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Faculty of Chemistry and Chemical Engineering
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SUMMARY OF THE DOCTORAL THESIS

INTERACTION OF NANOPARTICLES WITH BIOLOGICAL MEMBRANES

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TABLE OF CONTENTS

INTRODUCTION

Gold nanoparticles (GNPs) are used for delivery of anticancer drugs due to their low toxicity and high stability. For therapeutic uses of GNPs, their bioavailability is crucial and is a consequence of their interactions with cell constituents, lipids and proteins.

The subject of this study consists of the interaction of gold nanoparticles with biological membranes, such as cell membranes from **two** *cervical cancer* cell lines: **HeLa** and **CaSki**, and **two** human breast cancer cell lines, **MDA-MB-231** and **MCF-7**, as well as from tumor stem cells (isolated from glioblastoma), a GM1 cell **line**, and a ***normal* (*healthy*) cell line** derived from a **dental follicle, DF**.

The gold nanoparticles can be coated with various drugs, such as doxorubicin and/or biomolecules, e.g., resveratrol, R, piperine, P, icariin, Ic, or curcumin, CCM, with potential therapeutic activity and can penetrate the lipid membrane and the blood-brain barrier, BBB, implying their capability to target the interior of the cells. Most effects of the GNP nanoparticles, NPs, coated with cationic biomolecules, such as GNP@doxorubicin (GNP@D) at specific pH, are preferable to than those of individual GNP, due to their more positive charges. In this situation, a depolarization of the cell membrane can occur, which will open calcium channels, and calcium will reach the mitochondria and cause oxidative stress, ultimately killing especially cancer cells. Another effect could be penetration into the cell nucleus and killing cancer cells by apoptosis.

Another research direction could be related to the "blebbing" mechanism, which is responsible for the detachment of the cell membrane from the cytoplasm and facilitating the formation of blebs, followed by the destruction of cancer cells. GNP particles are biocompatible and do not affect normal cells.

In the future, additional animal investigations are necessary to check if the interactions of functionalized GNPs with drugs and natural biomolecules, as adjuvants, destroys cancer cells and at the same time is safe for the cell/organism and whether they can harmlessly infiltrate natural membranes, being representative for clinical applications.

The **first chapter** of this PhD thesis provides a comprehensive overview of the molecular pathways involved in the interactions between curcumin and its metabolites, as well as in brain vascular homeostasis. This review explores the current cellular and molecular aspects of curcumin-based effects, with a focus on metabolism and its impact on pathological conditions, such as neurodegenerative diseases, schizophrenia, and cerebral angiopathy. It also highlights the limitations imposed by the low bioavailability of curcumin and discusses ongoing efforts to overcome these impediments and realize its full therapeutic potential in neurological disorders.

Curcumin is among the best-studied natural substances, known for its biological actions on the central nervous system, its antioxidant and anti-inflammatory properties, as well as its benefits in human health. However, challenges persist in the effective use of curcumin, when considering metabolism and passage through the blood-brain barrier (BBB) in cerebrovascular disease therapies. Current challenges in curcumin applications focus on their effects in neoplastic tissues, along with the development of smart formulations to enhance its bioavailability. Formulations that include complexes of curcumin with phospholipids and proteins, or liposomal encapsulation, have been discovered. These novel strategies aim to

improve the bioavailability and stability of curcumin, as well as its ability to cross the BBB, thereby enhancing its efficacy in treating cerebrovascular diseases.

Chapter two discusses the functionalization of gold nanoparticles, GNP, with doxorubicin, D, an anticancer drug, both in the absence and in the presence of natural adjuvant biomolecules, such as piperine, P, resveratrol, R, resveratrol-piperine complex, RP, and icariin, I (Ic), which are therapeutic molecules with demonstrated anticancer and anti-inflammatory activity, forming well-stabilized colloidal dispersions. The green synthesis of GNP, on which self-assemblies of various selected biomolecules are loaded, adsorbed on their surface as a shell (core-shell assemblies), was confirmed by observing the surface plasmon resonance at approximately 538 nm. Moreover, resveratrol-stabilized gold nanoparticles, GNP-R, are functionalized with selected biomolecules: D, P, R, RP, and I, in different concentrations, resulting in D/P/R/RP/I@GNPs-R composite nanoparticles with different compositions. Another series of stabilized colloidal dispersions is generated using GNP-R1, where initial GNP-R are centrifuged and washed and then dispersed in aqueous solutions and further functionalized with the respective selected biomolecules. This study demonstrates the functionalization of gold nanoparticles, as highly stable composite nanoparticles, in the presence of phosphate buffered saline, PBS, as confirmed by the UV-VIS spectra of their aqueous colloidal dispersions.

Chapter three evaluates the anticancer activity of functionalized GNP_C using the *MTT assay* on ***four human cell lines***: breast cancer cell lines, MDA-MB-231 and MCF-7, tumor stem cells (isolated from glioblastoma), a **GM1** cell line, and a normal (healthy) cell line derived from a **dental follicle, DF**. GNP_C functionalized with R, P, or Ic exhibited comparable anticancer activity to GNP_C functionalized with doxorubicin for low concentrations of gold and natural compounds, thus *reducing the side effects* of the anticancer drug. Natural compounds such as trans-resveratrol, R, piperine, P, and icariin, Ic, have antioxidant and anti-inflammatory properties, as well as potential anticancer activity. Gold nanoparticles, GNP, are biocompatible and can be used as carriers for the delivery of biomolecules, improving their performance even at a low dose. The aim of the present study was to synthesize sodium citrate reduced GNP, denoted GNP_C (or GNP-C), and improve their stability and anticancer activity by functionalization with R, P, Ic, asparagine, A, and doxorubicin, D, as a standard drug. The obtained GNP cores, loaded with the selected biomolecules, adsorbed on their surface as shells, were characterized by various methods: UV-Vis spectroscopy, XRD, AFM, TEM and particle size analysis. These remarkable results require further examinations using various cell lines and animal models, for ***clinical applications***.

Chapter four explores an innovative nanocarrier system with gold nanoparticles (GNP) as the inner core, which aims to load a drug, doxorubicin (D), alone or accompanied by natural agents, such as trans-resveratrol (R), piperine (P) and/or icariin (Ic), enhancing the therapeutic effect of doxorubicin on cervical cancer, for example on HeLa and CaSki cell lines. Multifunctional GNP loaded with D, R, P, Ic (GNP@D/R/P/Ic), was prepared and characterized by UV-Vis absorption band (surface plasmon resonance: SPR), TEM, XRD, zeta potential and AFM. Their toxicity properties were evaluated on two cervical cancer cell lines, HeLa and CaSki, using the *MTT assay and phase contrast microscopy images*. Mono-functionalized GNP nanoparticles (e.g., GNP@D, GNP@R, GNP@P, GNP@Ic), and bi-functionalized (e.g., GNP@D/R), tri-functionalized (e.g., GNP@D/R/P), or multi-functionalized (e.g., GNP@D/R/P/Ic), were successfully prepared and characterized by UV-Vis absorption band,

TEM, HR-TEM, AFM and zeta potential. In vitro evaluation showed that the obtained GNP@D/R/P/Ic exhibited well-established stability and high anticancer efficacy. The nanoparticles for drug delivery were realized by combined strategies as well as multifunctionalized GNP, opening a new perspective for the design of future combination therapies against tumors. The proposed synergistic drug delivery strategy and chemical treatment of selected cancer cells improved the cytotoxicity and efficacy of doxorubicin on cervical cancer cells, offering advantages over monotherapeutic treatment with doxorubicin or resveratrol, or the other selected natural compounds.

