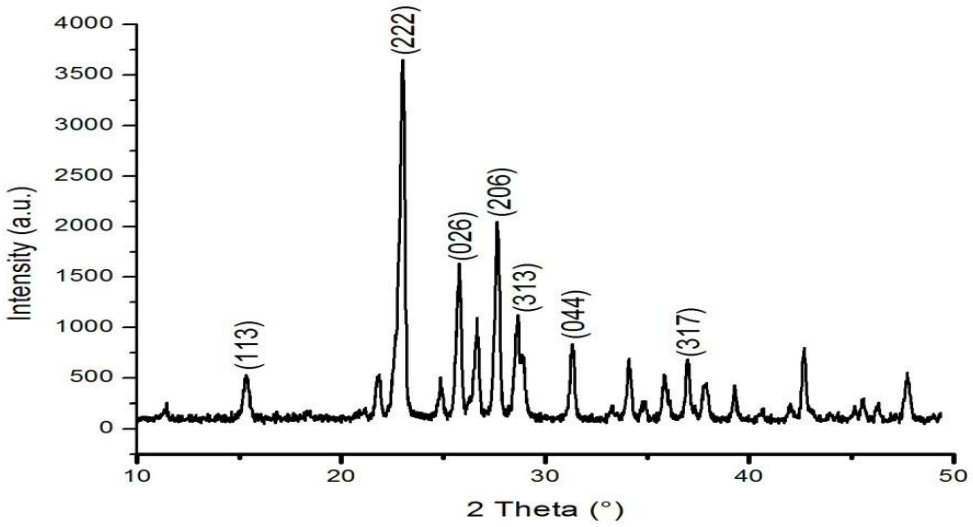

Fig. 1. XRD pattern of the sulfur nanoparticles.

3.3. Analysis of scanning electron microscope (SEM)

Fig. 2 show the SEM images of sulfur particle synthesize by 1M of HCl catalyzed in the presence of TOAB surfactants. From the figures it is clear that the sulfur particles are almost spherical shape and uniform size.

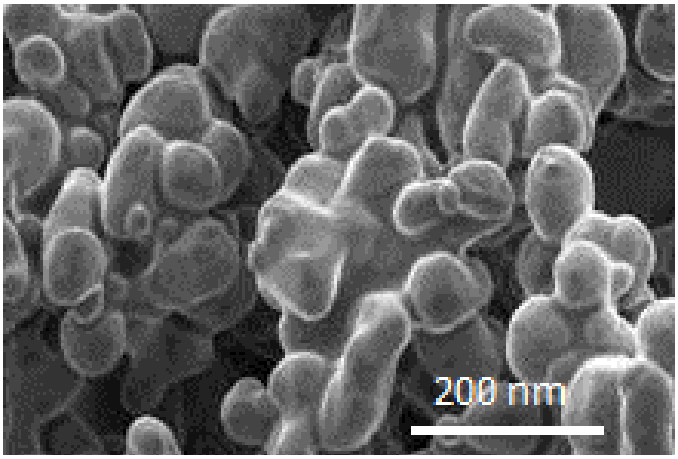

Fig. 2. SEM image of the sulfur nanoparticles.

3.4. Analysis of transmission electron microscope (TEM)

The morphology of the sulfur nanoparticles synthesized by a quick precipitation method was analyzed using TEM (Fig. 3). The mean size of S NPs was 5.7 nm, with spherical like shape. This again confirms the formation of lower size particle in TOAB media.

3.5 Antibacterial activity

In the current study, sulfur nanoparticles (5.7 nm) showed antimicrobial activity against *Staphylococcus aureus* reference strain (Gram-positive bacteria), where the MIC value was 5.47μg/ml. On the other hand, sulfur nanoparticle failed to show antimicrobial activities against Gram-negative

bacteria isolates of the present study at 0.68 to 350 μg/ml concentration. The examined Gram-negative bacteria were *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). In addition, no antimicrobial activity was found when Gram-negative bacterial strains of present study were tested with serially diluted (2-fold) sulfur nanoparticle at concentration from 1.56 to 800μg/ml.

