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DOCTORAL SCHOOL  
PHARMACY DOMAIN**



**SYNTHESIS METHODS FOR THE PHARMACO-  
TOXICOLOGICAL EVALUATION OF METALLIC  
NANOPARTICLES WITH OR WITHOUT ATTACHED  
BIOLOGICALLY ACTIVE NATURAL COMPOUNDS**

**ABSTRACT**

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**Timișoara  
2024**

The current thesis, entitled “***Synthesis methods for the pharmaco-toxicological evaluation of metallic nanoparticles with or without attached biologically active natural compounds***”, encompasses the most significant part regarding the research activity that I performed in the last 10 years, since I finished my first PhD thesis, also summing up my academic carrier.

Iron oxide nanoparticles (IONPs) have emerged as a significant class of metallic nanoparticles, particularly in the domain of targeted drug delivery and cancer therapy. Their unique magnetic properties, biocompatibility, and ability to be functionalized for specific applications make them suitable candidates for various biomedical applications, including imaging and therapeutic interventions. The versatility of IONPs allows for the integration of multiple functionalities, such as drug loading, magnetic targeting, and real-time monitoring of drug release, which are crucial in enhancing the efficacy of cancer treatments while minimizing systemic side effects.

Silver nanoparticles (AgNPs), another type of metallic nanoparticles, have gained significant attention due to their diverse applications, including antimicrobial functions, bioimaging, and wound dressings. These metallic nanoparticles have shown promising results in various in vitro studies, demonstrating their potential to inhibit the replication of the Hepatitis B virus, exerting anti-HIV activity by acting as a virucidal agent or inhibiting viral entry, and exhibiting cytotoxic effects on cancer cells. Moreover, AgNPs have shown antibacterial properties against pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as biofilm-inhibiting effects.

Metallic nanoparticles can be synthesized by chemical and physical methods, but they are quite expensive and potentially dangerous for the environment as they involve the use of toxic chemicals, which are responsible for various biological hazards. Currently, the development of experimental processes through biological mechanisms for metallic nanoparticle synthesis is evolving into an important branch of nanotechnology (green nanotechnology). The green synthesis methods have gained popularity due to their eco-friendly nature. Studies have demonstrated the successful green synthesis of metallic nanoparticles using plant extracts, fruit extracts, microorganisms, bacteria, fungi, algae, viruses, yeasts, etc. One of the primary advantages of green synthesis is the ability to control the size, shape, and crystallinity of metallic nanoparticles, which directly affects their magnetic properties. The synthesis of metallic nanoparticles, particularly IONPs, typically involves methods such as co-precipitation, thermal decomposition, and solvothermal techniques, which allow for control over particle size and surface properties. The

superparamagnetic iron oxide nanoparticles (SPIONs) are particularly noted for their low toxicity and high surface area, which facilitate efficient drug loading and release mechanisms. The surface of these nanoparticles can be modified with various biocompatible polymers or ligands to enhance their stability and targeting capabilities.

One of the most promising applications of IONPs is in the field of cancer therapy, where they can be used to deliver chemotherapeutic agents directly to tumor sites. This targeted approach is facilitated by the application of an external magnetic field, which allows for the precise localization of the drug-loaded nanoparticles at the tumor site, thereby enhancing the therapeutic efficacy and reducing off-target effects. Studies have demonstrated that IONPs can be conjugated with specific targeting ligands, such as folic acid, which binds to folate receptors overexpressed in many cancer cells, further improving the specificity of drug delivery systems.

Exploring active principles derived from plants has garnered significant attention in both traditional and modern medicine. These active compounds, often called phytochemicals, are bioactive substances that exhibit a range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Moreover, the role of phytochemicals in cancer prevention and treatment has been the subject of extensive research. Compounds such as flavonoids and alkaloids have been identified as having anti-inflammatory and immunomodulatory effects, which are crucial in managing several conditions. The therapeutic effects of these compounds are attributed to their ability to modulate various biochemical pathways involved in inflammation and cell proliferation, thereby offering a natural alternative to synthetic drugs with potentially fewer side effects.

