"Al. I. CUZA"UNIVERSITY OF IAŞI
FACULTY OF CHEMISTRY

ADRIANA ADOCHIȚEI

PhD Thesis

ABSTRACT

Peptides and metal-peptide complexes with biomedical implications and biological research

SUPERVISER
Prof. dr. GABI DROCHIOIU

2012

„We must be modest except in our aims"

(Otto Loewi)

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as superviser,
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and advices that gave during this research
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POSDRU/88/1.5/S/47646 *Project*
for providing me the schlarship to study
My family
for encouragement
- ***all my love-***
All ***my friends*** *and* ***coleagues***
who have supported me
- ***all my friendship-***

PhD thesis entitled *Peptides and metal-peptide complexes with biomedical implications and biological research* includes 166 pages and is presented in the following structure:

TABLE OF CONTENTS

Keywords: peptides conformation, peptide-metal complexes, circular dichroism (CD), FT-IR spectroscopy, metal-nanoparticles, biological activity.

The paper is accompanied by 138 references. The present summary includes a brief form of personal research results, general conclusions and an extract from the bibliography. I kept the numbering of chapters, tables, charts and figures included in the original thesis.

INTRODUCTION

In present, it is not known quite well the formation of metal-peptide complexes and their role in neurodegenerative diseases, especially due to variations in physiological pH. Therefore, the objectives pursued in this thesis are:

- Study of conformational changes of polypeptides in presence of dinitrophenol pesticides;
- Obtaining short peptide sequences using continuous solution-phase synthesis method;
- Synthesis of mutant peptide sequences of amyloid peptide Aβ 1-40;
- Obtaining metal complexes with natural peptides and synthetic ones;
- Peptides and their complexes characterization using FT-IR and circular dichroism (CD) techniques;
- Peptides and peptide-metal complexes studies by atomic force microscopy (AFM);
- Behavior study of natural peptides in the presence of metal ions at physiological pH variations, for elucidating the pathological and physiological aspects of degenerative diseases.
- Synthesis of gold and silver nanoparticles stabilized by short peptides;
- Nanoparticles characterization by UV-VIS spectroscopy, FT-IR, AFM;
- Biological activity testing of metal complexes and obtained nanoparticles on plants and microorganisms.

The thesis is divided into two main parts: Part I - "Reference study" and Part II - "Original results".

The research results are reported in 18 tables, 85 figures, 5 schemes and are presented in 4 original published papers and 3 ones submitted to ISI journals.

PART II. Original results

CHAPTER II. Synthesis and characterization of polypeptides adducts

DICHROISM CIRCULAR (CD)STUDIES

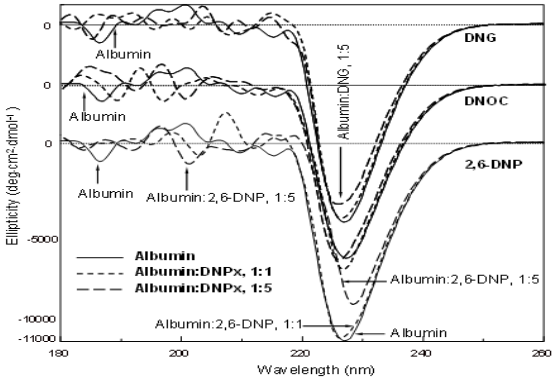

Figure II.5.1.1. Circular dichroism spectra wich show the effect of 2.6-DNP, DNG and DNOC on albumin conformation (solid line bold: molar ellipticity of albumin, dotted line: molar ellipticity of 1/1 adduct 2,6-DNP, DNG, DNOC with albumin,segmented line: ellipticity of 5/1 adduct of 2,6-DNP,DNOC DNG with albumin) (Drochioiu *et al*., 2009).

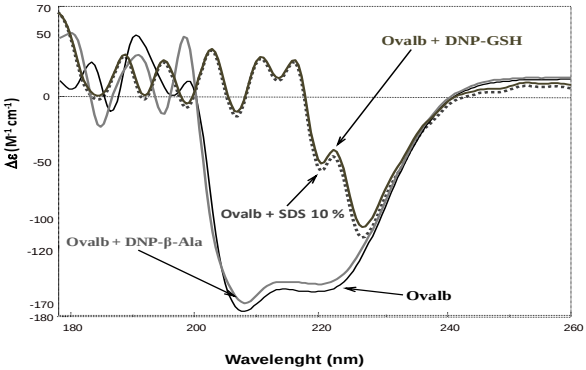

Figure II.5.1.4. Circular dichroism spectra wich show the effect of DNP-Glutathione (DNP-GSH), DNP-β-Alanine (DNP-β-Ala) and sodium dodecyl sulphate (SDS) on ovalbumin (Ovalb) conformation

