Nanotechnology and Nanoscience
ISSN: 0976-7630 & E-ISSN: 0976-7649, Volume 3, Issue 1, 2012, pp.-55-58.
Available online at http://www.bioinfo.in/contents.php?id=89

ASSESSMENT OF ANTIFUNGAL ACTIVITY OF MICRONIZED AND NANOSIZED ELEMENTAL SULFUR

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Received: July 30, 2012; Accepted: August 07, 2012

Abstract- Investigations of antifungal activity of micron and nanoscale forms of sulfur on ten pathogenic fungi have been studied. As micronized sulfur used crushed in a roller mill, and as nanosized sulfur precipitated from a solution of sodium polysulfide. The size and shape of the particles of sulfur were characterized using a laser analyzer and probe microscope, the structure by X-ray diffractometer. Biological (antifungal) effects of sulfur particles in Sabouraud medium has been studied. It is found that in all cases, antifungal activity of nanosized sulfur with an average particle size of 25 nm, 4-9 times higher than the sulfur micron with an average particle size of 8 microns. The results can be used to create more effective than conventional sulfur ointment formulations.
Keywords- Sulfur, nanoparticles, dispersion, fungicide.

Citation: Massalimov I.A., et al. (2012) Assessment of Antifungal Activity of Micronized and Nanosized Elemental Sulfur. Nanotechnology and Nanoscience, ISSN: 0976-7630 & E-ISSN: 0976-7649, Volume 3, Issue 1, pp.-55-58.

Introduction

At the moment a more extensive application of nanoparticles and nanomaterials in various areas of human activity is observed. Medicine also is not an exception. Introduction of nanotechnologies is considered to be able to dramatically change the situation connected with use of medicines containing nanosized active components [1]. The idea of using nanosized medicines is based on the fact that nanosize substances possess properties, different from those of macrodispersed substances. Particularly, large specific surface area of nanomaterials result in that surface phenomena (absorption -desorption, adhesion) begin to play the main role in their reactions with macro substances, macromolecules and biological objects. Consequently, biological activity effectiveness of traditional preparative forms increases; even small concentrations of nanoparticles without any significant toxic effect are able to produce high effect on live organisms.

Sulfur is one of the substances whose biocide properties (including antibacterial, antifungal and protocidal) have been indeed recognized for a long time. Presently, sulfur is a component of numerous cosmetics, for example, soap, crèmes, ointments, lotions. Sulfur ointment and other ointments, containing sulfur, sulfur-salicylic ointment, sulfur-tar ointment, sulfodekortem, Wilkinson ointment and others are used for treatment of various skin diseases of parasitic, mycotic, allergic and other nature-scabies, mycosis (keratomikosis, dermatophytes, etc.), psoriasis, seborrheic dermatitis and several others [2]. It is considered one of the safest medicines for treatment of the above mentioned diseases, seldom causing minor skin dryness. At the same time such antiexudative effect is an important component of anti-inflammatory therapeutic effect of sulfur-containing dermatological medicines. Medical drugs, based on elemental sulfur, are well demanded nowadays due to there high efficacy, absence of long-term effects and low cost.

Nanotechnology progress aroused additional interest to the problem of obtaining sulfur nanoparticles by a method allowing its further practical application. Progress is evident in this direction, though there are few works dedicated to obtaining sulfur nanoparticles in water medium and microemulsion [3-6]. There are even fewer reports on examining biological properties of sulfur nanoparticles. Only authors in [3] of the aforementioned papers [3-6] present investigation of sulfur nanoparticle microbicidal properties. In [3] it has been experimentally determined that applying nanoparticles of average particle size 10-15 nm on bacteria, yeast and microscopic fungi strains is more effective than those of 80-100 nm. Thus, size difference significantly changes biological activity. As it is relevant for many other prospective drugs, studies directed at effectiveness increase of drugs, based on highly dispersed sulfur are urgent nowadays, including investigations of biological properties of sulfur nanoforms. An interesting comparison is that of biological activity of nano and micronized sulfur particles, which are generally used as components of well-known sulfur ointments. One of the works, which compared the biological properties of micron-and nanosize sulfur is article [7]. It was experimentally established in work [7] dedicated to evaluating effect of sulfur nanoparticles on wheat spouting, that growth of shoots and roots was significantly promot-

ed by applying 20-25 nm sulfur nanoparticles. Simultaneously 8 μm average size sulfur particles did not prove effective in stimulating growth of plants.