Chapter five presents a comprehensive analysis of current research on gold nanoparticles (GNP), including their synthesis, characterization, and applications in cancer therapy. GNP are synthesized by various **chemical** and **biological** methods, each contributing to diverse applications. *Cytotoxicity* plays a critical role in determining their practical utility, with distinct considerations depending on the context: in *medical applications*, high biocompatibility with normal living cells is essential, while in targeting pathogens and cancer cells, induction of *apoptosis* is desirable. Thus, ***optimizing the concentration*** of GNP for each specific application is of paramount importance. Characterization techniques for GNP, their functionalization using biomolecules, and their subsequent applications in cancer therapy are highlighted, underlining their potential to bring about a breakthrough in therapeutic strategies.

Chapter six evaluates the syntheses of gold nanoparticles (GNP) and silver nanoparticles (nanoAg) with a focus on controlling the size, shape, and stability of nanoparticles (NPs). Various reducing and capping agents of NPs from the fields of chemistry and biology have been identified along with their role in the synthesis and properties of controlled NPs. These NPs have been characterized with a variety of methods to determine the activities of nanoparticles and their real-life applications. In addition, the carriers of these NPs, in vitro and in vivo investigations, and nanoscale interaction models are presented. This review also systematically addresses the biomedical applications of GNPs and AgNPs considering the current challenges and prospects in this field of research.

CHAPTER 1. SYNTHESIS OF CURRENT ASPECTS OF THE EFFECTS DUE TO CURCUMIN IN NEURODEGENERATIVE, NEUROINFLAMMATORY AND CEREBROVASCULAR DISEASES

Curcumin is among the best-studied natural substances, known for its biological actions on the central nervous system, its antioxidant and anti-inflammatory properties, and its benefits in human health. However, challenges persist in the effective use of curcumin, when considering metabolism and passage through the blood-brain barrier (BBB). This Chapter 1 provides an original overview of the current state of knowledge related to curcumin-based therapies (Figure 1), highlighting their potential as a complement to conventional medicine.

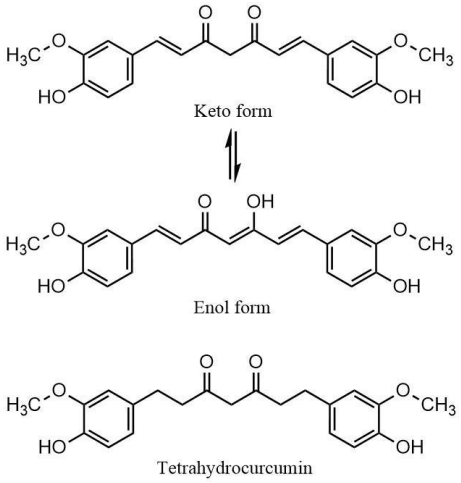

Figure 1. Curcumin in its keto and enol forms, and the structure of tetrahydrocurcumin (TC).

The multiple actions of curcumin make it a promising therapeutic candidate, for example in reducing metal-induced neurotoxicity. However, its limited bioavailability highlights the need for advanced formulations, such as nanoparticles or liposomal curcumin, to enable effective clinical application. Such effective clinical applications are presented in Table 1.

Table 1. Neuroprotective effects of curcumin in neurodegenerative diseases presented in terms of mechanisms, disease-specific impacts, and beneficial outcomes

	Mechanism/Effect	**Physiological Effects**
Anti-inflammatory	Inhibits pro-inflammatory cytokines (TNF-α, IL-1β) and NF-κB signalling.	Reduces chronic neuroinflammation, a key factor in AD
Antioxidant	Scavenges free radicals, enhances glutathione, SOD, and catalase activity	Protects neurons from oxidative damage in PD
Amyloid aggregation inhibition	Binds to Aβ, preventing their formation and facilitating disaggregation	Mitigates the hallmark pathology of AD
Mitochondrial protection	Preserves mitochondrial membrane integrity, reduces oxidative stress within mitochondria	Prevents energy deficits and apoptotic signalling, critical in PD
Metal chelation	Binds metals like iron and copper, reducing metal-induced oxidative damage	Decreases oxidative stress in AD and PD
Autophagy modulation	Enhances autophagic processes, promoting clearance of damaged proteins	Prevents accumulation of toxic aggregates in diseases like AD and PD
Neurogenesis stimulation	Increases BDNF expression	Supports synaptic plasticity and neuronal survival in various neurodegenerative contexts
Apoptosis inhibition	Downregulates caspase-3 expression	Prevents neuronal loss in diseases such as PD

There are two main perspectives on the actions of curcumin: its bioavailability and the pleiotropic effects mentioned in this chapter (regulation of pro-apoptotic factors such as caspase-3, inhibition of pro-inflammatory cytokines (such as TNF-α, IL-1β) and NF-κB signaling, increased expression of neurotrophic factor (BDNF) and reduction of heavy metal-induced neurotoxicity).

CHAPTER 2. GOLD NANOPARTICLES FUNCTIONALIZED WITH ANTICANCER BIOCOMPOUNDS

This study focuses on the functionalization of gold nanoparticles (GNP) with doxorubicin (D), an anticancer drug, both in the absence and presence of natural adjuvant biomolecules such as piperine (P), resveratrol (R), resveratrol-piperine complex (RP), and icariin (Ic), which are therapeutic molecules with demonstrated anticancer and anti-inflammatory activity, forming well-stabilized colloidal dispersions. The surface plasmon resonance at approximately 538 nm was used to confirm the green production of GNP as cores onto which self-assemblies of various selected biomolecules are loaded and adsorbed. Furthermore, the resveratrol-stabilized gold nanoparticles, GNP-R, are functionalized with selected biomolecules: D, P, R, RP, and Ic, at varying concentrations, yielding D/P/R/RP/Ic@GNP-R composite nanoparticles of varying compositions. Another series of stabilized colloidal dispersions is generated as GNP-R1, where the initial GNP-R are centrifuged and washed and then dispersed in aqueous solutions and further functionalized with the respective selected biomolecules.

The procedure described by Mohanty et al. was followed, but for the HAuCl4 concentration indicated by the authors (4 mM) we did not obtain a colloidal gold solution. The color of the reaction mixture quickly turned red and after 30 minutes an absorption peak was observed at 551 nm; but then a cloudy yellowish-brown suspension resulted; consequently, a more dilute $HAuCl_4$ solution (10^{-3} M) was used [27]. The color of the reaction mixture quickly changed to red, signifying the formation of gold nanoparticles, GNP, with their surface covered by resveratrol molecules, denoted GNP-R, and the maximum absorbance increases in the first hour. The maximum absorption is observed at approximately 538 nm. This GNP-R colloidal solution was stable for more than a year.

