UNIVERSITATEA LUCIAN BLAGA DIN SIBIU

DOCTORAL SCHOOL OF ENGINEERING AND MATHEMATICS

DOCTORAL FIELD: INDUSTRIAL ENGINEERING

SUMMARY

DOCTORAL THESIS

THE DESIGN AND THE SYNTHESIS OF A HYDROGEL BASED ON FUNCTIONALIZED NANO AND MICROPARTICLES

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Sincere acknowledgements,

I would like to express my deepest appreciation to all those who have been by my side during these years, who encouraged me and who made my dream possible.
Thank you for everything!

Words cannot express my gratitude to my supervisor professor ***Prof. Dr. habil Dan Chicea*** *from Lucian Blaga University of Sibiu, Research Center for Complex Physical Systems who guided me along the doctoral years and who taught me that everything is possible when you believe in it. I have the deepest respect for him.*
Dear Professor, you have been a tremendous mentor for me. You believed in my dream since our first meet, you advised, encouraged, helped me in every moment and you allowed me to become a real research scientist. I'm extremely grateful for all your implication, for all the scientific content you transferred to me, for your patience and for all the good jokes.
I look forward to continue our journey!

I am also thankful to my advisory committee, ***Associate Professor Dr. Angela Maria Bănăduc****,* ***Professor Dr. Eng. Sever Gabriel Racz*** *and* ***Biologist Dr. Alexandru Burcea*** *for their time and for their advice during these doctoral years.*
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I am also grateful to my parents, ***Doina and Costin****, who supported and loved me unconditionally for my entire life, who sustained me on schools and college and who understand me at every choice helping me reach this far today.*
Thank you, mom and dad!

PhD candidate Eng. Alexandra Maranciuc

DOCTORAL THESIS CONTENT

PREFACE

Before you lies the doctoral thesis “The design and the synthesis of a hydrogel based on functionalized nano and microparticles”. It was written to fulfil the doctoral program in Industrial Engineering field from Lucian Blaga University of Sibiu, Romania under the guidance of Professor Dr. Dan Chicea. I was engaged as a PhD student in this doctoral program since October 2020 to October 2023. The research career as research assistant in molecular biology and engineering which I followed right after graduation, since 2018 up to present, helped me to fulfil all the challenges of this thesis.

My desire to obtain products for helping people using materials science came true since my bachelor’s degree when I fabricated a lyophilized dressing starting from raw polymeric materials. In this thesis I wanted to approach an interest subject since the infections from patients’ injuries from hospital bacteria or from a personal carelessness it’s a worldwide problem appeared long time ago which still affects a significant number of people. The challenges from this thesis made me gained more experience with nanomaterials and hydrogel production. I learned how to solve the problems appeared in the fabrication process in the laboratory, so I realized that struggling is part of a successful result. This doctoral thesis taught me to have patient and to improve my professional and personal skills.

The thesis is my personal approach for the development of an innovative technique for nanoparticles characterization called Direct Particle Tracking and for the synthesis of the chitosan-based hydrogel with chitosan functionalized silver nanoparticles as final application with the potential to become an industrial medical device in mild infections of injuries or burns.

The motivation of this thesis lies in the need of antimicrobial products easy to handle and affordable for a large category of people. Burns or infected wounds, sensitive trauma that cause pain, are a worldwide problem. In Romania, the problems with patients infected with bacteria after a trauma is well known. Therefore, to avoid the antibiotic strategy which doesn’t offer results in all type of patients or grafting procedure strategy which is not available for all patients’ categories, the development of these alternative hydrogels produced using engineering can improve humans’ healing in case of a mild infection. Their purpose is to offer support since they are based on polymers while acting as antibacterial agents due to the silver nanoparticles.

The results obtained in my doctoral thesis possess innovative contributions to the fields of materials science and industrial engineering. Their dissemination was achieved through articles published in WoS journals, articles in other databases and through participations at international conferences mostly with oral presentations and to different international programs as PhD student. Another successful achievement of this thesis was the participation in many research projects as team member.

Sibiu, August 2023

Eng. Alexandra MARANCIUC

INTRODUCTION

Skin is an important organ of humans' bodies exposed to possible accidents very often. Wounds and burns can appear from various factors, such as injuries, heat or diseases. Even if our body is designed to heal the skin damage, the process is a long one and requires time. During this healing process, infections can appear, therefore the time is precious and every alternative method can make the difference.

At national and international level, the scientific community is struggling to overcome the problems caused by bacterial infections and their resistance, one of the main causes of death worldwide. Therefore, the medical engineering started to produce medical devices to offer treatment for patients in need since the actual strategies are insufficient.

The antibacterial application solution proposed in this thesis is based on chitosan functionalized silver nanoparticles embedded in a chitosan hydrogel as final application. Silver nanoparticles proved over time antibacterial properties on many bacteria species. These nanoparticles are able to attach and pass through the bacterial membrane using a specific mechanism, to induce reactive oxygen species formation and alter the microbial replication pathways of bacteria. In this way, the biofilm formation is inhibited and the antibacterial effect is exerted. Their properties depend on many physical-chemical factors, such as size, shape, concentration or surface coatings. The silver nanoparticles functionalization with chitosan is the strategy chosen in this doctoral research since it increases their biocompatibility. Their integration in the chitosan-based hydrogel will offer a longer release time for functionalized silver nanoparticles while offering also support for the potential patient. According to most literature studies, the antimicrobial effect of silver nanoparticles is stronger once the particles are smaller. Due to the importance of dimensions, advanced techniques were developed over time for their characterization. Electronic microscopy or physical dimensional techniques are used the most, but the development of a new technique could improve the theoretical information background known until now about nanoparticles behaviour.

One of the innovative theoretical and applied research parts of this doctoral project is the development of a new tracking analysis technique for nanoparticles' size determinations, called *Direct Particle Tracking*. It assures the investigation of silver

nanoparticles using an innovative optical pathway and a real-time system in order to prove the nanoscale range of particles and therefore to suggest their antibacterial effect.

Other innovative applied parts of the thesis are related to the functionalization of silver nanoparticles which was performed using a protocol developed in the laboratory and to the development of the hydrogel itself which was designed and synthesized entirely by a particular protocol.

The principal aim of the project is the fabrication of the polymeric chitosan hydrogel based on functionalized silver nanoparticles using chitosan which can be placed at the wound/burn infection to reduce the bacteria proliferation. The high content of water and chitosan will prevent it from sticking, so comfort will be assured. It will offer an antibacterial effect due to the silver nanoparticles which will be slowly released from the chitosan matrix of the hydrogel.

The second aim of this project is related to the investigation of silver nanoparticles by the innovative technique *Direct Particle Tracking* proposed and described in this thesis. This real-time technique has the huge advantage of offering information about dimensional characteristics of each individual silver particle and about how silver nanoparticles interact in suspensions.

To accomplish the purposes proposed in this doctoral thesis, some research objectives have been proposed:

- ***O1.*** *The development of Direct Particle Tracking technique in the laboratory for silver nanoparticles investigation.*
- ***O2.*** *The silver nanoparticles synthesis using two different syntheses.*
- ***O3.*** *The physical-chemical silver nanoparticles characterization and the results discussion presentation regarding their dimensions.*
- ***O4.*** *The silver nanoparticles functionalization using chitosan performed by two different syntheses.*
- ***O5.*** *The physical-chemical characterization of chitosan-functionalized silver nanoparticles and the results discussion presentation.*
- ***O6.*** *The determination of antibacterial effect of functionalized silver nanoparticles.*
- ***O7.*** *The design and fabrication of a hydrogel with chitosan-silver nanoparticles integration.*

To accomplish these objectives proposed, the doctoral thesis was divided into six main chapters with their related subchapters.

Chapter I, ***State of the art in nanoparticles and hydrogels approaches***, summaries theoretical information about each material type used in this research namely silver nanoparticles, chitosan and hydrogels. The information concentrated in this chapter has a significant impact in the background existed nowadays in literature. The chapter contains recent information about their properties, their syntheses methods and characterization techniques and their implications in applications as medical devices.

Chapter II, ***Experimental research regarding the innovative method proposed for silver nanoparticles size determinations: Direct Particle Tracking,*** exposes the development of the innovative technique proposed for this doctoral thesis. The chapter describes the physical phenomena occurring within the method, it presents the mathematical background of the method and it explains how the equipment is used in real experiments. The results presented in this chapter are related to simulations performed for silver nanoparticles. The simulations are made based on the developed software and showed that the method is valid to be used in nanoparticles sizing in future experiments.

Chapter III, *Experimental research regarding the synthesis and characterization of silver nanoparticles,* presents the two synthesis methods proposed for silver nanoparticles fabrication. The distinction between the syntheses based on the use of two different reduction agents for the chemical reductions proposed, Trisodium citrate dihydrate versus D-glucose, leads to differences in the characterization results presented in this chapter. Therefore, the results regarding their physical-chemical characterization focused on their dimensions are exposed in chapter III.

Chapter IV, *Experimental research regarding the functionalization of silver nanoparticles with chitosan,* exposes the functionalization approaches for silver nanoparticles. Therefore, in this chapter are presented the methods proposed for functionalization with chitosan using two different approaches, the results obtained after the physical-chemical characterization of functionalized silver nanoparticles and the antibacterial test performed on *Escherichia Coli* strain and *Staphylococcus Aureus* strains. Furthermore, the chapter exposes the innovative protocol proposed in this thesis for silver nanoparticles functionalization and its results compared to a standard method described in literature.

