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THE SiO_2/ZnO COMPOSITE MATERIALS FOR COSMETIC CREAMS

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Zinc oxide nanoparticles are known to have an effective antibacterial activity and silica based materials are widely used in many medical and cosmetic applications. Therefore, the SiO_2 composite materials already synthesized through different methods and by including ZnO, will print an antibacterial activity to the cosmetic creams. The aim of this paper is to obtain cosmetic creams for solar protection from these antimicrobial composite materials based on SiO_2/ ZnO. The composite materials were characterized by FTIR, SEM and by studying the antibacterial activity against *S. aureus.*

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1. Introduction

Recent studies in the field of nanotechnology, particularly nanoparticles have been received an important attention due to their unique physical and chemical properties [2], and because of their size and shape, they led to the development of new biocidal agents. Nano-materials are also called "a wonder of modern medicine" [1]. The past years, it has been discovered that the metal oxide nanomaterials are among the most studied products because of their wide applications such catalysis, sensors, environmental remediation, medicine, varistors, solar cells, rubber, concrete, foods, cosmetics and personal care products [2], but they have been studied also because of their exclusive antimicrobial, wound healing and anti-inflammatory properties [1].

Among the metal oxide nanoparticles, ZnO is interesting because it has excellent applications in various areas such as optical, piezoelectric, magnetic [1] and recently has been found new ways to include it in drug delivery, cosmetics (sunscreens), medical devices, dentistry and orthopedics [2]. Shaath [5] claims that the solar radiation contain certain amounts of UV-absorbing substance, so ZnO and TiO_2, microparticles named also sunscreen agents, can mask the skin in white color [7]. Silica is well-known for medical applications and it is one of the most used helpful agent in many cosmetic formulations because of its nontoxic, anti-caking, opacifying and emollient properties [3]. Silver and silver nanoparticles are recently used to enhance antibacterial properties of textiles materials [9], but also can be mentioned as silver based compounds designed for medical applications, silver based core/shell structures, Ag^+ substituted hydroxyapatite and Ag^+ complexes [4]; can be an example also SiO_2 /ZnO core-shell nanostructures or composite materials that were already synthesized for various applications such as catalysis, absorbent or photo-luminescence [3].

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The most common cosmetic products that protect against radiation are sunscreens. Sunscreens are complex formulations based on water in oil emulsion, containing mixture of organic/ inorganic and lipophilic /hydrophilic compounds. Before commercialization, sunscreens are submitted to rigorous assessments for effectiveness and human safety, in particular skin penetration, skin tolerance, cytotoxicity, toxicity, photo-stability and carcinogenity [6].

The FDA standard for SPF testing requires application of 2 mg/cm^3 to protect the skin. Sunscreen ingredients are generally divided into inorganic (physical blockers) and organic agents (chemical absorbers). Major inorganic agents used today are zinc oxide and titanium dioxide which are photo-stable and require thick application to achieve adequate reflection. Iron oxide, physical blocker is closer to natural skin color and often added to sunscreen mask the opacity of titanium and zinc oxide [21].

Therefore, also is developed the concept of incorporating an antimicrobial agent into dressing materials. So collagen, a wound healing matrix protein gained important clinical acceptance, being a safe material (Ramshaw et. al., 2009). Has been reported with success, that collagen scaffolds prints antimicrobial activity to tissue regeneration and has proven to be effective in wound treatment [8]. Collagen has been chosen due to its biocompatibility, high porosity, and facility to combine with other materials, low antigenicity, easy processing and absorbability in the body [10].

It has been shown that the nanoparticles are frequently found commercially in cosmetics and sunscreens (TiO_2 and ZnO) but unfortunately, toxicological studies made on ultrafine particles ($d<100nm$) possessed serious problems to the lungs [22].

Lately, it has been attracted an increasing interest on wound dressing materials but because don't have antimicrobial activity, needed to overcome this deficiency by developing a new way to synthesize and impregnate silver nanoparticles on bacterial cellulose; therefore, this Ag NP-BC will have intrinsic properties which will make it an attractive new wound dressing material [11].

