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SYNTHESIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SULFUR QUANTUM DOTS: A REVIEW

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ABSTRACT: Day by day it is challenging to prepare a chemical reagent against antimicrobial resistant pathogenic for pharmaceutical and food sector. There are several types of quantum dots are invented among them Sulfur Quantum Dots (SQDs) a new type of luminescent quantum dots which is prepared from unused sublimed sulfur can be replaced for these purposes. SQDs are highly water soluble, low toxicity and have an antimicrobial and antioxidant activity. There are several uses of this quantum dots, but in this review the synthesis method and their comparison, antibacterial and antifungal activity and antioxidant activity are described from its invention to present state. SQDs with packaging film exhibited effective antimicrobial effects especially against foodborne microorganisms caused by the production of reactive oxygen species (ROS). SQDs could be a potential agent for food packaging, disinfectant for fabric and increase the shelf life of food.

INTRODUCTION: Element sulfur is one of the most abundant and extensively used substances on earth. There is a huge demand of sulfur for various fields, such as sulfuric acid production, medicine, rubber production, lithium-sulfur batteries and agriculture. However, a large number of sulfur has not been used fully utilized, which produce waste of sulfur resources. So, it is a demand of time to develop novel pathway for the utilization of elemental sulfur in an efficient way for the economic and environmental perspectives [1, 2].

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Element sulfur is used as antimicrobial agent from the ancient. Microorganisms adsorb or uptake the elemental sulfur and generate hydrogen sulfide or pentathionic acid, which can impair a cluster of essential enzymes (especially –SH) responsible for microbial respiration or denature certain proteins and lipids [3]. Sulfur nanomaterials have low toxicity, good solubility and excellent photo-luminescent property and superior antimicrobial or antifungal activity than carbon nanomaterials [3, 4].

Quantum dots are a new type of fluorescent nanomaterials have unique physical and chemical properties, including high stability, narrow range of emission, and high quantum yield. Funtionalized quantum dots have an antibacterial activity. There are three molecular mechanism for antibacterial activity of quantum dots: (1) destruction of cell walls/cell membranes; (2) production of reactive

oxygen species (ROS) to destroy the cells; (3) binding with nucleic material (DNA/RNA) to inhibit cell proliferation [5]. In the past 5 years, sulfur quantum dots (SQDs), a novel class of metal-free fluorescent quantum dots, have better water solubility, low toxicity and stable photoluminescent property. Nowadays, SQDs are using various fields, like fluorescence detection, bioimaging, light-emitting diodes, fluorescent polymer composites, photocatalysis and antibacterial materials [6]. Not more than one decay ago, it was firstly invented and just only 5 years ago a facile and strong synthesis method was invented [7, 8]. Like sulfur nanoparticles, SQDs exhibit antibacterial activity that was firstly reported in 2021 against food borne pathogenic [9]. Later, negatively charged S-dots, exhibited potential antibacterial activity against pathogenic bacteria and found better than conventional antibiotics with suitable solubility and low toxicity [10]. Recently, alginate and chitosan based packaging materials containing S-dots showed antibacterial and antifungal activity and antioxidant activity, which added new dimension for packaging systems for the longer food self-life [11, 12]. Visible light-mediated photoactivated SQDs showed antibacterial activity on polycotton fabric which could be as a source of disinfectant agent [13]. This review present the synthesis, characterization, photoluminescence property, antimicrobial activity and antioxidant activity. After nearly 10 years invention of SQDs, several facile and time consuming with high photoluminescent quantum yield synthesis process were invented. Herein, we tried to discuss all synthesis process briefly and their limitation and comparison. The antimicrobial activity and antioxidant activity are vital factor for food packaging sector that's why we discussed about all research of SQDs for these.

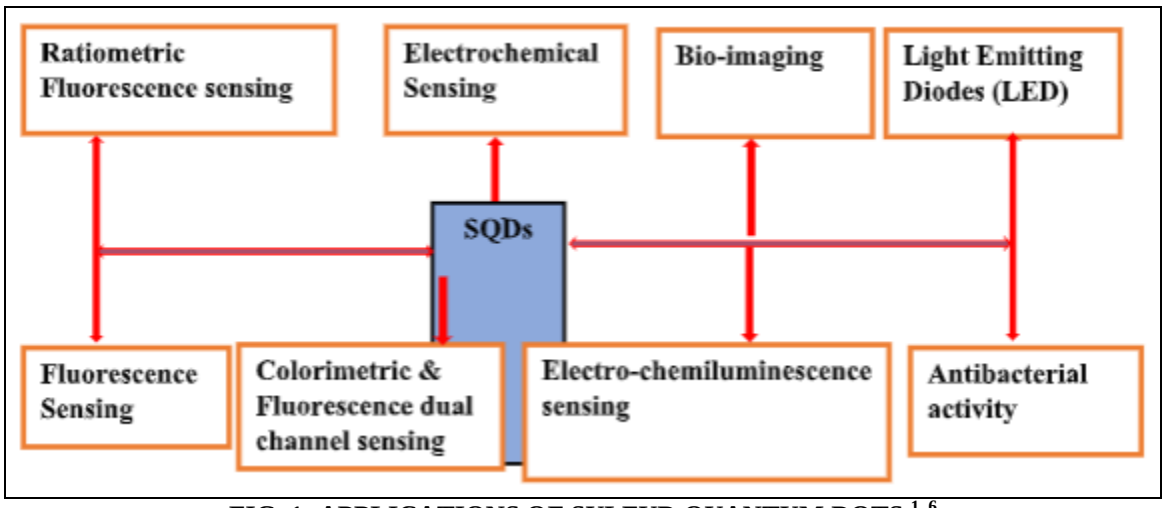

FIG. 1: APPLICATIONS OF SULFUR QUANTUM DOTS [1, 6]

Synthesis of Sulfur Quantum Dots: The synthesis method of Sulfur quantum dots include 1) Acid Etching Oxidation Method, 2) Assembly-Fission Method, 3) Hydrogen Peroxide-Etching Method, 4) Oxygen-Accelerated Method, 5) Ultrasonication and Microwave Method, 6) One step Hydrothermal method 7) Other Methods [1]. The first synthesis method of sulfur quantum dots was invented in 2014 and prepared by using CdS quantum dots. This process include three steps: physical contact, phase interfacial reaction, and in situ precipitation and dissolution. 1.5 mm $CdCl_2$ was added to 10 ml of oil acid and heated to 90 ºC to generate Cd-oil complexes, after that 0.75 mm of sulfur powder in 5 mL of oleyamine was injected into hot solution and heated up to 140 ºC for 20 h. 50 mL of ethanol was added to precipitate CdSQDs. The centrifuged precipitate dissolved in 50 ml n-hexane. Then HNO_3 was mixed with the diluted CdSQDs with slowly stirring for 36 h at room temperature. The first luminescent SQDs was highly water soluble and low toxicity but had low quantum yield of 0.5%. The first invented technique is now avoid due to low quantum yield, high cost and harsh reaction condition [7].