Nanotechnology has emerged as a promising approach to improve the bioavailability and bioactivity of herbal drugs. Various nanocarriers, such as solid lipid nanoparticles (SLNs) and nanoemulsions, have been developed to enhance the pharmacokinetic properties of herbal extracts, thereby increasing their therapeutic potential. These advancements not only improve the efficacy of herbal formulations but also address challenges related to the solubility and stability of active ingredients, which are often critical factors in the development of effective pharmaceutical products.

Integrating nanotechnology with phytochemicals has opened new avenues for drug delivery systems. Nanoparticles can enhance the solubility and bioavailability of phytochemicals, allowing for more effective therapeutic applications. This approach not only improves the pharmacokinetic profiles of these compounds but also minimizes potential side effects, as many phytochemicals exhibit lower toxicity compared to their synthetic

counterparts. The encapsulation of phytochemicals in nanocarriers has been shown to facilitate targeted delivery, particularly in cancer therapy, where precision is crucial for minimizing damage to healthy tissues.

The exploration of pharmaceutical formulations based on active principles derived from plants has gained considerable attraction in the biomedical and pharmaceutical fields. The complexity of these compounds, including their interactions with biological systems, underscores their potential as active pharmaceutical ingredients (APIs) in drug formulations. For instance, phytochemicals have been identified as key players in anticancer therapies, influencing multiple biochemical pathways and presenting a viable alternative to synthetic drugs. One of the primary advantages of utilizing plant-derived compounds is their inherent bioactivity, which can be harnessed through various extraction methods. The choice of extraction solvent is critical, as it can significantly affect the yield and efficacy of the bioactive compounds extracted from plant matrices. The extraction process itself is pivotal in isolating the active principles from plant materials. Furthermore, the synthesis and characterization of silver complexes with pharmaceutical agents have shown promise for clinical applications due to their cytotoxic profiles. The incorporation of plant extracts into pharmaceutical formulations often requires the use of excipients, which can enhance the delivery and efficacy of the active ingredients.

Pentacyclic triterpenes (PCTs) are a diverse class of secondary metabolites found in various plant species, characterized by their unique structure comprising five interconnected carbon rings. This natural compounds that have garnered significant attention in recent years due to their wide-ranging therapeutic effects. These compounds, which include well-known members such as oleanolic acid, ursolic acid, and betulinic acid, are primarily derived from various medicinal plants and have been shown to exhibit a plethora of biological activities, including anti-inflammatory, anticancer, antiviral, and antimicrobial properties. Their structural diversity and biological significance make them a focal point in pharmacological research, particularly in the context of developing new therapeutic agents.

In conclusion, the green synthesis of metallic nanoparticles represents a promising approach that combines environmental sustainability with advanced material science. The utilization of plant extracts and other biological materials for metallic nanoparticle synthesis not only enhances the biocompatibility of the resulting products but also offers a cost-effective and eco-friendly alternative to traditional methods. As the field of nanotechnology continues to evolve, the integration of green chemistry principles will play a crucial role in

developing sustainable and efficient methods for nanoparticle production. The potential applications of green-synthesized metallic nanoparticles in biomedicine, environmental remediation, and beyond will likely expand, and open new avenues for innovative solutions to contemporary challenges. The uses of pharmaceutical formulations based on herbal active principles represent a dynamic intersection of traditional knowledge and modern science. The multifaceted benefits of plant extracts, including their biological activity, antioxidant properties, and potential as excipients, underscore their significance in drug development. While the therapeutic potential of these formulations is well recognized, challenges related to safety, efficacy, and standardization must be addressed to fully integrate them into contemporary medical practice.

My main areas of research have been in cancer therapy, in particular in magnetic hyperthermia due to the magnetic properties of IONPs, and the analysis of some compounds of natural origin, attached or not on the surface of metallic nanoparticles, with therapeutic potential to highlight the mechanism of action associated with the antitumor effect. These directions were established after completing the doctoral studies and obtaining the title of PhD in Pharmacy. The doctoral studies represented the fundamental basis of these research directions, given the fact that, during the doctoral studies, I developed IONPs by the combustion method, the method optimized by the research group I was part of at the time.