So, it has been shown that the sunscreen filters, which block UV radiation are often applied in cosmetic products to protect the skin. And, however, formulations based on nanoparticles are delivery vehicle for screen compounds but also have advantages: this study showed that nanoparticles encapsulated in sunscreens can detain on the skin, don't penetrate the epidermal layer of the skin and attenuate UV radiation [18].

For a long time, scientists used antimicrobial drugs to inhibit and kill bacteria or other microbes but however they have been developed a specific microbial resistance over time. There have been made tests to overcome, reduce this growing problem, so one of the most promising strategy to get through this microbial resistance was to use nanoparticles [13].

In this perspective, it have been shown in previous tests that zinc oxide and titanium dioxide particles do not permeate the skin but the evidence of non-permeation was observed only by using tape stripping and electron microscopy. The investigations implied that the particles were detected only on the external surface of the skin (stratum corneum) [16].

Even if there have been made experimental and clinical studies about using vitamin E in new formulations such cosmetics and sun care products, there still a lack of enough indications if has a clinical benefit [17]. So, there are many research works that had studied skin tissue regeneration. In recent years, it has been used medicinal plants to induce regeneration of the skin. An example is aloe-vera, due to its therapeutic properties, such anti-inflammatory, antibacterial and the ability to improve the collagen to promote the skin repair [14].

S.aureus is the commonest cause of SSTI (skin and soft tissue infections) [25,26]. Methicillin-resistant *Staphylococcus aureus* (MRSA) (also called oxacillin-resistant *Staphylococcus aureus*) is a Gram (+) bacterium responsible for several difficult to treat skin infections. MRSA developed, through the process of natural selection, resistance to beta-lactam antibiotics (methicillin, dicloxacillin, nafcillin, oxacillin) and the cephalosporins. However, concern about the gradual development of resistance have turned attention to the development of new antibacterial agents active against Gram (+) bacteria. [27,28]

In this work, it was encapsulated nanocomposite materials based on SiO_2 / ZnO in collagen to produce new formulations aimed to protect the skin against UV radiation and regenerate skin. *In vitro* microbiology tests showed a good resistance against *Staphylococcus aureus* (*S.aureus*) of the

composites with zinc oxide nanoparticles/ silica, so were able to print an antimicrobial activity to the cosmetic products also.

The synthesis process and structure of Collagen/ SiO_2& ZnO were investigated by FT-IR, SEM and antibacterial tests. The antibacterial activity *S. aureus* was demonstrated by the Kirby-Bauer disk-diffusion method.

2. Materials and methods

Silicon dioxide was obtained starting from sodium silicate (Sigma Aldrich, reagent grade) and TEOS (Fluka, puriss; >99%). Zinc oxide was synthesis by precipitation starting from zinc acetate dehydrate (Sigma-Aldrich, ACS reagent). All other chemicals were reagent grade and were used without further purification [3].

The SiO_2/ ZnO composite materials were obtained according to Figure 1. Two synthesis routes can be identified, the first one (route A) can be assimilated with a sol-gel followed by alkaline precipitation while the second route (route B) consists into an acidic precipitation of induced by the addition of HCl 1M into the Zn^{2+} and sodium silicate precursors [3].

