„ALEXANDRU IOAN CUZA" UNIVERSITY OF IAȘI

Faculty of Chemistry

"Chemistry and Life and Earth Sciences" Doctoral School

LAURA ION

DOCTORAL THESIS

ABSTRACT

Compounds with peptide bonds and their biomedical applications

Scientific coordinator,

Prof. Dr. Gabi Drochioiu

IAȘI

2015

„ALEXANDRU IOAN CUZA" UNIVERSITY OF IAŞI

Mr/Mrs……

We inform you that on **27.11.2015**, **11:00 a.m**. in **502** room, 5th floor, UAIC building, Lăpuşneanu Street, Miss **Laura ION**, will hold a public session, the doctoral thesis entitled **"Compounds with peptide bonds and their biomedical applications"** in order to obtain the scientific title of **Doctor in science**, chemistry domain.

Doctoral Committee composed of the following:

President:

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Faculty of Chemistry, „Alexandru Ioan Cuza" University of Iași

Scientific coordinator:

Prof. dr. Gabi DROCHIOIU
Faculty of Chemistry, „Alexandru Ioan Cuza" University of Iași

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Prof. dr. Ionel MANGALAGIU,
Faculty of Chemistry, „Alexandru Ioan Cuza" University of Iași

We are sending the abstract with the request to communicate in writing, in duplicate, any comments and feedback.
We invite you to attend the public thesis presentation.

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ABSTRACT

Compounds with peptide bonds and their biomedical applications

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"The most important thing is to not stop asking questions. Curiosity has its own reason for existence"

Albert Einstein

I am grateful to Prof. Dr. Gabi Drochioiu for his support in carrying out this research, discussions and guidance that helped in completion of scientific research and my training as well.

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I am grateful to ***Biochemistry Laboratory*** *members for confidence and friendship.*

I am grateful to ***my family*** *for moral support during my studies and not only.*

Table of contents

Keywords: peptide compounds, aggregation, Alzheimer diseases, peptide heavy metal complexes, mass spectrometry, circular dichroism, zeins, electrophoresis.

The paper is accompanied by **154** bibliographic index. The summary includes personal research results, conclusions and a selective bibliography in a brief form. The experimental results are subject to **18** tables, **105** figures and **3** scientific papers published in journals with impact factor and 4 published in conferences volumes.

Introduction

Proteins are compounds with peptidic bonds and macromolecular structure, that are necessary for the structure, functioning and cell adjustment inside living organisms. The proteins possess several biological functions: catalyzing various biochemical reactions, oxygen transportation within the blood, influencing growth and development of various tissues, organism protection against diseases. In the latest few decades, proteins are the principle pions for biomedical researches due to their involvement in various pathological states.

Therefore, the thesis` *main objectives* are the following:

i. The solid phase peptide synthesis, using Fmoc strategy for normal and mutant amyloid peptides synthesis – which are important for the study of Alzheimer neurodegenerative disease;

ii. Peptides separation and purification by using high-performance liquid chromatography and electrospray mass spectrometric (ESI-MS) and matrix assistent laser desorbtion ionization – time of flight (MALDI –TOF);

iii. The study of synthesized peptides conformational changes in the aggregation process caused by fast photochemical oxidation. Techniques such as circular dichroism, fluorescence and mass spectrometry;

iv. Obtaining peptide complexes of various heavy metals and characterization by mass spectrometry and atomic force microscopy;

v. Extraction and characterization of the peptide compounds, such as zein using one-dimensional electrophoresis and mass spectrometry;

The thesis is divided into two parts: **Part I** - Study of literature (Chapter I) and **Part II** - Original results (Chapters II , III , IV and V) which includes the experimental part, conclusions and references.