The following years after completing my doctoral studies, were decisive regarding the clearer definition of my future research directions, i.e. I developed the obtaining of IONPs through a method as simple as the combustion method, but safer and ecological from the point of view of environment protection. Moreover, we have developed pharmaceutical formulations based on solid-lipid nanoparticles (SLNs), with a magnetic or metallic core (Ag) and a shell consisting of natural bioactive compounds (derived from plants), on whose surface synthetic compounds with a strong antitumor activity were/or not embedded (e.g. oleanolic acid, lupeol, etc.).

The research carried out was also facilitated by gaining two internal grants, funded by UMFVBT, one doctoral (4DOC/1276/30.01.2020 – *Magnetic nanoparticles as a support for triterpenic antitumor active principles*) and the other post-doctoral (ctr. no. 1682/26.01.2024 – *New advances in the development of smart magnetic nanoparticles used in cancer pathology*). For each synthesized sample or pharmaceutical formulation, both the in vitro and in vivo safety profile as well as the in vitro, in vivo, and in ovo therapeutic effects were established.

The collaborations established during this period added new research directions tangent to the initial directions, to which I contributed by applying the technical skills developed, and the knowledge accumulated throughout the entire scientific activity.

Based on these considerations, the habilitation thesis was structured as follows:

- the first chapter is dedicated to scientific achievements, in which I describe my scientific activity in detail. Here are included the most relevant research regarding my area of expertise, as well as the results obtained (books, articles, participation in scientific events, including awards).
- another chapter refers to my achievements regarding the didactic activity, followed by a detailed presentation regarding my professional activity (education and training, courses, respectively professional experience).
- the habilitation thesis ends with a generous chapter on the didactic and scientific perspectives that I propose further.

Regarding my area of expertise, I present in detail the application of the combustion method as well as the green method for obtaining IONPs. Moreover, the green method is also described for obtaining other types of metallic nanoparticles (namely AgNPs). Each subchapter ends with a detail of the medical and industrial applications of these metallic nanoparticles. The last sub-chapter talks about the synthesis, characterization, and applications in the biomedical field, of preparations based on plant extracts, or of pharmaceutical formulations containing encapsulated metallic nanoparticles obtained, with the surface loaded with either natural bioactive compounds extracted from plants, or compounds with therapeutic effect, chemically synthesized.

The scientific results obtained after completing the doctoral thesis consisted of:

- ❖ 76 ISI-indexed full-text articles;
- ❖ 5 ISI indexed proceedings full-text articles;
- ❖ 9 ISI-indexed abstracts;
- ❖ 25 abstracts published in a volume of abstracts with ISBN;
- ❖ 2 BDI-indexed full-text articles;
- ❖ 3 published book chapters in international publishing houses;
- ❖ 2 published books in national publishing houses
- ❖ 1 - patent
- ❖ 69 - communications at international/national conference/workshop;
- ❖ 18 - research projects: (as manager - 2 internal national projects; as a member - 14 national projects and 2 international projects)

The recognition and impact of my entire activity are underlined by the 76 papers detected by the ISI system, more than half of them are cited in ISI Thomson Reuters-rated journals, totaling a number of 1160 citations (without self-citations), accumulating a Hirsch index  $H = 19$ . Therefore, the research activity I have carried out as a member of the research team conferred me the ability to work in a multidisciplinary team and good communication skills.

In addition to the didactic and research activities carried out, I also performed administrative tasks at the Faculty / University level; therefore, I am an active member of more than 10 commissions established to resolve internal issues regarding the Faculty of Pharmacy.

The scientific perspectives that I want to develop soon, encompass the development of new smart iron oxide-based nanomaterials for biomedical applications. I propose to explore the biomedical use of MIONPs synthesized by two synthesis methods - the biological (green synthesis) and the chemical (combustion) methods, as well as by a technique involving the combination of the two methods.

With an awareness of my own learning needs and processes, I will draw on the knowledge and experience gained to use and apply skills in a variety of contexts. Career building was and is based on: feedback from both collaborators and colleagues, transparency, openness to novelty, communication and last but not least teamwork. I aim to achieve as many as possible of the necessary didactic and scientific objectives that are my responsibility as a teacher, researcher and colleague, using as tools in the fulfillment of the development plan both the maintenance and increase of academic and professional excellence standards and the direct collaboration with researchers, teachers and students.