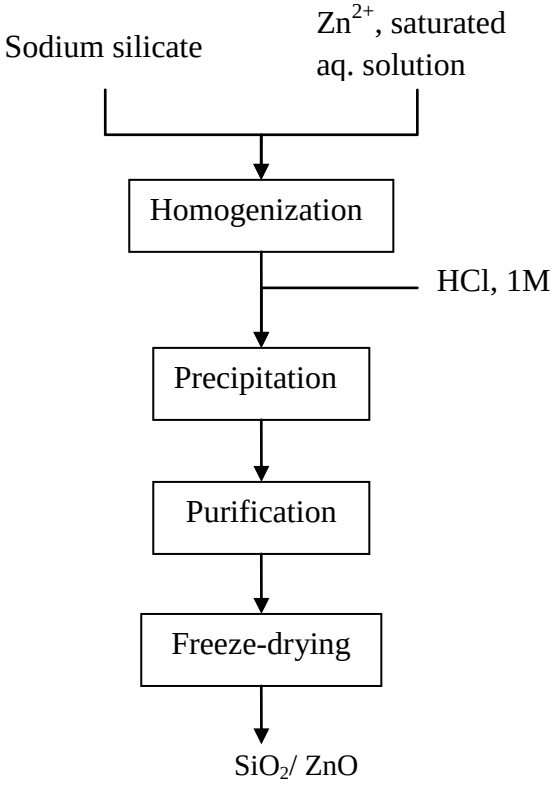

Figure 1. Synthesis of SiO_2/ ZnO composite materials - flow chart – route A [3].

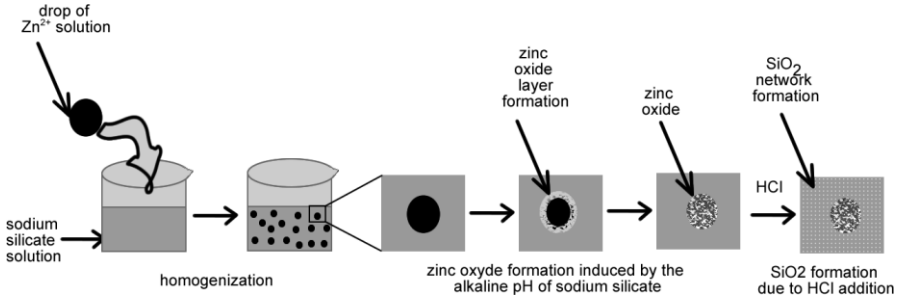

Fig. 2. Schematic representation of SiO_2/ ZnO composite material formation (route B)[3].

From the SiO_2/ ZnO composite materials obtained by precipitation was added ~0.25 g along with 10g of bovine collagen type I (2.42%) [32] and 13 mL water, mixed through rapid agitation. Final weighing was ≈25mL of the sample but for the synthesis was used only 15 mL. On the 15mL sample was added glutaraldehyde (GA) 200 μL (0.66%) and mixed untill it becomes homogeneous; samples were placed in Petri dishes and cooled for 24h then submitted to lyophilization process (Martin Christ Alpha 2-4 LSC).