Since different dosage forms of drugs often have side effects, including toxic, allergic and other undesirable effects, very important problem is to reduce active substance concentration without lowering its efficacy. Therefore this paper presents comparison of fungicide properties of different micronized sulfur concentrations, obtained by grinding in roller mill, and nanoparticles obtained by precipitation from sodium polysulfide. In both cases we have searched for such sulfur concentrations at which fungi growth could be completely prevented in nutrient medium for fungi cultivation (Sabouraud's dense medium) with simultaneous fungal growth in test samples of nutrient medium with no sulfur added.

Activity of both sulfur types (micro- and nanosize) was assessed towards three kinds of microorganism colonies, which contained ten clinical fungi strains. Two species of conditionally pathogenic mould fungi (Penicillium notatum and Aspergillus niger) were selected. Penicillium notatum are highly allergic and cause such diseases as bronchial asthma, rhinitis, atopic dermatitis. Aspergillus niger, causing a disease called black mold on certain fruits and vegetables, such as grapes, onions, pea-nuts. It can also cause lung disease, aspergillosis, otomycosis (fungal ear infection) with humans. The second type of colonies widely used yeast-like fungi - Candida albicans, causing fungous diseases of skin, mucosae, nails, internal organs have also undergone our examination. Along with the above pathogenic fungi have been studied seven types of clinical strains of fungi of the class dermatophytes: Trichophyton mentagrophytes var. gypseum (seu granulosum) strain 182, Trichophyton mentagrophytes var. gypseum (seu granulosum) strain 430, Trichophyton verrucosum strain 154, Trichophyton anthroponotic pathogen, Microsporum canis strain 4220, Trichophyton mentagrophytes var. interdigitale strain 214. Dermatophytes - a group of filamentous fungi affecting dead skin cells keratinocytes (epidermal corneal layer, nails and hair) was the first species of clinical fungi strains. The study of dermatophytes especially important for Russia, because this disease suffer from millions of inhabitants, the incidence continues to rise, and the medicamentous treatment of many forms of this infection is still not perfect.

Materials and Methods

For carrying out the research we used the following reagents of «chemically pure» qualification were used: elemental sulfur S, ground in a roller mill, sodium hydrate (NaOH), ethyl alcohol (C_2H_5OH) and hydrochloric acid (HCl). Also «chemically pure substance, citric and ant acids were used. All the chemicals were used as those were received without any further purification. For preparation of a nutrient medium the white powder an agar an agar which represented the natural product taken from a White sea alga of Ahnfeltia was used. In work the strains of fungi cultivated by Research Technological Institute of Herbicides and Plant Growth Regulators of the Academy of Sciences Bashkortostan Republic were used.

As micronized sulfur we used the elemental sulfur crushed in a industrial roller mill. Particle size distribution of sulfur powder particles was determined by Shimadzu SALD - 7101 laser particle size analyzer. We determined size and shape of sulfur particles using Solver Pro M Scanning Probe Microscope also. The analysis of structural characteristics of samples was carried out on x-ray diffractometer by Rigaku Ultima IV.

Results

Obtaining Sulfur Samples in Micron and Nano Ranges

Integral and differential particle size distributions of sulfur particles received after crushing in a roller mill is presented on Fig. 1 by circles painted in black color (●). From data presented on Fig. 1 it is visible that distribution of particles in this case lies in the range from 2 microns to 50 microns, and the average size is equal 8 microns.

