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PREPARATION, STUDY OF THE PROPERTIES AND POTENTIAL APPLICATIONS OF SELECTED NANOMETALS (Au, Ag, Cu)

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Abstract

Metallic nanoparticles, such as copper, gold, and silver nanoparticles, are becoming the subject of intensive research due to their antibacterial effects and a wide range of applications in medicine and industry. The testing of their bactericidal properties is coming to the forefront of interest precisely because of their potential use in combating pathogenic organisms. This topic is gaining importance in context of increasing microbial resistance to antibiotics and emergence of multi-resistant strains associated with increased mortality.

The research conducted within this dissertation focuses on preparation and characterization of the aforementioned metallic nanoparticles and testing of their antimicrobial effects using standard methods employed for testing antibacterial effects (dilution and disk diffusion methods). An important factor is stability of the prepared metallic nanoparticles, which affects their behavior in biological systems and influences their potential therapeutic applications. Properties of nanoparticles, such as size, shape, and surface functionalization, play a key role in examining their interaction with biological systems. Testing the antimicrobial effects of nanoparticles using classical methods thus encounters a complex problem related to the behavior of nanoparticles in the test environment.

We focused on this problem when testing the stability of silver nanoparticles in media used for ecotoxicity testing. The stability of the prepared nanoparticles was tested depending on the properties of the test medium. Describing the behavior of metallic nanoparticles in different environments is also relevant for assessing their potential ecotoxicity.

In the context of bone tumor treatment, specifically osteosarcoma, targeted therapy is currently gaining importance, which can utilize the functionalization of nanoparticles to optimize their distribution and effector properties. The uptake process of nanoparticles in target cells is crucial for their therapeutic effect and requires a detailed understanding of the mechanisms of interaction between nanoparticles and biological structures. For this purpose, gold nanoparticle systems of different sizes (5 nm, 50 nm) were prepared and functionalized with signaling peptides (CPP, NLS) and the uptake by osteosarcoma tumor cells was examined.

The results of the work suggest that metallic nanoparticles have a potential to represent a revolutionary approach in various medical applications, including cancer treatment, but require further research focused on their safety and efficacy.

Abstrakt

Kovové nanočástice, jako jsou nanočástice mědi, zlata a stříbra, se stávají předmětem intenzivního výzkumu díky svým antibakteriálním účinkům a široké škále aplikací v medicíně a průmyslu. Do popředí zájmu se dostává testování jejich baktericidity právě pro jejich potenciální uplatnění v boji proti patogenním organismům. Toto téma nabývá na významu v kontextu narůstající mikrobiální rezistence na antibiotika a vzniku multirezistentních kmenů spojených s nárůstem mortality.

Výzkum provedený v rámci této disertační práce se zaměřuje na přípravu a charakterizaci výše uvedených kovových nanočástic a testování jejich antimikrobiálních účinků standardními metodami využívanými pro testování antibakteriální účinků (diluční a disková difúzní metoda). Důležitým faktorem je stabilita připravených kovových nanočástic, která ovlivňuje jejich chování v biologických systémech a má vliv na jejich potenciální terapeutické aplikace. Vlastnosti nanočástic, jako jsou velikost, tvar a funkcionalizace jejich povrchu, hrají klíčovou roli při zkoumání jejich interakce s biologickými systémy. Testování antimikrobiálních účinků nanočástic klasickými metodami proto naráží na komplexní problém související s chováním nanočástic v testovacím prostředí.

Na tento problém jsme se zaměřili při testování stability stříbrných nanočástic v médiích používaných pro testování ekotoxicity. Byla testována stabilita připravených nanočástic v závislosti na vlastnostech testovacího média. Popis chování kovových nanočástic v různém prostředí je aktuální i pro hodnocení jejich potenciální ekotoxicity.

V kontextu léčby kostních nádorů, resp. osteosarkomu nabývá v současnosti na významu cílená léčba, která může využívat funkcionalizaci nanočástic za účelem optimalizace jejich distribučních a efektorových vlastností. Proces uptake nanočástic v cílových buňkách je zásadní pro jejich terapeutický účinek a vyžaduje podrobné pochopení mechanismů interakce mezi nanočásticemi a biologickými strukturami. Pro tento účel byly připraveny nanočásticové systémy zlata různých velikostí (5 nm, 50 nm) a funkcionalizovány signálními peptidy (CPP, NLS) a zkoumán uptake nádorovými buňkami osteosarkomu.

Výsledky práce naznačují, že nanočástice kovů mají potenciál představovat revoluční přístup v různých zdravotnických aplikacích včetně onkologické léčby, avšak vyžadují další zkoumání zaměřená na jejich bezpečnost a efektivitu.

Keywords

Metallic nanoparticles, copper nanoparticles, gold nanoparticles, silver nanoparticles, antibacterial effects of metallic nanoparticles, bactericidal testing, stability of metallic nanoparticles, bone tumors, targeted therapy, osteosarcoma, nanoparticles uptake.

Klíčová slova

Kovové nanočástice, nanočástice mědi, nanočástice zlata, nanočástice stříbra, antibakteriální účinky kovových nanočástic, testovaní baktericidity, stabilita kovových nanočástic, kostní nádory, cílená léčba, funkcionalizace nanočástic, buněčné linie.

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1 Introduction

Metal nanoparticles exhibit highly specific properties that distinguish them from their macroscopic forms. Metal nanoparticles have unique physical and chemical properties due to their size, which ranges from units to tens of nanometres. Understanding these properties is crucial for the further development of nanotechnology, as well as for potential applications in areas such as biomedicine, electronics, optics, catalysis, sensing or environmental technologies, among others.

At a time of increasing antibiotic resistance associated with the emergence of multidrug-resistant bacteria and the threat of increasing mortality from infectious diseases associated with them, metal nanoparticles are being tested as potential antimicrobials. Metal nanoparticles, when interacting with bacterial cell, offer a different mechanism of action than previously used antibiotics and thus could be a suitable candidate for treatment of infections. However, there is no uniform standardised procedure for testing the antimicrobial effects of nanoparticles. The actual application of metal nanoparticles in the treatment of infections requires understanding of behaviour of nanoparticles in the test environment, or their interactions with bacterial cells or their interactions in the whole organism.

With massive increase in production and application of nanoparticles, the question of their safety, both at the level of interaction with the human body and in the event of their release into the environment, arises. Cytotoxicity and ecotoxicity testing of not only metallic nanoparticles are still a hot topic and research challenge, where the stability and behaviour of nanoparticle systems under given test conditions must be taken into account.

Due to the large surface to volume ratio of metal nanoparticles, metal nanoparticles offer unique opportunities to functionalize their surface with different molecules. Thus, gold nanoparticles, due to their relatively low toxicity and excellent affinity for e.g. thiol groups, offer an excellent substrate for functionalization of their surface with various signaling molecules or chemotherapeutics. Such nanoparticles can be used, for example, for targeted cancer therapy. Compared to standard chemotherapy and radiotherapy, radiosensitization treatment using gold nanoparticles could be more gentle for the patient and thus reduce the total radiation dose applied.

2 Theoretical part

2.1 Nanotechnology and nanomaterials

Nanotechnology refers to a branch of science and engineering devoted to materials with dimensions of 100 nm or less. Nanomaterials are defined as materials that consist of nanoparticles of which at least 50 % have one or more external dimensions between 1 and 100 nm.[1–3]

These new nanomaterials exhibit different physicochemical behaviour compared to their "macro" counterparts. This difference can be attributed to the high

surface to volume ratio of the nanomaterials. The surface atoms and surface energy are strongly related to the properties of the nanomaterials, leading, for example, to a decrease in the lattice parameter values or to a decrease in the melting point values. The high number of surface atoms and high surface energy of nanoparticles strongly influence their catalytic properties. If the solid contains fewer atoms, then a lower number of orbitals contributes to the band formation. This effect leads to changes in the conduction bands in nanoparticles, and these changes are similar to changes in the size of the forbidden band in semiconductors and depend on the dimensions of the nanomaterial.[1]

Nanoparticles were studied a decade ago primarily for their special size-dependent physical and chemical properties, allowing for greater surface functionality, giving nanoparticles special physical, electronic, optical and magnetic properties[2]. Currently, nanoparticles have entered commercial research[3] and in recent years nanotechnology has spread to many industries. Applications in many areas include: electronic storage systems, photovoltaics, gas storage, catalysis, electrical and optical devices, magnetic separation and preconcentration of target analytes, biotechnology, diagnostics, but also in the areas of targeted drug and gene delivery[3–8]. Given the wide range of applications, such nanosystems have the potential to have a profound impact on our society.[2]

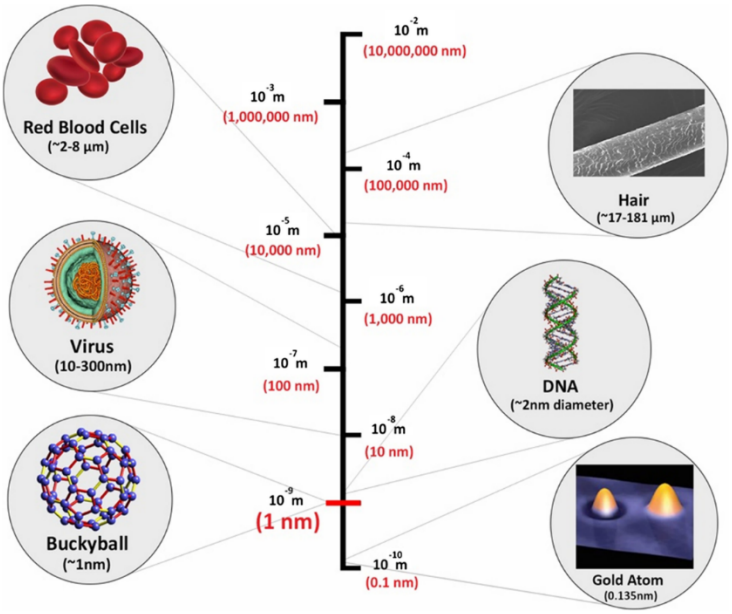

Figure 1: *Comparison of the size of nanomaterials (on a logarithmic scale) with biological objects and definition of the sizes "nano" and "micro".*[9]

The aforementioned properties also make nanoparticles an excellent candidate for biomedical applications, not only because various biological processes take place at the nanometer scale. Living organisms are made up of cells whose usual dimensions are approximately 10 µm. However, parts of cells are much smaller and are of sub-micron range. Proteins are even smaller with a typical size of only 5 nm, which is

comparable to the size of the smallest man-made nanoparticles[3]. This simple size comparison (Figure 1) gives an idea of the use of nanoparticles as very small probes that would allow us to study cellular mechanisms without introducing too much interference themselves[10]. Understanding biological processes at the nanoscale level is a strong driver for the development of nanotechnology.