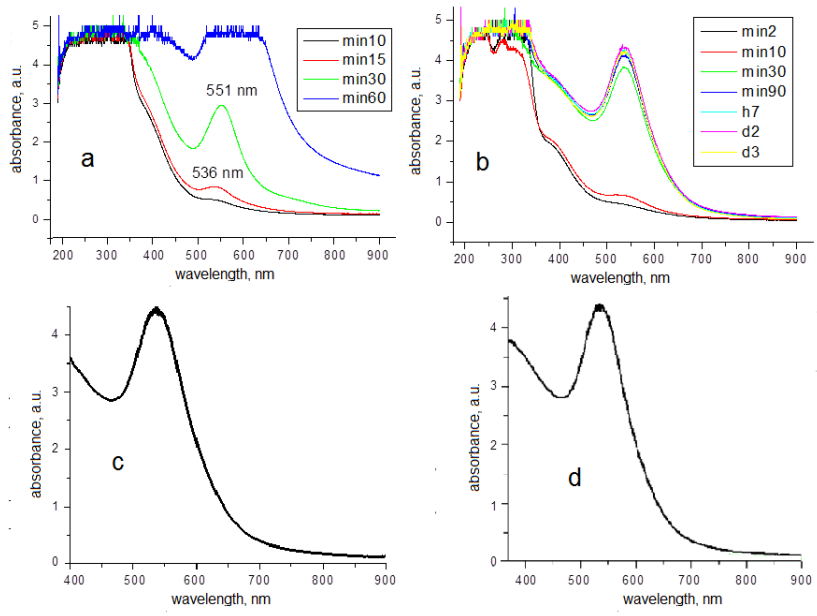

Figure 1. Time evolution of the UV-Viz spectrum for a mixture of 4 mM $HAuCl_4$ and resveratrol, in NaOH solution (pH 12) at room temperature (a); 1 mM $HAuCl_4$ solution and resveratrol (b); UV-Vis spectrum of a GNP-R1 solution after 1 year (d).

The strongest effect on the absorption band is manifested for the piperine (P) solution and for the resveratrol-piperine (RP) complex, both initially in PBS solutions. The UV-Vis spectra of the mixtures formed by the GNP-R1 colloidal solution and various solutions of resveratrol (R), doxorubicin (D), piperine (P) and resveratrol-piperine (RP) complex, at different volume ratios, are presented in Figure 7.

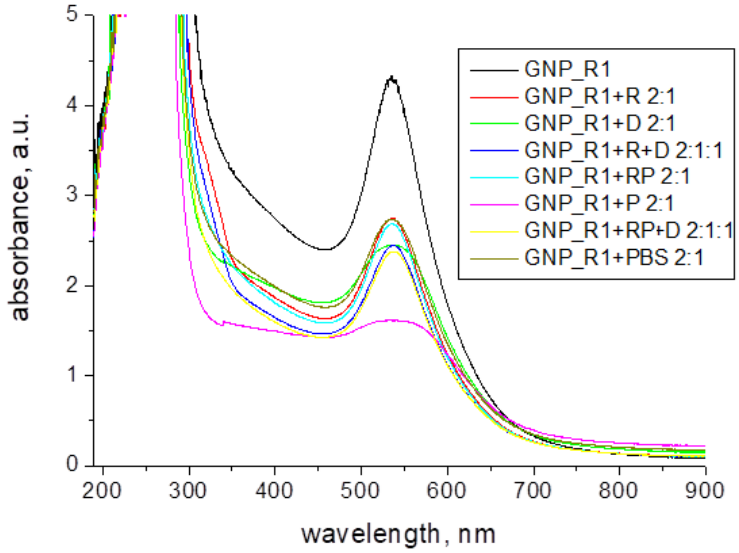

Figure 7. UV-Vis spectrum of the aqueous dispersion of GNP-R1 (Au 179 mg/L); of the R@GNP-R1 nanocomposite obtained from GNP-R1 and R, with R solution (30 mg/L) in PBS, of the D@GNP-R1 nanocomposite from GNP-R1 with D aqueous solution (42 mg/L), of the D/R@GNP-R1 nanocomposite from GNP-R1 with R solution (30 mg/L) in PBS and D, with D aqueous solution (42 mg/L), of the RP@GNP-R1 dispersion, from GNP-R1 and RP solution (50 mg/L) in PBS, of the P@GNP-R1 nanocomposite from GNP-R1 with P solution (40 mg/L) in PBS, of the D/RP@GNP-R1 nanocomposite from GNP-R1 mixed with RP solution (50 mg/L) in PBS and aqueous solution D (42 mg/L), and of the aqueous dispersion of GNP-R1 (Au 179 mg/L) with PBS solution, at different volume ratios, all together in the final dispersions, given in the inset of Figure 7.

A major objective of this study was to optimize the functionalization of GNP to be beneficial in achieving a multifunctional arrangement of multi-therapeutic components, within the adsorbed coating on the exterior of the nanoparticles, intended to achieve strong stability in various aqueous dispersions, including cell cultures.

CHAPTER 3. THE EFFECT OF GOLD NANOPARTICLES SYNTHESIZED WITH SODIUM CITRATE AND FUNCTIONALIZED WITH NATURAL COMPOUNDS WITH ANTICANCERIGENIC ACTION ON SOME TUMOR CELL LINES

The goal of this study was to synthesize GNP with sodium citrate, denoted GNP_C (or GNP-C), and to improve their stability and anticancer activity by functionalization with R, P, Ic, asparagine, A, and doxorubicin, D, as standard drugs. The GNP obtained as cores, loading selected biomolecules, adsorbed on their surface as shells, were characterized by various methods: UV-Vis spectroscopy, XRD, AFM, TEM and particle size analysis.