Chapter V, *Polymeric hydrogel synthesis based on functionalized silver nanoparticles,* includes the fabrication process for the final application of this research, the chitosan-based hydrogel with functionalized silver nanoparticles. The chapter exposes the synthesis method proposed for the hydrogel dressing and its characterization using optical microscopy.

Chapter VI, *Final conclusions, personal contributions and future directions of this research,* describes general conclusions of this research. It is highlighted the author personal contribution to the research field and there are described the future directions of the research based on the results from this doctoral thesis.

Key words: silver nanoparticles, size, chemical synthesis, DLS measurements, AFM size determination, chitosan, functionalization, antibacterial effect, hydrogel

I. STATE OF THE ART

1. STATE OF THE ART: NANOSCIENCE AND NANOPARTICLES

Nanotechnology, a research field developed constantly since 1959, represents an unique and innovative research strategy [1, 2]. Described first in 1974 by Norio Taniguchi and then experimented in 1981 by two IBM researchers who developed the scanning tunneling microscopy [2], the field of nanotechnology has gained proportion in science field. The big potential of this science is related to the ingress in atoms and molecules' investigation. The possibility of creating machines at nanoscale dimensions and the fabrication of devices with a specific atom distribution become a priority for certain researchers or engineers. Nanotechnologies developed until now create low dimensional materials with unique properties which offer the ability to be used in many domains such as, textile industry, medicine, electronics or automatics. Nanotechnology can be considered the innovative field of materials science due to the advantages offered. In latest years, the economy has shown improvements regarding the need for smaller and better materials in all domains. Beside the small dimension, nanomaterials have gained attention for their physical-chemical properties such as conductivity or optic activity [3].

Nanomaterials have changed the classic models investigated. The need for light and sustainable products, targeted therapies, optimized diagnostic devices have allowed the discovery of beneficial contributions of nanomaterials. In medicine, these nano dimensions are studied more and more. Their most important property, a larger surface-volume ratio compared to the bulk size, increases their activity in biological systems [4].

Nanoparticles, an important field of nanotechnology, are materials with nanometric dimensions less than 100 nanometres considered 0-D materials. The specific and well-defined properties of nanoparticles offer them advantages in variable engineering applications. The form and the dimension are the main factors studied in the research community. These factors can influence the physical-chemical and optical properties of nanoparticles. The concentration of nanoparticles solutions, the density or the colour are other factors which induce modifications in nanoparticle-based materials. Each application requires certain properties, so the dependence of size or shape to the final application is recognized though the literature studies.

Nanoparticles can be synthesized through different approaches. In general, there are two directions: “top-down” and “bottom-up” methods. Depending on the precursors and materials used, the two approaches are based on different technologies. The “top to bottom” method is based on a bulk material transformation into small particles, while the “bottom to top” method involves the production of nanoparticles using chemical reagents able to assembly atoms to “seed” nuclei which will grow further into clusters and particles of nanometric dimensions, as it can be observed in Figure 1 [5-7]. Organic particles are usually obtained through bottom-up methods such as chemical reduction, sol-gel, emulsification or self-assembly processes. These methods lead in most cases to nanoparticles fabrication in a spherical form and in a polydisperse size distribution due to the inherent surface tension that makes itself manifest. Besides organic particles, inorganic nanoparticles in different shapes can be obtained through bottom-up techniques, as well, for example by a nucleation process [8].

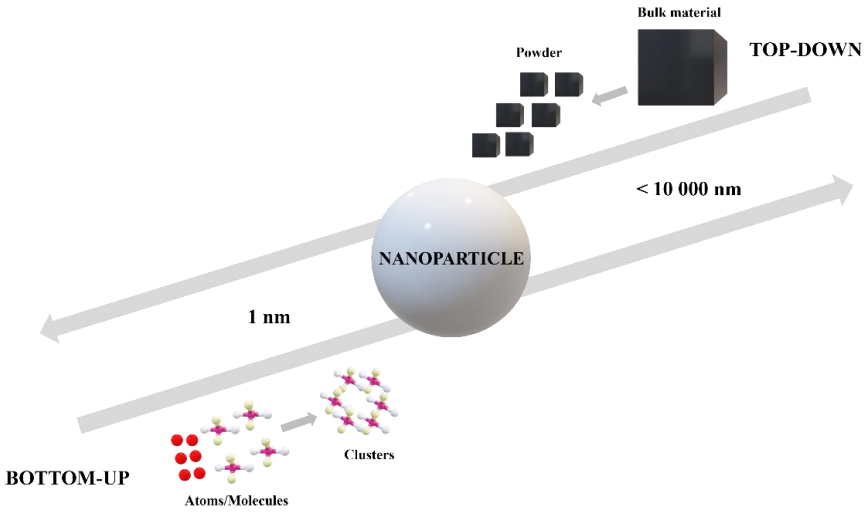

Figure 1. Top-down methods versus bottom-up methods from nanoparticles perspective

2. SILVER NANOPARTICLES

Silver is a soft, shiny metal [9] used from many years in medical applications due to its benefits. Evidences described in history showed the presence of silver in ancient Egypt and Rome, there being considered an excellent storage material [10]. Since the time of Hippocrates, silver has been used in wound healing, ulcers and infections. Silver begun to be used either by external administration or by ingestion in order to heal intern infections [10]. In the Indian culture, silver was integrated in creams or local

drugs for its reaction on bacteria. Along time, silver has proved a strong antimicrobial character being used in wound healing and prevention from diseases. In late 1900's, treatments based on colloidal nanosilver against infections proposed by the United States led to a deeper investigation of silver-based materials used also in nowadays [10]. The evolvement of the world led to the discovery of silver nanomaterials which seem to possess improved properties. Silver nanoparticles (Ag NPs) possess a high large-surface area volume which increases their activity inside the human body. The higher surface allows them a better exposure in bacteria strains, so the antibacterial effect is higher [11]. Besides these, once the nanometre scale is achieved, silver particles show better optical and physical properties compared to the bulk size. Their thermal conductivity, which is found in a high amount among the metals found, led to integration of silver nanoparticles in industrial devices as final application [12]. Their unique properties made them an interesting subject also in biomedical applications where are intensively studied as antibacterial agents.

The shape, a property described for nanoparticles, influences the surface to volume ratio and their distribution in the body. In the case of Ag NPs, the shape is a property able also to modify the bacterial response. The large surface of such nanosized particles is beneficial for drug delivery applications, cancer therapy, tissue regeneration or photocatalytic applications [13]. In case of human or animal cells, the spherical form is preferred in many biomedical applications. Since they are integrated in a biomaterial, prevention of side effects should be taken into consideration when the shape is chosen. A large area to volume ratio and a right shape, without corners, can led to a proper distribution and diffusion of nanoparticles in the body and can prevent the cells apoptosis around the tissues in medical applications [13]. However, in case of bacteria, there are literature studies which sustain the use of triangular shape of nanoparticles since they exhibit a high killing rate [14-16].

The small size allows the particles to flow in the circulation system once they are ingested by oral administration or to stay in the dermal layer when they are applied on the skin. Size-dependent effect was proved by Osonga et al. [17] which compared the antifungal activity of Ag NPs versus Au NPs synthetized with the same reducing agent. The study was performed on different types of fungi as also on Gram-negative or Gram-positive bacteria. After a period of incubation, a higher concentration of 10 μM of reducing agent proved to suppress almost the total growth of fungi, while at 4 μM a continues growth could be identified. Besides the concentration importance, the size also proved to be essential. The smallest particles identified using TEM images

(around 9 nm) showed a total inhibition in case of fungi and bacteria, while at higher dimensions, the antimicrobial activity decreased [17].

The surface of nanoparticles is related to their reactivity [18] since the Ag ions can easily create electrostatic interactions with other molecules or compounds. In the case of bacteria, the supposed mechanism of suppression is also correlated to the affinity of silver for sulfate group which is found in proteins or enzymes at the bacterial membrane level.

Silver nanoparticles, along with zinc for example, have antimicrobial properties for different microorganisms. Silver nanoparticles are extremely versatile regarding their characteristics. In case of silver nanoparticles approach, the mechanism of bacterial cannot identify them as an antibacterial agent, therefore their actions are guided under many changes: modifications of efflux plumps, alteration of membrane so that they could enter, an increased number of particles, leakage of intracellular content and the final alteration of DNA with the production of ROS (reactive oxygen species) which will destroy the pathogen [19]. So, silver nanoparticles destroy the microorganisms by altering their DNA and by inducing the appearance of ROS [19].

3. HYDROGELS

In tissue engineering, there are some gradual approaches which include treatments based on cells or biological molecules, biological molecules and hydrogels or only hydrogels for wound treatments. These products of tissue engineering are classified according to their composition focused on polymers. Natural or synthetic polymer, hydrogels offer specific properties related to the final application. As a natural material with multiple benefits, the interest for chitosan-based devices and their derivates has increased gradually until nowadays. It offers a higher biocompatibility of inert or metallic materials, it is biodegradable and it is a good support the human’s cells regeneration [20].