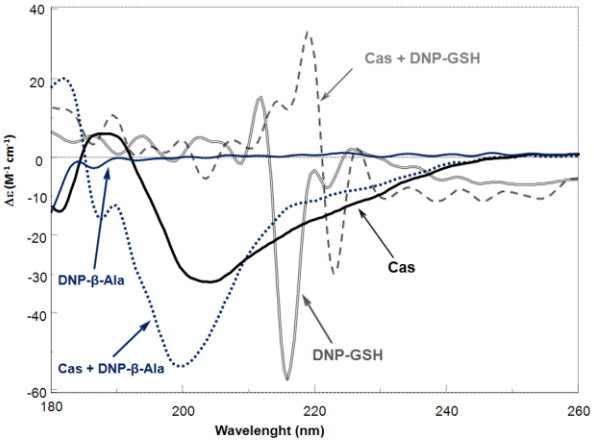

Figure II.5.2. Circular dichroism spectra wich show the effect of DNP-Glutathione (DNP-GSH), DNP-β-Alanine (DNP-β-Ala) on casein (Cas) conformation

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From the data presented in this chapter can make the following conclusions:

- Comparing the results obtained for ovalbumin and bovine serum albumin was found that the effect of dinitrophenols is strongly manifested when polypeptides have a high content of α-helix forms.
- Also, casein presented total conformational changes in the presence of DNP-glutathione.
- Our results suggest that albumin could be a protective agent against pesticide dinitrophenols, and this ones can may be considered disruptive factors of secondary structure.

CHARACTERIZATION OF PEPTIDES AND THEIR COMPLEXES BY CIRCULAR DICHROISM (CD)

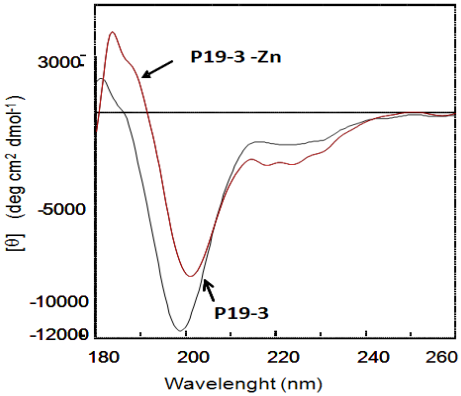

Figure III.5.1. Circular dichroism spectra of P19-3 peptide, and its complexes with zinc (Murariu *et al.*, 2009).

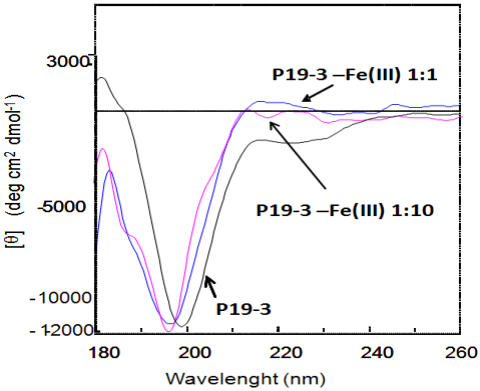

Figure III.5.2. Circular dichroism spectra of P19-3 peptide, and its complexes with iron (III), molar ratio 1:1 and 1:10

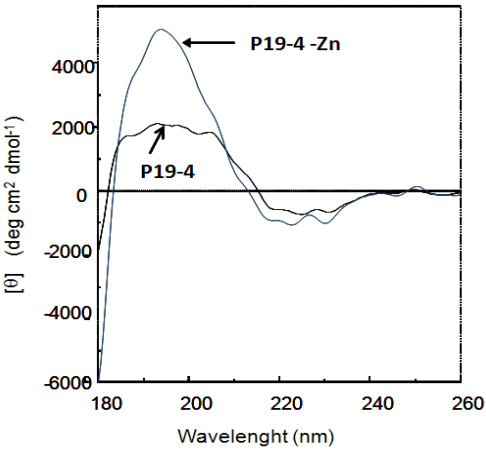

Figure III.5.3. Circular dichroism spectra of P19-4 peptide, and its complexes with zinc (Murariu *et al.*, 2009).