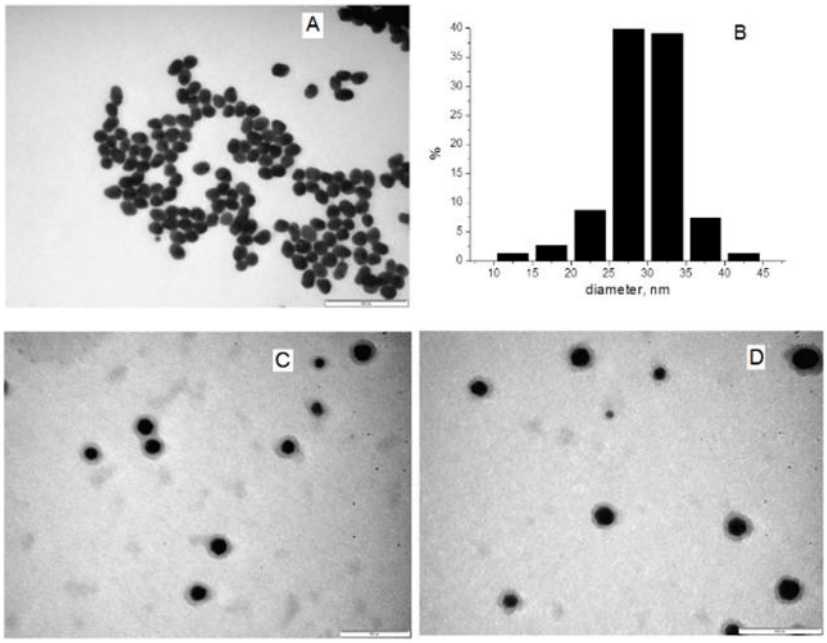

Figure 2. TEM image of the synthesized GNP_C nanoparticles; the bar is 200 nm (A) and the size distribution histogram for GNP_C (B). TEM image of the functionalized GNP, used in sample composition (3) GNP-C 3.9 μg/mL, P 0.67 μg/mL in Fig. 7 and in sample (2) in Fig. 8, is shown in C; and TEM image of sample composition (4) GNP-C/A 3.9 μg/mL, D 0.21 μg/mL used in Fig. 7 and as sample (3) in Fig. 8, is shown in D; the bar is 100 nm (C and D).

The anticancer activity of functionalized GNP_C was evaluated using the MTT assay on model human cell lines: MDA-MB-231 and MCF-7 (breast cancer cell lines), tumor stem cells (isolated from glioblastoma), a GM1 cell line, and a normal (healthy) cell line derived from a dental follicle, DF.

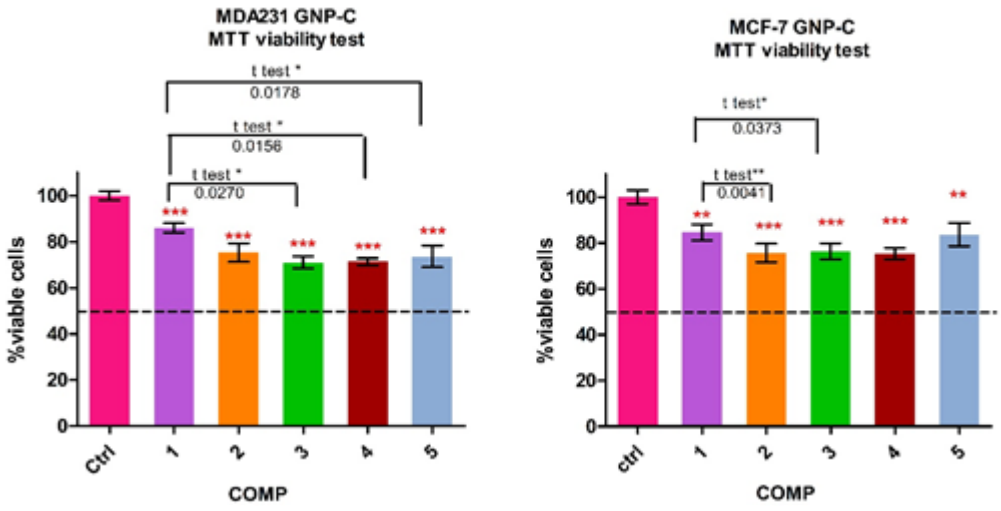

Figure 7. Cell viability (viable cells in % of Control (Ctrl), from MTT assay): Control (untreated cells); 1) GNP-C; 2) GNP-C, R; 3) GNP-C, P; 4) GNP-C/A, D; 5) GNP-C, Ic at different concentrations on MDA231 and MCF-7 cell lines.

GNP_C functionalized with R, P or Ic showed comparable anticancer activity to doxorubicin-functionalized GNP_C for low concentrations of gold and natural compounds, thus reducing the side effects of the anticancer drug.

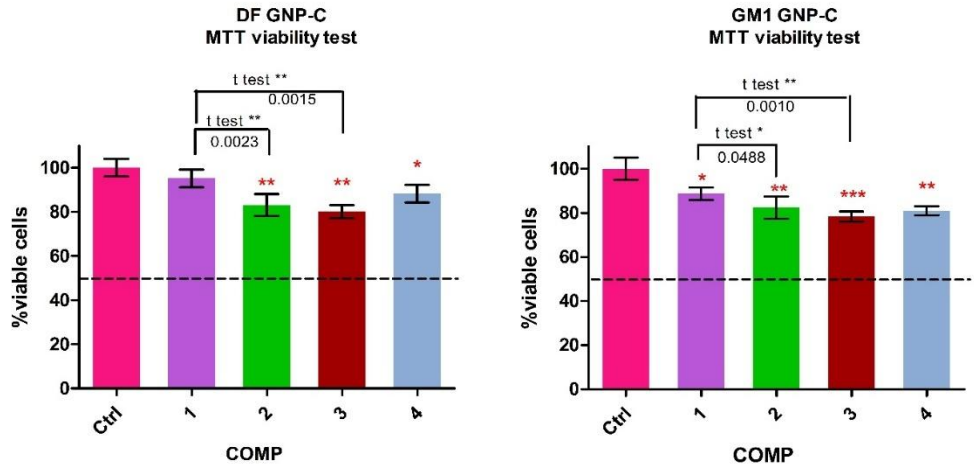

Figure 8. Cell viability (% viable cells of Control, from MTT assay): Control (Ctrl); 1) GNP-C; 2) GNP-C / P; 3) GNP-C/A / D; 4) GNP-C / Ic at different concentrations on DF and GM1 cell lines.

The cytotoxicity efficacy plays a crucial role in determining their practical utility in medical applications, also having high biocompatibility with normal living cells, while targeting cancer cells by inducing apoptosis. This study highlights the characterization techniques for GNP, their functionalization using biomolecules and their potential applications in cancer therapy, highlighting their potential in advancing therapeutic strategies.

CHAPTER 4. IMPROVED STABILITY OF MULTIFUNCTIONALIZED GOLD NANOPARTICLES WITH POTENTIAL ANTICANCERIGENIC EFFICACY ON HUMAN CANCER CELLS

The drug delivery nanoparticles were realized by combined strategies as well as multifunctional GNP, opening a new perspective for the design of future combination antitumor therapies. The proposed synergistic drug delivery strategy and chemical treatment of selected cancer cells enhanced the cytotoxicity and efficacy of doxorubicin on cervical cancer cells, offering advantages over monotherapeutic treatment with doxorubicin or resveratrol, or the other selected natural compounds.

Multifunctional GNP loaded with D, R, P, Ic (GNP@D/R/P/Ic) were prepared and characterized by UV-Vis absorption band (surface plasmon resonance: SPR), TEM, XRD, zeta potential and AFM. Their toxicity properties were evaluated on two cell lines, HeLa and CaSki, using MTT assay and phase contrast microscopic imaging.