CHAPTER III

Obtained results:

Peptides synthesis and characterization

III.2.1. $A\beta_{1-40}$ peptide purification and characterization

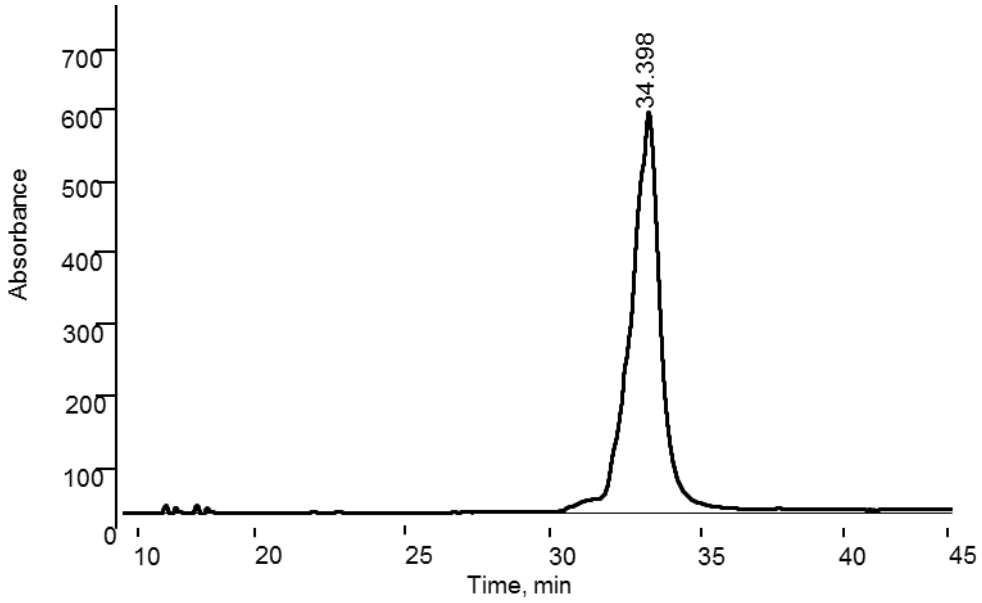

Figure III.14. *RP-HPLC spectra of $A\beta_{1-40}$ peptide purified on a C_4 column*

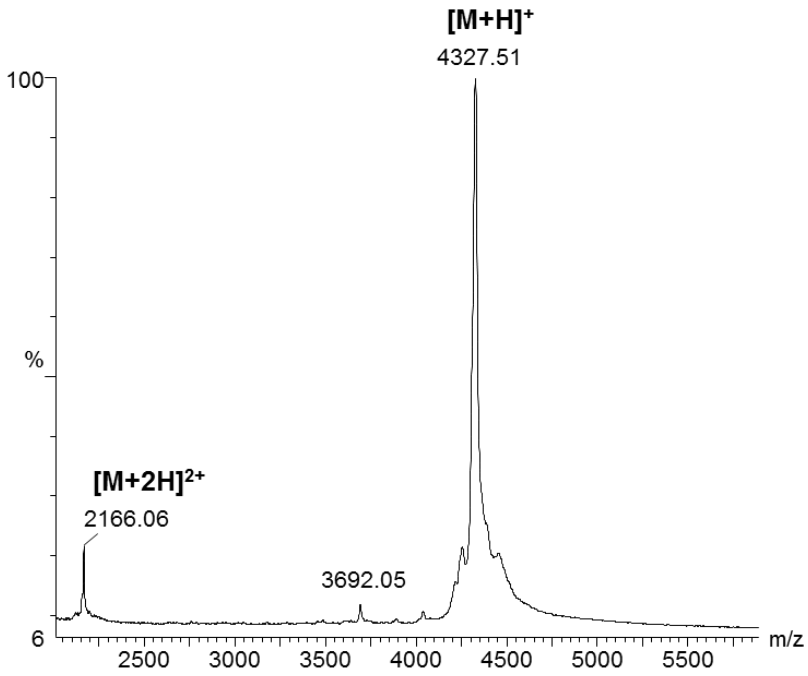

Figure III.15. *MALDI ToF mass spectrum of the peptide $A\beta_{1-40}$ after purification*