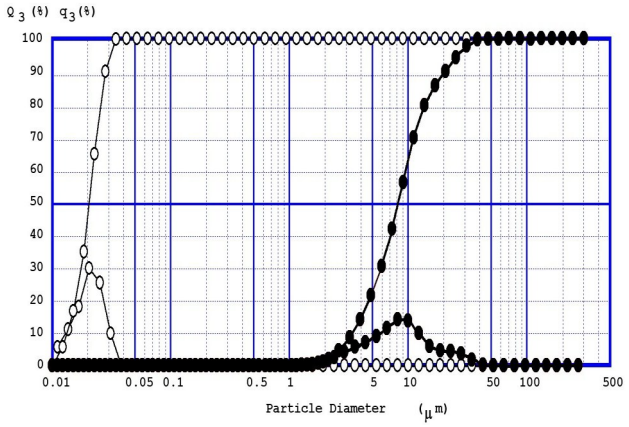

Fig. 1- Integral and differential particle size distributions of sulfur particles obtained: (○) - from sodium polysulfide, average particle size is 25 nm; ● - after grinding in roller mill, average particle size is 8 micron.

For obtaining of sulfur nanoparticles the method of chemical sedimentation from solution of sodium polysulfide in detail described in article [8] was used. Sodium polysulfide in aqueous solution was obtained by reacting finely-ground elemental sulfur with barium hydroxide water solution. Transparent cherry red sodium polysulfide aqueous solution was obtained with density of 1.355 g/cm^3. At the next stage sulfur powder was isolated from it by gradually adding 10% hydrochloric acid to sodium polysulfide solution until complete sulfur precipitation. Precipitated sulfur formed a pale yellow flocculation. Obtained thus powder was first washed with plenty of water to remove sodium chloride contained in aqueous solution, and secondly - with ethyl alcohol to remove water remainders. The powder was then stored under ethyl alcohol layer.

Integral and differential distribution of sulfur particle distribution in aqueous medium was measured. Fig. 1 shows particle size distribution for sulfur nanoparticles by not painted over circles (o). As seen from Fig. 1 average size of the particles ground in a roller mill is 8 μm, while that of the particles obtained by chemical precipitation is 25 nm.

Along with measuring particle size distribution using laser particle size analyzer, we determined size and shape of sulfur particles using Solver Pro M Scanning Probe Microscope see Fig. 2b. From data presented on Fig. 2b it is visible that sulfur nanoparticles are of spherical shape. By using of the special program of processing of images it was established that sizes ranging from 10 to 50 nm, and the average size of particles is equal 25 nanometers show Fig. 2a just as in Fig. 1.

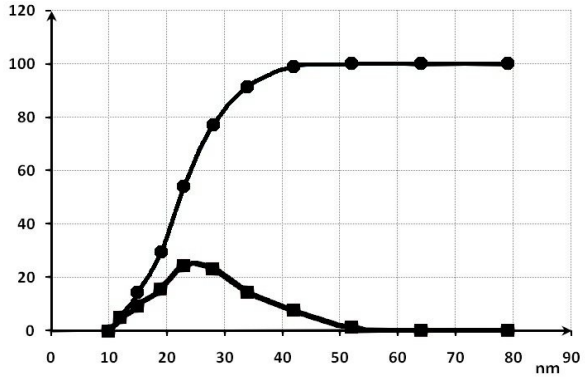

Fig. 2a- Particle size distribution of sulfur particles (a) obtained by using powder, isolated through mixing sodium polysulfide and chloride acid

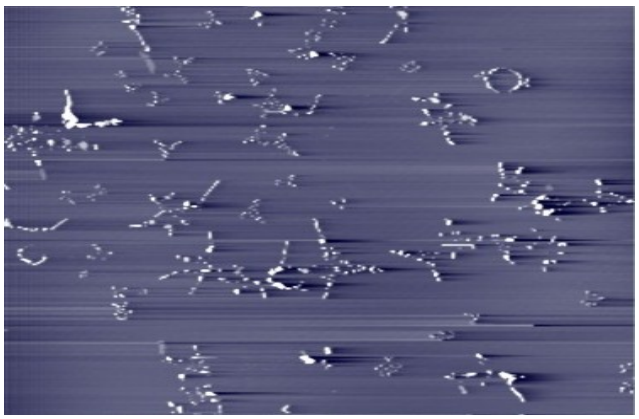

Fig. 2b- Particle size distribution of sulfur particles probe-microscope image of sulfur nanoparticles