2.2 Nanoparticles

The term "nanoparticle" or the prefix *"nano"* comes from Greek and means "dwarf or small". It refers to a size of 10^{-9} metres. The first references to nanoparticles date back to the 4th-5th century BC in Egypt and China. Back then "soluble gold" was discovered and used for aesthetic or medical purposes[11]. Perhaps the most famous example of nanotechnology is the Lycurgus Cup, made between 290 and 325 AD in Alexandria or Rome. The glass of the cup is dichroic, which means that in incident light it looks like jade with an opaque greenish-yellow tone, but in transmitted light it changes to a ruby colour. This property is imparted to the glass by dispersed nanoparticles of gold and silver.[12] In 1857, Faraday prepared colloidal gold by reducing an aqueous solution of tetrachloroauric acid[13]. The term colloid comes from the Greek words "*kolla*", which means "glue", and "*eidos*", which means "like"[14]. It was first used by Thomas Graham in 1861[15] to refer to mixtures such as starch and gelatin in water[16]. A colloid usually refers to a dispersion with particle sizes ranging from 1 nm to 1000 nm (or 500 nm).

Compared to larger particles, nanoparticles typically have a 35-45% higher surface-to-volume ratio.[17] This high ratio value also affects various intrinsic properties of nanoparticles such as their high surface reactivity.[17]

Nanoparticles exist in a variety of chemical compositions from micelles to metals (or their oxides), from synthetic polymers to large biomolecules. Various classifications of nanomaterials have been introduced over the last two decades. One way of classification is the division into natural and synthetic nanoparticles. Another way is to classify them according to their spatial arrangement into 3D, 2D, 1D and 0D. A practical classification was proposed by Tuominen et al.[18] who divided nanomaterials into five groups: metal-based materials, carbon-based materials, polymeric nanomaterials, dendrimers and composites.

Each of these materials has a completely different chemistry. Already when preparing the actual nanoparticles, their further application must be considered. Nanoparticles can be prepared by different methods to achieve the desired sizes and shapes and thus the desired chemical and physical properties[4,19]. Size and shape affect the capacity for nanoparticle functionalization, fluid resistance and diffusion, optical properties and cellular uptake of nanoparticles. Surface charge, in addition to controlling the stability of the colloidal suspension and its tendency to aggregate, plays a major role in shaping the interactions between nanoparticles and the surrounding environment.[1]

A key issue that hinders the use of nanoparticles in industry is their reproducibility. However, this problem is partly inherent in nanoparticles, as the

product of synthesis is always prone to polydispersion, sometimes with a wide distribution of sizes, shapes and defects[1].

The characterization of nanoparticles is therefore a crucial step needed to fully understand the behavior of nanoparticles and subsequently translate their performance advantages from the laboratory to specific real-world applications. Determining the physicochemical properties of nanoparticles and exploring their structure-function relationships is a challenge for science nowadays. These efforts are limited by our ability to fully explore the nanoscale domain - different characterization techniques are based on different physical properties of the materials, and therefore each of these analytical methods provides only partial information about a given parameter.[1]

2.3 Nanoparticles synthesis methods

Nanoparticles synthesis methods may be divided into "bottom up" approaches, which are based on the construction of nanoparticles "from below", i.e. atom by atom, respectively. In contrast, "top down" methods are based on the gradual division of bulk samples into smaller particles - dispersion methods (grinding, etching, ablation, cutting, sputtering, thermal decomposition, etc.)[8,9,17,20]

Although nanoparticle preparation by top-down methods is relatively simple, it is not very suitable for the preparation of irregularly shaped nanoparticles and small-sized nanoparticles[17]. Disadvantages of top down approaches include surface structural defects arising during some synthesis methods. These defects have a negative impact on the physical properties and surface chemistry of metal nanoparticles[17].

2.3.1 Physical methods

Physical methods usually lead to particles with a large variation in diameters. Colloidal particles prepared by these methods often have diameters greater than 10 nm and are less reproducible[17].

For example, nanoparticles can be prepared by mechanical grinding of lump materials and stabilizing them by adding capping agents. Incorporated nanoparticles are usually polydisperse and may be contaminated with the grinding material[8,17]. While these methods are advantageous for their simplicity, they are energy intensive and some methods are less suitable for preparing particles of the desired size.

Other techniques include e.g. laser ablation providing very pure nanoparticles, but in small quantities[21,22]. The size of the nanoparticles can be varied by the used laser energy[22]. Other methods include sputtering and PVD (physical vapor deposition).[8,17]. Current requirements for nanoparticles include the achievement of a defined diameter and composition of their surface, as well as the requirement for reproducibility of the synthesis itself, properties and ease of separation of the nanoparticles.

2.3.2 Physical - chemical methods

There are methods using, for example, decomposition of suitable organometallic compounds, pyrolytic methods, electrolytic methods, hydrothermal synthesis, radiation-assisted reduction reactions - photochemical synthesis, radiolysis, microwave or ultrasonic methods, and others.[7,8,17,23].

2.3.3 Chemical methods

Chemical methods of preparation of metal nanoparticles are most commonly used in practice as they allow easy and controllable preparation of nanoparticles of defined size, crystallinity and composition[21]. In addition, plasmon absorbance and fluorescence can also be controlled to transmit electronic and photonic signals by appropriately chosen nanoparticle size. A disadvantage may be the use of reducing agents that are toxic (hydrazine, borohydride, diborane, silanes, etc.).

The most widespread and very easy to perform method for the preparation of colloidal metal suspensions consists in the reduction of transition metal salts. The synthesis of metal nanoparticles in solution requires a metal precursor, most often in the form of the appropriate salt of the nanometal being prepared, a reducing agent and a stabilizing agent. A wide range of reducing agents are used for this purpose, e.g. hydrazine, hydrides ($NaBH_4$), reducing sugars e.g. glucose, galactose, fructose (Tollens method[24,25])[8], salts of organic acids - e.g. sodium citrate (Turkevich method)[26,27], ascorbic acid (vitamin C)[28], phenolic substances such as hydroquinone[29], pyrogallol[30] or gallic acid[31] and many others. This method allows preparation of colloidal metal nanoparticles of desired size with a small variation up to gram quantities.

2.4 Metal nanoparticles

2.4.1 Silver nanoparticles AgNPs

Silver-based nanomaterials in various forms such as particles, wafers and wires are used as components in a variety of products and applications. Silver nanoparticles are one of the most studied nanomaterials due to their wide range of applications. These materials are of great interest due to their strong antimicrobial properties against bacteria, viruses and fungi. In addition, silver nanoparticles can be used as disinfectants and show synergistic effects with antibiotics.[2,21]

Silver nanoparticles are used as antibacterial agents in cosmetics, medical products, bandages, textile fabrics and coatings, as well as in clinical applications such as the treatment of chronic wounds, burns and diabetic defects. Silver nanomaterials (especially those with diameters $\leq$ 10 nm) are toxic to many human cell lines. Their toxicity is dependent on factors such as size, exposure time and dose. In order to minimalize this problem, immobilization of these structures on various support materials such as polymers, activated carbon, metal oxides and graphene oxide has been investigated.[2,21]

2.4.2 Gold nanoparticles AuNPs

Gold-based nanomaterials with antimicrobial properties can be created in different ways. Nanomaterials containing gold include nanodots, nanodot clusters and nanoshells. Optimal properties of these materials may be achieved by conjugating them with other compounds or by modifying their nanostructure.[21,32,33]

Antimicrobial activity can also be achieved by conjugating gold nanoparticles with antibodies and various antimicrobial agents. Gold nanoparticles conjugated with antibiotics exhibit potent antimicrobial activity against various bacteria and antibiotic resistant strains. Nanoclusters of metals such as silver, copper and gold are used as antibacterial agents.[2,21,34]

Gold nanoclusters exhibit excellent properties in imaging, detection and biomedical applications. Gold nanoclusters conjugated with various surface ligands are widely used for therapeutic purposes due to their easy modifiability, pleiotropic effects, photothermal stability and high biocompatibility. In pharmacology, gold nanoparticles offer antiangiogenic, anti-HIV, antimicrobial and antiarthritic effects. Biomedical applications of these materials include gene therapy, drug targeting, diagnostics and catalysis. [8,11,35,36]