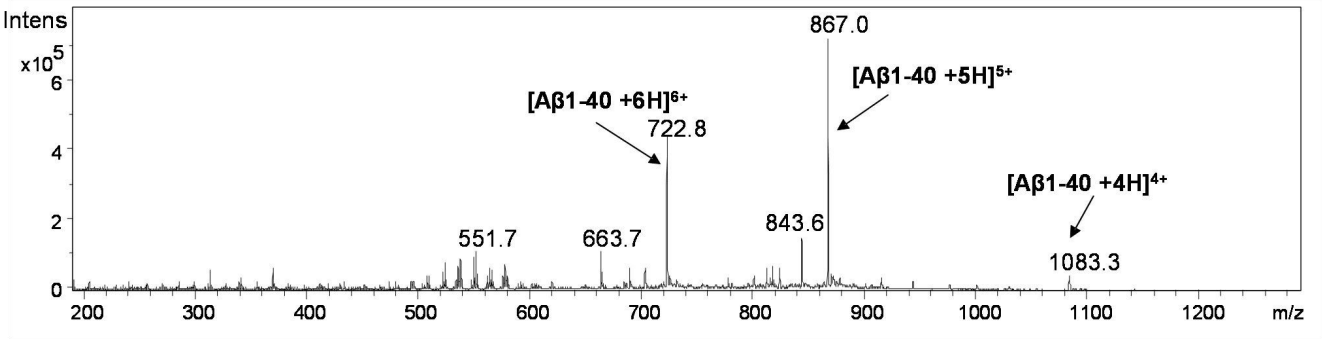

Figure III.16. *ESI-MS spectrum of $A\beta_{1-40}$ peptide after purification*

III.2.4. Mutant peptide purification and characterization derived from $A\beta_{1-16}$: $A\beta_{1-16}$ (F→G), (F→G, H→A) and (F→G, H→S) respectively:

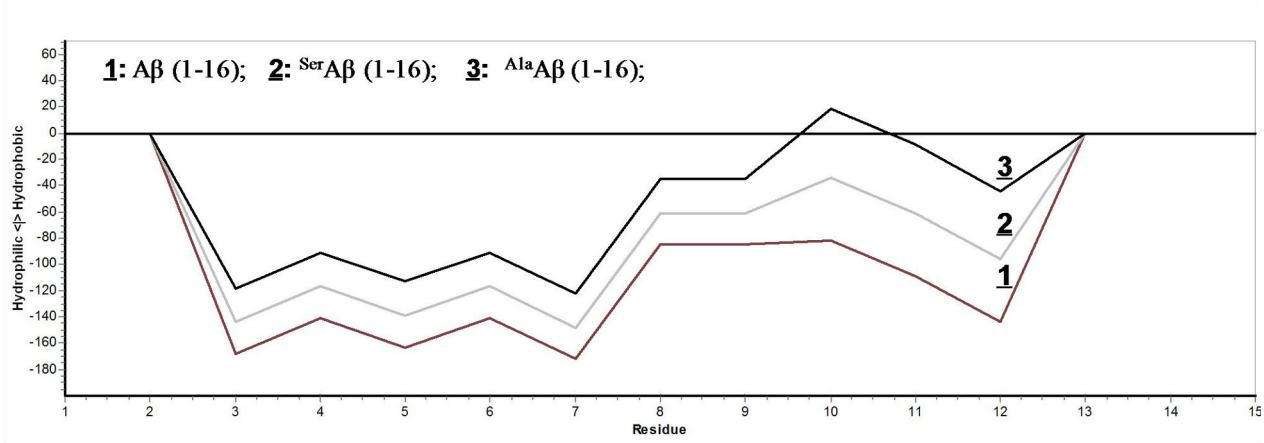

Figure III.21. *Schematic representanion of hydrophilic/hidrophobic character of $A\beta_{1-16}$ mutant peptides*

Table III.1. *Retention time and specific mass of $A\beta_{1-16}$ mutant peptides*

No.	Code	Peptide sequence	HPLC Retention time (minutes)	ESI-Ion trap m/z $[M+H]^+$
1	$A\beta_{1-16\ (F\rightarrow G)}$	DAE**G**RHDSGYEVHHQK	17.88	1864.85
2	$A\beta_{1-16(F\rightarrow G,\ H\rightarrow S)}$	DAE**G**R**S**DSGYEV**SS**QK	21.90	1714.91
3	$A\beta_{1-16(F\rightarrow G,H\rightarrow A)}$	DAE**G**R**A**DSGYEV**AA**QK	25.24	1665.87