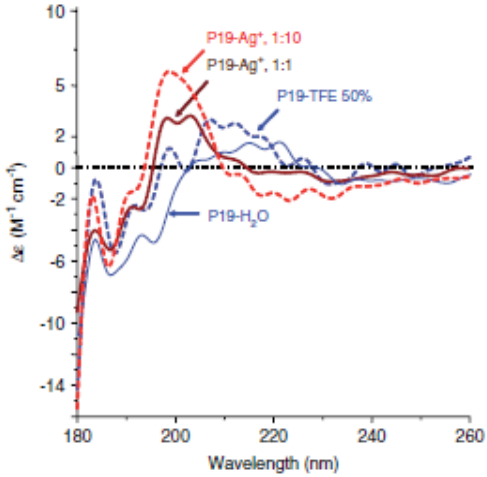

Figure III.5.4. Effect of silver ions on the secondary structure of peptide P19 (Murariu *et al.*, 2011).

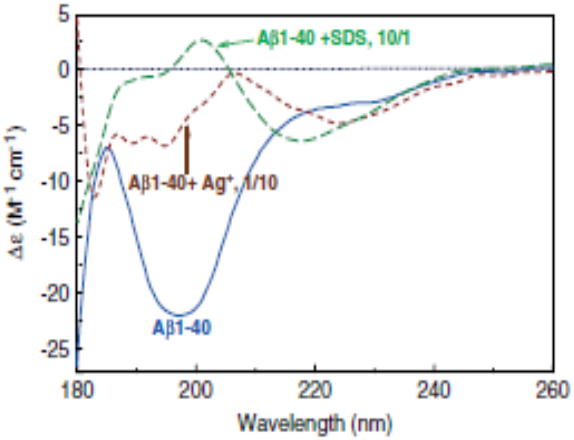

Figure III.5.6. CD spectra of amyloid-β peptide 1–40 (Aβ, 0.2mM) and in presence of silver ions and SDS (1:10 and 10:1 molar ratio) (Murariu *et al.*, 2011).

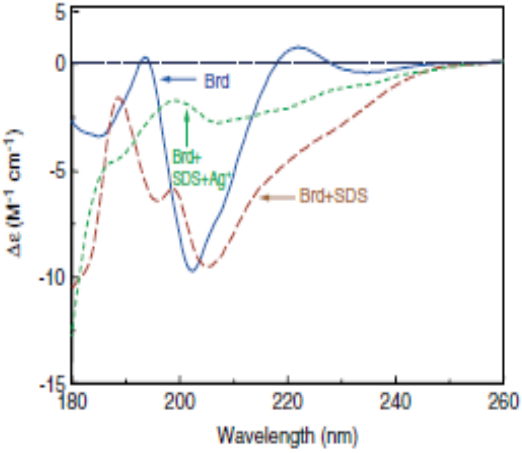

Figure III.5.7. Conformational changes of bradykinin (Brd) in presence of SDS (Brd+SDS, 1:10) and silver ions (Brd+SDS+Ag+, 1:5:5) (Murariu *et al.*, 2011).

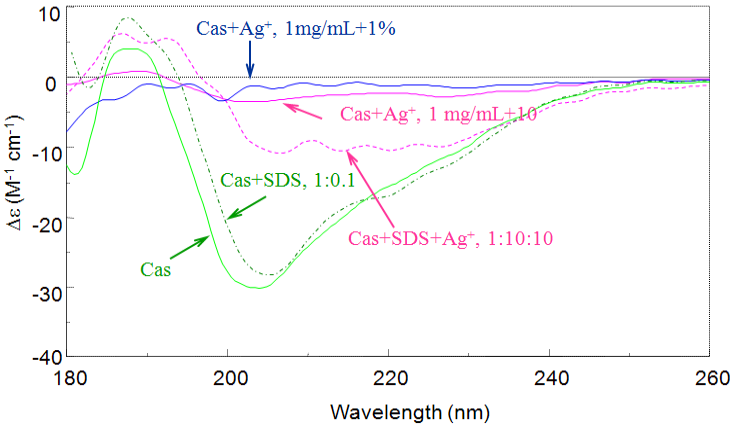

Figure III.5.8. Effect of silver ions (Ag^+), sodium dodecyl sulfate (SDS), and their mixture on the conformation of casein (Cas).

FT-IR SPECTROSCOPY CHARACTERIZATION OF PEPTIDES AND THEIR COMPLEXES

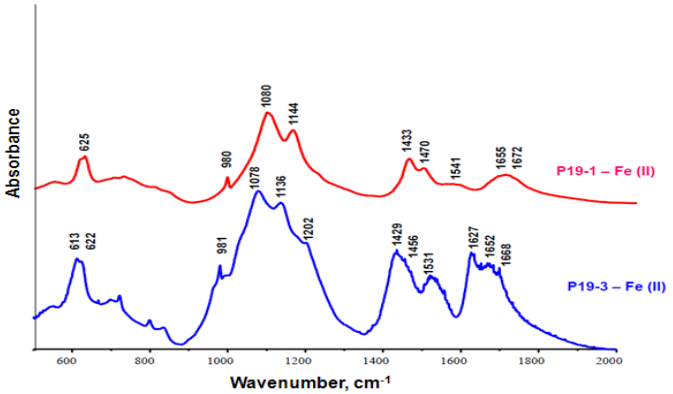

Figure III.6.1. FT-IR spectra of P19-1 and P19-3 complexes with iron (II) metal ions.