2.4.3 Copper nanoparticles CuNPs

Nanomaterials based on copper and copper oxide are of great interest due to their unique properties that enable their use in many fields such as sensors, optics, solar cells, catalysts, electronics, remediation applications and antimicrobials. Copper is a highly conductive material and is cheaper than materials such as gold and silver. Since copper oxide is thermodynamically more stable than elemental copper, most synthesized copper nanoparticles have a surface oxide layer. Copper oxide nanostructure is also a p-type semiconductor with a monoclinic structure and high dielectric constant. The generally accepted mechanism of antibacterial action of copper-based nanomaterials is based on the release of copper cations. Copper cations can damage the bacterial cell membrane and enter the cells where they interfere with the function of enzymes, DNA, leading to death of the bacterium. Copper nanoparticles are highly reactive antimicrobial materials due to their high surface to volume ratio. Among metal oxide nanoparticles, copper oxide nanoparticles are of great importance because they are the simplest member of the copper group and they have important antimicrobial properties that inhibit the growth of viruses, fungi, bacteria and algae.[5,37–39]

2.5 Properties of metal nanoparticle colloids

One of the most important aspects in the field of metal colloids is the mechanism of stabilization of metal nanoparticles in the dispersion medium. Nanoparticles are generally unstable and tend to agglomerate because van der Waals, electrostatic, magnetic and other forces act on the short distances between the particles. In the absence of repulsive forces, nanoparticles aggregate, agglomerate, or

undergo coalescence processes. Exemplarily, these repulsive forces can be achieved by electrostatic or steric stabilization.[40]

Table 1: *Forces affecting the stability of nanoparticles in solution.*[41]

Powers	**Impact**
Van der Waals interactions	Electromagnetic force between short range NPs, attractive nature of interaction.
Electrical double layer	Electrical interaction between NPs by overlapping an electrical double layer, usually repulsive.
Hydrating forces	Interaction between water molecules on hydrophilic NPs, repulsive nature.
Hydrophobic forces	Attractive interaction between hydrophobic NPs in water.
Steric, electronic and electrostatic forces	Adsorption of inorganic ions, surfactants, polymers and polyelectrolytes on the NP surface. Polymers can form osmotically acting bridges and lead to interpenetration of chains. Surface layers may have attractive or repulsive effects.

The particles of the dispersion phase, which carry an electric charge, move towards the dispersion medium due to the induced electric field. This phenomenon is generally known as electrokinetic phenomena. Electrokinetic phenomena can be divided into two main groups. The first group consists of the mechanical motion caused by an external electric field, which includes electrophoresis and electroosmosis.[42] The second group consists of phenomena where mechanical motion causes the generation of an electric field, such as the sedimentation potential (called the Dorn potential - it is the inverse of electrophoresis) and the flow potential. These phenomena are caused by the existence of an electric charge at the phase interface between the dispersed phase particles and the dispersion medium and the arrangement of the charge near this interface. [43]

Interfacial interactions are caused by both internuclear interactions and interactions between ligands and between ligands and solvent. Thus, their extent depends on the chemical and physical properties of both the nanoparticles (core and surface ligands) and the medium in which the nanoparticles are located. The combination of these factors contributes to the overall stability of the nanoparticles or their ability to aggregate[40,44,45].

The interest in controlling the assembly of nanoparticles into specific extended structures has greatly promoted the development of strategies that allow the control and manipulation of interactions between them using a wide range of external stimuli. The ability to reversibly aggregate nanoparticles in solution depends on thermodynamic and kinetic parameters. In terms of thermodynamics, the interactions between nanoparticles are controlled by the total pair potential, which is the sum of attractive and repulsive interfacial forces[45]. When the repulsive interactions between the particles dominate, the nanoparticles are stable in solution, while when the

attractive forces dominate, they are unstable and aggregate or may occur in the reversible aggregation band. Knowledge of these phenomena offers an understanding of nanoparticle interactions and enable their controlled synthesis. [40,45]

Aggregation instability is typical for very small particles, the smaller the particles are and the higher their concentration, the more the system tends to aggregate.[42] Therefore, for a colloidal system to be stable, we must create a sufficient energy barrier to prevent the particles from aggregating and thus the surface energy from decreasing. Stabilization of metal nanoparticles is most often performed by methods based on electrostatic repulsion or the steric effect.

2.5.1 Potential ζ

For colloidal metal nanoparticles, the Stern model is the most applicable. It is the electrostatic repulsion between the particles that is the key factor affecting the stability of colloidal solutions. In an electrolyte solution, the interaction of free ions with the surface charge of the particle can lead to the screening of the electrostatic potential of the nanoparticle and, as a result, lead to undesirable aggregation of the nanoparticles. The difference in electrical potential between the stationary charge layer surrounding the particle and the solution potential is referred to as the ζ (*zeta*) potential.[46]

The layer of liquid tightly surrounding the particle is divided into two parts; an inner region, called the Stern layer, where the ions are strongly bound, and an outer, diffusion region, where the ions are less tightly bound. Within the diffusion layer, there is a theoretical boundary within which the ions and particles form a stable unit. When the particle moves (e.g., due to gravity), the ions inside the boundary move with it, but any ions beyond the boundary do not move with the particle. This boundary is called the surface of hydrodynamic shear, or the plane of slip. The potential that exists at this boundary is known as the ζ potential.[46]

The magnitude of the potential ζ indicates the potential stability of the nanoparticle colloidal system. If all particles in suspension have a large negative or positive potential ζ, they tend to repel each other, and there is no tendency for them to aggregate. However, if the particles have low values of ζ potential, then there is no repulsive force to prevent the nanoparticles from aggregating.

The ζ potential of particles in solution is affected by the ionic strength of the solvent, the presence of charged or uncharged molecules that can adsorb to the particle surface, and the pH of the solution. Especially when working with particles that have (de)protonatable groups on their surface, the pH value at the pH zero charge point (pzc) is important.[46]

2.6 Characterization of metal nanoparticles

Nanoparticles and nanomaterials have received a lot of attention in recent years due to their wide application potential and their special properties. It is a rapidly growing class of materials. This is linked to the need for their detailed characterisation.

The characterization of nanoparticles is a vast and complex discipline. Many methods can be used to characterize the size, crystal structure, elemental composition and many other physical properties of nanoparticles.[1]

The four basic properties, size, shape, surface charge and porosity of nanoparticles are closely related to their functionality and effects on health and the environment.

The measurement of these quantities is important for the realization of the potential benefits of nanomaterials in specific applications. Characterization is also the first step to ensure that the desired properties of the synthesized particles are reproducible.

In particular, the identification of interactions between ions or molecules within a porous nanoparticle in a liquid medium is still a very unexplored topic. An important lesson from the last decade is that in biological fluids, proteins cluster with nanoparticles to form a protein corona that gives the nanoparticles a "biological identity". By making the application of nanoparticles in a biological context a key technology, knowledge of the physicochemical properties of nanoparticles under physiological conditions is of paramount importance.[1]

2.6.1 Nanoparticle size

The size and morphology of nanoparticles can be measured at the single particle level at subnanometer resolution using high-resolution microscopy techniques such as electron or scanning probe microscopy, which provide very detailed information about the shape of the nanoparticle.

These characterization methods are based on the interaction between the atomic structure and the incident electron beam (TEM, SEM) or scanning probe microscopy (AFM). Their disadvantage is that they are not ensemble techniques, which raises questions e.g. how representative and statistically relevant the data obtained are.[1]

Light scattering, diffusion and sedimentation methods are commonly used for routine analysis of colloidal suspensions. These methods usually do not give us direct information about the shape of the nanoparticle. The value obtained for the diameter of the nanoparticle is reported as the spherical diameter of the particle, which under given conditions would be consistent with the behaviour of the sample under investigation. To translate this information into actual nanoparticle dimensions, knowledge of the nanoparticle shape is required. Indirectly, we can infer the shape of a nanoparticle using methods that provide information about the mass/electron composition of the nanoparticle. These methods include static scattering methods using either light or X-rays. The aim is to synthesize monodisperse nanoparticles, but real nanoparticle samples often exhibit some degree of polydispersity. [1]

Nanoparticle size distribution is a measure of the control and quality of the nanoparticle synthesis process. Therefore, in practice, the term particle size distribution is used to describe the size. The particle size distribution expresses the percentage distribution of particles with respect to size. The particle volume distribution is characterised by the volume distribution. Information on the shape and

anisotropy of particles in solution can also be obtained by techniques based on radiation scattering, e.g. methods combining static and dynamic light scattering. The above mentioned methods for the analysis of nanoparticles in solution only allow the derivation of the anisotropy factor of the particles, and the detailed study of the morphology of the particles remains limited to high-resolution microscopic methods. However, qualitative information on the shape of nanoparticles obtained by light scattering-based methods is often necessary to confirm microscopy results, since sample preparation and the result obtained by electron microscopy may be affected by sample agglomeration or damage to the particle structure may occur during the process. [1]

2.6.2 ζ potential of nanoparticle colloids

Measurement of the zeta potential can provide very valuable information on the behaviour, fate and toxicity of nanomaterials, especially in relation to biological and environmental purposes.

There are a number of studies that correlate the ζ-potential with particle behaviour in solution or with bioavailability and potential toxicity. An accurate and comparable measurement of the ζ potential could allow the development of correlations to predict behaviour between different types of nanoparticles based on their measured zeta potential.[46]

The magnitude of the ζ potential was used as an indicator of the aggregation stability of the dispersion. Zeta-potential values above ±30 mV were usually considered to be moderately stable colloids against aggregation, with the notion that electrostatic repulsive forces were high enough to prevent aggregation. This derivation was originally described for the behavior of colloids with sizes in the hundreds of nm, and was probably not entirely appropriate for nanomaterials.[46]

The lack of standards for measuring the ζ-potential of nanoparticles hampers current efforts to use the ζ-potential for comparison between studies.