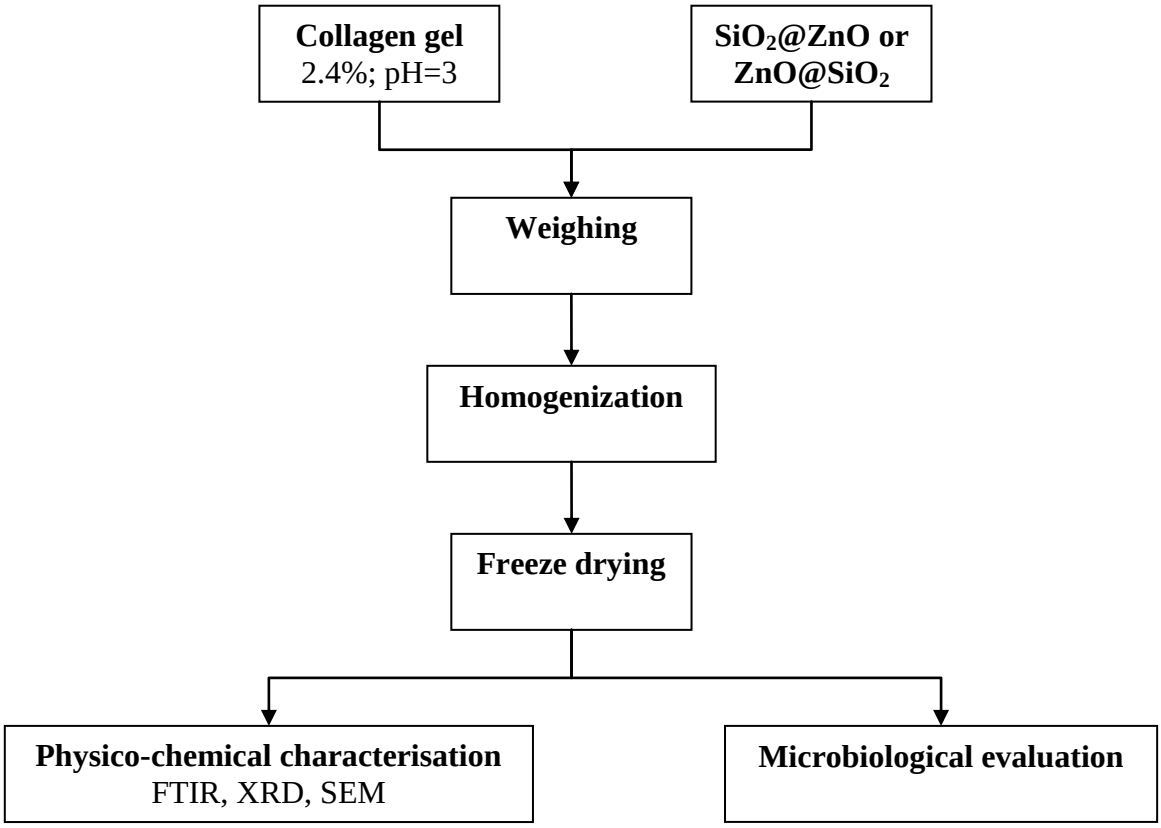

Fig. 3. Schematic representation of Collagen/ SiO_2&ZnO composite materials formation

The synthesized SiO_2/ ZnO composite materials were investigated by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) as well as by determining the, *in vitro,* antimicrobial activity against *S. aureus* [3].

Infrared spectroscopy (IR) measurements were performed on an iN10 MX mid infrared FT-IR microscope operated in transmission, reflection or Ge-ATR mode. The spectra were recorded over the wavenumber range of 400–4000 cm^{-1} by co-adding 32 scans with a resolution of 4 cm^{-1} on finely crushed samples [3].

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu K_α radiation from a Cu X-ray tube (run at 15mA and 30 kV) was used. The powdered samples were scanned in the Bragg angle, 2θ range of 10 – 70^o, with a sampling interval of 0.02^0. SEM images were recorded on a HITACHI S2600N instrument with an EDS probe. Before imaging, all samples were covered with a thin gold layer.

For antibacterial assays *S. aureus* ATCC 25923 (Gram (+) bacterium) was grown in Luria Bertani Agar (LBA) plates at 37°C with following composition: peptone (Merck), 10 g/L; yeast extract (Biolife) 5 g/L, NaCl (Sigma-Aldrich) 5 g/L and agar (Fluka) 20 g/L.

The Kirby-Bauer disk-diffusion method was performed to exploit antibacterial activity of the tested samples [29, 30] .

Briefly, *S. aureus* was inoculated onto each plate by adding 1 mL inocula and spread with a glass spreader. The samples (0.5 cm^2) were sterilized with ultraviolet irradiation for 30 min. and placed in a LBA medium; All the plates were incubated under anaerobic conditions at 37^o for 24 h.

After passing the time the inhibition zone was measured (in millimeters) on the agar surface around the samples. In this study triplicate plates were prepared for each sample. The mean zone of inhibition was calculated with standard deviation procedure; standard deviation was calculated as the square root of variance using STDEV function in Excel 2010.

3. Results and discussion

The purpose was to achieve nanocomposite materials based on Collagen/ SiO_2& ZnO to produce new formulations aimed to protect the skin against UV radiation and regenerate skin. The nanomaterials were characterized and tested from a physical and chemical point of view in order to see the possible antimicrobial applications. It has been found that the collagen does not modify the antimicrobial activity, in fact increases the biocompatibility. This type of materials can be used as antimicrobial implant because of the hardness structure of silica.