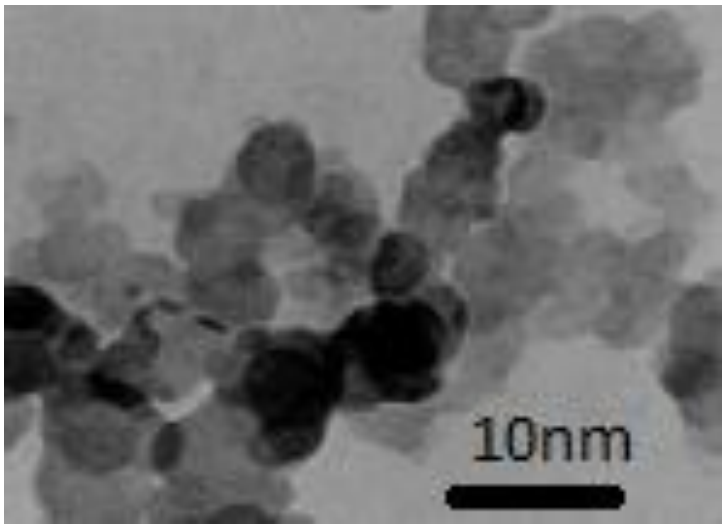

Fig. 3. TEM image of the sulfur nanoparticles.

In one recent study [12] using plate assay, sulfur nanoparticles suspension (150 μg/ml) produced larger inhibition zone (30 mm) in *Staphylococcus aureus* (Gram-positive) than that (25 mm) in *Pseudomonas aeruginosa* (Gram-negative). In agreement to our study, Choudhury et al [6] used broth microdilution method and found that custom-made sulfur nanoparticles failed to impart any antimicrobial activity against multidrug-resistant Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Enterobacter aerogenes*). However, in the same study they detected antimicrobial effect when polyethylene glycol-stabilized sulphur nanoparticles were applied.

In a previous study [12], antibacterial activity against Gram-negative bacteria was detected when a suspension of sulfur nanoparticles was applied. In the current study, complete solubility of sulfur nanoparticles was achieved using 100% DMSO to make sure that the nanoparticles are responsible for antimicrobial activity. In broth microdilution method conditions, DMSO showed inhibition to all studied bacterial strains at 50, 25, 12.5, and 6.25% DMSO concentrations. Therefore, inhibition of bacterial growth by sulfur nanoparticles solutions containing 6.25 to 50% DMSO was not considered an indication of sulfur nanoparticles antibacterial activities.

In the present study, sulfur nanoparticles exhibited antimicrobial activities against Gram-positive and not Gram-negative bacteria. This can be explained by the fact that Gram-negative bacteria possess outer membrane [17], which creates a barrier that prevents or limits penetration of sulfur nanoparticles into bacterial cell and may enhance self-aggregation between nanoparticles.

It's clear that evaluation of antimicrobial activity of sulfur nanoparticles is exposed to crucial factors. For example, rate of diffusion and bioavailability of nanoparticles in different media (broth and agar) may be different for certain organisms [18]. In addition, the species of bacteria as well as the strain examined is expected to cause variation in the results.

Conclusion

Synthesis of sulfur nanoparticles by oxidation precipitation method using sodium thiosulphate and tetraoctylammonium bromide as ionic stabilizer in conc. hydrochloric acid at 40 ^{o}C.The described process gives highly crystalline pure a-sulfur with uniform shape and size of 5.7 nm of sulfurnanoparticles. The structure and the size of prepared nanoparticles were characterized on the base of XRD, SEM and TEM. The synthesized nanoparticles are showing very high antibacterial activity against *Staphylococcus aureus.*

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