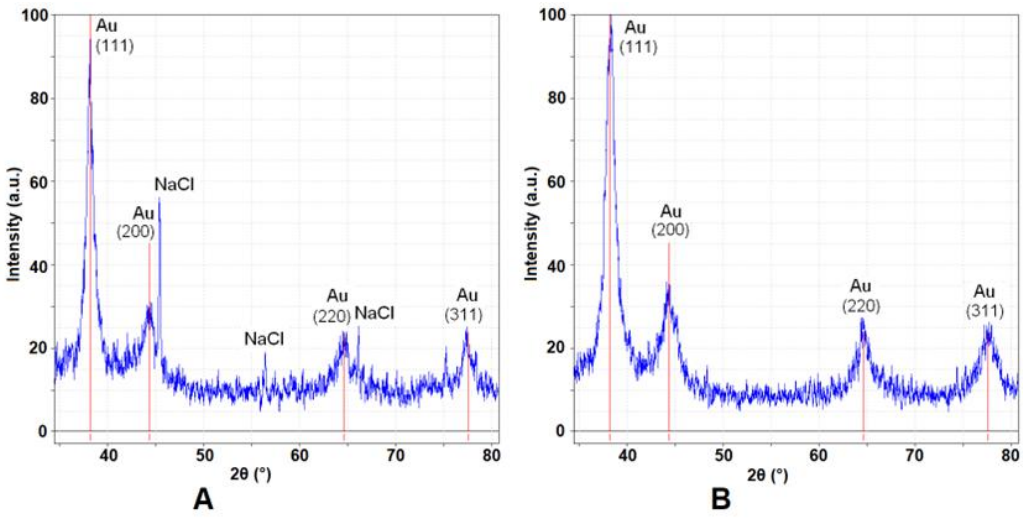

Figure 3. X-ray diffraction patterns for the synthesized GNP_R (GNP-R) nanoparticles (panel A) and GNP_R1 (GNP-R1) nanoparticles (after centrifugation and washing, panel B); [00-0000] experimental patterns, blue curves; PDF: [89-3697] red vertical bars indicate the positions of the diffraction lines for the ordered crystalline domains of Au; XRD radiation with the wavelength, λ, of Cu-Kα X-rays is 1.541874 Å. The 2θ(°) values correspond to the diffraction planes of the gold crystallites and confirm their formation and presence. θ is the Bragg angle.

The aim of this research is to explore a new nanocarrier system with gold nanoparticles (GNP), as the inner core, which aims to load a drug, doxorubicin (D), alone or accompanied by natural agents, such as trans-resveratrol (R), piperine (P) and/or icariin (Ic), improving the therapeutic effect of doxorubicin on cervical cancer, for example on HeLa and CaSki cell lines.

Cell viability (% viable cells from Control, from MTT assay) versus composition for HeLa cells at 24 and 48 hours is presented in Figure 8, and for the CaSki cell line in Figure 9. In both cases, a drastic reduction in cell viability was detected compared to control cells which are untreated, with the effect becoming pronounced after 48 hours.

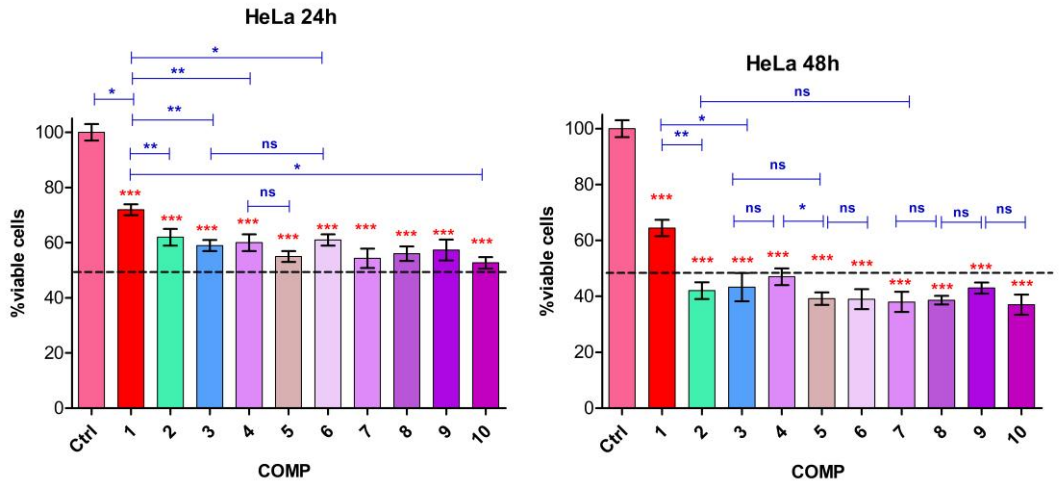

Figure 8. Control (Ctrl); 1-D; 2-R, D; 3-P, D; 4-R, P, D; 5-Ic, D; 6-GNP_R1, D; 7-GNP_R1, R, D; 8-GNP_R1, P, D; 9-GNP_R1, Ic, D; 10-GNP_R1, R, P, Ic, D at different concentrations.

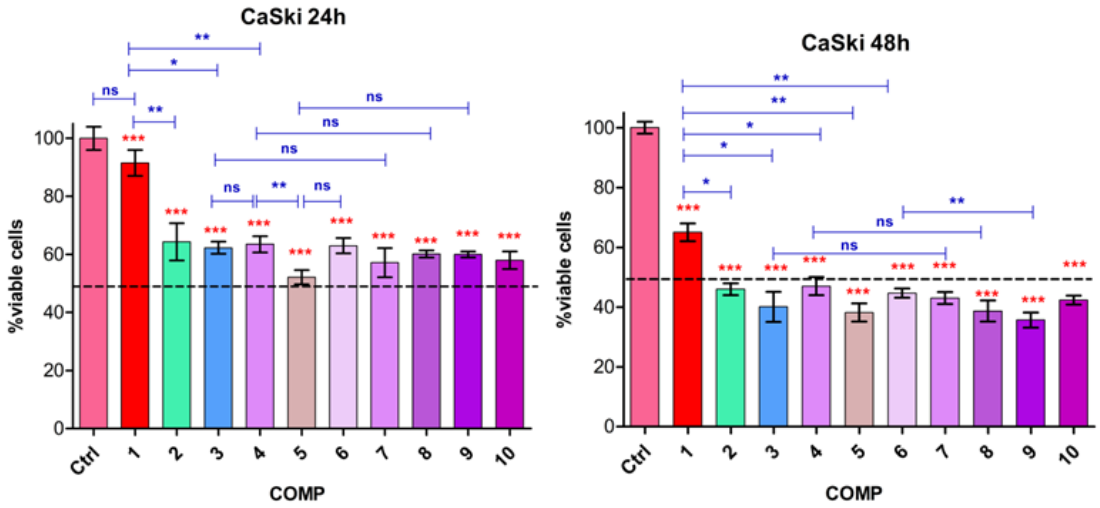

Figure 9. Control (Ctrl); 1-D; 2-R, D; 3-P, D; 4- R, P, D; 5-Ic, D; 6-GNP_R1, D; 7-GNP_R1, R, D; 8-GNP_R1, P, D; 9-GNP_R1, Ic, D; 10-GNP_R1, R, P, Ic, D at different concentrations.