2.6.3 Transmission electron microscopy

Transmission electron microscopy (TEM) is undoubtedly one of the most important techniques for characterizing nanoparticles. TEM uses an electron beam focused on a thin (typically less than 200 nm) sample to produce electrongrams of nanomaterials with high lateral spatial resolution. Current electron microscopes achieve resolutions of less than 0.05 - 0.1 nm by reducing image distortion through bias correction, thus providing high quality images with atomic resolution. Due to its high spatial resolution and selectivity, TEM allows the study of size, shape and crystal structure at the single particle level. [1]

2.6.4 Scanning electron microscopy

Scanning electron microscopy allows to obtain an image of the surface by detecting electrons secondarily emitted during interaction with the incident electron beam. Compared to TEM, SEM uses lower beam energy, resulting in lower depth penetration, making SEM sensitive only to the surface of the sample. However, this surface interaction means that SEM is useful in the analysis of "thick" (>100 nm) samples, which is not possible with TEM. [1]

2.6.5 Atomic force microscopy

Atomic force microscopy is a scanning electron microscopy technique that can be used to examine and image the surface of objects of nanometric or even atomic dimensions (and to describe other properties of the sample associated with atomic forces). Depending on the mode of detection, vertical or lateral deflections of the cantilever, or changes in the amplitude, frequency or phase of the cantilever oscillation are recorded. In general, atomic force microscopy uses three modes: contact, non-contact and tapping. If the nano-objects are located directly on the surface of a (ideally smooth and flat) substrate, no specific preparation is needed for the measurement. The particles in suspension need to be placed on a smooth surface (such as mica or silicon). In contrast to scanning tunneling microscopy, no sample conductivity is required in the case of atomic force microscopy. The scanned image directly shows the size and morphology of the nano-objects. [1]

2.6.6 X-ray diffraction

X-ray diffraction is a versatile technique for studying a wide range of structural properties of crystalline samples. The range of obtainable information includes both microscopic properties of the sample, such as the arrangement of the crystal components, and macroscopic ones, such as the shape and size of the crystal. This information can be obtained from the analysis of the peak width at half height (FWHM), the half-width of the diffraction line. As the diffraction angle increases, the broadening of the diffraction lines increases sharply due to the microstrain, which thus affects the back reflection (at higher angles θ) the most. Small D crystallite sizes below 500 nm have a similar effect on the broadening of the diffraction line profiles. [1]

2.6.7 Dynamic light scattering

In general light scattering, light scattered from different particles is in random phase so that there is no interference. When a laser is used, the light is coherent and interference occurs when it interacts with the particles. As the small particles in the liquid move due to Brownian motion, the distance the scattered light must travel to the detector changes. The scattered waves can interfere depending on the distance between

the particle and the detector. This results in fluctuations in the intensity of the scattered radiation around the average intensity value. [1]

Certain discrepancies in the results may be attributed either to the difference between the hydrodynamic radius (which may include the solvation envelope or the adsorbed layer on the particle surface) and the actual radius, or to changes in particle size resulting from the preparation of the sample for the electron microscope (drying) or changes resulting from electron irradiation.[1]

2.7 Metal nanoparticles and their antibacterial applications

Resistance arises due to natural resistance in some species of bacteria (present before the discovery of antibiotics), genetic mutations in microbes, by one species acquiring resistance from another, and selection pressure due to the use of antibiotics, which gives a competitive advantage to mutant strains. Sub-optimal doses of antibiotics, especially as a result of misuse of antibiotics, facilitate the gradual selection of resistance. Examples of important resistant pathogens in the world are penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and multidrug-resistant Gram-negative bacteria (MDRGNB).[47] Hopes are being pinned on nanoparticles that could effectively combat multidrug-resistant strains through mechanisms of action different from those of antibiotics.[32,48,49]

The continuous emergence of bacterial resistance has challenged the research community to develop novel antibiotic agents. Among the most promising of these novel antibiotic agents are metal NPs, which have shown strong antibacterial activity in an overwhelming number of studies. Generally, antibiotic-resistant bacteria appear in a relatively short period of time even when new antibiotics are released to the market. However, it is hypothesized that NPs with antibacterial activities possess the potential to reduce or eliminate the evolution of more resistant bacteria because NPs target multiple biomolecules at once avoiding, the development of resistant strains.[33]

2.7.1 Mechanisms of antibacterial action of nanometals

Nanoparticles can attach to and penetrate bacterial cell membranes, causing structural damage and leading to cell death. Several kinds of nanoparticles, such as silver and copper generate reactive oxygen species, which can damage cellular components like DNA, proteins and lipids. Metal nanoparticles, such as silver nanoparticles, release metal ions that can interact with bacterial enzymes and proteins, disrupting their function. Some nanoparticles can bind to bacterial DNA, preventing replication and transcription, which inhibits bacterial growth. Nanoparticles can prevent the formation of biofilms, which are protective layers that bacteria form to shield themselves from antibiotics.[33]

2.8 Study of the silver nanoparticles behavior in the liquid media with different ionic strength

Silver metal nanoparticles are of continuing interest because of their special properties compared to bulk metal and their potential applications. Relatively low-cost manufacturing and a wide spectrum of antibacterial activity of silver nanoparticles are reasons for their incorporation into a variety of products including toys, fridges, food packaging, plastics, cosmetics, keyboards, textiles etc.

An increasing amount of artificial products containing silver nanoparticles results in their inevitable discharge to the environment. For this reason, there is increasing number of publications dealing with toxicity testing. Toxicity of AgNPs is affected by intrinsic nanoparticle features like particle size, surface chemistry, capping agents, but also by environmental factors such as pH, redox state, presence of ligands, ionic strength and ionic composition of liquid medium.

The aim of this work was to determine important physical-chemical parameters affecting behavior of silver nanoparticles in liquid media within the fish eco-toxicity test and discus methods applicable for their cost effective and low time consuming measurement.

2.9 Application of gold nanoparticles to osteosarcoma cells for potential use in radiotherapy and targeted cancer treatment.

Different types of gold nanoparticles are becoming a very interesting and effective tool in cell biology and tumor biology.

Nanoparticles can not only be selectively targeted with appropriate modification, but also used to transport suitable drugs (chemotherapeutics) directly into the tumor cell. Targeting anticancer therapy directly to tumor cells thus allows to reduce the systemic effects of chemotherapy. Chemotherapeutics that are normally insoluble or unstable in aqueous media can also be delivered into cells using nanoparticles. By binding to nanoparticles, we can also extend their biological half-life.[35]

Another advantage for potential anti-cancer therapy is that in tumors that have immature and permeable blood vessels with fenestrations wider than normal mature blood vessels, gold nanoparticles have been shown in *in vivo* experiments to have an increased accumulation capacity and to be retained in tumor tissue[50,51]. This phenomenon is described as the enhanced permeability and retention effect (EPR).

Another treatment strategy is the use of gold nanoparticles for radiosensitization. The aim should be to reduce the radiation burden and increase the effectiveness of radiation treatment. Especially in radioresistant tumors, radiosensitization could be beneficial. When cells are irradiated with high-energy photons in *in vitro* and *in vivo* experiments, gold nanoparticles show the ability to sensitize tumor cells.[52] Treatment can therefore be well localized and targeted to the site of their high deposition.

3 Experimental part

3.1 Preparation of metal nanoparticles

The chemical reduction of the corresponding metal salts was used to prepare these metal nanoparticles. The aim was to prepare stable colloidal systems suitable for the intended testing of interaction with biological material. The formation of an electrical bilayer is part of the electrostatic stabilization of the colloid, but the reagents used in the preparation of the nanoparticles themselves can influence the behavior of the prepared nanoparticles. Therefore, reducing agents and stabilizers that are non-toxic are preferably used. However, sometimes the use of potentially toxic reducing agents cannot be reliably avoided. Another pitfall in the preparation of stable colloids may be the lack of electrostatic stabilization, especially in the intended applications where pH or ionic strength may be altered. Therefore, it is also advantageous to use steric stabilization using a suitable polymer/surfactant.

3.2 Testing the antimicrobial properties of metal nanoparticles

The topic of testing antimicrobial properties in the context of the application potential of nanoparticles is still a hot topic. There is still no uniform recommendation on how to test the antibacterial effect of nanoparticles. Due to the nature of nanoparticles themselves, testing their antimicrobial properties is difficult and influenced by a number of factors

The antimicrobial properties of the prepared colloidal metal nanoparticle systems were tested for these purposes by modified agar diffusion disk method and modified dilution method.

The antimicrobial properties of metal colloids are influenced by a number of factors such as their stability of interaction with the test medium itself and the effect of the antimicrobial effects of nanoparticles may not be dose dependent.

3.3 Study of the silver nanoparticles behavior in the liquid media with different ionic strength

The colloidal system obtained by glucose reduction showed a high temporal stability and contained spherical 40 nm nanoparticles. This colloid had the best combination of parameters critical for reliable studying of nanoparticle agglomeration using laser diffraction and thus was chosen for subsequent experiments.

The changes of hydrodynamic diameter and ζ-potential were described in demineralized water and in liquid media prescribed for acute fish toxicity test by OECD guideline 203 in Ag concentration range 1-250 μM. In demineralized water, the rate of nanoparticle agglomeration was very slow and systems were not at steady-state upon 13 000 min. OECD 203 liquid medium significantly promoted the nanoparticle agglomeration as e.g. for 250 μM Ag solution, the hydrodynamic diameter increased from 37.5 to 650 nm within 80 min. Influence of ionic strength of medium on nanoparticle agglomeration was studied for ionic conductivity range (7-680 $\mu S.cm^{-1}$).[53]

The influence of nanoparticle concentration and ionic strength of liquid media on rate and level of particle agglomeration has to be taken into consideration during the design of aquatic toxicity test methods.

3.4 Application of gold nanoparticles to osteosarcoma cells for potential use in radiotherapy and targeted cancer treatment.