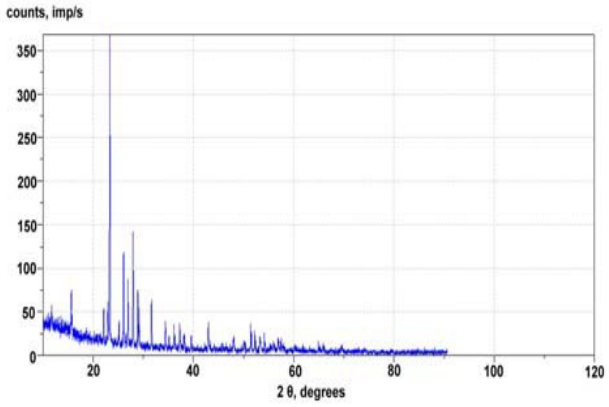

Fig. 3- X-ray pattern of sulfur powder separated from sodium polysulfide within 50 nm size range in Fig. 1.

Sulfur in the form of bulk material and micron powders is known to possess orthorhombic structure. At the same time, nanoparticle formation is known to induce formation of structural states uncharacteristic of bulk materials. Therefore, structure of powder particles was examined by X-ray diffraction along with measuring curves of particle size distribution. As a result in Fig. 3, it was determined that sulfur nanoparticles possess orthorhombic structure as well, which agrees with carried out earlier our research work data [8] and with data of other researchers [3,6].

Antifungal Activity

Antifungal activity investigations of medical preparations containing sulfur nanoparticles and sulfur, which was ground in roller mill, were carried out in relation to complete delay of growth in fungal test-cultures at inoculation on jell (agar) Sabouraud medium, containing respective preparations obtained according to [9,10]. To achieve this 0.5 ml of dispersion composed of preparation powder and ethyl alcohol (40 g of powder to 40 ml of ethyl alcohol) was added to the test tubes with melted and cooled (up to 50°C) Sabouraud agar medium up to medium final concentrations of 1-500 mg/ml. The mixture was stirred then until a homogeneous state and cooled to room temperature. After gel formation fungal test cultures, pre-incubated in a thermostat at 28° C for 30 days, were inoculated onto the medium containing medicinal preparations and to test samples (preparation free, with 0.5 ml ethyl alcohol added).

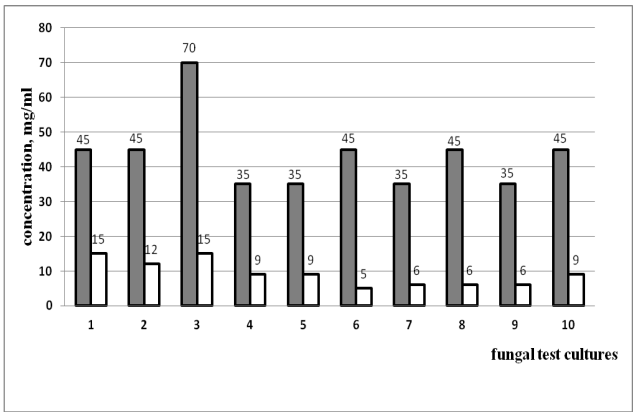

Fig. 4- Concentrations of preparations, containing micronized particles (■) and nanoparticles (□), leading to a full growth inhibition of the following fungi: 1- Penicillium notatum; 2**-** Aspergillus niger; 3**-** Candida albicans; 4- Trichophyton mentagrophytes var.gypseum (seu granulosum) strain 182; 5- Trichophyton mentagrophytes var.gypseum (seu granulosum) strain 430; 6- Trichophyton verrucosum strain 154; 7- Trichophyton anthroponotic pathogen; 8- Trichophyton mentagrophytes var. interdigitale strain 214; 9**-** Trichophyton rubrum strain 212; 10**-** Microsporum canis strain 4220.