Hydrogels are biomaterials organized in the form of a three-dimensional network of polymer chains used mostly in tissue engineering applications due to their superior properties compared to other dressings: biocompatibility, biodegradability, elasticity, porosity, water uptake and good sweeling properties in exudate absorption from wounds [21]. They are 3D materials with high percentage of water and solvents in their structure. They can be easily manipulated under many forms, are comfortable and versatile depending on their destination, so they are a perfect choice for skin wounds.

II. EXPERIMENTAL RESEARCH

EXPERIMENTAL RESEARCH REGARDING THE SYNTHESIS AND PHYSICAL-CHEMICAL CHARACTERIZATION OF SILVER NANOPARTICLES

1. AIM OF THE EXPERIMENTAL RESEARCH

The purpose of this chapter is to determine the proper synthesis for Ag NPs fabrication. Since their dimensions are an important aspect for the final antibacterial application proposed in this thesis, the size determination is the main aspect to be considered in nanoparticles fabrication.

Ag NPs were synthesized using two approaches based both on chemical reduction. This synthesis method was chosen since it offers a high yield of reaction in short time and in simple laboratory conditions. Also, the costs for it are low. Further, there will be exposed two chemical syntheses performed using different reducing agents for the same silver precursor. So, using silver nitrate as precursor, trisodium citrate dihydrate (TSC) (Ag NPs-TSC) and D-glucose (Ag NPs-Glucose) were compared in two reduction reactions. The aim of the comparison is to determine which method can provide smaller nanoparticles for the final hydrogel application proposed so that a potential bactericidal effect could be indicated. Also, it will be concluded how the reagents used interact with the metal salt for Ag NPs formation.

The Ag NPs fabricated using the further methods were characterized by many techniques in order to validate the best chemical synthesis performed in the laboratory. Various techniques were performed for structural and dimensional analysis in both samples. The characterization is an essential step in this field of biomaterials since any material or device produced must accomplish some specific criteria once it is fabricated. The integrity and the compatibility of materials proposed are essential since the final product has a medical purpose.

2. SYNTHESIS METHODOLOGY

For this work, the materials used in both chemical syntheses were purchased from Sigma-Aldrich, Germany and VWR International, America, according to Table 1. The materials were used without any further purification.

Table 1. Materials used in this research for Ag NPs synthesis

Reagent	Chemical formula	Purity	Form
Silver nitrate	$AgNO_3$	≥99.8% ACS	powder
Trisodium citrate dihydrate	$C_6H_5Na_3O_7 \cdot 2H_2O$	99%	powder
Anhydrous alpha-D-glucose	$C_6H_{12}O_6$	96%	powder
Sodium hydroxide	NaOH	97%	powder
Sodium chloride	NaCl	>99%	powder
Acetone ultrapure	C_3H_6O	≥99.5% ACS	liquid

2.1 Ag NPs SYNTHESIS METHODOLOGY USING TSC AS REDUCING AGENT

The first synthesis proposed was based on TSC. In this procedure, TSC was used as a reduction agent as well as a stabilizing agent in Ag NPs formation, so no other chemical reagent was introduced in the reaction.

A total of 50 mL of 10 mM Ag NO_3 was heated in an Erlenmeyer glass until the boiling point was reached. In parallel, 1% TSC solution was obtained and added drop by drop to the $AgNO_3$ solution by heating and stirring for 10 min. The mixing was stopped when the colour changed to yellow, as can be observed in Figure 2 and the Ag NPs solution was left to cool at room temperature.

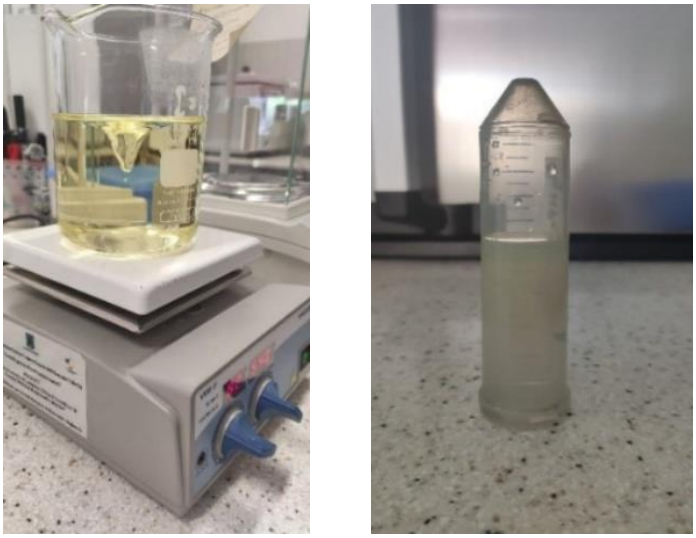

Figure 2. The addition of TSC 1% to $AgNO_3$ as the reduction reaction and the Ag NPs suspension

The silver nanoparticle solution was filtered using Millex Syringe Pores of 0.22 µm from Sigma-Aldrich. The final suspension was maintained at room temperature for the next analysis in a 50 mL tube, as can be observed in Figure 3.

Figure 3 shows the methodological flow of the synthesis performed.

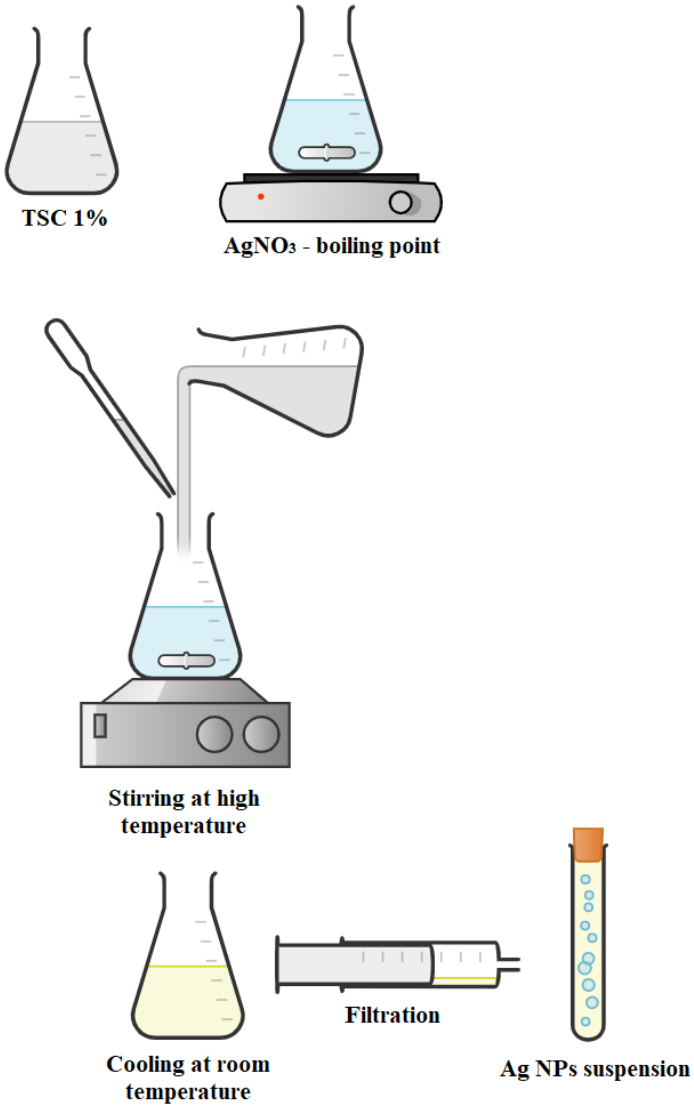

Figure 3. TSC reduction in Ag NPs synthesis

2.2 Ag NPs SYNTHESIS METHODOLOGY USING D-GLUCOSE AS REDUCING AGENT

This synthesis was based on the chemical reduction of silver nitrate ($AgNO_3$) using D-glucose anhydrous 96% and sodium hydroxide (NaOH), as can be observed in Figure 4. D-glucose reduces the silver ions only in an alkaline medium; therefore, NaOH helps the chemical reduction without interfering with the other chemical reagents.

Initially, 100 mL of 5 mM $AgNO_3$ solution was prepared by completely dissolving the silver powder in ultrapure water for 10 min at room temperature. The second step was the preparation of the reduction solution based on D-glucose ($C_6H_{12}O_6$) and NaOH. A total of 0.01 M D-glucose and 0.25 M of NaOH were dissolved in 400 mL ultrapure water under magnetic stirring at 82°C for 30 minutes. The silver solution was added slowly to the reduction solution until it turned to a light-yellow colour. After 15 minutes of mixing, 1.28 M NaCl was added to the solution as a stabilizing agent. The final part of the synthesis was based on the washing steps, realized with ultrapure acetone, before drying the suspension at room temperature.