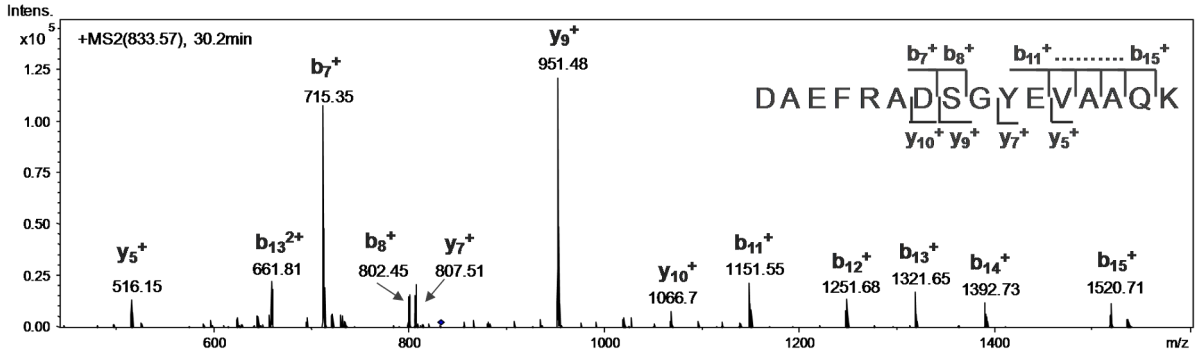

Figure III.22. *MS/MS spectrum of Aβ1-16 (F→G, H→A) peptide*

III.3. Conclusions

i. The following peptides were synthesized: **Aβ1-40, Aβ25-35, Aβ1-16, Aβ1-16(F→G), Aβ1-16(F→G, H→A)** and **Aβ1-16(F→G, H→S)** respectively;

- The obtained peptides were separated and purified by high performance liquid chromatography using C4 and C8 columns (stationary phase) and acetonitrile: water (0.1% TFA) (mobile phase).
- Peptide purity was confirmed by MALDI ToF and electrospray mass spectrometry. Synthesized peptides experimental molecular weights were similar to their theoretical masses;
- Histidine residue modification in the peptide sequence leads to significant changes in peptide character.

CHAPTER IV

Obtained results:

Peptides conformational and aggregation studies

IV.1. Aggregation studies of the β-amyloid peptide by rapid photochemical oxidation and mass spectrometric characterization

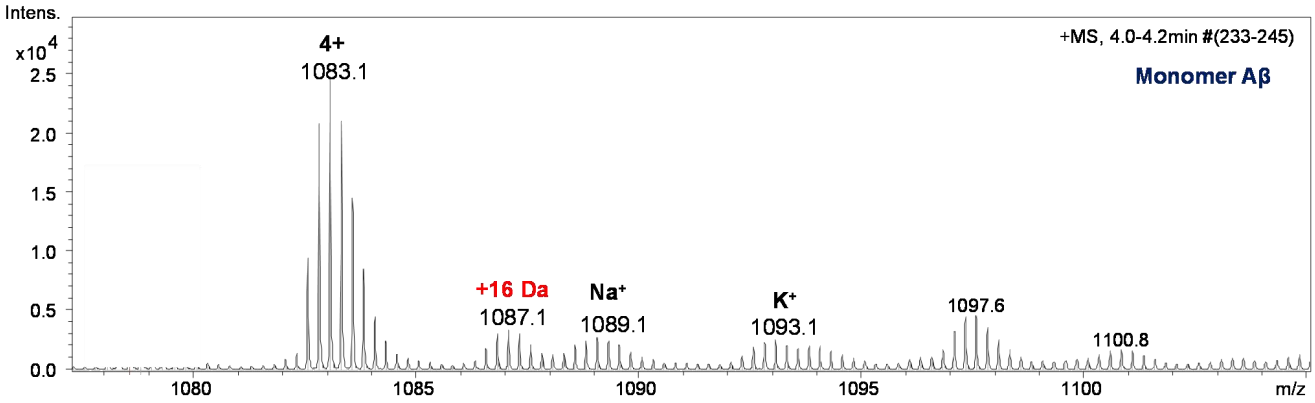

Figure IV.2. *MS spectrum of $A\beta_{1\text{-}40}$ peptide at starting point (T_0)*