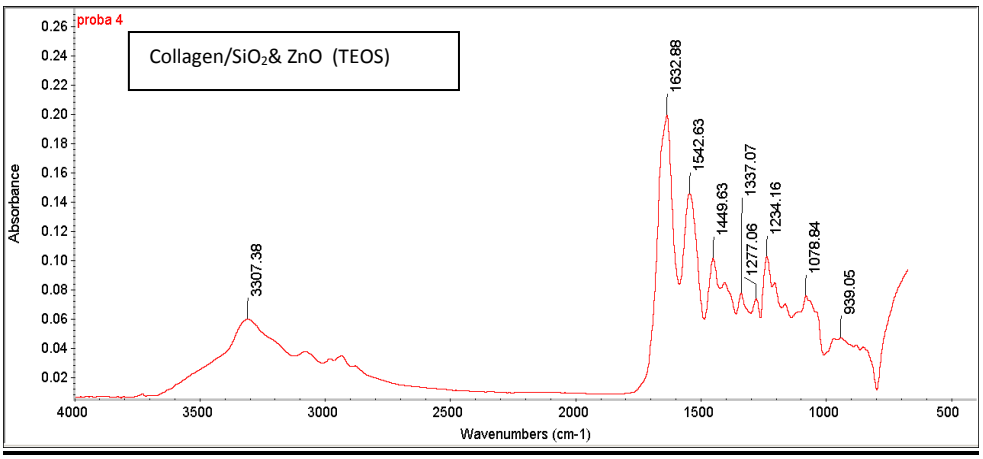

Fig. 4. FTIR analysis of Collagen/ SiO_2 &ZnO from TEOS

In FTIR analysis, the most characteristic IR bans are caused by denatured collagen. At 1234,16 cm^{-1} we can still find IR bands due to collagen and the one from 1078,84 cm^{-1} is present because of silica. The ZnO can't be seen in this analysis because has length of 400 cm^{-1}.

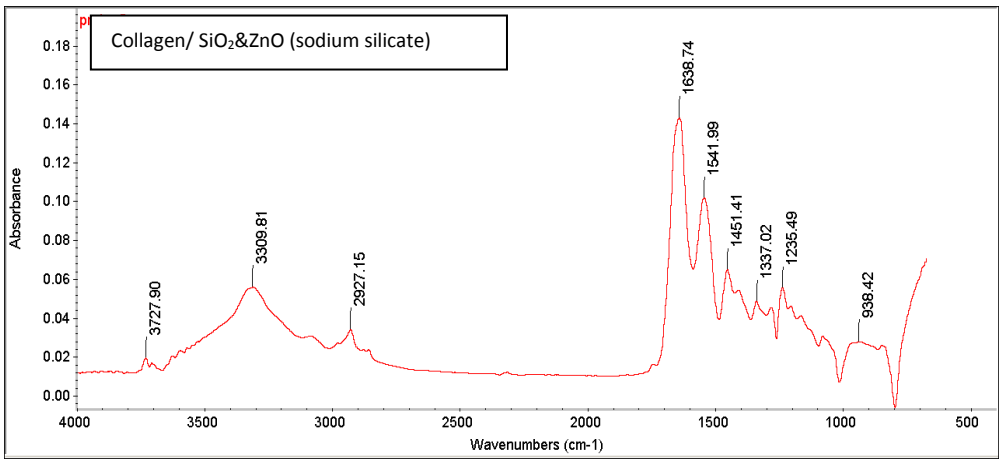

Fig. 5. FTIR analysis of Collagen/ SiO_2 &ZnO from sodium silicate

The analysis is similar with the previous case which means that the characteristics bands seen are due to denatured collagen and the one from 938,42 cm^{-1} is visible because we have silica is present in the structure.

The samples were analyzed by SEM and in all cases agglomeration occurred. Even at 250x magnification smooth surface can be seen which means that these agglomerates are formed from small particles, and also at the 500x we can observe agglomerates.