As mentioned above for the study of the antifungal action of micronized and nanosized elemental sulfur were used three types of known species of pathogenic fungi: dermatophytes (7 species of fungi), mould fungi (Penicillium notatum, Aspergillus Niger) and yeast-like fungi Candida a1bicans.

The results were registered in relation to colonization absence in fungi test cultures and fungal growth detection in test samples. Thus, yeast-like fungi were detected on 2nd-5th day, dermatophytes - on 5th -15th day, moulds - on 5th day.

The results presented on Fig. 4 confirm that sulfur is an effective and universal preparation having antifungal activity. Upon reaching a certain concentration of sulfur in the nutrient medium completely inhibited growth of all examined species of fungi in the work. The Fig. 4 also shows that for the complete suppression of growth in different species require different concentrations of sulfur. The results show high efficiency of sulfur in the nanosized state. The transition in nanoscale form leads to a decrease in sulfur concentration required for complete suppression of growth in the number

from three to nine times. For example, to suppress the most resistant to sulfur fungus Candida albicans required 70 mg / ml micronized sulfur, as in the case of nanoscale sulfur enough 15 mg / ml. When exposed to the mould fungi, the concentration required to inhibit the colony is reduced by 3-4 times. But the most effective nanoforms sulfur exhibits when exposed to dermatophytes, in this case the concentration of sulfur decreases by 4-9 times. Thus, the results obtained in this work indicate the universal and high efficiency of sulfur nanoparticles to combat fungal diseases.

Conclusions

Comparing antifungal potency of medical preparations composed of nano and micron size sulfur particles we have discovered that the efficacy of the former is in excess of the latter by a factor of 3-9 show Fig. 4 for all different types of studied fungus. Thereby achieved results are consistent with general concept of applying nanoparticles in modern technologies, wherein usage of dispersed substances in nanosize range fundamentally change many (physical, chemical, biological) of their properties. This fact allows at one time to gain such a biological effect for nanoparticles, which is not observed with micronized particles, at another - to considerably reduce (in some times) active substance effective dosage. The results achieved can be effectively used for preparation of sulfur containing ointments and medicines with sulfur nanoparticles as biology active substance.

Acknowledgements

This work was supported by grants № 12-03-97034 «The study of the sulfur nanoparticles formation and evaluation of their biological properties» from the fund by the Russian Foundation for Basic Research. We are also thankful to the Academy of Sciences Bashkortostan Republic for financial assistance in performing the research work.

References

[1] Kewal K. Jain (2008) *The Handbook of Nanomedicine*, 404.
[2] Mashkovsky M.D. (2002) *Pharmaceuticals Moscow*, Novaya Volna, 2(14), 608.
[3] Deshpande A.S., Khomane R.B., Vaidya B.K., Joshi R.M., Harle A.S. and Kulkarni B.D. (2008) *Nanoscale Res. Lett*., 3, 221-229.
[4] Guo Y., Zhao J., Yang S., Yu K., Wang Z. and Zhang H. (2006) *Powder Technology*, 162(2), 83-86.
[5] Xie X.E., Zheng W.J., Bai Y. and Liu J. (2009) *J. Mat. Lett*., 63, 1374-1376.
[6] Chaudhuri R.G. and Paria S. (2010) *J. of Colloid and Interface Science*, 343, 439-446.
[7] Abdrakipova L.F., Massalimov I.A. and Mustafin A.G. (2011) *Quarantine and Plant Protection*, 9, 33-35.
[8] Massalimov I.A., Mustafin A.G., Shangareeva A.R., Khusainov A.N. and Zaynitdinova R.M. (2012) *J. Chem. Chem. Eng*., 6, 233-241.
[9] Kashkin P.N. and Sheklakov N.D. (1978) *Guide to Medical Mycology. M. Medicine*, 325.
[10] Kashkin P.N. and Lisin V.V. (1983) *Practical Guide to Medical Mycology, M. Medicine*, 190.