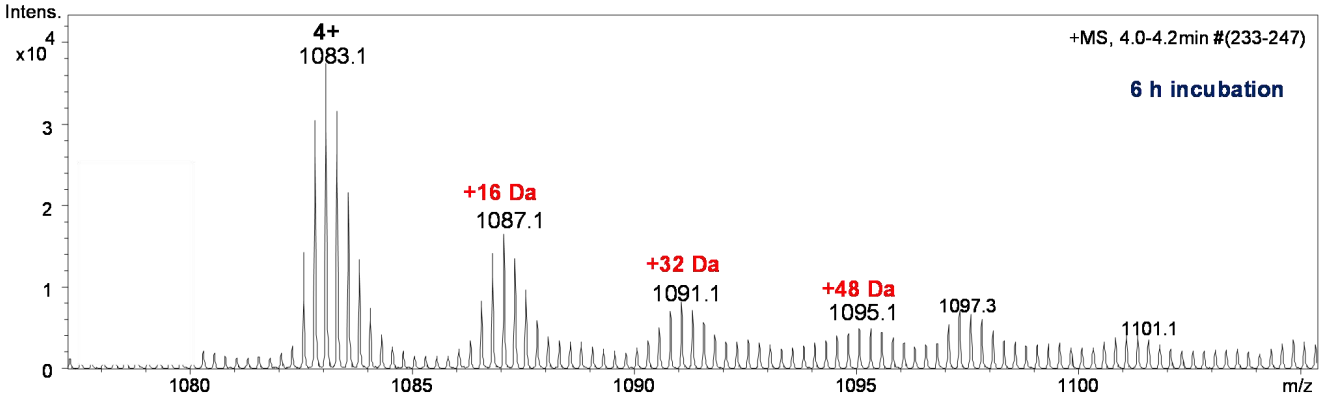

Figure IV.4. *MS spectrum of $A\beta_{1\text{-}40}$ peptide after 6 hours incubation*