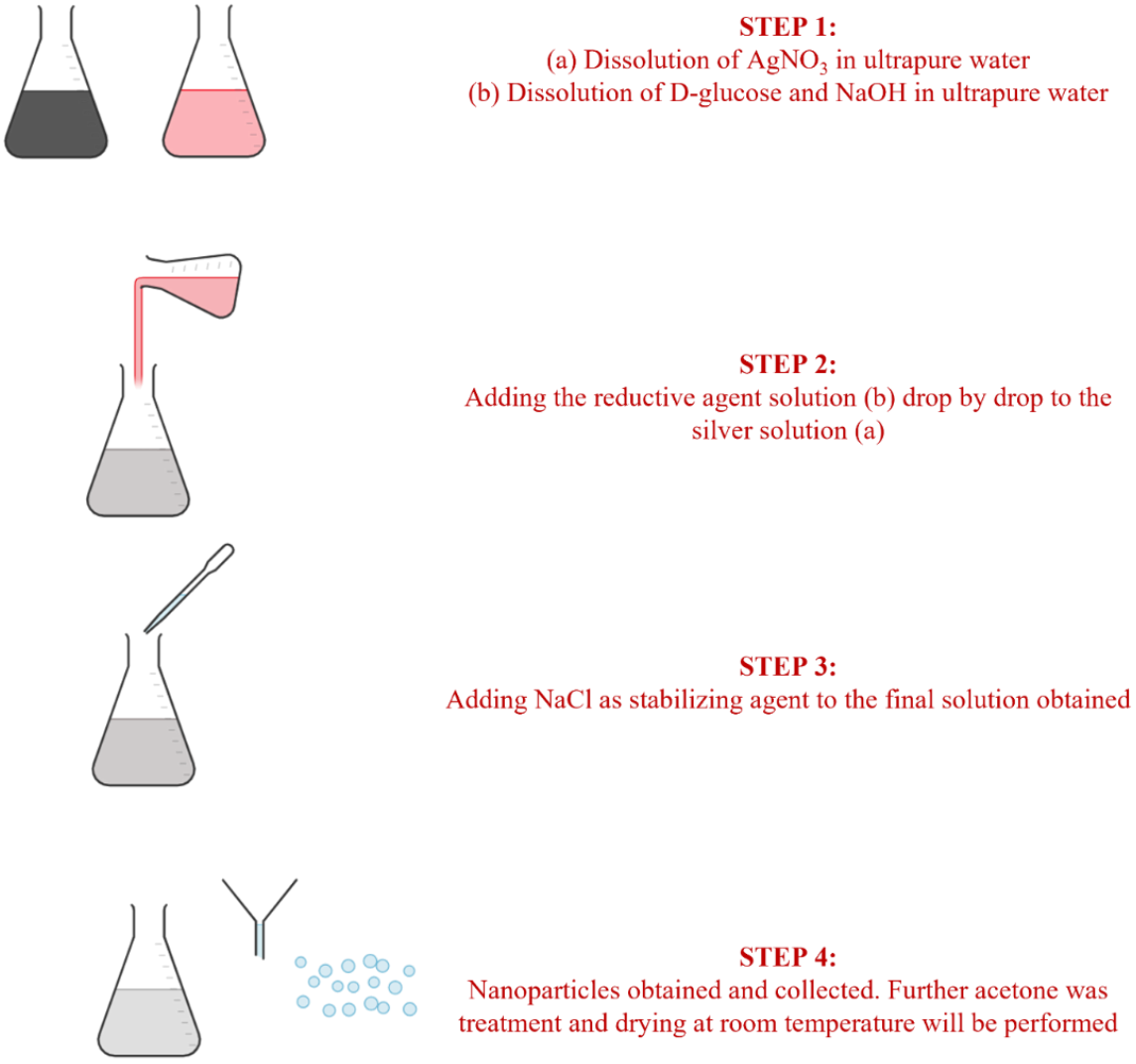

Figure 4. D-Glucose reduction in Ag NPs synthesis

The solution was not filtered since, in our attempt to complete this step, the Millex Syringe Pores with 0.22 µm size pores we used quickly clogged. The silver nanoparticle solution was maintained at room temperature for subsequent analysis.

3. RESULTS AND DISCUSSIONS: COMPARISON BETWEEN TSC AND D-GLUCOSE AS REDUCING AGENTS FOR Ag NPs SYNTHESIS

This part is based on a comparison of the two synthesis methods proposed in this thesis in order to observe which synthesis is the best to remain in the nano range in case of Ag NPs. The characterization methods were performed in both methods to compare the chemical and physical differences appeared, the sizes and to see if nanoparticles can be different fabricated in the nano range.

3.1 ATR-FTIR RESULTS FOR Ag NPs-TSC AND Ag NPs-GLUCOSE

ATR-FTIR (Fourier-transform infrared spectroscopy with ATR crystal) analysis was performed to identify the various functional groups and their implications in Ag NPs synthesis, as can be observed in Figure 5 and Figure 6.

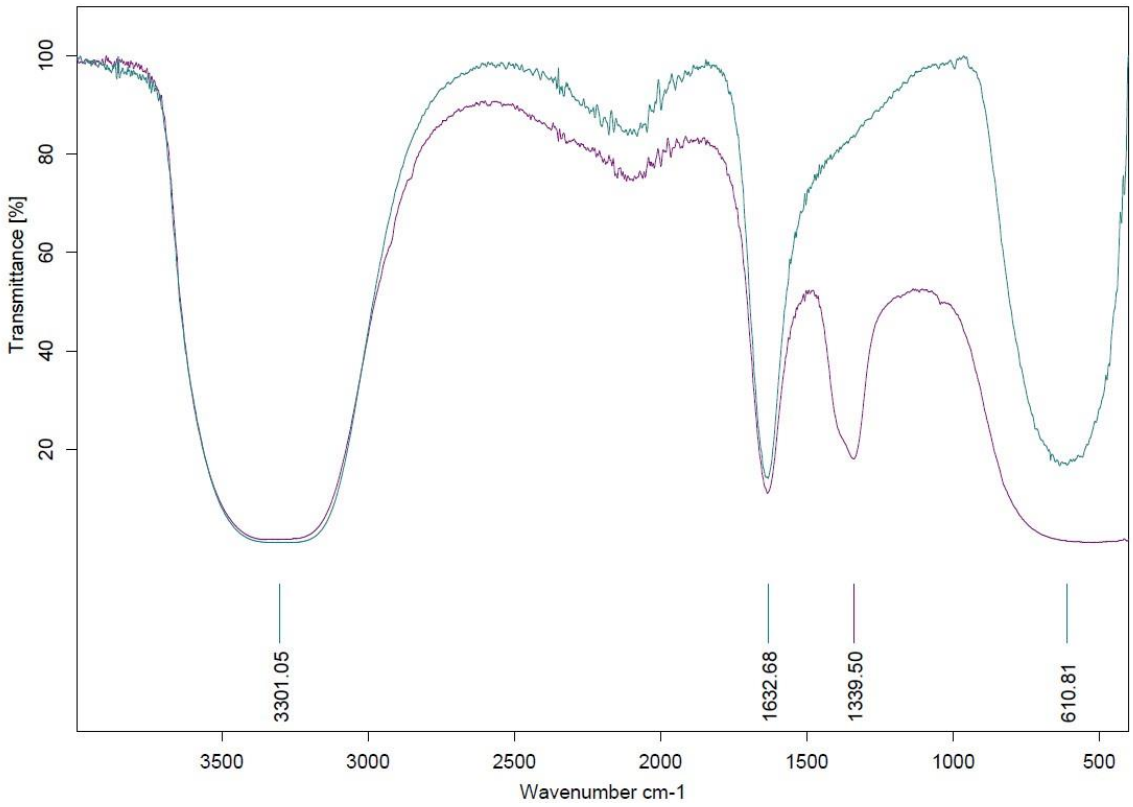

Figure 5. The ATR-FTIR spectra of $AgNO_3$ (purple) and Ag NPs-TSC (green) aqueous suspensions

In the Ag NPs-TSC and $AgNO_3$ spectra, at 3301 cm^{-1} O-H or N-H stretching vibrations are recorded, since they are found in reaction's solvent. The sharp peak observed in 1628 cm^{-1} is correlated with the C=O molecule vibration that appears when

the reducing agent acts and forms strong bounds around Ag nanoparticles, or it can be associated with a -N-H- bonding from an amine group. The peak found at 1339 cm^{-1} is a specific peak that can be observed in the case of $AgNO_3$. The most important aspect that stands out is the disappearance of this peak in the case of Ag NPs-TSC sample. This result strongly suggests that nanoparticles were obtained and the $AgNO_3$ was reduced. For the last peak at 610 cm^{-1}, there are several studies in the literature that suggest an association with an Ag network based on Ag–Ag interaction [22,23], but in our opinion this network is quite difficult to be obtain and identify through FTIR analysis, so the peak can be associated with C-H bondings out of the plane, which are found in the molecules [24].