Later, a simple top-down method was applied to synthesis water dispersed sulfur quantum dots from bulk sulfur. Sublimed sulfur powder (1.4 g), ultrapure water (50 mL), polyethylene glycol-400 (PEG-400) (3 mL) and sodium hydroxide (4g) were heated 70 ºC up to 125 hours. Here, PEG-400 acts

as passivation agent, PEG-400 is important to improve the photo luminesce activity of sulfur dots. Here, researchers proposed an "assemble-fission" mechanism for the formation of sulfur dots. The formation of S dots includes three steps: dissolution, assembling, and fission. The process follow the below reaction

$3S + 6\ NaOH = 2Na_2SO_3 + 2H_2O$

$(x\text{-}1)\ S\ Na_2S = Na_2S_x x = 2\text{-}5$

At first elemental sulfur react with sodium hydroxide and formed sodium sulfide (Na_2S), which further reacted reacted with elemental sulfur, leading to the formation of unstable intermediate sodium polysulfide (Na_2S_x), which lastly underwent a fission reaction to release sulfur particles. It is a time consuming procedure, it requires more than 100 hours to reach a dynamic equilibrium and monodispersed sulfur quantum dots are obtained. The quantum yield found 3.8% and high stability over two months. Although some constrains, it is the first facile method for synthesis of SQDs and it creates groundbreaking step to synthesis more efficient SQDs [8].

A highly quantum yield containing SQDs up to 23% synthesized by using H_2O_2 etching assisted top-down method. This method was similar as like [8], sublimed sulfur powder, PEG and NaOH were mixed together and stirred at 70 ºC. After injecting H_2O_2 into the non-luminescent S dots green emission, after more added H_2O_2, it change to blue emission. According to the amount of H_2O_2 and time, quantum yields of green and blue photoluminescence was 16% and 23% and the simultaneous decrease size in 5 to 3.5 nm^2. A rapid synthesis process was developed by means of hydrothermal method. 0.3 g of sulfur, 0.8 g of NaOH and 0.5 mL of PEG were mixed in 10 mL water and transferred in a Teflon-lined autoclave chamber and heated at 170 ºC.

This process produced 4.02% quantum yield at 4 h but it is a high energy consuming and low quantum yield [14]. A facile short time consuming synthesis method was applied for the preparation of SQDs. In this method, sublimed sulfur powder (0.1 g), ultrapure water (40 mL), PEG-400 (2.5 ml), and sodium sulfide (7.0 g) were added, which was allowed to react under ultrasonication. After 3 h reaction, a weak green emission appeared under uv light. Extension of the ultrasonication time resulted in a lighter color and achieving quantum yield of 2.1% after 12 h [15]. After using Cu^{2+} etchant agent instead of H_2O_2 into similar solution described by [8] found highly photoluminescence quantum yield of 32.8%. This caused by the effective surface etching using $Cu^{2+}$and the suppression of the nonradiative recombination transitions. However, this process is limited for the synthesis of SQDs especially for bioimaging purpose due to the toxicity of copper [16].

A facile SQDs synthesis method was invented under pure-oxygen atmosphere, monodisperse size 1.5-4 nm and high fluorescence quantum yield of 21.5%. In this procedure, sublimed sulfur, NaOH, and PEG were added in water and the mixture was stirred at 90 ºC under O_2 atmosphere. After 8-10 h, the transparent solution emit bright blue and cyan fluorescence under illumination with 365 and 395 nm light. It was the first time to discover that highly fluorescent SQDs prepared from elemental sulfur based on the oxidation of divalent polysulfide ions into zero-valent sulfur under pure O_2 atmosphere [17].

A microwave assisted top-down method was invented with high quantum yield. Sulfur powder and PEG-400 were mixed with NaOH aqueous solution vigorously stirred for 1 h and then heated to 90 ºC via microwave irradiation within 5 minutes and the solution was kept at the 90 ºC for 30 min for the formation of more sulfur core and the solution was kept at least 5 h at 70 ºC temperature. As a result, there was a breakthrough in the SQDs synthesis process with a high photoluminescence quantum yield of 42.9%. After treating with 3 wt.% H_2O_2 lead to producing quantum yield of 49.25% [18]. A first synthesis process was carried out by using sodium thiosulfate (S2 oxidation state) and etching of sulfur (in situ formed) by NaOH in an aqueous medium. In this process, sodium thiosulfate and oxalic acid were added in DI water followed by PEG-400. Then the mixture was allowed to stir at 70 ºC for 2 h and then added NaOH and heated this mixture 4 h and a very pale yellow produce luminescent sulfur dots. But this method produce a low photoluminescent quantum yield of 2.5% [19]. By changing the passivating agent CMC instead of PEG a blue luminescent SQDs was prepared. Here, 1 g of CMC

was dissolved in 80 mL water, then 1.6 g sublimed sulfur and 4 g of NaOH heated at 95 ºC with continuous stirred for 24 h under O_2 atmosphere. The absolute fluorescence quantum yield found 7.1%, which is higher than many previous reported SQDs [20]. Tan *et al.* prepared S-dots by following multi-step H_2O_2 assisted synthesis process. Sublimed sulfur, PEG-400 and NaOH solution heated at 70 ºC for 72 h, then centrifuged to remove larger particles. After that, H_2O_2 was added as an etchant and the prepared SQDs showed a low quantum yield of 11% [21].