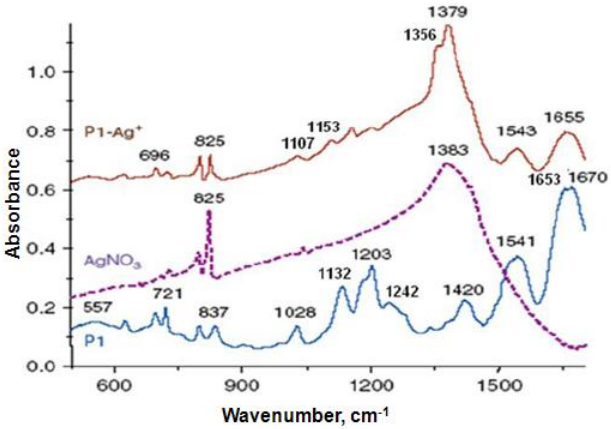

Figure III.6.2. FTIR spectra of peptide P19-1 (P1) and its complex with silver ions (Murariu *et al.*, 2011).

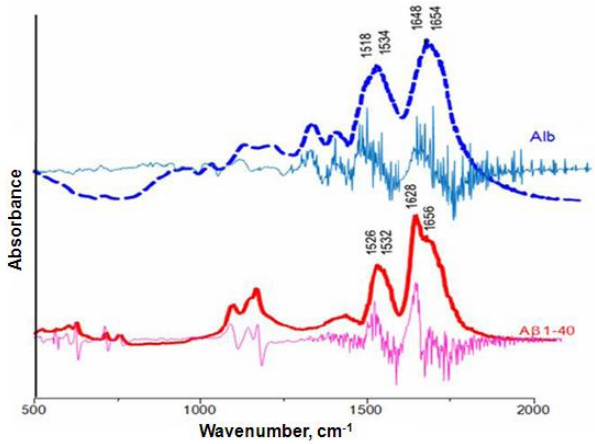

Figure III.6.3. FTIR spectra (500-2000 cm^{-1}) of amyloid-β peptide (Aβ1-40) and ovalbumin (Alb) and second derivative spectra (Adochiței and Drochioiu, 2011)

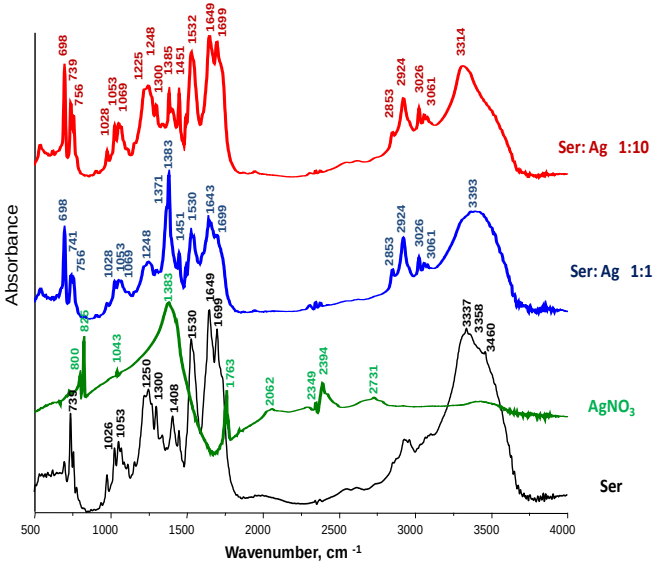

Figure III.6.4. FT-IR spectra (500-4000 cm^{-1}) of serinon

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With the data obtained in this chapter can conclude the following:

- CD and FT-IR spectra showed completely different conformations for peptides P19-3 and P19-4 and showed that only P19-3 is able to bind heavy metal ions to form complexes with an altered conformation.
- The results obtained for casein by circular dichroism and infrared spectroscopy confirmed that there is a synergistic process between sodium dodecyl sulphate (SDS) and silver ions to induce conformational changes from β-folded forms in α-helix ones.
- Serinon proved to have similar results with glutathione.
- AFM images obtained for P19 peptides showed roughness at addition of mercury ions, the formations appeared on film showed fibrils and specific plaques, results that are correlated to the data obtained by circular dichroism.