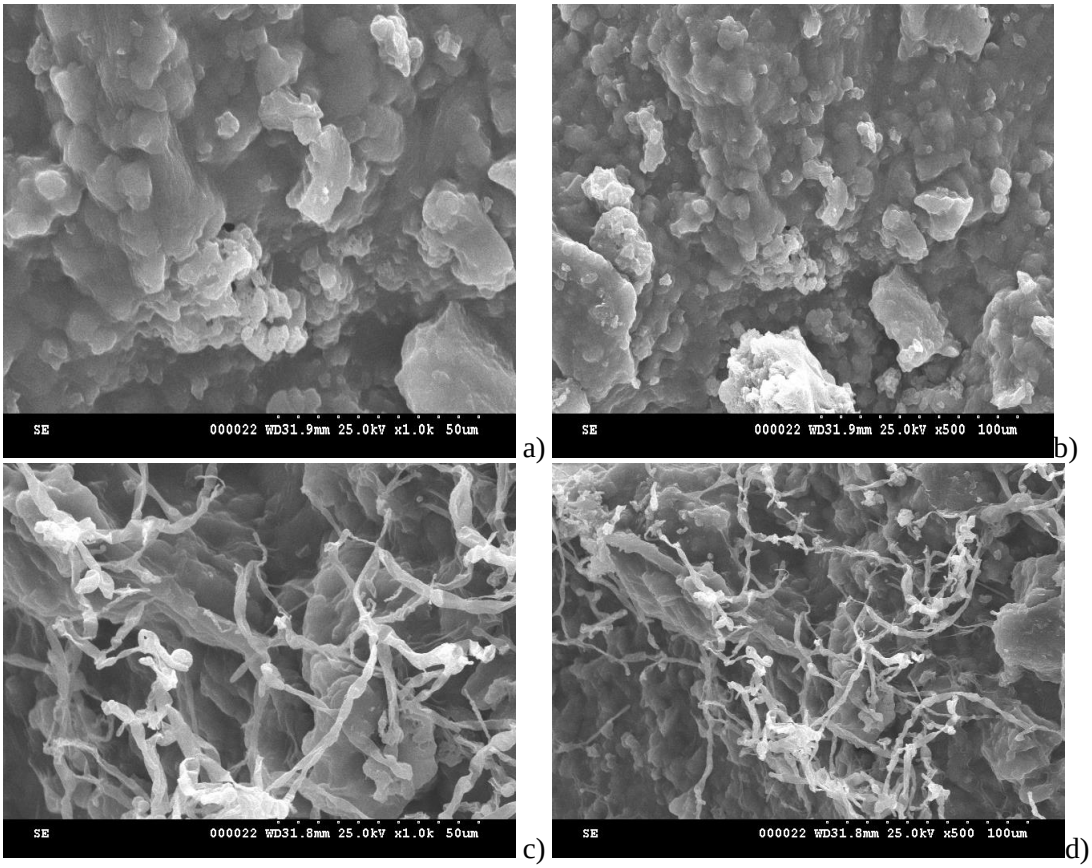

Fig. 6. SEM images of Collagen/ SiO_2& ZnO from TEOS

SEM morphology of nanocomposite showed in Figure.6.a) and b) describes spherical shapes, smooth surface with heterogeneous appearance that forms agglomerates, but in c) and d) it can be seen the presence of interconnected pores between the fibril network structures and some small amounts of agglomerates. The fibril structures were caused by the presence of mineral compounds (collagen). This interconnected porous layer like is benevolent for cell attachment and migration for skin regeneration but the porous nature can help oxygen to get through the skin. Particle size distribution showed that the microspheres were in the range of several tens of micrometers.