Different SQDs synthesis method was established by changing the passivating ligand. Liu *et al.* proposed a synthesis method by using polyvinyl alcohol (PVA). Sublimed sulfur, NaOH, PVA solution heated at 75 ºC for 12 h under pure O_2 atmosphere and found PLQY of 4.62% [22]. A more stable and excellent water dispersible SQDs was prepared from Hydroxypropyl-β-cyclodextrin (HP-β-CD). 2 g of HP-β-CD and 2 g of NaOH were dissolved in DI water and 0.7 g sublime sulfur added. This mixture was heated at 85 ºC in a pure O_2 atmosphere for 12 h. The monodispersed SQDs particle size 3.5-6.5 nm contain low quantum yield of 4.66% [23].

A facile, green and high efficient SQDs was prepared by ultrasound-microwave assisted etching on-pot synthesis method. Sublimed sulfur, NaOH and PEG-400 dispersed in water and the mixture was subject to ultrasound-microwave irradiation at 70 ºC for 1 h. Then 12 wt. % H_2O_2 solution was added and the reaction was allowed the same condition for 1 h. The smallest size 2.22 to 0.6 nm SQDs was prepared with high photoluminescent quantum yield of 58.6% and showed excitation independent emission [24]. With the help of bubbling assisted synthesis process SQDs was prepared in a short time. Sulfur, NaOH, PEG-400 solution heated at 70 ºC for 5 h with an air pump to keep the ventilation of the reaction system. The bubbled air is critical for the formation of SQDs, by providing O_2 to transfer divalent sulfur ions into the elemental sulfur core and etching the surface species deactivates nonradiative paths. It is a time suitable method but have low quantum yield of 8% [22]. A negatively charged, highly antibacterial and biocompatibility to humans SQDs but low quantum yield of 5.13% was prepared by employing poly (sodium 4-styrenesulfonate) (PSS) by etching of H_2O_2. Sulfur, NaOH, and PSS were mixed and heated at 70 ºC for 12 h. Then H_2O_2 injected into the mixture and turned colorless luminescent SQDs [10]. A mid-level photoluminescent quantum yield of 17.6% SQDs was prepared by employing low molecular weight PEG-200 instead of PEG-400. PEG-200 endow the SQDs more hydroxyl groups. PEG-200, NaOH and Sublimed sulfur powder were added in DI water and the mixture was heated at 30 ºC for 0.5 h under O_2 atmosphere, then agitated at 90 ºC for 10 h.

The S-dots with multiple hydroxyl groups showed superior water dispersibility, strong and tunable emission and low toxicity [25]. A low photoluminescent quantum yield of 4.8% SQDs was prepared by mechanochemical method. In this procedure, 500 mg NaOH moist powder, 0.4 mmol sodium thiosulfate and PEG-400 were grinded in a morter and pastle for 10-15 min and light yellow powder produced. Then, extended the grinding time and found a whitish powdered sample. After that, dispersed in distilled water for 1 h and dialyzed and found SQDs [26].

A highly stable blue luminescent quantum dots were rapidly synthesized by a solvothermal method using sublimed sulfur and β-cyclodextrin as the passivator. Sublimed sulfur (0.3 g), NaOH (0.3 g), and β-cyclodextrin (0.15 g) were dissolved in 25 mL ethanol and sonicated for 20 min. After that, the brown-yellow mixture was transferred to autoclave and heated a 180 ºC for 4 h.

The fluorescence quantum yield of β-SQDs 14% [27]. Gao et al. reported a simple and effective method for mass-producible synthesis of ultra-bright fluorescent SQDs on the basis of one-pot solvothermal treatment of S-ethylenediamine (S-EDA) solution. Sublimed sulfur dissolved in S-EDA solution at 170 ºC for 5 h and produced a dark brown solution. The produced S-dots could be readily purified and collected by direct precipitation in ethanol and followed by centrifugation. A highly quantum yield of 87.88% SQDs synthesized by a short time and facile purification process. A possible reaction mechanism was proposed that the S can dissolve in EDA to form an open chain alkylammonium polysulfide at room temperature [28].

$$2H_2NCH_2CH_2NH_2 + S_x \longrightarrow (H_2NCH_2CH_2NH_3^+)\,(H_2NCH_2CH_2NH\text{-}S_x)$$

On the other hand, the addition of H^+ could regenerate zerovalent sulfur ion from the alkylammonium polysulfide and this reaction has been employed to synthesis sulfur nanoparticles.

$$(H_2NCH_2CH_2NH_3)\,(H_2NCH_2CH_2NH\text{-}S_x^-) \xrightarrow{H^+} (H_2NCH_2CH_2NH_3^+) + S_x\downarrow$$

Futhermore, several groups have also reported the H_2S would be produced from the S-EDA solution at high temperature by the following reactions.

$$2H_2NCH_2CH_2NH_2 + S_x \longrightarrow (H_2NCH_2CH_2NH)_2\,S_{x-1} + H_2S$$

$$H_2NCH_2CH_2NH_2 + ¼\,S_8 \longrightarrow H_2NCH_2CSNH_2 + H_2S$$

SQDs with size around 2 nm was synthesized by a microwave-assisted method using sulfur powder as precursor. In this method, 1 g NaOH, 0.175 g sulfur powder and 1.5 ml PEG -400 mixed under stirring at 70 ºC to form a yellow solution. Then, this solution was placed in a microwave oven and reacted at 200 W for 1.5 h. Finally, the mixture was centrifuged for 15 min at 5000 rpm and found quantum yield of 4.3% SQDs [29]. By using sodium hypochlorite as the etching agent, Lu *et al.* were prepared a SQDs. Sulfur powder and PEG-400 were added into 10 mL of NaClO aqueous solution. Then the mixture was transferred to a Teflon-lined autoclave and placed in a muffle furnace at 180 ºC for 12 h. A low PLQY of 2.90% was produced with 1.68 to 1.24 nm particle size S-dots [30].

A fluorescent tunable SQDs were obtained by one-pot hydrothermal method and by H_2O_2 assisted etching after hydrothermal treatment. Sublimed sulfur, NaOH and PEG-400 mixture sonicated for 20 min and heated for 4 h at 180 ºC. After removing precipitate and filtering, the solvent was dried by rotary evaporator drying at 60 ºC and Green-SQDs were obtained. By slowly added H_2O_2 dropsies to the Green-SQDs, found a colorless supernatant Blue-SQDs. They found PLQY of respectively 6.3% and 8.1% for Green-SQDs and Blue-SQDs [31].