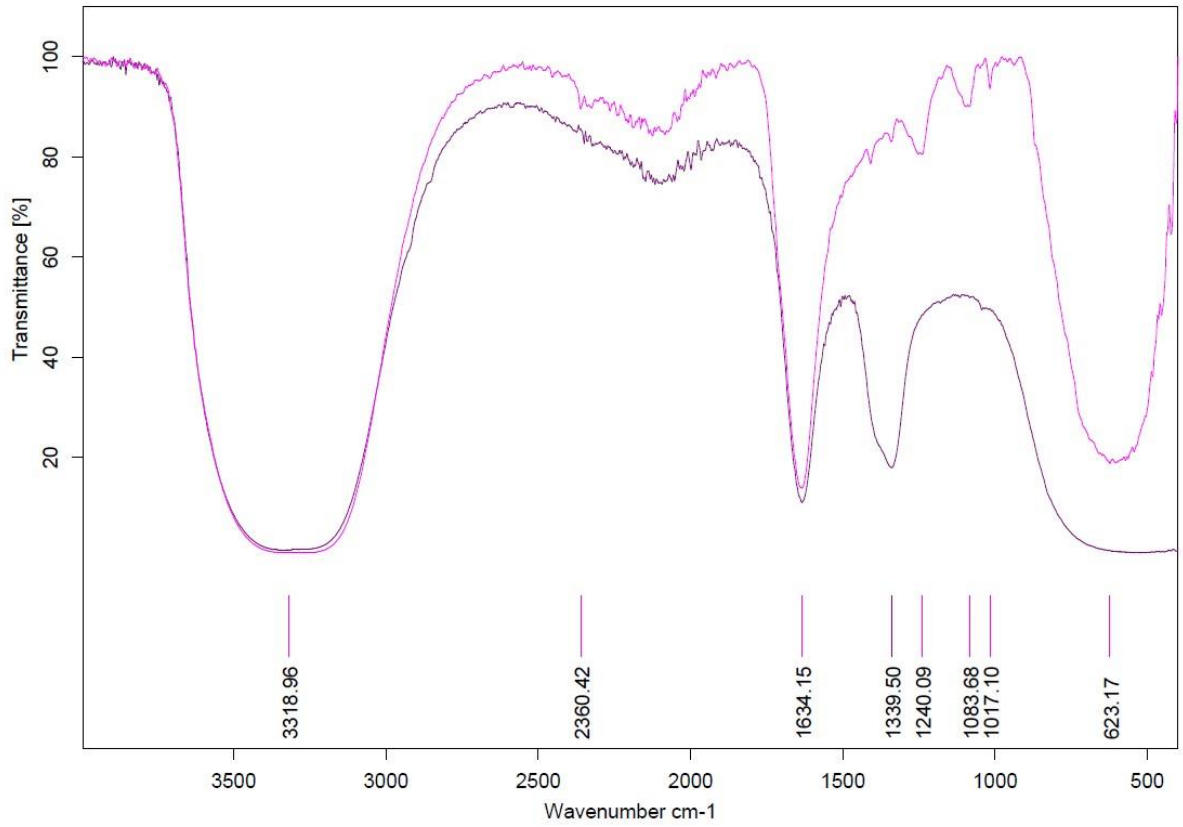

Figure 6. The ATR-FTIR spectra of $AgNO_3$ (purple) and Ag NPs-D-Glucose (pink) aqueous suspensions

The spectrum of Ag NPs-Glucose sample shows a very large peak at 3318 cm^{-1} associated with the free OH group found in the reduction agent for Ag^+ to Ag^0 [25, 26]. In case of $AgNO_3$, the bonds can be identified as -NH- molecule vibrations. At 1634 cm^{-1}, the sharp peak is assigned to C=O group vibrations or to a -NH- amine group, while at 1240 cm^{-1} the C-O- stretching can be found in case of Ag NPs-Glucose. The specific peak at 1339 cm-1 is identified only in case of $AgNO_3$, result which also

suggest the formation of Ag NPs. These effects occur in D-glucose structure and its interaction with Ag^+ [23, 24, 27]. The reduction in D-glucose with NaOH is well-known as the Lobry de Bruyn-van Ekenstein rearrangement in chemistry, since the reaction provides a mixture of D-glucose, D-mannose and D-fructose. The presence of OH molecules and C=O groups is also explained by the appearance of these isomers in the reduction reaction [28, 29]. The last peak found at 623 cm^{-1} is associated with C-H bending. Compared to previous sample, the one based on Ag NPs-TSC and comparing to $AgNO_3$, in case of Ag NPs-Glucose it can be observed that between 1240 cm^{-1} and 1017 cm^{-1} the transmittance curve is not that smooth. Therefore, the remaining of a small quantity of unreduced silver ions in reaction or the fabrication of different type of nanoparticles is a possible explication.

The FTIR results proved differences in both chemical syntheses and in the reduction solution used. The results showed that the peak from 1339 cm^{-1}, only appeared in $AgNO_3$ is not present in Ag NPs suspensions, so the chemical reduction was achieved in both cases. The other peaks were attributed to specific chemical groups according to literature. Furthermore, the spectrum obtained in case of Ag NPs-TSC showed more promising results since well-defined and specific peaks for silver nanoparticles solutions were recorded compared to Ag NPs-Glucose.

3.2 UV-VIS RESULTS FOR Ag NPs-TSC AND Ag NPs-GLUCOSE

UV-VIS (UV-VIS spectroscopy) was performed to highlight the surface plasmons for silver nanoparticles and to show that in Ag NPs silver ions are absent. This difference in ions presence shows that nanoparticles were formed and the precursor is no longer found in the samples.

Figure 7 illustrates the absorption spectra of $AgNO_3$ and both Ag NPs suspensions investigated. The red line is associated with $AgNO_3$ spectrum, the green curve is associated with Ag NPs synthesized using TSC and the pink curve is representing Ag NPs synthesized using Glucose.

Examining Figure 7, it can be noticed that the spectra for the samples are different from the $AgNO_3$ spectrum, which was an expected result. The vertical axis represents the absorbance of the suspension and the horizontal axis represents the wavelength. $AgNO_3$ has a strong absorption at about 300 nm which disappears in both samples' absorptions. This result indicates that silver particles were formed during synthesis and

the $AgNO_3$ was reduced in this regard. This result confirms the FTIR analysis performed in which reduction of $AgNO_3$ was also observed.

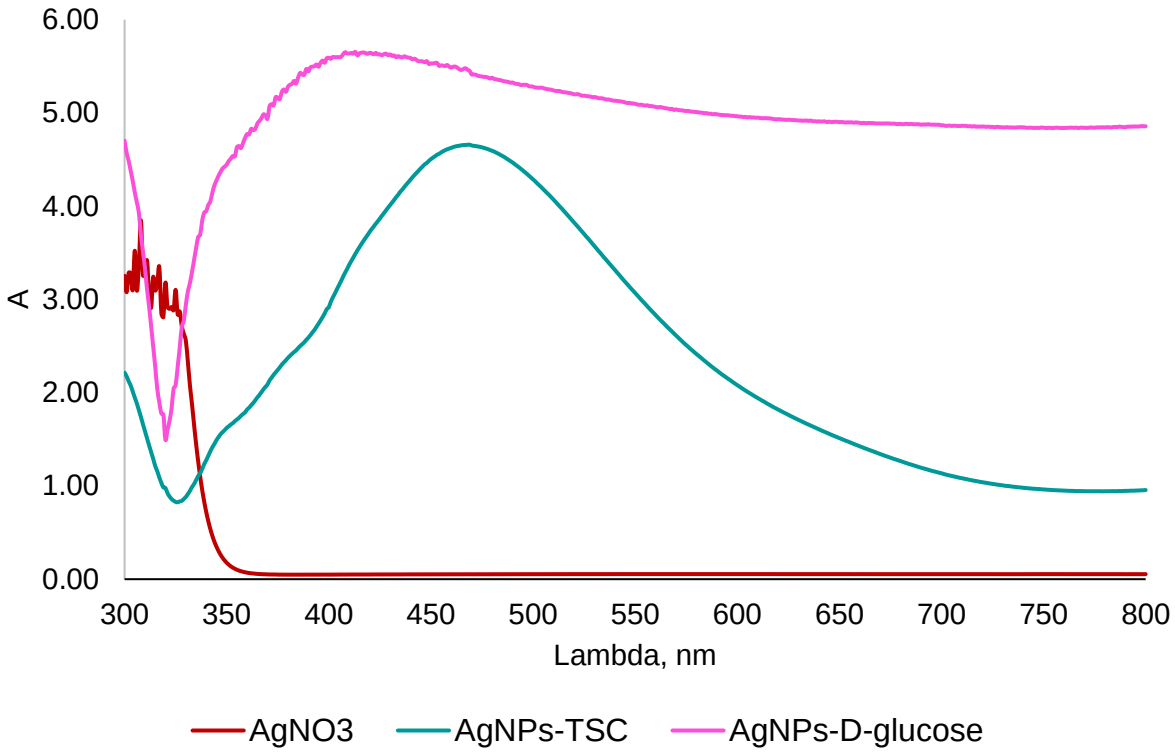

Figure 7. The absorption spectra of $AgNO_3$ and both Ag NPs aqueous suspensions

Both Ag NPs suspensions present a wide absorption peak, yet different in width. The Ag NPs-TSC sample has a strong absorption starting from 350 nm with a maximum peak at around 460 nm. The wavelength shown in the spectrum strongly suggests the formation of Ag NPs since in this range since the majority of literature studies also reported this result. Our result is consistent with the position of the absorption peak reported in other references [12, 30-33].

Ag NPs-Glucose sample shows a different absorption with a larger peak which suggests a polydisperse suspension, with various dimensions of particles. However, the absorption is found at around 400 nm, which also suggests that silver particles were formed. Based on these affirmations, the UV-VIS spectrum for Ag NPs-Glucose showed a mix of particles, both micro and nanoparticles.

The false shift appeared on smaller wavelengths on the pink line could induce the idea of smaller nanoparticles in case of Ag NPs-Glucose than Ag NPs-TSC, but the peak shape which is not well defined. The further analyses performed will show that this hypothesis is not sustain.