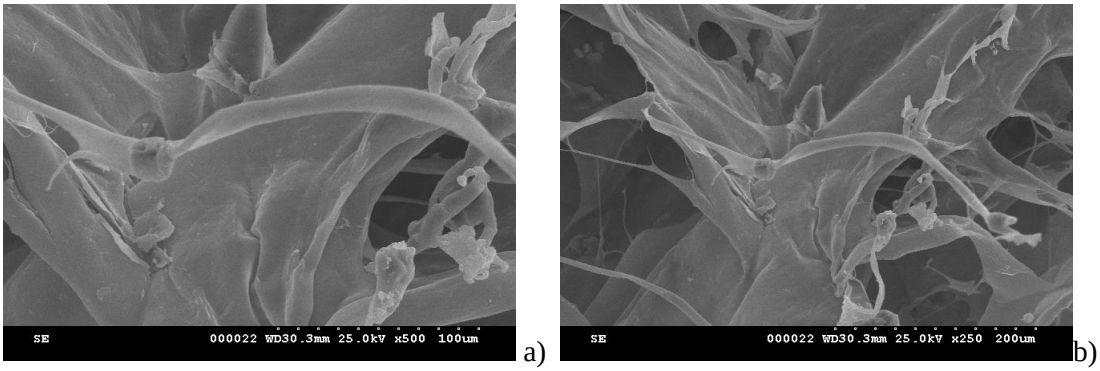

Fig. 7. SEM images of Collagen/SiO_2&ZnO from sodium silicate

The SEM imagines from above shows that it has been formed an intertwined stratification of fibril structures which is due to collagen.

The bacteriological experiments performed *in vitro* demonstrated effectiveness of ZnO as well on Collagen/ SiO_2& ZnO composite materials inhibiting the growth of *S. aureus* bacterium. Analyzing the antibacterial activity of the samples it can conclude that the sample which has Collagen/ SiO_2&ZnO (sodium silicate) has a better antibacterial activity then the sample with Collagen/ SiO_2& ZnO (TEOS) against *S. aureus*. The antibacterial activity of Collagen/ SiO_2& ZnO (sodium silicate) is higher perhaps of the higher content of ZnO encapsulated into the SiO_2 network as presented below, in Figure. 8.

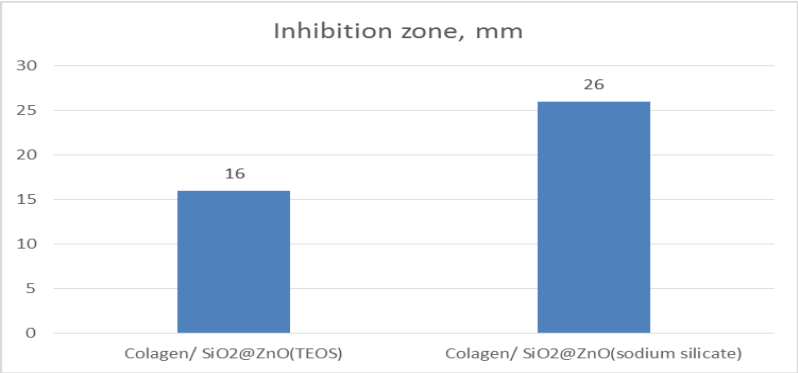

Fig. 8 Antibacterial activity against S. aureus for tested samples

As is well known, Gram (+) bacteria were much more difficult to destroy, due to the more complicated cell wall structure consisting of several layers of peptidoglycan [31]. Despite this fact, these tested samples were highly antibacterial activity against S. aureus strain.

4. Conclusion

Collagen/ SiO_2& ZnO composite materials with antibacterial activity were obtained by two routes starting from TEOS and sodium silicate as precursors. Based on FTIR analysis it can reveals the presence of denatured collagen in both samples (starting from TEOS and sodium silicate), and silica bonds. From the SEM morphology it can observe the porosity and the appearance of these interconnected fibrils which are due to the presence of collagen. After the antibacterial experiments the Collagen/ SiO_2& ZnO composite materials exhibit antibacterial activity against *S. aureus*. Even if the bacteria was very hard to destroy due to its complicated cell structure and despite all that, the samples still exhibited high antibacterial activity.

Acknowledgements

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