By using hyperbranced polyglycerol (HPG) as a ligand to direct the synthesis of dendritic HPG-SQDs from cheap elemental sulfur. Under the O_2 atmosphere mixture was heated at 95 ºC for 24 h and low PLQY of 6.8 % SQDs were produced [32]. A facile one-pot synthesis with a quantum yield of 7.04 % approach was employed to transfer sulfur into SQDs by using ethanol solvent instant of alkali NaOH. Sulfur powder, H_2O_2 and PEG-400 was successively added to ethanol and ultra-sonicated for 10 min and reacted at 220 ºC for in a Teflon-lined autoclave for 36 h. The obtained SQDs displayed higher photoluminescent properties than those observed in water and NaOH. This is possibly because ethanol could efficiently disperse bulk sulfur and provide sufficient contact between the etching agent and the sulfur [33]. There are found one article that SQDs were prepared from tioacetamide (TAA) as a sulfur source.

It is well known that TAA hydrolyzes at 100 ºC under acidic or neutral conditions to produce hydrogen sulfide (H_2S) and dissociated into reductive sulfide ions (S^{2-}). In this process, 0.25 g carboxy methyl cellulose (CMC) was added in 12 mL water, then 6 mL of 50 mM TAA and 2 mL 3% H_2O_2 were added and heated at 100 ºC for 4 h with continuous stirring. A blue fluorescence with good water solubility with PLQY of 9.31% S-dots were produced [34]. Recently, L-cysteine was employed as capping reagent for the first time to prepare green fluorescent SQDs. Sublimed sulfur powder 0.7 g, 2 g NaOH, and 3.5 g L-cysteine were added in 25 ml DI water, heated at 70 ºC for 24 h. Next, 5 mL of H_2O_2 added dropwise and reacted for 108 h and finally deed red color found. The supernatant after centrifugation was treated with ion-exchange resi step by step and filtrated. In this process, excellent optical stability, low biological toxicity and good fluorescent property in the light with quantum yield of 13.87% SQDs were produced [35].

Priyadarshi et al. were prepared SQDs with quantum yield of 79.8% modified by the method of [28] and recycle 70% of the solvent and > 50% of water. They used water instead of ethanol to resuspend the SQDs after EDA removal. The conversion efficiency was 21% than the 15% of reported Gao *et al* [36]. Polyethyleneimine (PEI) using as a passivator for the synthesis of SQDs, found good water solubility and stablility with a quantum yield of 5 %. 4.0 g of NaOH, 5 mL of PEI and 4.0 g of sublimed sulfur powder dissolved in 50 mL DI water and heated at 75 ºC for 120 h [37].

The bovine serum protein (BSA)-capped SQDs are synthesized by an H_2O_2-assisted chemical etching reaction with fine water dispersibility and good optical stability with quantum yield of 5.74%. The resultant BSA-capped SQDs are endowed with abundant carboxy and amino groups on the surface, exhibits excellent water dispersibilty and improving surface chemical activity [38].

A novel chitosan oligosaccharide SQDs (COS-SQDs) were prepared by mixing sublimed sulfur powder, KOH and COS solution with by adding of H_2O_2. 0.7 g sulfur and 2.805 g KOH added in 25 mL water and heated at 70 ºC for 3.5 h. Then, COS 0.075 g was added and continuously stirred for 1.5 h. Next, 20% H_2O_2 was added drop wise at 70 ºC and found yellowish SQDs with quantum yield of 8.45% [39].

A green and facile ozone-assisted top-down approach was introduces for the rapid synthesis of SQDs. The formation of SQDs involves the dissolution of bulk sulfur powder into small particles in an alkaline environment, followed by the oxidation of polysulfide ions (S_x^{2-}) into zero-valent sulfur (S^0) by ozone.

The bulk sulfur, NaOH and PEG-400 solution were heated at 90 ºC for 3 h and then the solution treated with ozone for 1 h and produced light yellow SQDs. A strong blue fluorescent obtained under UV lamp and found PLQY of 9.26% [40].

A facile and short time consuming sonication assisted with high quantum yield of 10.4% method was employed for S-dots synthesis. In this approach, 0.3 g sulfur, 2 g NaOH, 0.5 mL oleic acid and 20 mL water were mixed and the mixer was allowed to react for 3 min under high amplitude ultrasonic waves using a probe sonication instrument. The obtain forthy mixture was allowed to condense by heating at hot plate at 190 ºC for 12 h and a pale-yellow S-dots produced within very short time [41]. In the current study, SQDs were synthesized directly from sublimed powder *via* a one-pot solvothermal method by using sucrose as a stabilizer to enhance the stability and biocompatibility. In this method, 0.795 g sucrose was dissolved in 15 mL H_2O_2 (10% wt.) and then 20 mg sublimed sulfur powder was added to the solution and heated at 220 ºC for 24 h. They found low toxicity, good water solubilty and potential biodistribution for renal clearance with in high PLQY of 21.5% [42].

Functionalized SQDs were prepared by using *p*-phenylenediamine as the precursor and tetraethylene glycol (TEG) as the stabilizer for getting highly antibacterial activity. Sublimed sulfur and *p*-phenylenediamine as the ratio of 1:1 were taken and hydrothermally heated at 200 ºC for 24 hours in the presence of TEG. For getting neutral charge hydroxyl group was incorporated, carboxyl group was incorporated for negative charge and quaternary ammonium group was incorporated for positive charge [43].

First time SQDs with dual emission was invented by using rhodamine-B (rhB) as a passivator. 160 mg sodium heparin, 1 mg rhB, and 1molL^{-1} NaOH were dissolved in 20 mL pure water, and then 560 mg of sublimed sulfur powder was added gradually and it was stirred at 70 ºC for 24 hoursunder pure oxygen atmosphere [44]. Multicolor emitting SQDs were prepared by using H_2O_2etching method. For blue-SQDs, sublimed sulfur powder (0.6 g), 1,2 ethylenediamine (EDTA) 3 mL added at 20 mL ethanol and refluxed for 6 hours 70 ºC.