CHAPTER IV. SYNTHESIS AND CHARACTERIZATION OF METAL-NANOPARTICLES CONJUGATED WITH PEPTIDES

AFM CHARACTERIZATION

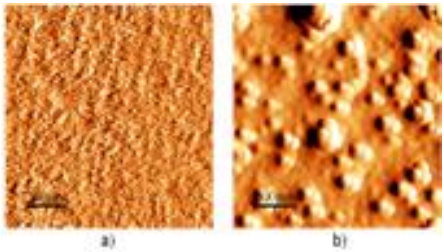

Figure IV.5.1. AFM images: a) glutathione; b) silver nanoparticles.

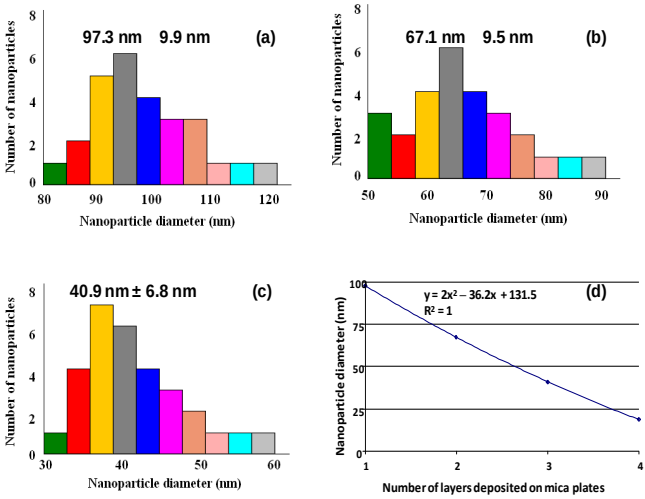

Figure IV.5.2. Nanoparticle diameter as determined from AFM images of multilayers of Au-NPs. Histograms of (a) monolayer, (b) double layer and (c) triple layer of Au-NPs, after dabbing with blotting paper. An average diameter of 18.7 nm (d) was obtained for four layers of Au-NPs using the equation $y = 2x^2-36.2x+31.5$, where x = 4, y = nanoparticle diameter.

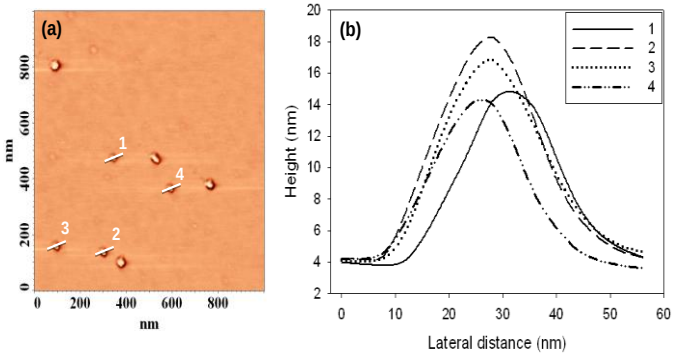

Figure IV.5.3. AFM phase images of some selected Au-NPs (a), and corresponding cross section profiles (b).

BIOLOGICAL TESTS

Figure IV.7.1. Biological activity of gold nanoparticles: 1-Control; 2-$CuSO_4$, 3 mM; 3-Au-NPs, from 3 mM Au^{3+}; 4- GS-Au-NPs; 5- Au-NPs:Cu(II); 6- GS-Au-NPs:Cu(II).