CHAPTER 5. GOLD NANOPARTICLES: FROM SYNTHESIS TO FUNCTIONALIZATION AND BIOMEDICAL APPLICATIONS

Nanoparticles with sizes between 1 and 100 nm are mainly used for clinical and commercial purposes for the reason of their large surface area and unique physicochemical, mechanical, and electrical properties [1,2]. Nanotechnology, the science that focuses on molecular-scale processes, is at the heart of these advances [3-9]. It has rapidly integrated disciplines such as physics and engineering [3], biomedicine [4-8], pharmaceutical sciences [7,8], and nanomedicine [9,10], with applications in molecular biology, biophysics, and bioengineering [9]. In addition, the field of nanotoxicology examines the relationships between the physicochemical properties of nanoparticles and their toxicological profiles [3,8,10]. Among nanoparticles, gold nanoparticles (GNP) are particularly significant in biomedicine due to their distinctive optical and electronic properties [1-3,8,11,12].

Over the years, researchers have refined and improved the methods for synthesizing GNP, with three classic approaches attributed to Turkevich, Brust, and Martin [34-36]. The Turkevich method, introduced in 1951, uses trisodium citrate to reduce gold ions (Au^{3+}) to gold atoms (Au^0), yielding spherical nanoparticles. In addition to chemical methods, biological synthesis techniques involving bacteria, plants, biomolecules, algae, and fungi have been reported in recent years. These methods offer economic and environmental advantages [37-44].

Considering the incidence of cancer as a major health challenge in this century, this section focuses on the anticancer properties of gold nanoparticles. GNP can be functionalized with various biomolecules to enhance their properties, with doxorubicin, piperine, resveratrol, and icariin being notable examples.

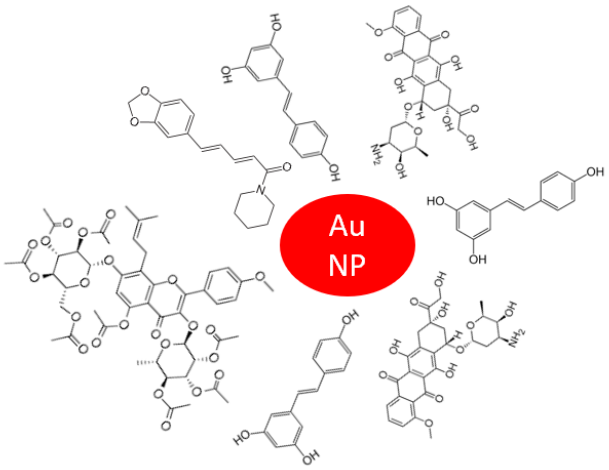

Figure 3. Functionalization of GNP with resveratrol, doxorubicin, piperine and icariin

All cell membranes are composed of lipids, usually arranged in a bilayer structure. A defining characteristic of these lipids is their amphipathic nature, being constituted from a hydrophobic tail and a hydrophilic head group [86]. Cell membranes play an important role in maintaining the integrity of the cell, providing both protection and facilitating essential functions of the cell. [87] The interface between the cell interior and the outer lipid layer serves as a dynamic site where lipids and proteins interact to drive diverse biochemical processes [88].

Model membranes serve as simplified in vitro representations of biological membranes, offering the significant advantage of providing qualitative insights into the physicochemical properties of lipids. Thus, they are useful tools in research [95–98]. These membranes are usually fabricated on solid support and can be monolayers or bilayers. [99-102] They are usually bilayers but can also exist as suspended or deposited vesicular layers. Additional types include bilayers, lipid bilayers with cushioned polymers, hybrid bilayers, and tethered lipid bilayers [93–103].

In cancer therapy, gold nanoparticles have shown promise as carriers for anticancer drugs, such as curcumin, a natural phenolic antioxidant. Studies have shown that GNP enhances the cytotoxicity of turmeric against cancer cells while protecting healthy cells [134]. This dual functionality highlights the versatility and potential of gold nanoparticles in therapeutic applications.

Metal nanoparticles are currently a significant area of interest, with extensive research aimed at translating them from experimental to practical applications. Given this trajectory, nanoparticles are expected to gain even greater importance in nanoscience and everyday life in the near future.

CHAPTER 6. SILVER AND GOLD NANOPARTICLES: CHALLENGES AND PROSPECTS

It should be noted that both silver nanoparticles (AgNPs) and gold nanoparticles (GNPs) are of great interest for science, nanotechnology and medical applications. In general, from a clinical point of view, the use of nanoparticles is due to their large contact surface and physicochemical properties, as well as their toxic potential for the human body. AgNPs can be applied as an antifungal agent against Candida and Xanthomonas [254-256] strains and also in combination with antifungal agents, such as amphotericin B [205]. Another area of application of silver nanoparticles is in the treatment of infections and cancer (Figure 4).

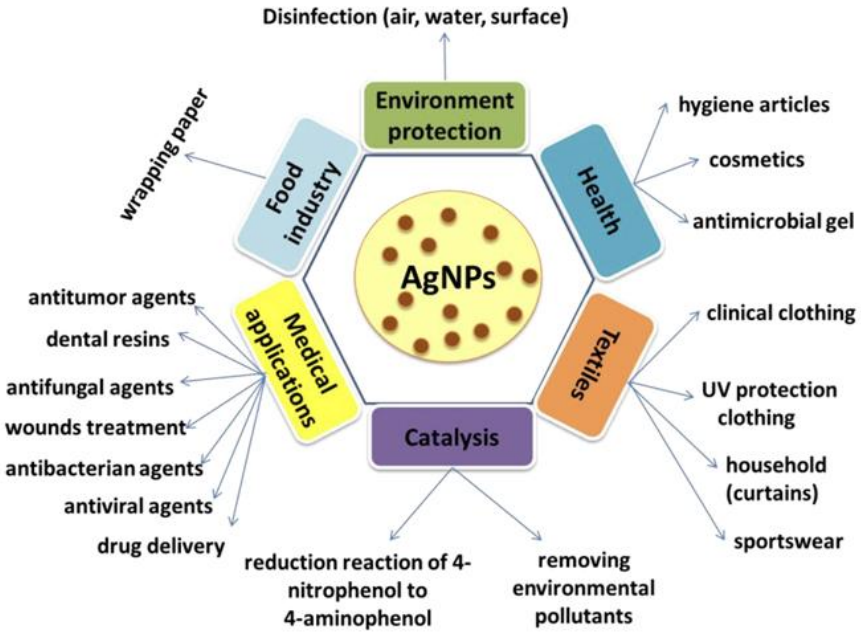

Figure 4. Applications of silver nanoparticles

CHAPTER 7. GENERAL CONCLUSIONS

This PhD thesis provides a comprehensive presentation of the effect exerted by gold and silver nanoparticles functionalized with different active compounds, represented by anticancer drugs or natural products of plant origin, on tumor cells.