After cppling 5 mL of H_2O_2 (7.75 wt%) were added as droplet upon agitation. For cyan emitting SQDs, sublimed sulfur powder (0.6g), 1,3 diaminopropane (PDA), 3 mL added at 20 mL ethanol and refluxed for 24 hours 70 ºC. After cppling 7 mL of H_2O_2 (7.75 wt %) were added as droplet upon agitation. For green-SQDs, sublimed sulfur powder (0.6 g), 1,4 butanediamine (BDA),, 3 mL added at 20 mL ethanol and refluxed for 24 hours 70 ºC. After cppling 7 mL of H_2O_2 (7.75 wt%) were added as droplet upon agitation [45].

TABLE 1: SQDS SYNTHESIS PROCESS AT A GLANCE

Synthesis Methods	Materials	Temperature ºC	Time (h)	PLQY %	References
HNO_3 acid Etching Method	Sulfur powder, $CdCl_2$, oil acid, HNO_3	25	36	0.5	[7]
Assemble-Fission method	Sublimed sulfur powder, NaOH, PEG-400	70	125	3.8	[8]
H_2O_2 etching method	Sublimed sulfur powder, NaOH, PEG-400, H_2O_2	70	5	23	[2]
Hydrothermal method	Sublimed sulfur powder, NaOH, PEG-400,	170	4	4.02	[14]
Ultrasonication promoted method	Sublimed sulfur powder, sodium sulfide, PEG-400,	Room temperature	12	2.1	[15]
Cu^{2+} etching method	Sublimed sulfur powder, NaOH, PEG-400, Cu^{2+}	70	72	32.8	[16]
Oxygen accelerated method	Sublimed sulfur powder, NaOH, PEG-400 under pure oxygen	90	10	21.5	[17]
Microwave assisted method	Sublimed sulfur powder, NaOH, PEG-400	90 & 70	6 h 35 min	42.9	[18]
Situ reaction method	Sodium thiosulfate, Oxalic acid, NaOH, PEG-400	70	6	2.5	[19]
Oxygen assisted CMC method	Sublimed sulfur powder, NaOH, CMC	95	24	7.1	[20]
Multi-step H_2O_2 assisted method	Sublimed sulfur powder, NaOH, PEG-400, H_2O_2	70	72	11	[21]
Oxygen assisted PVA method	Sublimed sulfur powder, NaOH, PVA	75	12	4.62	[46]
Oxygen assisted HP-β-CD method	Sublimed sulfur powder, NaOH, Hydroxypropyl-β-cyclodextrin (HP-β-CD)	85	12	4.66	[23]
Ultrasound-microwave assisted H_2O_2etching Method	Sublimed sulfur powder, NaOH, PEG-400, H_2O_2	70	2	58.6	[24]
Bubbling air assisted method	Sublimed sulfur powder, NaOH, PEG-400	70	5	8	[22]
H_2O_2etching with PSS Method	Sublimed sulfur powder, NaOH, PSS, H_2O_2	70	12	5.13	[10]
Oxygen assisted PEG-200 method	Sublimed sulfur powder, NaOH, PEG-200	90	10	17.6	[25]
Mechanochemical method	Sodium thiosulfate, NaOH, PEG-400	Nil	1	4.8	[26]
Solvothermal method	Sublimed sulfur powder, NaOH, β-cyclodextrin	180	4	14	[27]
One-pot solvothermal method	Sublimed sulfur powder, S-ethylenediamine, ethanol	170	5	87.88	[28]
Microwave-assisted method	Sublimed sulfur powder, NaOH, PEG-400	70	1.5	4.3	[29]
Sodium hypochlorite etching method	Sublimed sulfur powder, PEG-400, NaClO	180	12	2.9	[30]
One-pot hydrothermal with H_2O_2 assisted etching Method	Sublimed sulfur powder, NaOH, PEG-400, H_2O_2	180	sonicated for 20 min, 4 h	6.3 Green &Blue 8.1	[31]
Oxygen assisted HPG method	Sublimed sulfur powder, NaOH, hyperbrancher polyglycerol (HPG)	95	24	6.8	[32]
Ultrasonicated assisted H_2O_2 etching method	Sublimed sulfur powder, ethanol , PEG-400, H_2O_2	220	36	7.04	[33]
H_2O_2 etching method	Tioacetamide, carboxy methyl cellulose (CMC), H_2O_2	100	4	9.3	[34]
H_2O_2etching with L-cysteine Method	Sublimed sulfur powder, NaOH, L-cysteine, H_2O_2	70	108	13.87	[35]
One-pot solvothermal method	Sublimed sulfur powder, S-ethylenediamine, ethanol	180	6	79.8	[36]

Surface etching method	Sublimed sulfur powder Polyethyleneimine (PEI), NaOH	75	120	5	[37]
H_2O_2etching with BSA method	Sublimed sulfur powder, bovine serum protein (BSA)NaOH	70	12	5.74	[38]
H_2O_2etching with COS method	Sublimed sulfur powder, chitosan oligosaccharide (COS), KOH	70	5	8.45	[39]
Ozone-assisted method	Sublimed sulfur powder, NaOH , PEG-400, under ozone atmosphere	90	4	9.26	[40]
Sonication assisted method	Sublimed sulfur powder, NaOH , oleic acid	190	12	10.4	[41]
One-pot solvothermal method	Sublimed sulfur powder, NaOH , sucrose, H_2O_2	220	24	21.5	[42]
Hydrothermal method	Sublimed sulfur powder, *p*-phenylenediamine, tetraethylene glycol (TEG)	200	24		[43]
H_2O_2 etching method for blue SQDs	Sublimed sulfur powder, 1,2 ethylenediamine (EDTA), ethanol, H_2O_2	70	6	14.22	[45]
H_2O_2 etching method for cyan SQDs	Sublimed sulfur powder, 1,3 diaminopropane (PDA), ethanol, H_2O_2	70	24	13.89	[45]
H_2O_2 etching method for green SQDs	Sublimed sulfur powder, 1,4 butanediamine (BDA), ethanol, H_2O_2	70	24	1.87	[45]
Oxygen assisted one-pot hydrothermal method	Sublimed sulfur powder, NaOH, rhodamine-B, sodium heparin and oxygen atmosphere	70	24		[44]