Two nanoparticle size systems of 5 nm and 50 nm were compared for targeted transport of gold nanoparticles into 143B osteosarcoma cells. The aim was to test whether smaller nanoparticles would be more suitable for targeting into the cell nucleus (smaller nuclear pore size) and therefore more suitable for radiosensitization of tumor cells. Nanoparticles of the required sizes had to be synthesized before starting the actual experiments with the cell lines. Several original procedures were developed for this purpose. All syntheses were based on tetrachloroauric acid as a source of gold ions. The tetrachloroauric acid was reduced with various reducing agents to achieve the desired nanoparticle sizes. The size of the as-prepared nanoparticles or their hydrodynamic diameter (Dh) was verified by measuring the particle size distribution by DLS using Zetasizer Nano instrument.[54]

4 Results and conclusions

The dissertation focuses on the preparation and characterization of selected nanoparticles of metals - silver, gold and copper. These nanoparticles have a wide application potential in many areas of human activity.

Proprietary synthetic procedures have been developed for the preparation of metal nanoparticles. Several different reducing agents and procedures have been used for this purpose. The hydrodynamic diameter of the prepared nanoparticles was measured and the stability of the prepared colloids was verified by measuring the zeta potential. For selected nanoparticles, microscopic techniques were used to verify their morphology. The selected nanoparticle colloids were stabilized with a polymer stabilizer (polyvinylpyrrolidone) and the parameters of the thus stabilized nanoparticles were measured.

Gold nanoparticles were prepared by reduction of tetrachloroauric acid with ascorbic acid. Ascorbic acid serves as a reducing and stabilizing agent for the gold nanoparticles. This procedure allows to reproducibly prepare nanoparticles of different sizes with respect to the reaction conditions. Prepared gold nanoparticles are suitable for potential applications in biology and medicine due to use of a non-toxic reducing agent.

Various stabilizers were tested to increase the stability of the prepared colloids. The prepared nanoparticles were sterically stabilized by addition of polymeric compounds/surfactants. For this purpose, different water soluble polymers (PVP - polyvinylpyrrolidone, PEG - polyethylene glycol, PVA - polyvinyl alcohol, Tween 20, Agar-agar) were tested.

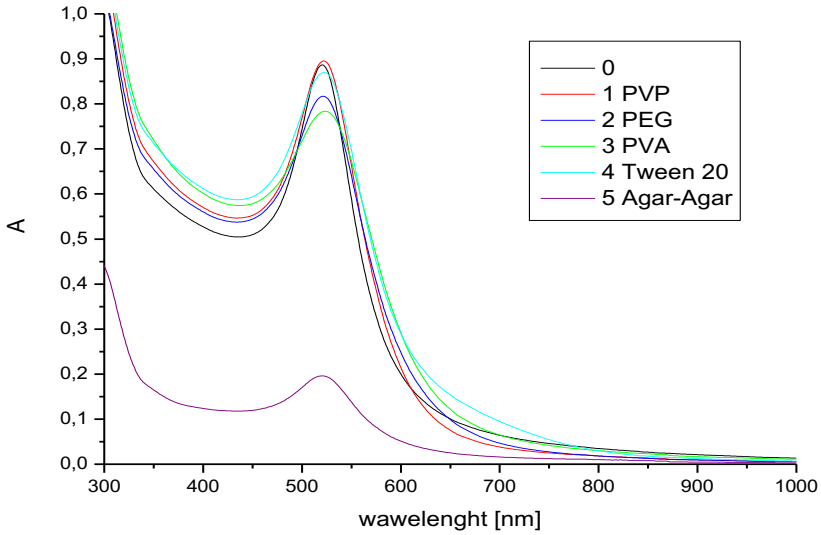

Image 2: *Absorption curves for ZA1 gold nanoparticles using different stabilizers.*

PVP and Tween 20 were the most suitable of the tested stabilizing additives (No.1, Figure 3), (No. 4, Figure 3), where even after 6 months no sedimented large particles are present at the bottom of the vials. For the colloid without the addition of stabilizer (No. 0, Figure 3), sedimentation due to aggregation of nanoparticles is evident at the bottom of the vial. The natural polysaccharide agar-agar was completely unsuitable, where coagulation of the prepared nanoparticles occurred after its addition to the colloid (No. 5, Figure 3). PVP was chosen to stabilize the prepared colloids as a non-ionic block copolymer, which is considered to be biocompatible[44], moreover, Tween 20 has the properties of a non-ionic tenside, which could further influence the eventual testing of nanoparticle properties.

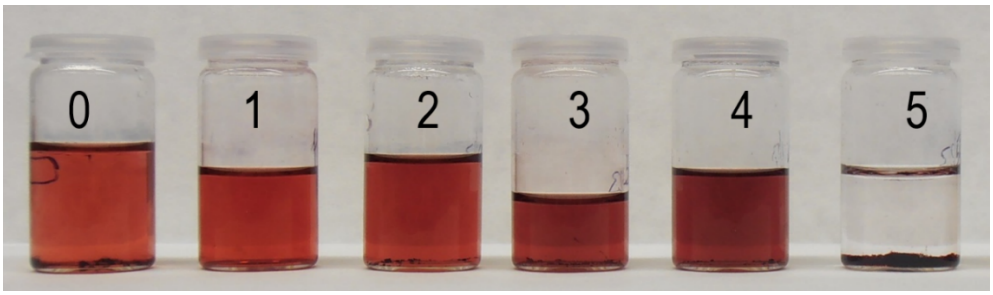

Figure 3: *Prepared colloids of ZA1 gold nanoparticles with different stabilizers - after 6 months.*

The size and ζ potential of the prepared nanoparticles were measured for both Au-labeled nanoparticles stabilized only by ascorbic acid and Au_PVP. The results are presented in the tables (Table 2), (Table 3).

Table 2: *Hydrodynamic diameter and ζ potential of Au gold nanoparticles.*

Name	Hydrodynamic diameter DLS D_h [nm]	Standard deviation [%]	ζ potential [mV]	Standard deviation [%]
Au1	43,78	2,62	-27,41	5,53
Au2	40,99	2,05	-32,56	3,53
Au3	45,15	2,03	-27,62	2,39

Table 3: *Hydrodynamic diameter and ζ potential of gold nanoparticles of Au_PVP.*

Name	Hydrodynamic diameter DLS D_h [nm]	Standard deviation [%]	ζ potential [mV]	Standard deviation [%]
Au1_PVP	57,01	1,95	-16,57	3,53
Au2_PVP	53,10	2,22	-17,53	2,22
Au3_PVP	53,46	2,59	-15,05	1,66

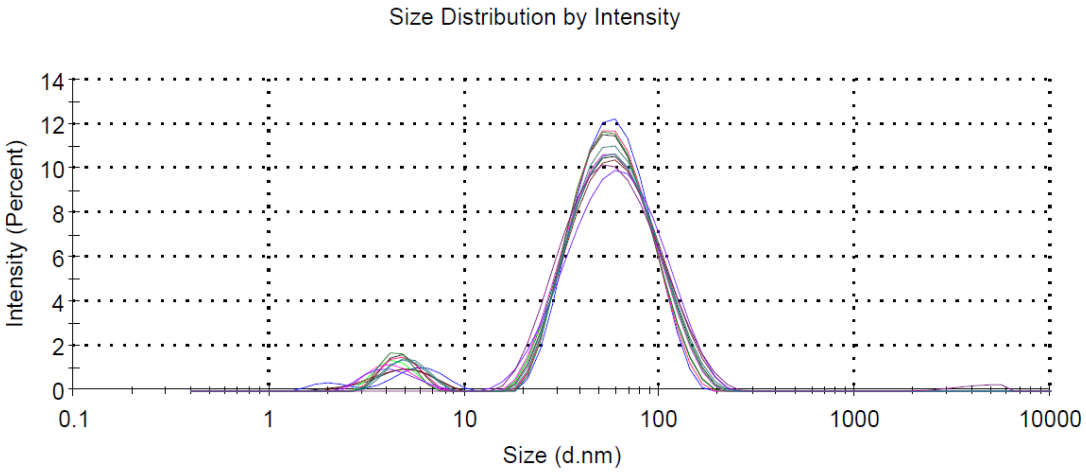

Figure 4: *Distibution curves for Au1 nanoparticles.*

From these values, it can be seen that the Au nanoparticles have a smaller hydrodynamic diameter than the nanoparticles stabilized by addition of PVP (Au_PVP).

The ζ potential of PVP-stabilized nanoparticles decreases, which could be explained by the potential shielding by the polymer layer. The shape of the distribution curve (Figure 4) shows two maxima, the smaller maximum corresponds to a gold nanoparticle fraction of about 5 nm. This bimodal shape of the particle size distribution could correspond to a condition where the smaller peak corresponds to primary nanocrystals and the larger one corresponds to larger nanoparticles formed by

aggregation growth. The observation of early bimodal patterns of nanocrystal size distribution should be attributed to aggregation growth rather than Ostwald ripening[55].

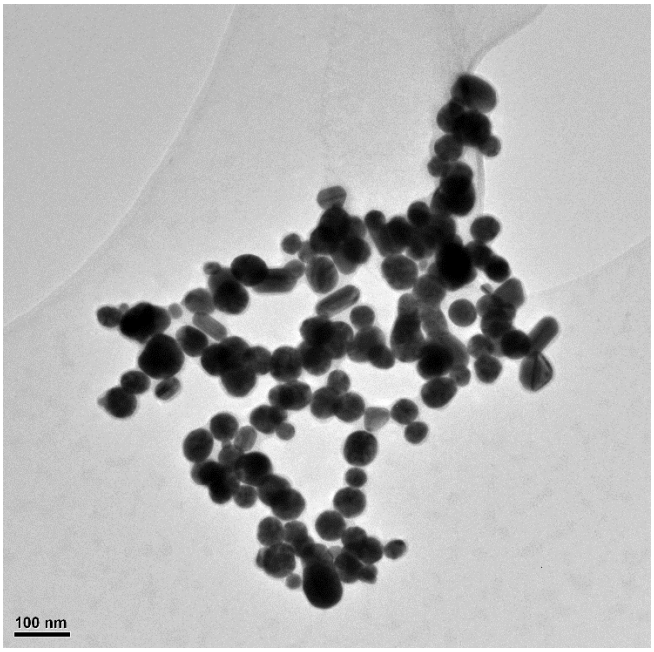

Image 5: *Au1 nanoparticles in SEM.*

For the Au1 nanoparticle sample, a SEM micrograph was also taken, which shows nanodots and nanotriangles in addition to spherical nanoparticles,

Silver nanoparticles prepared by reduction with sodium borohydride in the presence of sodium citrate were also prepared as a part of the dissertation thesis. The values of their hydrodynamic diameter and ζ potential are given in the table (Table 4) together with the values measured for silver nanoparticles prepared by the Tollens method.