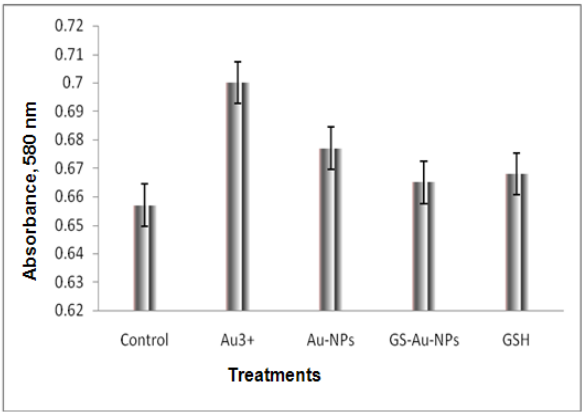

Figure IV.7.2.1. The effect of Au-NPs and GS-Au-NPs on *E. coli* growth

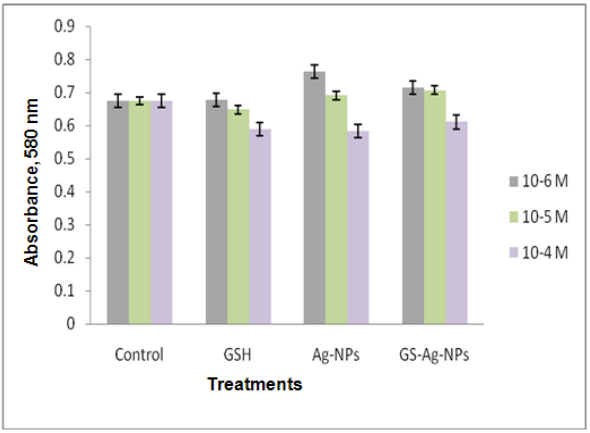

Figure IV.7.2.2. The effect of Ag-NPs and GS-Ag-NPs on *E. coli* growth

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From the data presented in this chapter can make the following conclusions:

- The Ag-NPs thus prepared tend to aggregate together upon addition of Ag^+ due to the strong coordination bond between Ag^+ and – NH_2, –COOH of glutathione modifier.

- Gold nanoparticles and gold nanoparticles containing copper showed inhibitory effect on the development of plantlets.
- Tests on gold and silver nanoparticles in the presence of *E. coli* showed aggregation process (even at 2 hours after inoculation with silver nanoparticles), an important process for understanding their biological properties.
- The results showed no toxicity of gold nanoparticles on bacterial growth.
- Silver nanoparticles stimulated the growth of microorganisms at 10^{-6} M concentration.

GENERAL CONCLUSIONS

Based on research presented in this paper we can highlight the following general conclusions:

1. We studied methods of peptide synthesis and we synthesized several new peptides, such as:
   - ✓ Fragments of amyloid peptide Aβ 1-40, respectively sequences 1-16, containing alanine and serine residues, using solid phase synthesis.
   - ✓ A tripeptide, glutathione analogue, by replacing the remaining cysteinyl residue with the serinil, using continuous solution-phase synthesis. The continuous solution-phase synthesis led to obtain pure peptides and higher productivity than peptides obtained by solid phase synthesis. Thus, was designed and synthesized by this method a tripeptide called serinon.
2. Characterization of newly synthesized peptides was performed by MALDI-TOF mass spectrometry type, respectively GC-MS.

3. In MS spectrum were identified the fragments Aβ 1-16-Ala and Aβ 1-16-Ser wich confirm obtained sequences.
4. Spectrometric measurements of peptide serinon proved that GC-MS can be applied to short peptide sequences (such tripeptide). Thus, we compared the results obtained by GC-MS of serinon with those of glutathione and were observed structural similarities.
5. We studied the interaction of polypeptides such as bovine albumin, ovalbumin and casein, with dinitrophenols and related compounds, as well as heavy metals, to understand more deeply the mechanisms of conformational changes of polypeptides, in different environments. The following conclusions are:
    - BSA had a slightly altered conformation in the presence of dinitrophenol derivatives, representing a return rate of conformers β-turn much lower compared to the control, which indicated the tendency to maintain polypeptide chain in linear form.
    - casein was more deeply affected by the presence of dinitrophenol derivatives, circular dichroism spectrum of its adduct with DNP-β-Ala presenting β-folded forms and disordered structures, although the presence of alanine would normally indicate α-helix conformers.
    - dinitrophenols are disruption factors of the secondary structure of polypeptides investigated.
6. We studied other peptides and their complexes with heavy metal ions, which were previously synthesized or purchased from specialized companies.
7. We obtained peptide-complexes with Zn, Fe (II), Fe (III), Ag, Cu (II) ions of peptides with 19 amino acid residues (sequences of glycine, histidine and alanine, respectively, in different positions), serinon-complexes, bradykinin, Aβ 1-40, ovalbumin and casein, and were

characterized by circular dichroism, infrared spectroscopy and atomic force microscopy.

8. FT-IR spectroscopy has been applied to the structural characterization of peptides and polypeptides, in different environments. Amide I band - between 1600 and 1700 cm^{-1} - was the most intense absorption band of all peptides and proteins investigated and it was associated with the extent of vibrational C = O group and directly related peptide or protein conformation.

   - The obtained data show that FT-IR spectra of ovalbumin peptide Aβ 1-40 and have much in common, but there are also important differences, which occur mainly in the region of amide I band.
   - Also, tripeptide serinon newly synthesized showed similar structure and some properties of glutathione, as well as its relationship with metals.
   - Bradykinin, which presented only β-sheet structures, the addition of SDS showed a certain proportion of α-helix. While the proportion of unordered conformers remained unchanged, a combination of SDS and silver ions had a synergic effect on the formation of both α-helical and β-turn conformers.
   - Aβ 1–40 in the fresh aqueous solutions was found as a mixture of random β-sheet and β-turn forms. On adding silver ions, the peptide conformation changed severely from β-sheet to α-helix. SDS had the same effect
   - FT-IR results for casein showed a synergic effect between SDS and silver ions on the formation of β-sheet to α-helix conformers.