Characterization of Sulfur Quantum Dots: Characterization of any products is essential for better understanding about the mechanism behind the growth dynamics, optical and photoluminescence property and the applications. The size of monodispersed SQDs depend upon applied reaction condition, reaction time and extent of etching agent. The particles size found 1.6 nm to less than 10 nm from the different synthesis method of SQDs. The high resolution TEM images recorded SQDs that the spacing between two lattice fringes is 2.16 A^0 which is different from (206) planes of orthorhombic S_8 phase. The XRD pattern showed different diffraction peaks from orthorhombic S_8 phase according to JCPDS file no. 83-2285 due to synthesized SQDs amorphous in nature [8]. After increasing the concentration of H_2O_2, particle size decreased from 6.5 to 3.5 nm and found quasi-spherical shape. HRTEM images showed clear lattice fringes of 0.23 nm which is different from (206) planes of orthorhombic S_8 phase [2]. However, the SQDs are in an amorphous state and their crystallinity can be improved by increasing the reaction temperature or pressure. The XRD pattern of SQDs matched well with that of an orthorhombic S_8 phase and the SQDs produced from hydrothermal and microwave-assisted methods gave more sharp peaks [47]. The chemical compositions and structure of the SQDs were also investigated by XPS. The XPS spectrum mainly composed C, O, and S elements. The XPS S2p peaks in the range of 161-165.5 eV usually the evidence of the presence of atomic sulfur [2, 7, 8, 14, 15, 17, 18, 20]. Moreover, the binding energy peaks more than 166 eV showed the presence of oxidized sulfur species. For example, the binding energy at 167.9 eV, 168.9 eV and 170.0 eV which represented respectively SO_2^- ($2p^{2/3}$), SO_2^- ($2p^{1/2}$) or SO_3^- ($2p^{2/3}$), and SO_3^- ($2p^{1/2}$) [7]. The binding energies at 167.45, 168.3 and 169.2 eV were attributed to the SO_2^- ($2p^{2/3}$), SO_2^- ($2p^{1/2}$) or SO_3^- ($2p^{2/3}$), and SO_3 ($2p^{1/2}$) respectively [8]. The binding energy peaks at 166.5, 167.4 and 170.1 eV can be assigned to SO_2^- ($2p^{2/3}$), SO_2^- ($2p^{1/2}$) or SO_3^- ($2p^{2/3}$), and SO_3^- ($2p^{1/2}$) respectively [2]. The Raman Spectrum for SQDs respectively showed three bands, the broad peaks appearing between 520 and 650 cm^{-1} for polysulfide, 800 cm^{-1} for C-O stretching vibration and 1100 cm^{-1} for S-O vibration [15]. In most cases PEG is used as passivating agent for the synthesis of SQDs for the getting of efficient stabilizing effect FTIR spectrum of SQDs can be employed to surface groups of SQDs. In the FTIR spectrum of PEG matched well with the molecular peak of PEG and indicated that there is no chemical interaction between PEG and SQDs. The characteristic peaks at 2870, 1452, 1097 and 946 cm^{-1} corresponding to the stretching vibration of C—H, bending vibration

of C-H, stretching vibration of C-O and O-H, respectively [48]. The SQDs showed so far characteristic light absorption in the ultra-violet region of 200-400 nm range. At first, SQDs reported 335 nm absorption peak at the UV/Vis absorption spectra due to the direct band gap transition [7]. Two absorption peak at 222 nm and 370 nm found when SQDs prepared by assembly-fission method and ascribed by authors that two peaks to the n→σ* transition and the existence of S_2^{2-} and S_8^{2-} species respectively [8]. SQDs prepared under pure O2 atmosphere showed two absorption peak at 216 nm and 334 nm, authors ascribed to the n→σ* transition of nonbonding electrons of S atoms and direct band gap transition of atomic sulfur. After increasing the reaction, two absorption peaks gradually blue-shifted to 213 nm to 329 nm respectively, possible caused by the effect of quantum confinement [17]. An intensive peak 220 nm was observed for n→σ* transitions during preparation of SQDs by adding H2O2 [2]. Two absorption peak 331 nm and 360 nm obtained for the preparation of SQDs by hydrothermal method [14]. The UV/vis absorption spectrum of SQDs showed a shoulder peak at 212 nm and a broad absorption band centered at about 340 nm, which is ascribed by the authors to the n→σ* transition of nonbonded electrons of s atoms and the direct band gap transition of zerovalent sulfur respectively [28].

Photoluminescence Property: Sulfur quantum dots exhibit photoluminescence property like quantum dots and this is related to the energy band gap between sulfur and sulfur quantum dots. Element sulfur band gap of 2.79 eV displays a characteristic semiconductor behavior resulting in a weak and broad photoluminescence emission peak around 500 nm. The emission results from the band to band radiative recombination of electrons (e^-) and holes (h^+). A blue emission was observed when the elemental sulfur converted sulfur quantum dots, attributed to quantum confinement effects. This effect is assumed due to the bandgap shifting from 2.79 eV to 3.7 eV respectively for sulfur and SQDs [49]. Firstly, inventor of SQDs found 428nm emission against 352 nm excitation and it was excitation dependent photoluminescence [7]. Later, researchers reported that photoluminescent intensity and excitation-dependent PL and excitation-independent PL depend on the reaction time and particle size distribution. By increasing reaction time, increase PL intensity and found excitation-independent emission. At 100 h, they found excitation-dependent PL and 125 h found excitation-independent PL [8]. During the preparation of SQDs under O_2 atmosphere, excitation-dependent emission found. The emission peaks tuned from 424 to 542 nm by adjusting the excitation wavelength from 320 to 460 nm and the maximum peak appeared 490 nm with excitation of 400 nm [17]. Both blue and green emissive SQDs showed an excitation-dependent emission, with the peak shifting towards 430 to 520 nm when the excitation wavelength from 340 to 440 nm. The photoluminescent quantum yield of SQDs strongly depend on the increasing heating time and etching agent concentration and the passivation agent. This could be attributed to more effective etching/passivation of surface polysulfide species, which reduced the nonradiative recombination rate via surface states and favored the nonradiative recombination channel [2]. An excitation-independent strong and high quantum yield of SQDs exhibited 423 nm emission wavelength although excitation wavelength changed from 300 to 370 nm. The strongest emission band is observed at 340 nm excitation [28]. Green and blue fluorescence found at emission wavelength at 520 nm and 420 nm upon excitation at 370 nm and 350 nm for Green-SQDs and Blue-SQDs respectively [31]. Recently, excitation-independent emission SQDs prepared by using PEI as passivator, at 120 h reaction and maximum excitation is around 310 nm [37]. Above all these are green or blue fluorescence, synthesis of SQDs with strong long wavelength emission like red or near infrared emission until now unavailable.