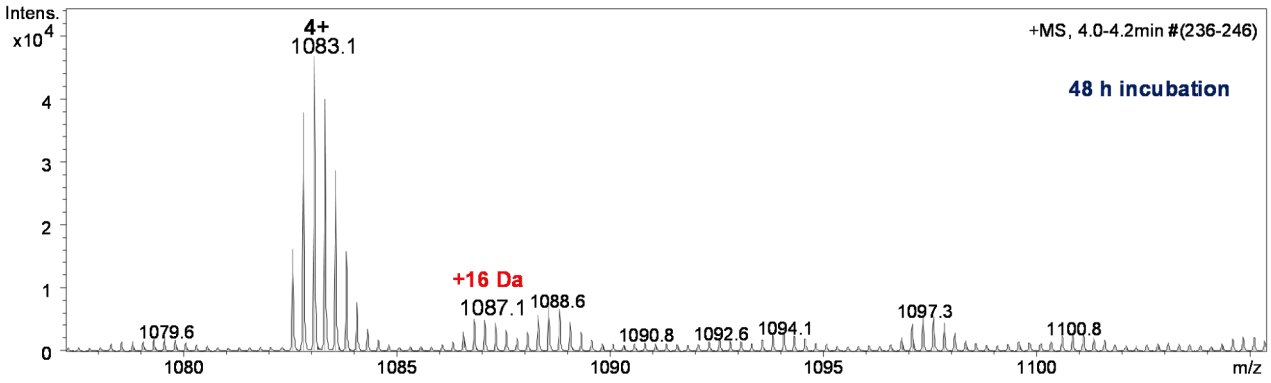

Figure IV.6. *MS spectrum of $A\beta_{1-40}$ peptide after 48 hours incubation*

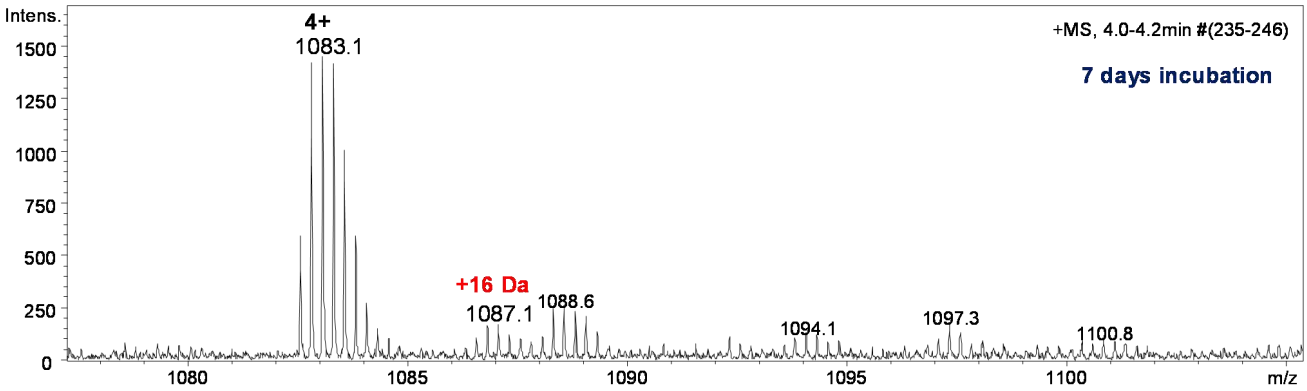

Figura IV.7. *MS spectrum of $A\beta_{1-40}$ peptide after 7 days incubation*

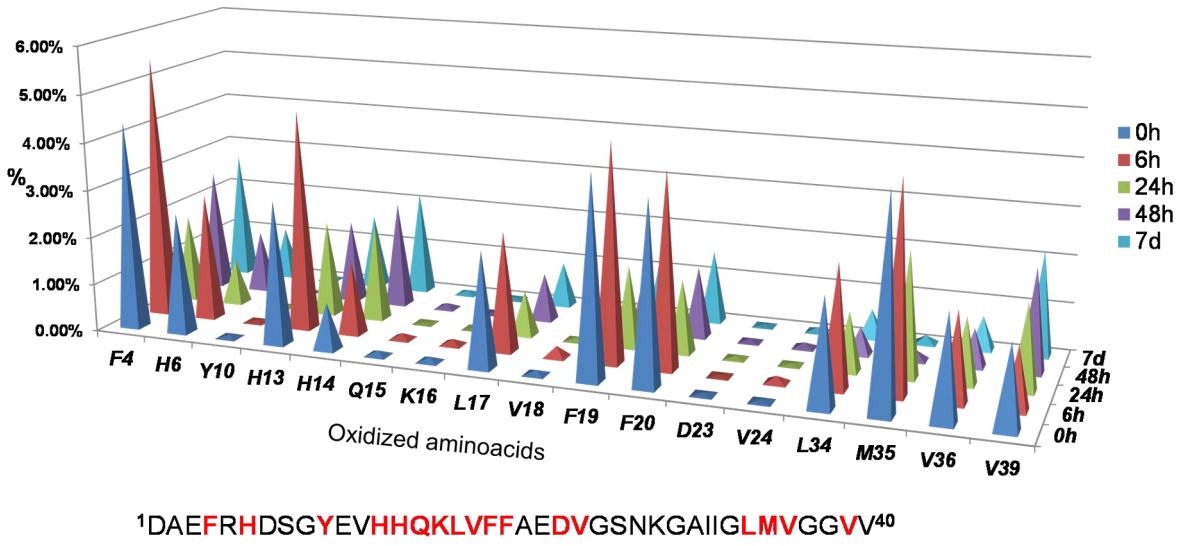

Figure IV.8. *Schematic representation of photooxidated aminoacids from $A\beta_{1-40}$ sequence*

IV.2. $A\beta_{1-40}$ peptide circular dichroism studies at various incubation times