3.3 AFM RESULTS FOR Ag NPs-TSC AND Ag NPs-GLUCOSE

AFM (Atomic Force Microscopy) was performed as a confirmation for DLS (Dynamic Light Scattering) size results. As the main result is obtained from DLS, AFM can offer another approach for this size measurements, yet being considered only a confirmation. DLS measurements offer an average diameter of the Ag suspensions, while AFM is applied on various areas of the sample, not on the entire surface.

Several scans were performed over different regions of the samples prepared as described earlier. A 3D topography of a scanned region of Ag NPs-TSC deposited on the mica substrate is presented in Figure 8. It shows a region of the sample with 2 nanoparticles identified in the field. Z represents the height of the cantilever tip during scanning and x represents the horizontal displacement of the cantilever.

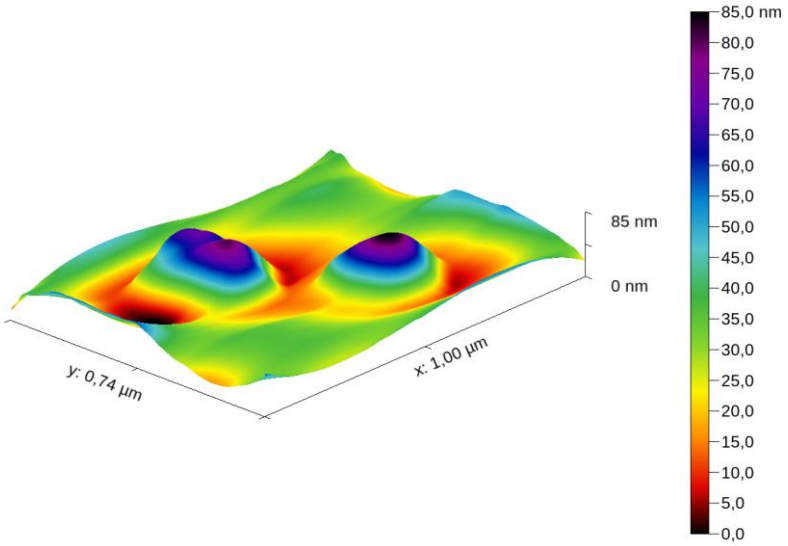

Figure 8. The topography of a region of the sample, illustrating two nanoparticles

Figure 9 illustrates several profiles extracted over different nanoparticles from the substrate after scanning them. It can be noticed that the height of the profiles is different from each other, yet in the range of tens of nm, the maximum being fixed at around 80 nm.

A total number of 29 profiles were carefully extracted from the sample and the height of each profile was assessed. The average height was 49 nm for Ag NPs-TSC sample and the standard deviation was 14 nm, therefore we can conclude that the AFM diameter dAFM is 49 +/-14 nm for Ag NPs-TSC sample.

The same procedure was used for Ag NPs-Glucose where the nanoparticles diameters were identified. The results are presented in Table 2.

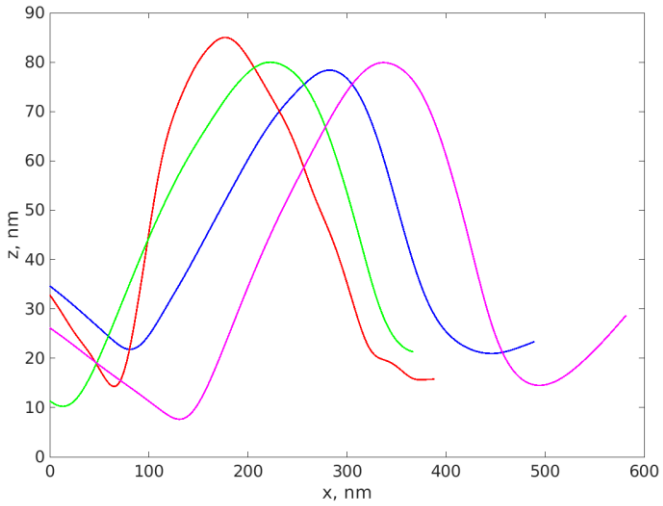

Figure 9. The plot of four profiles extracted over different nanoparticles from the scanned sample, located in different regions of the sample

3.4 DLS AND AFM SIZE RESULTS FOR Ag NPs-TSC AND Ag NPs-GLUCOSE

The DLS procedure was used to process the time series recorded on aqueous suspension of Ag NPs samples. Table 2 describes the average diameter assessed using the DLS and the AFM procedures.

Table 2. The DLS and AFM diameters; the error in assessing them

No.	Sample	d DLS, nm	Δd DLS, nm	d AFM, nm
1	**Ag NPs- Glucose**	1140	107	973
2	**Ag NPs-TSC**	58	6	49

Table 2 shows the results for both samples. It can be concluded that the two particle sizing techniques confirm the average diameters we found for the nanoparticles synthesized using the two chemical reduction procedures proposed. The results show that the synthesis based on TSC gives the smallest diameter of the particles, compared to the Glucose-based synthesis which proved to give us microparticles of silver, not nanoparticles. Both the techniques were consistent since in all the cases the results correlate.

These results are strongly encouraging for future experiments on antibacterial tests, since for the antibacterial effect particles in nano range are considered to have the strongest effect comparing to other particles sizes.

III. EXPERIMENTAL RESEARCH

EXPERIMENTAL RESEARCH REGARDING THE ANTIBACTERIAL EFFECT OF FUNCTIONALIZED SILVER NANOPARTICLES

1. AIM OF THE EXPERIMENTAL RESEARCH

The purpose of this work is to expose the antibacterial effect of functionalized Ag NPs. To accomplish this objective, the Ag NPs were functionalized using various chitosan concentration and using various synthesis strategies. The choice of chitosan is sustained by its incredible properties since it is a natural biodegradable product extracted from shells. The nanoparticles functionalization will increase their biocompatibility and their release time. The functionalization was performed using two protocols since one of them was established entirely in the laboratory for this doctoral thesis and the other is well-known in literature as synthesis method. The difference between them is made by the reduction agent introduced in the reaction for the silver precursor: TSC (Ag NPs-TSC-Chitosan) versus NaOH (Ag NPs-NaOH-Chitosan).

The susceptibility of microorganisms to Ag NPs-TSC-CH and Ag NPs-NaOH-CH was tested using the disc diffusion method.

2. RESULTS REGARDING THE ANTIBACTERIAL EFFECT

The disc diffusion analysis was conducted using sterile paper discs purchased from Merck company on two bacteria strains *Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus),* as it can be observed in Figure 10. The loaded discs were let to dry for 10 minutes before to be placed on the agar surface.

The process described in Figure 10 applies to controls and functionalized Ag NPs samples since for accurate results the controls must be performed in the same conditions as the samples. The controls represent all the reagents used in the reactions in the specific concentration. Once the test is completed, the inhibition zone appeared around the loaded disc is measured and it is established, based on the diameter of the inhibition zone, which sample has the most powerful effect on the bacteria tested. The higher the inhibition zone, the higher the inhibitory effect of Ag NPs is.

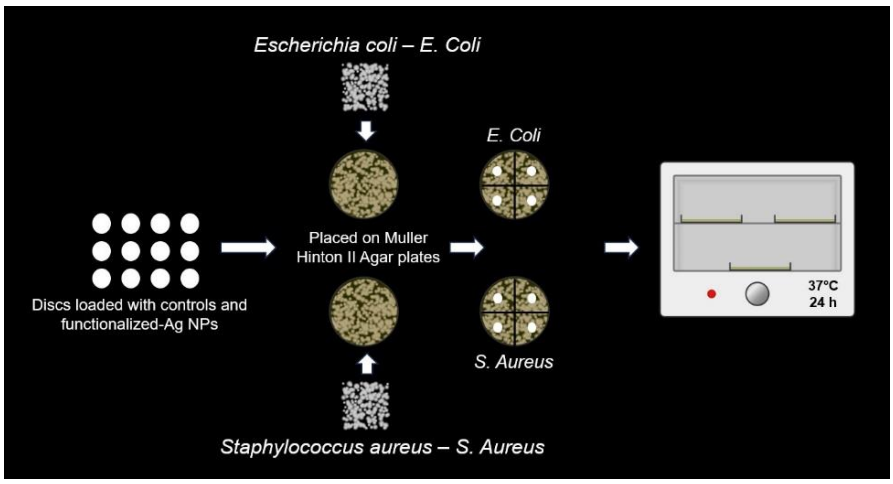

Figure 10. Disc diffusion method applied in antibacterial test for functionalized Ag NPs

The results of the antibacterial test proved that in case of controls, there is no bacteria inhibition in the plate, therefore the reagents used in the reactions don't have any effect on *E. Coli* or *S. Aureus*. The control test proved that any other antibacterial activity is due to the silver nanoparticles presence.

In contrast, the antibacterial test performed on all the samples described further in Table 3, using both syntheses approached in this thesis, showed a high inhibitory effect for all samples.

Table 3. Chemical composition and samples description for a total volume of 100 mL

Number	Sample	Chitosan, w/v (%)
1	**Ag NP-TSC-CH0.3**	**0.3**
2	**Ag NP-TSC-CH0.6**	**0.6**
3	**Ag NP-TSC-CH0.9**	**0.9**
4	**Ag NP-NaOH-CH0.3**	**0.3**
5	**Ag NP-NaOH-CH0.6**	**0.6**
6	**Ag NP-NaOH-CH0.9**	**0.9**

Once the antibacterial effect on *E. Coli* and *S. Aureus* was clearly confirmed, the diameters of inhibition zones were measured using a ruler for each bacteria strain and each sample. A higher diameter inhibition zone suggests higher antibacterial effect for the sample. Therefore, the results are presented in Table 4.