Antimicrobial Activity: Sulfur nano particles show the antimicrobial efficacy against both the conventionally sulfur resistant and sulfur susceptible microbial pathogens for fungi and bacteria [3]. There are several theories for the mechanism of the antimicrobial activity of nanosulfur: a) the destruction of metabolic processes of bacterial cell wall by the interaction of nanosulfur with target molecules on bacteria; b) the degradation of cellular components by the sulfur ions and H_2S which are produced from the decomposition of nanosulfur inside the cell; c) cell death caused by the interfering nanosulfur in DNA replication; d) the SH-enzymes present in bacterial

cell wall, which is responsible for normal intermediary metabolism of carbohydrates, fats and proteins blocked by nanosulfur and leading to cell death; e) reactive oxygen species produced by nanosulfur which degrade the bacterial cell wall [49]. Sulfur-based nano particles sulfur quantum dots is also expected to show same antibacterial activities.

Sulfur quantum dots synthesized from PEG-coated hydrothermal method exhibit strong antibacterial activity against food borne pathogenic bacteria *Listeria monocytogenes* and *Escherichia coli*. Research found MIC/MBC 256/512 µg/mL and 32/64 µg /mL respectively for *E. coli* and *L. monocytogenes*. They showed no cytotoxicity effect to L929 mouse fibroblasts up to 1000 µg/mL [9]. The negatively charged sulfur quantum dots was prepared by using poly(sodium 4-styrenesulfonate) (PSS) as a capping agent exhibit antimicrobial activity against Gram-positive Methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They found that excellent antimicrobial activity of SQDs against both test bacteria with a MIC of 1.2 mg/mL. In addition, the PSS-SQDs show a broad-spectrum antibacterial activity against other pathogenic Gram-positive and Gram-negative bacteria, including *Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia* and *Acinetobacter baumanni*. Futhermore, this agent exhibit strong bactericidal acivity than clinically used antibiotics, vancomycin for *Staphylococcus aureus* and piperacillin/tazobactam for *Pseudomonas aeruginosa*. The negatively charged PSS-SQDs have excellent hemocompatibility and low toxicity, which indicates as a potential biocompatible antibacterial agent [10].

Alginated film base-SQDs showed antibacterial and antifungal activity for food packaging purpose. Alginate/SQDs ehihibited bactericidal effect against *L. monocytogenes* completely stopped the growth after 9 hours exposure and reduction of *E. coli* after 12 hours exposure showing 2 Log CFU/mL. The antifungal activity of the Alginate/SQDs film exhibited against both *A. niger*and *P. chrysogenum* strains, showing inhibition regions of 14 mm to 18 mm. Authors told the mechanism action of antibacterial and antifungidial activity by a) the ROS produced by SQDs, formation of free radical such as hydroxyl free radicals (OH•), and superoxide anions ($\bullet O^{2-}$); b) smaller size SQDs easily penetrate the microbial cell wall; c) the PEG functionalized surfaces, interacting their surface functional groups with the biological surfaces. The alginated-SQDs film for food packaging to prevent the mold contamination of the bread even after storage of 14 days. So, packaging films with added SQDs can be used in active packaging applications, specially products highly susceptible to fungal contamination [11].

Shivalkar *et al.* first time explored the light driven antibacterial activity of water soluble SQDs. These SQDs exhibited excellent antibacterial activity for *E. coli* bacteria by generating ROS under sunlight or visible light. The concentration of SQDs 1.13 mg/mL showed >90% inhibition of bacteria under 6 hours sunlight irradiation. Researchers also found 42.93% inhibition of *Bacillus subtalis* bacteria at 0.068 mg/mL dose. SQDs applied to polycotton for the observation of inhibition of *E. coli* and found satisfactory result at 20 mg/mL of 10 µL [13].

A comparative antimicrobial activity of the sulfur based particles against foodborne pathogenic bacteria and fungi carried out and found that SQDs exhibited more strong antimicrobial activity than elemental sulfur and sulfur nanoparticles. The SQDs exposure eradicated most conidia and degradaded mycelial structure for both fungal strains [50]. By adding sulfur quantum dots into chitosan a multifunctional coating solution was prepared. This film exhibited good antibacterial agent against *E. coli* and *L. monocytogenes*. In addition, both *E. coli* and *L. monocytogenes* were completely eradicated by using 3% SQDs with chitosan by their synergistic effect. The functional coating solution was applied on the surface of enoki mushrooms, which totally prevented the growth of *L. monocytogenes* to secure the mushroom's safety. The coating solution of SQD and chitosan can solve *Listeria* outbreaks of enorki mushroom [12]. Sulfur quantum dots prepared by using polyvinyl alcohol(PVA) showed a promising antibacterial activity against Gram-positive and Gram-negative pathogens and obtained IC_{50} value was 2136 µg/mL for this activity. The Gram-positive strains *L. monocytogenes* and *B. cereus* exhibited similar MIC/MBC value of 566/1132 µg/mL, but *S. aureus* showed 1132/2256 µg/mL. On the other hand, the Gram-negative bacteria *E.*

coli, S. aeruginosa showed higher concentration MIC/MBC at 566/1132 µg/mL but *S. enterica* showed MIC/MIB at 2265/4531 µg/mL. The cell viability profile showed that the percentage of cell viability was 100% up to 500 µg/mL, and at 1000 µg/mL, the cell viability was reduced to less than 90% and further reduced to ~ 60% at 2000 µg/mL [51]. SQDs prepared by using EDA exhibited antibacterial activity against *E. coli, S. enterica, B. cereus*, and *L. monocytogenes*, where the MIC/MBC are respectively 1200/2500 µg/mL, 1200/2500 µg/mL, 75/150 µg/mL and 50/75 µg/mL. This prepared SQDs were non-toxic for human contact and biological applications up to an effective concentration 500 µg/mL with an IC_{50} value of 741.1 µg/mL [36]. Sulfur nanoparticles act against acne vulgaris causing multidrug resistant bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* and better activity than conventional antibiotics for acne vulgaris [52]. So, there is an opportunity to research for the preparation of external application for the acne eradicating by using SQDs.