The main conclusions that can be drawn from this scientific endeavour are the following:

1. The cerebrovascular actions of curcumin occur at high doses leading to the presence of its main metabolites capable of crossing the blood-brain barrier. Lower doses of curcumin can also be administered in nanomedicine, using advanced formulations such as nanoparticles, liposomes and polymer-based carriers to improve their stability, bioavailability and targeted delivery to the brain. In the context of stroke, curcumin appears to ameliorate inflammation and oxidative stress. It reduces ischemia and contributes to an optimal adaptive immune response by activating microglia Iba1, decreasing matrix metalloproteinase 9 and increasing Nrf2 expression. Curcumin also exhibits a vascular protective effect in type 1 and 2 diabetes, conditions linked to a higher risk of neurodegeneration due to the inhibitory effect of hyperglycemia on proteasomal degradation. These actions suggest a likely role for curcumin in the complementary management of Alzheimer's disease, Parkinson's disease (AD), PD, and microangiopathy, regardless of blood glucose concentrations.

2. The GNP-R and GNP-R1 colloidal solutions obtained by green synthesis via resveratrol reduction were used together with doxorubicin, piperine, resveratrol, resveratrol-piperine complex and icariin, in the synthesis of multifunctional GNP-anticancer drug composites, e.g., D/P/R/RP/Ic@GNP-R. These advanced composites were made by self-assembly of biomolecules on the surface of gold nanoparticles, already stabilized with a resveratrol coating. GNP has a great ability to be easily functionalized with biomolecules, as determined from surface plasmon resonance, SPR. The constructed multifunctional GNPs provide colloidal solutions of high stability in PBS under various conditions, which makes them useful for biological investigation in various cell cultures, as tools for pharmaceutical and medical applications.

3. Optimization of GNP functionalization and of their concentration for loading different biomolecules at their site of action is of particular importance and specific to each type of human cancer cell line. The cytotoxicity efficacy plays a crucial role in determining their practical utility in medical applications, also having high biocompatibility with normal living cells, while targeting cancer cells by inducing apoptosis. The characterization techniques for GNP, their functionalization using biomolecules and their potential applications in cancer therapy were highlighted, underlining their potential in advancing therapeutic strategies.

4. The nanoparticles used for drug delivery were made through a combinatorial strategy, as well as multifunctional GNP. The biocompounds used as adjuvants, resveratrol, piperine and icariin, added to the GNP composition, had similar cytotoxicity-inducing effects on cervical cancer cell lines, HeLa and CaSki, significantly increased in the presence of doxorubicin. Functionalized GNP_R1@D/Ic (GNP@D/Ic) or GNP_R1@Ic (GNP@Ic) induced more intense effects compared to resveratrol and piperine, especially on the HeLa cell line. Mono-functionalized Au nanoparticles (e.g., GNP@D or GNP_R1@D, GNP_R1@R, GNP_R1@P, GNP_R1@Ic), and bi-functionalized (e.g., GNP_R1@D/R), tri-functionalized (e.g.,

GNP_R1@D/R/P), or multi-functionalized GNP_R1@D/R/P/Ic) were successfully prepared and characterized by techniques such as UV-Vis, TEM, HR-TEM, AFM and zeta potential.

5. The proposed synergistic drug delivery strategy and chemotherapeutic treatment of cancer cells enhanced the cytotoxicity and efficacy of doxorubicin on cervical cancer cells, offering advantages over treatment with doxorubicin or resveratrol, or other individual natural compounds. The enhancement of the anticancer activity of doxorubicin through interaction with gold nanoparticles and the natural biomolecules, resveratrol, piperine, and icariin, may have clinical applications.

6. With the recent integration of GNP into medical preparations and devices, it has been necessary to clarify how these nanoparticles interact with cell membranes, both in model systems and in vivo. Metal nanoparticles represent a significant area of interest, with extensive research aimed at translating them from experimental to practical applications. Given this trend, nanoparticles are expected to gain even greater importance in nanoscience and everyday life in the near future.

7. A thorough characterization of nanoparticles is required to determine their morphology, size, shape, distribution and functional groups, as these determine their activity and potential applications. Functionalization of nanoparticles with various biomolecules such as antibiotics, anesthetics, anticancer compounds, fatty acids, amino acids and proteins ensures their biological activities against pathogens and cancer.

In future research, multifunctional GNP will be further used and developed in the study of their interaction with cancer cells and new formulations will certainly be adapted to obtain optimized therapeutic combinations and thus making them suitable for supporting medical applications. Furthermore, the interaction between molecules adsorbed on GNP could lead to highly ordered self-assemblies, which could also be maintained inside cells, modifying the mechanism of action of doxorubicin in cancer cells with resistance to anticancer drugs.

CHAPTER 8. SCIENTIFIC RESEARCH ACTIVITY

The scientific research activity is embodied in **seven (7) published scientific papers**, of which **four (4) are ISI-rated**: with a **total I.F. = 9.6;** three (3) papers published in *Annals. Series on Biological Sciences*, Academy of Romanian Scientists (AOSR); and eight (8) participations in scientific conferences.

1. LIST OF PUBLISHED ARTICLES

1. C.-A. Moldoveanu, M. Tomoaia-Cotisel, A. Sevastre-Berghian, G. Tomoaia, A. Mocanu, C. Pal-Racz, V.-A. Toma, I. Roman, **M.-A. Ujica**, L.-C. Pop, „A Review on Current Aspects of Curcumin-Based Effects in Relation to Neurodegenerative, Neuroinflammatory and Cerebrovascular Diseases", *Molecules*, **30**(1), 43 (2024). https://doi.org/10.3390/molecules30010043 (**I.F.=4.6**); **five (5) citations**.
2. **M. A. Ujica**, I. Mang, O. Horovitz, A. Mocanu, M. Tomoaia-Cotisel, „Gold nanoparticles functionalized with anticancer biocompounds", *Studia UBB Chemia*, **70**(1), 47-63 (2025). DOI: 10.24193/subbchem.2025.1.04 **(I.F.=0.5)**
3. **M. A. Ujica**, I. Mang, O. Horovitz, O. Soritau, G. Tomoaia, A. Mocanu, H.-R.-C. Benea, V. Raischi, C. Varhelyi, G. Borodi, M. Tomoaia-Cotisel, „The effect of gold nanoparticles synthesized by sodium citrate and functionalized with anticancer and natural compounds on cancer cell lines", *Studia UBB Chemia,* **70**(1), 65-87 (2025). DOI: 10.24193/subbchem.2025.1.05 (**I.F.=0.5**)
4. A. Mocanu, **M. A. Ujica**, O. Horovitz, G. Tomoaia, O. Soritau, C. R. Popa, A. Kun, C. T. Dobrota, H. R.-C. Benea, I. M. Mang, G. Borodi, V. Raischi, M. Roman, L.-C. Pop, M. Tomoaia-Cotisel, „Enhanced Stability of Multi-functionalized Gold Nanoparticles and Potential Anticancer Efficacy on Human Cancer Cells", *Biomedicines*, **13**, 1861 (2025). https://doi.org/10.3390/ biomedicines13081861 (**I.F.= 3.9**).
5. **M. A. Ujica**, C.-T. Dobrota, G. Tomoaia, C.-L. Rosoiu, A. Mocanu, M. Tomoaia-Cotisel, „Interaction of functionalized gold nanoparticles with biological membranes", *Annals. Series on Biological Sciences*, **14**(1), 139-168 (2025). DOI 10.56082/annalsarscibio.2025.1.139
6. **M. A. Ujica**, C.-T. Dobrota, G. Tomoaia, A. Mocanu, C.-L. Rosoiu, I. Mang, V. Raischi, M. Tomoaia-Cotisel, „Gold nanoparticles: from synthesis through functionalization to biomedical applications", *Annals. Series on Biological Sciences*, **13**(2), 145-167 (2024). DOI: 10.56082/annalsarscibio.2024.2.145
7. **M. A. Ujica**, G. A. Paltinean, A. Mocanu, M. Tomoaia-Cotisel, „Silver and Gold Nanoparticles: Challenges and Perspectives", *Annals. Series on Biological Sciences*, **9**(1), 97-139 (2020); **19 citations**. DOI: 10.56082/annalsarscibio.2020.1.97