Table 4: *Hydrodynamic diameter and ζ potential of AC and AG silver nanoparticles.*

Name	Hydrodynamic diameter DLS D_h [nm]	Standard deviation [%]	ζ potential [mV]	Standard deviation [%]
AC	60,82	1,29	-29,40	1,24
AG	36,35	1,183	-29,6	1,52

The morphology of selected nanoparticles was also evaluated by AFM.

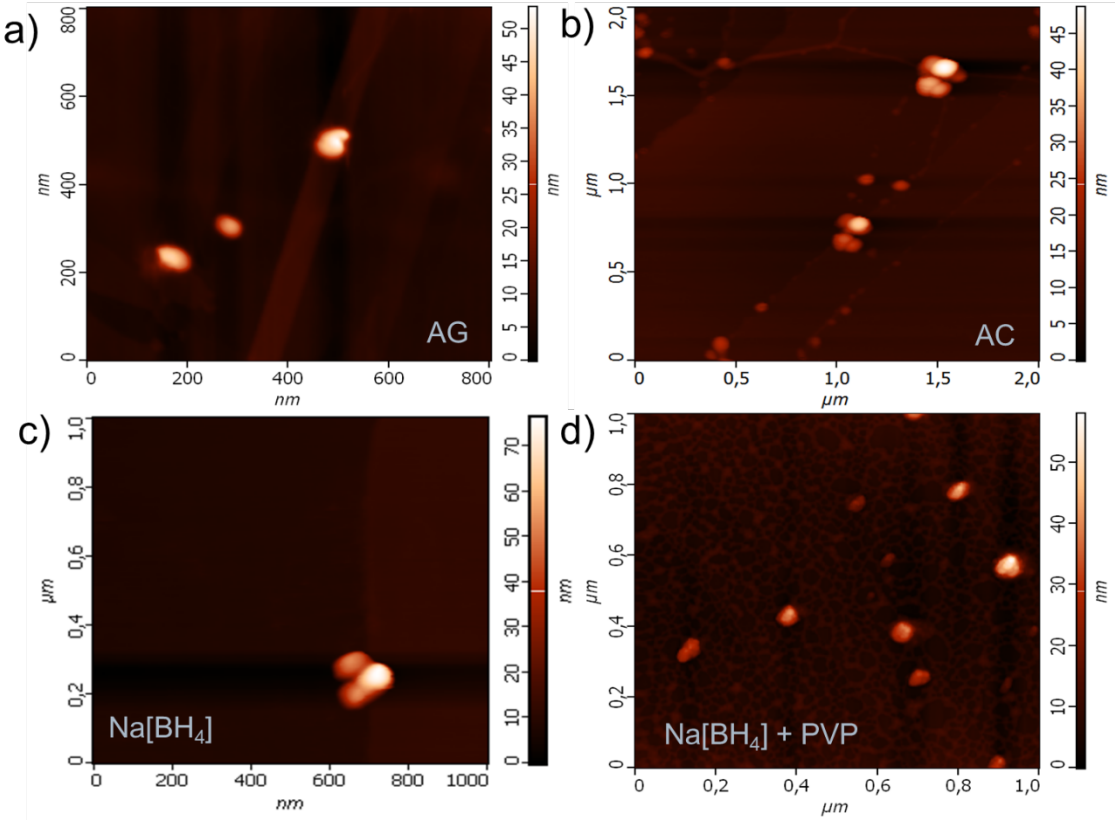

Figure 6: *AFM topograms of prepared silver nanoparticles.*

Silver nanoparticles obtained by Tollens reduction and sodium borohydride have a spherical shape according to AFM, while nanoparticles prepared by sodium borohydride reduction in the presence of sodium citrate have a disc-shaped shape. The dimensions are given in the table (Table 5).

Table 5: *Parameters of silver nanoparticles obtained by AFM.*

Name	AFM size [nm]	Shape	Note
AC	70-130 nm diameter, 40 nm height	disc-shaped/lens-shaped	highly polydisperse
AG	30-50 nm	spherical	-
Ag3	60-70 nm	spherical	-
Ag3_PVP	45-60 nm	spherical	rough surface, polymer micelles

The obtained dimensions of the silver nanoparticles are in good agreement with the values obtained by measuring the hydrodynamic diameter. Interesting result is obtained for silver nanoparticles prepared in sodium citrate as a stabilizer, which gives

lens/disc-shaped nanoparticles with the described polydispersity. The size fluctuation is also evident from the distribution curves for AC (Figure 7).

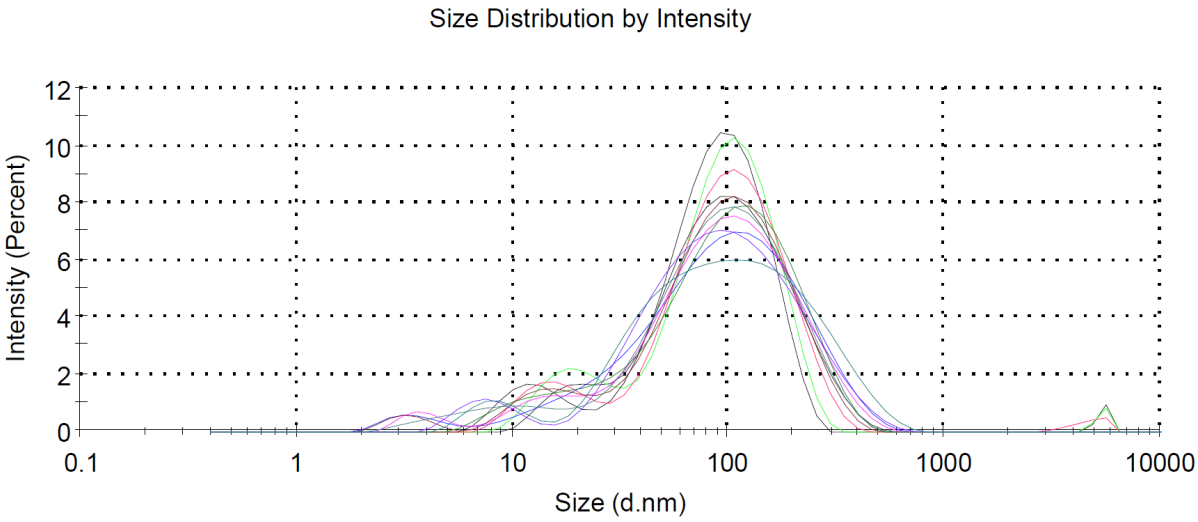

Figure 7: *Size distribution curves of AC nanoparticles.*

The morphological description of Ag3_PVP nanoparticles is different from other nanoparticles which had smooth surface. The PVP present in the nanosilver colloid after evaporation of water caused the surface to thicken with the presence of individual polymer micelles.

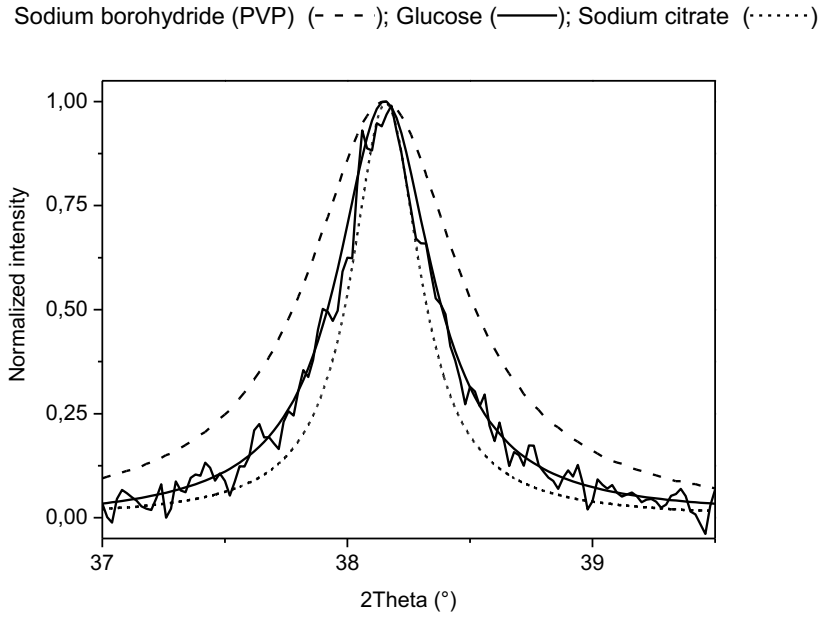

Figure 8: *XRD diffractogram - fitted data for silver nanoparticles prepared by reduction with sodium borohydride = Ag3_PVP, glucose = AG and sodium citrate.*

XRD was also used to verify the size of nanoparticles, according to the Scherer equation the bandwidth (full width at half max.) increases with decreasing particle size. The most intense diffraction of the Kα cubic structure was measured at 38.2°. The experimental data was fit with a Lorenz function. The half-widths decrease in order: Ag3_PVP>AG>AC. This shows that the Ag3_PVP particles, which are similar in size to the AG particles on the AFM, are actually the smallest (Figure 8).