9. Circular dichroism technique revealed the conformational changes occurring peptide molecules, even with the change of a single amino acid residue position, in the primary structure.
    - Circular dichroism spectra in the far UV indicate that albumin has a slightly altered conformation in the presence of dinitrophenol derivatives. The results suggest that albumin may be a protective agent against dinitrophenol pesticides.
    - CD spectra showed completely different conformations for peptide P19-3 and P19-4. Results suggest that peptide P19-3 is able to bind heavy metal ions to form complexes with altered conformation. Because of the rigid structure demonstrated by CD, P19-4 peptide cannot bind all kind of metal ions. Consequently, the spectra of P19-4/metal ion were similar to those of pure peptide.
    - In aqueous solutions, P19-1 showed β-turn conformers and unordered populations, and was found to contain almost only β-turn populations in a 50% TFE solution. On adding silver ions to P19 prepared in aqueous solutions, at pH 7.4, the peptide conformation severely changed. Silver ions transformed unstructured peptide molecules into α-helical conformers and stabilized β-turn structures.
10. Atomic force microscopy showed that the secondary structure of peptides and formation of nanostructures by self-assembling peptide conformation dependent on peptide conformation. By atomic force microscopy it has revealed similarities and differences between peptides. AFM images obtained for P19-3 and P19-4 peptides were different, depending on the metal used. Thus, on addition of mercury ions was observed fibrils and specific plaques.
11. We prepared and characterized gold and silver nanoparticles conjugated with short peptides (glutathione). Characterization of newly synthesized nanoparticles led to the following results:

-Morphological analysis of surfaces confirmed the formation of nanoparticles, which crowded when allowed to stay for 48 hours in aqueous suspensions, forming relatively large aggregates (30-150 nm) with different shapes.

- Height of gold nanoparticles (Au-NPS) was relatively constant, ranging between 14 and 18 nm, located in range of 20-30 nm diameter.

- SEM photographs confirmed that the average size of the gold nanoparticles was found to be about 20 - 50 nm, but some other aggregates sized over 200 nm.

-FT-IR results showed the formation of a S-Ag bond and a S-S one.

- Intense absorption of nanoparticles in the UV-VIS spectra suggested the aggregation process and plasmon absorption phenomen.

12. Results of biological activity of gold nanoparticles and nanoparticles conjugated with glutathione led to the following conclusions:
    - ✓ stimulates germination process;
    - ✓ stimulate further development of plantlets;
    - ✓ nanoparticles containing copper and gold nanoparticles with copper stabilized with glutathione, inhibit the growth of seedlings;
    - ✓ reveal their nature without toxicity on *E. coli*;
    - ✓ Au-NPs had a complex effect on *E. coli*, indicating an aggregation process at 2 hours after inoculation.
13. Results of biological activity of silver nanoparticles and nanoparticles conjugated with glutathione led to the following conclusions:
    - ❖ showed no toxic effect as long as they were kept in suspension for 48 hours at concentrations of 10^{-5} M or less;
    - ❖ stimulated the growth of microorganisms at a concentration of 10^{-6} M;

- ❖ caused a significant decrease in bacterial density, probably due to aggregation on the surface of nanoparticles.

14. The results of this paper are published in 2 articles ISI, 3 articles submitted to ISI journals, 2 articles published in CNCSIS journals and 7 presented papers on national conferences.

15. The objectives proposed were accomplished.

Scientific results

Papers published in ISI journals

1. Murariu, M., Dragan, E. S., **Adochiței, A.,** Zbancioc, G., Drochioiu, G. Silver-induced conformational changes of polypeptides: a CD study. *J. Pept. Sci.,* 17: 512–519, **2011** (IF = 1,954/2010).
2. **Adochiței, A.,** Drochioiu, G. Rapid characterization of peptide secondary structure by FTIR spectroscopy. *Rev. Roum. Chim.,* 56(7), 783-791, **2011** (IF = 0,311/2010).