Table 4. Inhibition zones for functionalized Ag NPs

No.	Sample	Inhibition zone for *E. Coli* strain	Inhibition zone for *S. Aureus* strain
1	**Ag NP-TSC-CH03**	**31 mm**	**35 mm**
2	**Ag NP-TSC-CH06**	29 mm	33 mm
3	**Ag NP-TSC-CH09**	30 mm	30 mm
4	**Ag NP-NaOH-CH03**	**31 mm**	**34 mm**
5	**Ag NP-NaOH-CH06**	29 mm	34 mm
6	**Ag NP-NaOH-CH09**	29 mm	30 mm

The data collected show that the highest antibacterial effect is confirmed in the samples with the lowest chitosan concentration, Ag NP-TSC-CH0.3 and Ag NP-NaOH-CH0.3 where the inhibition zones are the largest. Between the samples there are no major differences for the same bacteria strain. For example, in case of *E. Coli*, all the samples showed similar inhibition zones with diameters between 29 mm-31 mm. In case of *S. Aureus*, there are minor differences. The lowest concentration of chitosan showed an inhibition zone of 35 mm, while the highest concentration proved to have an inhibition zone of 30 mm.

A real difference is observed between the two bacteria strains. The antibacterial effect is higher for *S. Aureus* compared to *E. Coli*. For all samples, in case of *S. Aureus* the inhibition zones are higher, starting from 30 mm.

This qualitative and semiquantitative test realized proved that the samples obtained in the laboratory are able to kill bacteria organisms, therefore their integration in medical devices as antibacterial agents is a possible approach.

IV. EXPERIMENTAL RESEARCH

EXPERIMENTAL RESEARCH REGARDING THE HYDROGEL

1. AIM OF THE EXPERIMENTAL RESEARCH

The aim of this entire research was the design and the synthesis of a hydrogel based on functionalized silver nanoparticles for infected wounds and burns.

The need of hydrogel as final application is due to the possibility of industrialization. Being a product designed and produced in the laboratory, the hydrogel can be obtained on a macro scale with low cost. The low-cost can be justified by the small number of reagents needed, by the low cost of chitosan and by the small number of equipment needed for its synthesis.

2. POLYMERIC HYDROGEL

The hydrogel synthesis was performed using a chemical synthesis starting from chitosan powder. Using a protocol established in the laboratory, the hydrogel was prepared for several days and was dried at high temperature in the oven. Figure 11 shows the result of hydration of the polymeric hydrogel synthesized in this doctoral thesis as medical device for infected wounds. The results proved a high resistance of the hydrogel during manipulation, therefore the possible industrial product was achieved during all the syntheses.

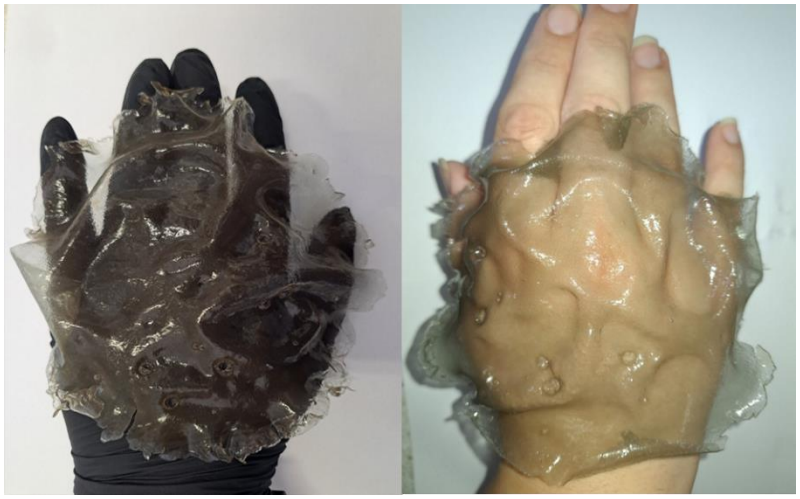

Figure 11. Hydrated polymeric hydrogel for wounds

FINAL CONCLUSIONS

The doctoral thesis addresses the fabrication of a polymeric hydrogel as a necessity in infections treatments. The wounds and burns infections are a worldwide problem, yet in Romania the number of hospitalized infections is a serious problem.

The purpose of this doctoral thesis was achieved by accomplishing all the objectives proposed.

Chapter II described the *Direct Particle Tracking* technique which was developed in this research project in the stage of mathematical simulations. The applied research performed in this step of the thesis was based on simulations realized on Ag NPs suspensions on which many sets of analysis were conducted. All three simulations sets performed showed that the method is valid for nanoparticles sizing. The simulations histograms showed a random distribution of nanoparticles using the same conditions as in the laboratory and the gaussian histograms proved that the nanoparticles simulated were obtained in the nano range. From 30 nm up to 160 nm, the histograms showed consistent distributions for the values used in the simulations, therefore the accuracy of the method was proved.

The procedure was tested on experimental data, as well, on silver nanoparticles suspended in ultrapure water in the laboratory. This research is a work in progress since in the sample cavity there are some convectional currents that appear leading to artifacts in the diffusion process. A possibility of the current appearance may be the laser power that hit the sample, so the next step is to reduce the dimension and the thickness of the sample cavity and the laser power.

Chapter III revealed a comparison of silver nanoparticles syntheses based on characterization results. There were investigated dimensional and chemical characteristics of the particles obtained. Silver nanoparticles were synthesized starting from precursor materials in two chemical approaches: a chemical reduction using trisodium citrate dihydrate and a chemical reduction based on D-glucose. The silver precursor was the same in both cases, namely $AgNO_3$. Through the comparative analysis, it was proved that nanoparticles obtained using trisodium citrate hydrate are smaller compared to the ones obtained using glucose. The ATR-FTIR and UV-VIS analysis confirmed the silver nanoparticles formation since the reduction of silver nitrate was sustained by modifications in FTIR spectrum. The results from UV-VIS spectra showed specific peaks in both syntheses, yet a sharper peak at around 460

nm was identified in case of silver nanoparticles synthesized using trisodium citrate dihydrate. DLS measurements showed a big difference between both methods. The nanoparticles based on trisodium citrate reduction were measured at around 58 nm, while the reduction using glucose showed nanoparticles up to 1 micron. This aspect was confirmed by AFM measurements, where the difference between nanoparticles was similar: 49 nm versus 943 nm. The small dimensions obtained through the reduction based on trisodium citrate encourage the use of these silver nanoparticles in biomedical applications, more precisely in antibacterial applications where the effect is higher once the nanoparticles are in nanometric sizes.

Chapter IV exposed the functionalization process of silver nanoparticles. For the functionalization two syntheses were proposed: a protocol established in this doctoral thesis and a classical protocol for chitosan functionalization. The choice of chitosan was due to an increase in nanoparticles biocompatibility since silver nanoparticles are metallic particles. There were established three chitosan concentrations in every synthesis to observe if they can modify the nanoparticles dimensions and their antibacterial effect: 0.3%, 0.6% and 0.9%. Both protocols are based on chemical functionalization, yet the reagents and the conditions are different. The first functionalization performed was based on chitosan and trisodium citrate dihydrate. The second functionalization, the classical treatment, was based on chitosan and NaOH. The comparison between both methods was performed on analysing the differences obtained after characterization. ATR-FTIR results showed that in both syntheses, $AgNO_3$ reduction occurred since its specific peak from 1339 cm^{-1} is no longer observed in samples spectra. Also, the functionalization with chitosan was suggested by FTIR, yet this result needs the confirmation of the other techniques performed. Therefore, UV-VIS analysis exposed the nanoparticles' formation since no silver ions were recorded in neither samples' spectrum. In case of Ag NPs-TSC-CH the peaks obtained in UV-VIS results proved the presence of silver nanoparticles since their absorption begins at 350 nm with a maximum at around 420 nm. For Ag NPs-NaOH-CH the peak for nanoparticles was recorded at around 450 nm, a specific value for synthesized Ag NPs, yet higher comparing to Ag NPs-TSC-CH. This observation already suggested a difference in nanoparticles sizes, so DLS measurements were performed. Since DLS is size determination technique, the viscosity of each suspension is needed. Therefore, the rheological properties of all six samples were tested and their viscosities were determined. It can be concluded that once the chitosan concentration increases, their viscosity also increases. DLS measurements confirmed the observations made in UV-