4.1 Testing the antimicrobial properties of metal nanoparticles

At a time of antibiotic crisis and the rise of multidrug-resistant bacterial strains, metal nanoparticles are offering themselves as potential means to combat resistant bacteria. Metal nanoparticles exhibit a different behavior from conventional antimicrobial agents, so we focused our work on this testing issue.

There are no recommendations for testing the antibacterial effects of metal nanoparticles, so the literature data are difficult to compare. We attempted to demonstrate the pitfalls involved by using classical methods of testing antibacterial agents. The diffusion disk tests showed that the prepared Ag, Au and Cu nanoparticles have bacteriostatic effects on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* (Figure 9). Further, the antibacterial effects of the prepared nanoparticles were tested using dilution method to demonstrate the bactericidal effects of the prepared nanoparticles. The performance of the experiment is dependent on many factors such as the pH of the solution, ionic strength of the test medium used and the tendency of the nanoparticles to aggregate or, on the contrary, to dissociate and form ions.

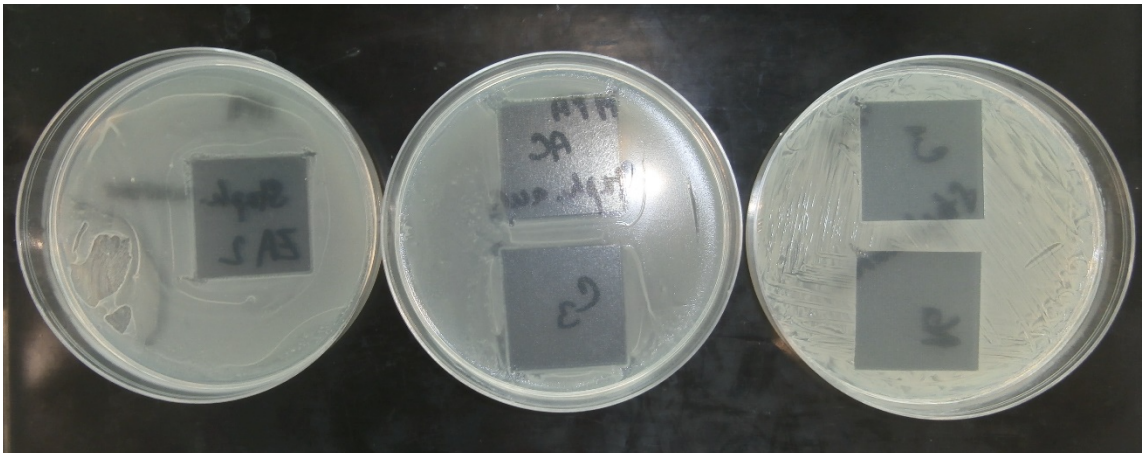

Figure 9: *Petri dishes - disk method for testing the antimicrobial effects of nanoparticles - growth inhibition.*

4.2 Study of the silver nanoparticles behavior in the liquid media with different ionic strength

In the following work[53], we therefore focused on the behaviour of silver nanoparticles in the test medium for ecotoxicity tests. The behaviour of silver nanoparticles in solutions in Ag concentration range 1-250 μM was studied for liquid medium prescribed for acute fish toxicity test by OECD guideline 203 and demineralized water.

In demineralized water, the rate of nanoparticle agglomeration was very slow. Systems were not at steady-state upon experimental time 13 000 min. For whole concentration range, the hydrodynamic diameter of nanoparticles increased only two times during experimental time. OECD 203 liquid medium for zebrafish testing significantly promoted the nanoparticle agglomeration. E.g. for 250 μM Ag solution, the hydrodynamic diameter increased from 37.5 to 650 nm within 80 min. Both, the agglomeration rate and steady-state hydrodynamic diameter significantly decreased with decreasing Ag concentration in OECD 203 liquid medium.

Influence of ionic strength of liquid medium on nanoparticle agglomeration was further studied in solutions with different ionic conductivity (7-680 $\mu S.cm^{-1}$) for selected concentration 250 μM Ag. While the size of agglomerates monotonically increased with solution ionic conductivity, the rate of agglomeration reached the maximum values in solutions with ionic conductivity ca 400 $\mu S.cm^{-1}$.

Our experiments demonstrated the significant influence of liquid medium ionic strength on agglomeration of dispersed silver nanoparticles. Different toxicity of agglomerates and primary nanoparticles is a well-known fact. From toxicological point of view it is even questionable to consider nanoparticles and agglomerates as the same chemicals. Thus it is possible to state that the difference between ionic strength of used liquid medium and potentially polluted natural water is the critical parameter for aquatic toxicity tests of nanoparticles. In our opinion, meaningful results are possible to obtain only with using the environmentally relevant experimental system, where the optimization of cultivation medium parameters has the same priority as a selection of appropriate biological model.

Concentration of Ag nanoparticles in liquid medium was another important parameter strongly influencing the agglomeration rate and level. Calculation of agglomeration rate constants could be important part of design of semi-static eco-toxicity test methods. The period for exchange of liquid media have to respect the dependence of agglomeration rate on corresponding nanoparticle concentration.[53]

4.3 Application of gold nanoparticles to osteosarcoma cells for potential use in radiotherapy and targeted cancer treatment.

The last part of the dissertation is focused on the use of gold nanoparticles for targeted treatment of tumors, which was carried out during the internship at the Mayo Clinic in the USA.

Our aim was to prepare gold nanoparticles and target them efficiently to radioresistant bone tumor cells in order to increase their radiosensitivity. Such

nanoparticles must be stable, not tend to aggregation and agglomeration, retain their properties in solutions of different ionic strength, pH and in environments where they would be exposed to interaction with numerous proteins and where they must be protected from protein corona formation and thus from recognition by immune system cells and uptake by reticuloendothelial system organs. This may extend their circulating half-life and make any treatment more effective. Only nanoparticles with these properties can find use not only in *in vitro* but also in *in vivo* applications.

In literature it has been described that cancer cells generally show higher uptake of nanoparticles than non-cancer cells. Our aim was, among others, to verify the size dependence of nanoparticle uptake by cells, which according to data in literature reaches the maximum at nanoparticle sizes around 50 nm. Since the nuclear pore size is 20-50 nm, we also wanted to verify whether nanoparticles with smaller size would be more suitable for targeting the cell nucleus (expected increase of radiosensitization effect). For this purpose, two nanoparticle systems with different sizes of metallic gold nanoparticles of 5 nm (BS) and 50 nm (ZA) were prepared and stabilized subsequently.

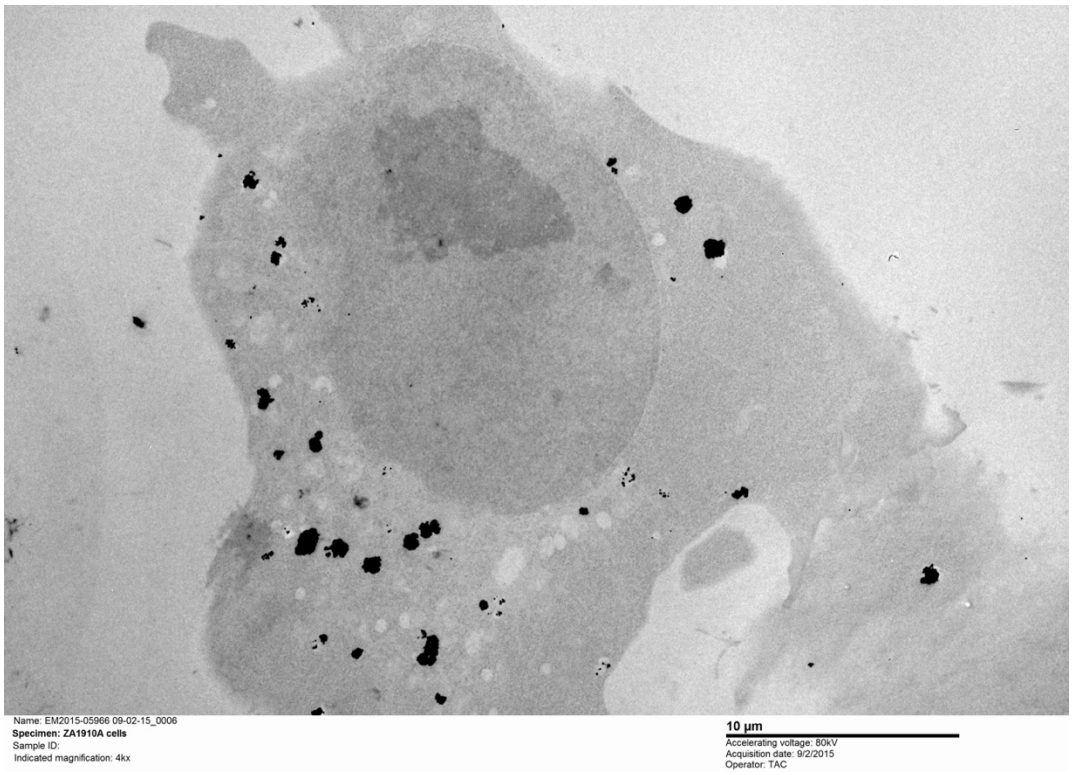

Figure 10: *Transmission electron microscope image showing 143B osteosarcoma cells after 24 h incubation with non-functionalized AuNPs (ZA), various magnifications, presence of nanoparticles in the cytoplasm, (Mayo Clinic, Rochester, MN)*

For the planned functionalization of the prepared nanoparticles with signal peptides, the size dependence of the hydrodynamic diameter of the prepared

nanoparticles on the amount of the used stabilizing agent (mPEG-SH) was first verified in an attempt to leave a free surface on the nanoparticles for the binding of signal peptides. To increase the uptake of nanoparticles by cells, mPEG-SH stabilized nanoparticles were further functionalized with signal peptides. For targeted transport into the cytoplasm we used signal peptide CPP and for targeted transport into the cell nucleus we used NLS. Low toxicity of prepared nanoparticle system was confirmed by MTS assay. [54]

Nanoparticles of desired size around 5 nm and 50 nm (TEM) were prepared by the above mentioned procedures. The uptake of functionalized and non-functionalized nanoparticles was verified on 143B osteosarcoma cell lines (Figure 10).