2. SCIENTIFIC CONFERENCE PARTICIPATION LIST

The scientific results from the Doctoral Thesis "Interaction of nanoparticles with biological membranes" were disseminated through seven (7) oral presentations at the National Conferences of the Romanian Academy of Scientists and a poster presentation at the International Conference "Processes in Isotopes and Molecules", PIM.

1. AOSR Spring Scientific Conference, "Science and Cultural Diplomacy - Factors of International Cooperation", May 23-24, 2025, Bucharest:
Oral presentation: **Biological Sciences** section.

1.1. C. T. Dobrotă, C. L. Roşoiu, M. A. Ujică, G. Tomoaia, A. Mocanu, O. Horovitz, M. Tomoaia-Cotişel, „Opportunities and challenges for optimal doxorubicin delivery based on the combination of gold nanoparticles and different natural biocompounds in cancer treatment", **Volume of abstracts** 2025, pag. 128-129, ISSN 2601-5102.

1.2. M. A. Ujică, G. Tomoaia, C. T. Dobrotă, O. Horovitz, C.-L. Roşoiu, A. Mocanu, M. Tomoaia-Cotişel, „Gold nanoparticles enhance doxorubicin delivery. Mechanism of action of doxorubicin in cancer therapy", **Volume of abstracts** 2025, pag. 129-130, ISSN 2601-5102.

Oral presentation: **Medical Sciences** section.

1.3. M. A. Ujică, C. T. Dobrotă, G. Tomoaia, O. Soriţău, A. Mocanu, O. Horovitz, C.-L. Roşoiu, M. Tomoaia-Cotişel, „Interaction of nanoparticles with biological membranes", **Volume of abstracts** 2025, pag. 155-157, ISSN 2601-5102.

2. AOSR National Autumn Scientific Conference, "The Role of Artificial Intelligence in the Sustainable Development of Romania", September 23-24, 2024, Iași:
Oral presentation: **Chemical Sciences** section.

2.1. M. A. Ujică, I. Mang, G. Tomoaia, A. Mocanu, M. Tomoaia-Cotişel, "Physical-Chemical Study of Gold Nanoparticles Functionalized with Anticancer Biocompounds", **Volume of abstracts**, Volume **18**(2), 48 (2024) ISSN 2601 – 5102.

Oral presentation: **Biological Sciences** section.

2.2. M. A. Ujică, I. Mang, G. Tomoaia, A. Mocanu, M. Tomoaia-Cotişel, "New doxorubicin formulation strategies in cervical cancer, HeLa and CaSki cell lines", **Volume of abstracts**, Volume **18**(2), 83 (2024) ISSN 2601 – 5102.

Oral presentation: **Medical Sciences** section.

2.3.M. A. Ujică, I. Mang, G. Tomoaia, O. Soritau, O. Horovitz, A. Mocanu, M. Tomoaia-Cotişel, "Enhancement of doxorubicin cytotoxicity in combination with gold nanoparticles, resveratrol, piperine, resveratrol:piperine complex, in cervical cancer cell lines, HeLa and CaSki", **Volume of abstracts**, Volume **18**(2), 101 (2024) ISSN 2601 – 5102.

3. National Autumn Scientific Conference of the Academy of Scientists of Romania (AOSR). Real Convergence Romania – European Union, CRUE, September 20-21, 2019, Braşov:
Oral presentation: **Medical Sciences** section.

3.1. M. A. Ujica, M. Tomoaia-Cotisel, A. Mocanu, O. Horovitz, “Gold and Silver Nanoparticles: From Synthesis to Applications”, **Integral Communications**, Volume **2**, 318-325 (2019) ISSN 978-973-618-430-7.

4. 12th International Conference, "Processes in Isotopes and Molecules", PIM, September 25-27, 2019, Cluj-Napoca, National Research and Development Institute for Isotopic and Molecular Technologies:

4.1. Poster T4-56: M. A. Ujica, A. Mocanu, D.A. Chira, M. Tomoaia-Cotisel, "Challenges in metallic nanoparticles synthesis and prospective applications".

3. LIST OF RESEARCH PROJECTS

Participation in scientific research contracts carried out in the Scientific Research Center of Excellence in Physical Chemistry, CECHIF Center:

1. **INA-MAT Project 2021-2023**
   PhD student M. A. Ujica participated in the scientific research project PN-III-P4-ID-PCE2020-1910, No. 186/2021, INA-MAT, during the period 2021-2023, carried out under the leadership of the Project Director Prof. Univ. Dr. Maria Tomoaia-Cotisel, in the Scientific Research Center of Excellence in Physical Chemistry, led by the Founder (2006) and Director (2006-present) of the Center, Prof. Univ. Dr. Maria Tomoaia-Cotisel, Faculty of Chemistry and Chemical Engineering; within Babeș-Bolyai University of Cluj-Napoca.

2. **NanoSilva Project 2017 -2019**
   PhD student M. A. Ujica participated in the scientific research project PN-III-P4-ID-PCE-2016-0875, NANOSILVA No. 83/2017, in 2019, carried out under the leadership of the Project Director Assoc. Prof. Dr. Aurora Mocanu, in the Center for Scientific Research of Excellence in Physical Chemistry, led by the Founder (2006) and Director (2006-present) of the Center, Prof. Dr. Maria Tomoaia-Cotisel, Faculty of Chemistry and Chemical Engineering; within Babeș-Bolyai University in Cluj-Napoca.

3. **Member of the Scientific Research Center of Excellence in Physical Chemistry (CECHIF Center)**
   PhD student Madalina Anca Ujica has been a member of the Scientific Research Center of Excellence in Physical Chemistry since 2018. CECHIF Center: Founder (2006) and Director (2006-present): Prof. Univ. Dr. M. Tomoaia-Cotişel, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University of Cluj-Napoca.