VIS analysis. For Ag NPs-TSC-CH were recorded smaller sizes for nanoparticles compared to the classical functionalization treatments: 53 nm for chitosan 0.3%, 72 nm for chitosan 0.6% and 55 nm for chitosan 0.9%. For the same chitosan concentrations, the samples Ag NPs-NaOH-CH showed dimensions of 14 nm, 73 nm, 317 nm, therefore a significant difference. Regarding their physical-chemical characterization, the samples Ag NPs-TSC-CH proved to be more suitable for the final application of this thesis since their dimensions were similar and the method was more accurate. However, antibacterial tests were performed for both syntheses' protocols. After the characterization, the antibacterial effect for both syntheses was tested using the diffusion disc method on two bacteria strains: a gram-negative strain, *Escherichia coli* and a Gram-positive, *Staphylococcus aureus*. In this antibacterial test, the first analysis was conducted on controls to exclude their possible implications in bacteria inhibition. The antibacterial test performed on TSC 1%, NaOH 0.1M and chitosan 0.3% showed in both bacteria strain that no inhibition zone appears, therefore the antibacterial effect is due only to the functionalized silver nanoparticles. The test on Ag NPs-TSC-CH showed that they are able to inhibit bacteria proliferation by obtaining a big halo around the impregnated discs in both *Escherichia coli* and *Staphylococcus aureus* Petri dishes. This halo is associated with their inhibition zone which was around 30 mm for both bacteria. The smallest chitosan concentration proved to have the strongest effect in both microorganisms since its inhibition zones were the biggest. The Ag NPs-NaOH-CH samples also exhibit a strong antibacterial effect on both bacteria strains since their inhibition zones were similar as diameters as Ag NPs-TSC-CH. From this point of view, there were not big differences between the two protocols, even if their dimensions are clearly different. Yet, an important difference was obtained between *Escherichia coli* and *Staphylococcus aureus*. In case of *Staphylococcus aureus,* the antibacterial effect was stronger since the inhibition zone was larger in diameter for both functionalization protocols. Once the characterizations were completed, the Ag NP-TSC-CH0.3 was chosen for further applications since it possesses the strongest antibacterial effect for both bacteria strains and the particles are still around 50 nm.

Chapter V described the final application of this research. A chitosan hydrogel with chitosan functionalized silver nanoparticles (Ag NP-TSC-CH0.3) was obtained and analysed using optical microscopy with a 4k camera attached on the microscope. Therefore, the real time observations and the videos realized on the hydrogel showed the shadows of nanoparticles in all layers. Unfortunately, the optical microscopy is not

enough to affirm that there are individual nanoparticles in the hydrogels, yet their presence was proved. Future tests will be made on the hydrogel obtained in this thesis to identify better its morphology and its properties.

FUTURE DIRECTIONS

Regarding the *Direct Particle Tracking* method, the future experiments will be focused on eliminating the currents appeared in the small cavity with the sample so only the movement of the diffused particle to be recorded since the software program proved to offer optimal results. Therefore, the experiments in the laboratory will be performed on the functionalized silver nanoparticles obtained.

Silver nanoparticles functionalization using many other different compounds and determination of their antibacterial effect is another strategy for future directions since it was proved that developing a protocol in the laboratory can lead to promising results.

Another strategy which will be continued is related to the hydrogel synthesis and testing. In this doctoral thesis a high dry temperature was used, so possible silver nanoparticles aggregation clusters may occur, therefore the reaction parameters modification is a future direction. Furthermore, a detailed characterization for the hydrogel will help us understand better how silver nanoparticles integrate in the chitosan matrix, so future research will clarify these aspects.

PERSONAL CONTRIBUTION

PERSONAL CONTRIBUTIONS TO THIS DOCTORAL THESIS

The present doctoral thesis has contributed to an improvement of materials science and industrial engineering fields through its relevant theoretical information and applied research in nanoparticles synthesis and functionalization and in the design of a possible commercial product based on hydrogel.

The main personal contributions resulted from this doctoral thesis are:

1. The development of an innovative characterization technique, *Direct Particle Tracking*, for nanoparticles precise size determinations based on their diffusion.

2. The experimental research regarding the comparison of two chemical syntheses for silver nanoparticles.

3. The physical-chemical size characterization using a custom DLS and AFM techniques since in general, electronic microscopy is used for size determinations. This thesis proposed another approach for size measurements.

4. The proposal of a personalized protocol based on trisodium citrate dihydrate and chitosan for silver nanoparticles functionalization.

5. The experimental research regarding a comparison of the physical-chemical characterization and of antibacterial effects for both functionalization approaches based on multiple chitosan solutions for silver nanoparticles.

6. The conception, design and fabrication of an easy handle, low-cost polymeric hydrogel which could be industrialized as a medical product.

PUBLICATION LIST, CONFERENCES AND RESEARCH PROJECTS PARTICIPATIONS

* LIST OF ARTICLES PUBLISHED IN WoS JOURNALS

1. **Nicolae-Maranciuc Alexandra**, Chicea Dan, Chicea Liana Maria. *Ag nanoparticles for biomedical applications - Synthesis and characterization - A review.* International Journal of Molecular Sciences **2022**, 23, 5778.
DOI: https://doi.org/10.3390/ijms23105778
INDEX: WoS, IF 5.924 Q1, AIS Q2

2. Chicea Dan, **Nicolae-Maranciuc Alexandra***, Doroshkevich Aleksandr S, Chicea Liana Maria, Ozkendir Osman Murat. *Comparative Synthesis of Silver Nanoparticles: Evaluation of Chemical Reduction Procedures, AFM and DLS Size Analysis*. Materials **2023**, 16, 5244.
DOI: https://doi.org/10.3390/ma16155244
INDEX: WoS, IF 3.4 Q2, AIS Q1
*Corresponding author

* LIST OF ARTICLES PUBLISHED IN OTHER DATABSES

1. Chicea Dan, Codescu Mirela Maria, **Nicolae Alexandra**, Doroshkevych Oleksandr, Islamov Akhmed, Kulik Miroslaw. *Nanoparticles Size Distribution Assessment During Early Synthesis Stages*. MATEC Web Conf. **2021**, 343 01005.
DOI: https://doi.org/10.1051/matecconf/202134301005

* LIST OF CONFERENCE PROCEEDINGS

1. Chicea Dan, **Maranciuc Alexandra**. *Direct Optical Particle Tracking for Particle Size Distribution Assessment*. 8th International Conference on Sensors Engineering and Electronics Instrumentation Advances (SEIA' 2022), 21-23 September **2022**, Corfu, Greece, p. 203-210, ISBN: 978-84-09-43854-9

* ORAL PRESENTATIONS AT INTERNATIONAL CONFERENCES PRESENTED BY THE THESIS AUTHOR

1. Chicea Dan, Codescu Mirela Maria, **Nicolae Alexandra**, Doroshkevych Oleksandr, Islamov Akhmed, Kulik Miroslaw. *Nanoparticles Size Distribution Assessment During Early Synthesis Stages*. 10th International Conference on Manufactoring Science and Education (MSE 2021), 02-04 June **2021**, Sibiu, Romania

2. Chicea Dan; **Maranciuc Alexandra**. *Ag-Nps for biomedical applications- synthesis and characterization.* 20th International Balkan Workshop on Applied Physics and Materials Science (IBWAP 2022), 12-15 July **2022**, Constanta, Romania

3. Chicea Dan; **Nicolae-Maranciuc Alexandra**. *Using direct particle tracking to assess Fe3O4*. 2nd World Conference on Materials Science and Nanotechnology, 26-28 May **2023**, Brussels, Belgium

4. Chicea Dan; Neague Petru.; **Nicolae-Maranciuc Alexandra**. *A dimensional and structural characterization of Ag-Nps*. 21st International Balkan Workshop on Applied Physics and Materials Science (IBWAP 2023), 11-14 July **2023**, Constanta, Romania

* POSTER PRESENTATIONS AT INTERNATIONAL CONFERENCES PRESENTED BY THE THESIS AUTHOR

1. Chicea Dan; **Maranciuc Alexandra**. *Direct Optical Particle Tracking for Particle Size Distribution Assessment.* 8th International Conference on Sensors Engineering and Electronics Instrumentation Advances (SEIA' 2022), 21-23 September **2022**, Corfu, Greece

2. **Maranciuc Alexandra**; Chicea Dan; Codescu Mirela Maria. *A comparison of silver micro and nanoparticles with different synthesis methods*. 5th International Conference on Emerging Technologies in Materials Engineering (EmergeMAT), 27-28 October **2022**, Bucharest, Romania

* POSTERS

1. Chicea Dan; **Maranciuc Alexandra**; Olaru Sorin. *Nanoparticles size distribution assessment by Direct Optical Particle Tracking*. TIM 22 Physics Conference, 23-25 November **2022**, Timisoara, Romania

* PARTICIPATION IN INTERNATIONAL PROGRAMS FOR PhD STUDENTS

1. Visiting PhD student in an international exchange programme at **Institute of Human Genetics PAS in Poznan, POLAND** from 28 March to 1 April **2022**.

2. Participation at **European School On Nanosciences & Nanotechnologies (ESONN 2023) at Université Grenoble Alpes in Grenoble, FRANCE** from 27 August to 9 September **2023**: participation in lectures and practical works.

* RESEARCH PROJECTS PARTICIPATION AS MEMBER IN THE RESEARCH TEAM

1. International project between Joint Institute Nuclear Research, Dubna and Romania (**2021**):

Study of spatial distribution of particles in liquid media with different water content and energy parameters of ZrO_2 nanopowder dispersion system using DLS and SANS methods

2. Excellence Hasso Plattner (LBUS-HPI-ERG-2020-07) grant from Lucian Blaga University of Sibiu (**2021-2022**):

Development of advanced numerical algorithms and robust computational protocols for dimensional and structural characterization of nanostructures

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