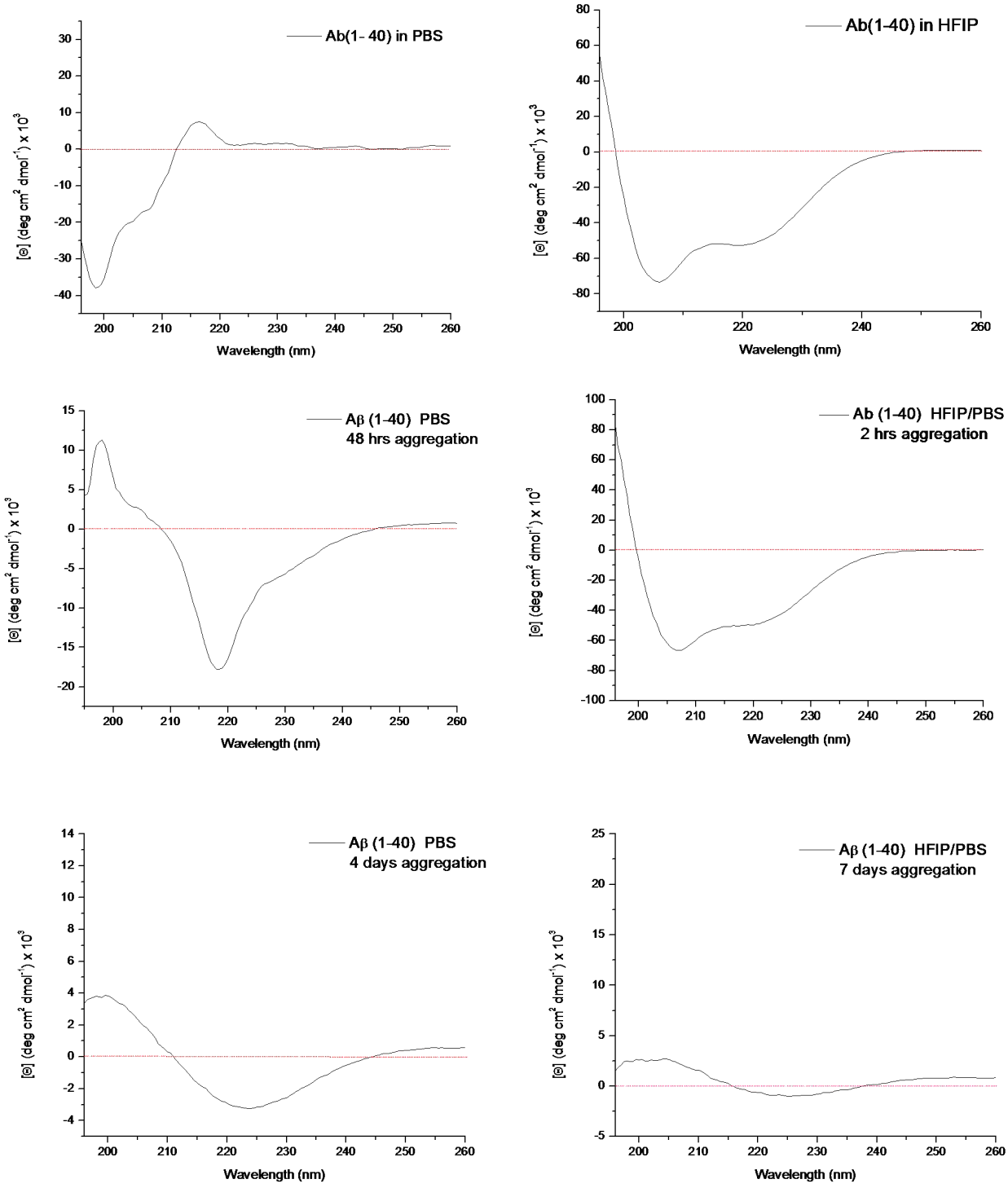

Figure IV.9. Circular dichroism measurements of *$A\beta_{1-40}$ peptide in PBS (a), HFIP (b), after 2 hours (c), after 48 hours (d), after 4 days (e) and after 7 days (f)*