Functionalized SQDs were prepared by using ligands synthesized with different head groups containing different charges. There was high antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* by using positively charged SQDs with a very low concentration of 10-25 ng/mL[43].

TABLE 2: ANTIMICROBIAL ACTIVITY OVERVIEW OF SQDS

Types of SQDs	Susceptible Microorganisms	MIC/MBC or Zone of Inhibition	References
Gelatin/Agar based-SQDs Film	*Listeria monocytogenes*	256/512 µg/mL	[9]
	Escherichia coli	32/64 µg/mL	
PSS-SQDs	*Staphylococcus aureus*	1.2 mg/mL	[10]
	Pseudomonas aeruginosa	1.2 mg/mL	
	Listeria monocytogenes	2LogCFU/mL	[11]
	Escherichia coli	2LogCFU/mL	
Alginated-SQDs Film	*A. niger*	12 mm	
	P. chrysogenum	18 mm	
Light mediated-SQDs	*Escherichia coli*	1.13 mg/mL	[13]
	Bacillus subtalis	0.068 mg/mL	
PVA-SQDs	*L. monocytogenes*	566/1132 µg/mL	[51]
	B. cereus	566/1132 µg/mL	
	S. aureus	1132/2256 µg/m	
	E. coli	566/1132 µg/mL	
	S. aeruginosa	566/1132 µg/mL	
	Salmonella enterica	2265/4531 µg/mL	
EDA-SQDs	*Escherichia coli*	1200/2500 µg/mL	[36]
	Salmonella enterica	1200/2500 µg/mL	
	B. cereus	75/150 µg/mL	
	Listeria monocytogenes	50/75 µg/mL	
	Staphylococcus aureus	>5/5µg/mL	[43]
TEG-SQDs	Enterococcus faecalis	>5/5µg/mL	
Positive-SQDs	*Staphylococcus aureus*	25/25 ng/mL	
	Enterococcus faecalis	10/80 ng/mL	
Negative-SQDs	*Staphylococcus aureus*	50/50 ng/mL	
	Enterococcus faecalis	60/100 ng/mL	
Neutral-SQds	*Staphylococcus aureus*	125/225 ng/mL	
	Enterococcus faecalis	140/140 ng/mL	

Antioxidant Activity: Ruchir Priyadarshi *et al.* at first reported the antioxidant activity of SQDs. The concentration dependent 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of SQDs compared to the widely used antioxidant ascorbic acid . At a concentration of 75 µg/mL of ascorbic acid , the antioxidant activity was 100% against ABTS , whereas the activity was ~ 90% against DPPH. On the other hand, SQDs exhibited 100% elimination of ABTS free radicals at a concentration of 25 µg/mL, while showed ~ 65% antioxidant activity against DPPH at a concentration of 75 µg/mL. So,

the antioxidant activity of SQDs are weaker than ascorbic acid against DPPH [9]. A proposed reason for this is that the high hydrophilicity of SQDs makes them more active in aqueous ABTS than in methanol solution of DPPH. Furthermore, ascorbic acid is soluble in both aqueous and polar organic solvents. In spite of SQDs exhibited a threefold higher free-radical scavenging activity than ascorbic acid in aqueous system and showed a high potential for using as antioxidants in biological systems [49]. Alginate-based SQDs showed antioxidant activity against ABTS and DPPH. The free radical scavenging effects of ~ 50% and ~100% respectively against DPPH and ABTS. It has been proposed that free radical scavenging activity of SQDs is due to the presence of surface hydroxyl groups conferred by PEG capping. The •H transfer from the hydroxyl groups to the ABTS• and DPPH• radicals produced in quenched forms of ABTS-H and DPHH-H. Alginate/SQDs film is expected to prevent oxidative degradation of packaged foods due to its high antioxidant activity [11].

The chitosan film containing SQDs exhibited strong antioxidant activity against ABTS and DPPH. Only chitosan film showed free radical scavenging activities of 29.2% and 5.5% respectively for ABTS and DPPH. By adding SQDs 3 wt. % with chitosan film, exhibited the highest antioxidant activity of free radical scavenging of ABTS and DPHH of 78.6% and 20.5% respectively. On the other hand, the hydrophilic nature of SQDs and increased contact with free radicals explain the higher antioxidant activity values for ABTS free radicals compared to DPPH. This film showed adequate antioxidant activity and were considered to extend the self-life of food by potentially preventing oxidative degradation of packaged/coated foods [12]. Recently, the antioxidant activity of the SQDs was studied against ABTS and DPPH simulating aqueous and organic media. At a concentration of 75 µg/mL of SQDs fully scavenged ABTS free radicals, whereas 100 µg/mL could scavenge~ 66 % DPPH free radicals. However, the antioxidant potential of SQDs are similar to that of ascorbic acid. The outstanding ability of SQDs to scavenge oxidative free radicals in aqueous media, a component of biological systems, is considered as potential antioxidant activity of SQDs in biological and related systems [36].

CONCLUSION: In recent years, many synthesis method with high photoluminscent quantum yield and time consuming were developed for the preparation of SQDs. Mainly SQDs were prepared from sublimed sulfur, which had a sustainable impact for solving unused sulfur from petroleum industry. It is a unique quantum dots have better water solubility, stability, but low toxicity unlike others quantum dots. For the reason of antibacterial and antifungal and antioxidant activity, it could be used as pharmaceutical sector and food industry. In the food packaging system, SQDs might be used as efficient agent with polymers for the protection of microorganisms and enhanced the shelf-life. However, there is no sufficient study about the antimicrobial test by using SQDs. So, there is a great opportunity to study antimicrobial activity especially antifungal study against acne vulgaris, candida species for the preparation of external application of SQDs. During COVID-19 period there was a huge demand of efficient, non-resistant disinfectant for fabric; SQDs could be potential agent but more study require for this purpose.

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