According to TEM images, higher spontaneous uptake is observed for non-functionalized ZA nanoparticles (50 nm).

Uptake of gold nanoparticles is enhanced by their functionalization with CPP peptide. Nanoparticles functionalized with both signal peptides CPP and NLS penetrate through nuclear pores to the cell nucleus. In toxicity tests, no decrease in viability of the tested cells was observed after exposure to the nanoparticles.

ZA nanoparticles with a size of 50 nm seem to be the most suitable for targeted transport of nanoparticles into 143B osteosarcoma cells.[54]

5 Conclusion

The dissertation focuses on preparation and characterization of silver, gold, and copper nanoparticles, which have wide application potential in various fields. Original synthetic procedures were developed, using different reducing agents and methods. The hydrodynamic diameter and stability of the nanoparticles were measured, and their morphology was verified using microscopic techniques.

Gold nanoparticles were prepared by reducing tetrachloroauric acid with ascorbic acid, making them suitable for biological and medical applications due to the non-toxic reducing agent. Various stabilizers were tested, with PVP proving to be the most effective. The size and ζ potential of the nanoparticles were measured, showing that PVP-stabilized nanoparticles had a larger hydrodynamic diameter and lower ζ potential due to polymer layer shielding. Silver nanoparticles were prepared using sodium borohydride, sodium borohydride/sodium citrate combination and Tollens method, with their morphology evaluated by AFM. Copper nanoparticles were prepared using sodium borohydride with ascorbic acid.

The antimicrobial properties of the silver, copper and gold nanoparticles were tested, showing antibacterial effects on *Staphylococcus aureus* and *Escherichia coli*.

The prepared silver, gold, and copper nanoparticles were tested using the disc diffusion method and the dilution method. These nanoparticles exhibited bacteriostatic effects in the disk diffusion test and bactericidal effects in the dilution method. Testing the antibacterial effects of nanoparticles has its challenges, and there is currently no clear recommendation that would allow comparison of results among different authors. The dissertation discusses the challenges of nanoparticle stability, which significantly affects the obtained results.

The behavior of silver nanoparticles in ecotoxicity tests was studied, highlighting the significant influence of liquid medium ionic strength on nanoparticle agglomeration.

Gold nanoparticles were also targeted to osteosarcoma cells for potential use in radiotherapy and targeted cancer treatment. The aim was to increase the radiosensitivity of radioresistant bone tumor cells. The nanoparticles were stabilized and functionalized with signal peptides for targeted transport into the cytoplasm and cell nucleus. Low toxicity and effective uptake of these nanoparticles suggest their potential for targeted drug delivery and radiosensitization in cancer treatment. This method offers a relatively simple procedure for the preparation of functionalized gold nanoparticles of the desired size, which are stable in solutions of different ionic strength, pH and in the presence of proteins of the cell medium. These nanoparticles could find applications for targeted transport of drugs (chemotherapeutics) into cancer cells or for their radiosensitization. Their use for testing under *in vivo* conditions is also proposed. Penetration of gold nanoparticles into the cell nucleus of tumor cells

could enhance the biological effect of radiation therapy specifically in radioresistant tumors such as osteosarcoma.

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7 List of Publications

Publications in peer-reviewed journals and journals with IF

1. Bures, Z., Mamo, T., Vlcek, M., Lu, L., & Yaszemski, M. J. (2020). **Signal protein-functionalized gold nanoparticles for nuclear targeting into osteosarcoma cells for use in radiosensitization experiments**. *Neoplasma*, 67(3), 576-583. (IF 2,575)
2. Oprsal, J., Bures, Z., Vlcek, M., Knotek, P., Pouzar, M., & Benes, L. (2013). **A Study of Silver Nanoparticles Behavior in Liquid Media for Ecotoxicity Tests**. *Advanced Science, Engineering and Medicine, 5*, 589-592. (IF 0,34)
3. Bures Z., Vlcek M. (2011). **Preparation and stabilisation of nanogold and nanosilver**, *Journal of Nanocomposites and Nanoceramics,* 2(1), 17-20.

Other publications in peer-reviewed journals and journals with IF

1. Bajer, M., Fortunato, J., Bureš, Z., Havel, J., Maňák, J., Blaha, V. **Hypertriglyceridemic Crisis Associated with Severe Acute Pancreatitis - Case Series**. *Atherosclerosis* 2023, 379, S142-S143. (IF 5,3).
2. Friedova, N., Pelclova, D., Bures, Z., Krijt, J., & Kohout, P. (2021). **Response to the questions related to the article: "Osmium absorption after osmium tetroxide skin and eye exposure"**. *Basic & clinical pharmacology & toxicology*, *128*(4), 555-556. (IF 4.08)
3. Bureš Z., Albrecht D. (2021), **A serious diagnosis in a geriatric patient - metformin-associated lactic acidosis, do we think about it enough?**, *Geriatrics and Gerontology*, 10(2), 92-100.

Lectures

1. Bureš Z., Vlček M.: **Biological Aspects of powder materials and nanomaterials**,
   15th KSAP-PM, Pardubice, 26 September 2013, pp. 13-15, ISBN: 978-80-7395-604-2.
2. Bureš Z., Palarčík J., Vytřasová J, Vlček M.: **Preparation, characterization and microbiological properties of nanocopper, nanogold and nanosilver**, Development of Materials Science in Research and Education, Kežmarské Žľaby, Vysoké Tatry, Slovakia, 9.9. - 13.9. 2013, ISBN 978-80-970896-5-8.
3. Bureš Z., Vlček M**., Preparation and potential applications of silver and gold nanoparticles**, Development of Materials Science in Research and Education, Lednice, Czech Republic, 3.9. - 7.9. 2012, ISBN 978-80-2547-237-8.
4. Bureš Z., Vlček M., **Stabilisation of metallic nanoparticles**, Development of Materials Science in Research and Education, Kežmarské Žľaby, Vysoké Tatry, Slovakia, 29.8. - 2.9. 2011, ISBN 978-80-8134-002-4.

Posters and contributions in proceedings

1. Dědková K., Bureš Z., Palarčík J., Vlček M., Kukutschová J., **Acute aquatic toxicity of gold nanoparticles to freshwater green algae**, NANOCON 2014: 6th International Conference, November 5th-7th 2014, Hotel Voronez I, Brno, Czech Republic, EU. Ostrava: Tanger, 2014. p. 682-685. ISBN 978-80-87294-53-6
2. Bureš Z., Vlček M., **Microbiological aspects of nanocopper, nanosliver and nanogold**, International Days of Material Science 2013 - ReAdMat, Pardubice.
3. Bureš Z., Vlček M., **Stable nanodispersed metals**, 10th International Conference Solid State Chemistry 2012, University of Pardubice, 10.6.-14.6. 2012, pp. 126, ISBN: 978-80-7395-499-4.
4. Bureš Z., Vlček M., **Preparation and characterisation of metal nanoparticles**, International Days of Material Science 2012 - ReAdMat, Pardubice, 60, ISBN: 978-80-7395-524-3

Other lectures

1. Bureš Z., **Nutrition in the elderly and its specifics**, 28th National Gerontological Congress, Hradec Králové, 26.4. - 27.4. 2023.
2. Bureš Z., Symposium New Generation of Non-Terminal Nutrition Tailored to Patients - Upward to Strength and Beyond, lecture "**Can Special Nutrition Help Us Fight Sarcopenia?**", SKVIMP Congress, New Adalbertinum Hradec Králové 1.6. - 3.6. 2023.
3. Bureš Z., Symposium - New evidence - pharmaconutrients for better nutritional support of oncological patients, lecture **"Role of β-hydroxy-β-methylbutyric acid (HMB) in clinical nutrition"**, SKVIMP Congress, Nové Adalbertinum Hradec Králové, June 2 - 4, 2022.

Other posters and contributions in proceedings

1. Albrecht D., Bureš Z., Novák V., Víšek J., **Unusual cause of severe condition**, XXXI Congress of the Czech Society of Internal Medicine, 30 October - 2 November 2024, Prague.
2. Horská J., Havel J., Trčková E., Skořepa P., Bureš Z., Albrecht D., Maňák J., Blaha V., **Protracted disorder of consciousness with septic shock with MODS in a patient with highly suspected haemophagocytic lymphohistiocytosis (HLH)**, XXXth Congress of the Czech Society of Internal Medicine, 8-11 November 2023, Brno.
3. Klein L., Bureš Z., Habalová J., Hošek F., Sobotka L., **Use of the chemical necroctomy method in polymorbid patients,** 9th Central European Burn Congress and 23rd Annual Conference of the Society of Burn Medicine, Proceedings pp. 34-36, 21-22 September 2023, Košice, SR.

Consulted bachelor theses:

1. HOUDEK, Jakub. ***Preparation and study of properties of metal nanoparticles*.** Bachelor thesis. University of Pardubice, Faculty of Chemical Technology. 2014.

2. HOLÍK, Michal. ***Bactericidal effects of nanosilver*.** Bachelor thesis. University of Pardubice, Faculty of Chemical Technology. 2014.

Research Internship

- **Mayo Clinic, Rochester, Minnesota, USA, 6/2015-9/2015**

Department of Orthopedic surgery and Department of Biomedical Engineering