Papers published in CNCSIS journals

3. Murariu, M., Dragan, E. S., **Adochiței A.,** Hăbăşescu, L., Zbancioc, G., Pui, A., Drochioiu, G. Conformational changes of peptides on binding metals: CD and FT-IR studies. Bull. Polytechnic Inst. Iaşi, Tom LV (LIX), Fasc. 4, 89-94, ISBN: 978-973-621-255-0, **2009**.
4. Drochioiu, G., **Adochiței A.,** Hăbăşescu, L., Zbancioc, G., Pui, A., Grădinaru, R., Rusu, E., CD and FT-IR study of some adducts of albumin with toxic dinitrophenyl derivatives. Bull. Polytechnic Inst. Iaşi, Tom LV (LIX), Fasc. 4, 23-28, ISBN: 978-973-621-255-0, **2009**.

Papers submitted to ISI journals

5. Murariu, M., **Adochiței, A.**, Gradinaru, R., Stoica, I., Drochioiu, G., Biological activity of some glutathione-stabilized silver and gold nanoparticles. *Journal of Experimental Nanoscience*, **2012**.
6. Murariu, M., **Adochiței, A.**, Stoica, I., Drochioiu, G., Gold nanoparticles as Possible In vivo Detoxification Agents in Copper Overloading. *Chemistry Central Journal*, **2012**.
7. Drochioiu, G., **Adochiței, A.**, Murariu, M., Metal-induced conformational changes of casein. *Food Chemistry*, **2012**.

Posters

1. Murariu, M., Dragan, E. S., **Adochiței A.,** Hăbăşescu, L., Zbancioc, G., Pui, A., Drochioiu, G. *Conformational changes of peptides on binding metals: CD and FT-IR studies.* Zilele Facultății de Inginerie Chimică şi Protecția Mediului, Ediția a VI-a, "Noi frontiere în chimie şi inginerie chimică", Iaşi, Romania, 18 – 20 noiembrie **2009**.
2. Drochioiu, G., **Adochiței A.,** Hăbăşescu, L., Zbancioc, G., Pui, A., Grădinaru, R., Rusu, E., *CD and FT-IR study of some adducts of albumin with toxic dinitrophenyl derivatives*. Zilele Facultății de Inginerie Chimică şi Protecția Mediului, Ediția a VI-a, "Noi frontiere în chimie şi inginerie chimică", Iaşi, România, 18 – 20 noiembrie **2009**.
3. P.S.V.6 - **Adriana Adochiței**, Ecaterina Stela Dragan, Manuela Murariu, Iuliana Stoica şi Gabi Drochioiu, *Conformational changes of peptide-metal complexes: CD, FT-IR and AFM studies.* A XXXI-A National Conference of Chemistry, Râmnicu Vâlcea, Secțiunea V-Chimie Analitică şi Protecția Mediului, 6-8 octombrie **2010**.
4. P60 - **Adriana Adochiței**, Manuela Murariu, Gabi Drochioiu, *Metal binding to peptides: CD, FT-IR and AFM studies.* Zilele Universității „Al. I. Cuza", Faculty of Chemistry, 12-13 noiembrie **2010**.

5. P17- **Adriana Adochiței**, Manuela Murariu, Gabi Drochioiu, *Glutathione-based silver nanoparticles: structure and biological activity.* Zilele Universității „Al. I. Cuza", Faculty of Chemistry, 28 octombrie **2011**.
6. P-S1-21 - **Adriana Adochiței**, Manuela Murariu, *Tehnici spectroscopice de dicroism circular si FT-IR utilizate in analiza conformațională a unor peptide şi proteine: studiu comparativ.* Zilele Facultatii de Inginerie Chimică şi Protecția Mediului, Ediția a VIII-a „MATERIALE ŞI PROCESE INOVATIVE", Iaşi, 17-18 noiembrie **2011.**

Oral Communication

7. S1. 4 – Laura Hăbăşescu, **Adriana Adochiței**, Gabi Drochioiu, *Studii FT-IR şi UV-VIS ale complecşilor dipeptidelor GLU-GLU şi SER-GLY cu ioni de cupru (II).* Zilele Facultății de Inginerie Chimică şi Protecția Mediului, Ediția a VII-a, „90 de ani de la naşterea Academicianului Cristofor Simionescu", 17-19 noiembrie **2010**.

It was realized one research stage, 1 May – 31 July **2012** (3 months), at University of Chemical Technology and Metallurgy, Sofia, Bulgaria.

References

1. Chen, Y. R., Huang, H. B., Chyan, C. L., Shiao, M. S., Lin, T. H., Chen, Y. C., The effect of Abeta conformation on the metal affinity and aggregation mechanism studied by circular dichroism spectroscopy, *J. Biochem*., 139, 733–740, **2006**.

2. Danielsson, J., Pierattelli, R., Banci, L., Gräslund, A., High-resolution NMR studies of the zinc-binding site of the Alzheimer's amyloid beta-peptide., *FEBS J,* 274(1):46-59, **2007**.
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