IV.3. $A\beta_{1-40}$ peptide interaction with sodium dodecil sulfate

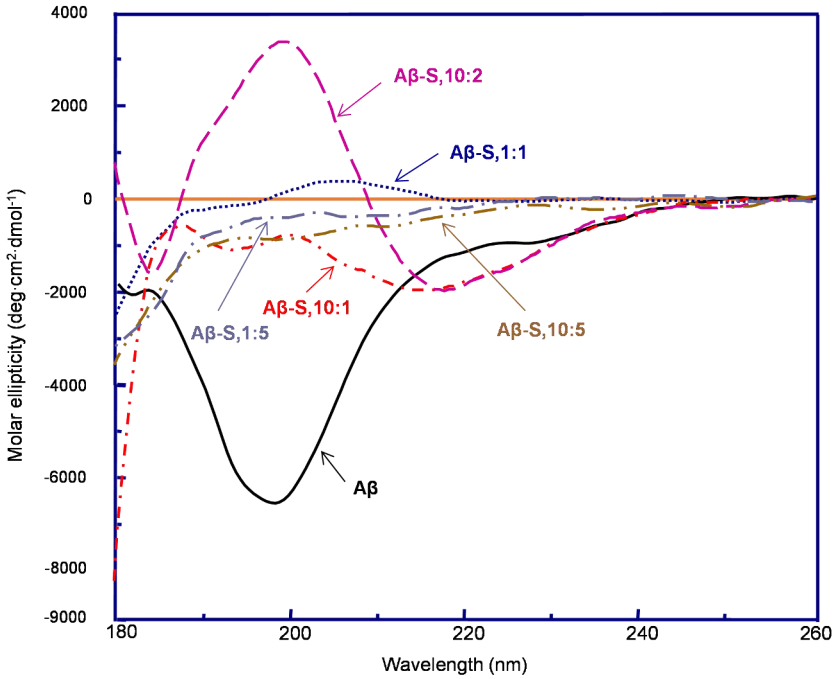

Figure IV.10. *Conformational modification of $A\beta_{1-40}$ peptide in presence of SDS (S):* 10: 1; 10: 2; 10: 5; 1: 1; 1: 2; 1: 5 *molar ratio*

IV.4. $A\beta_{1-40}$ peptide interaction with stearic acid

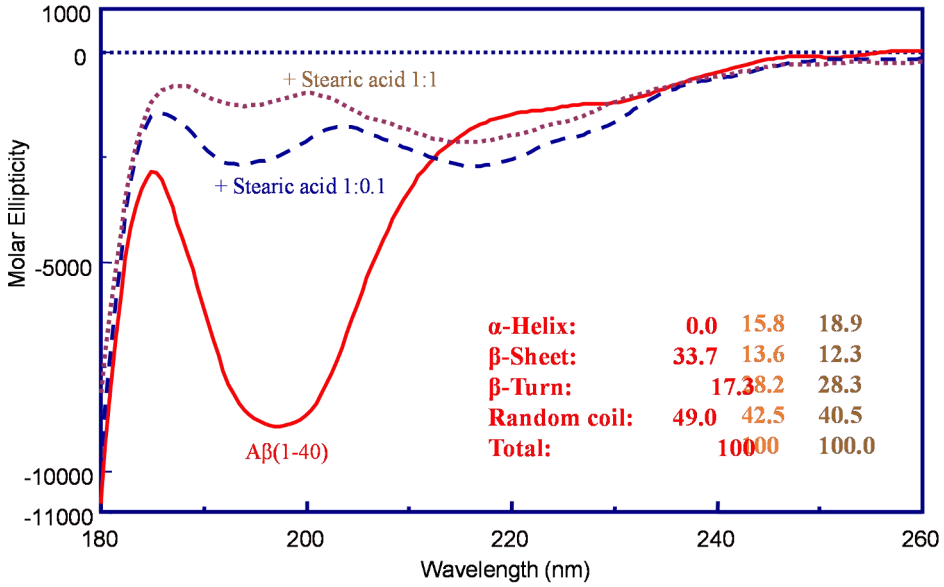

Figure IV.11. Conformational modification of *$A\beta_{1-40}$ peptide in presence of stearic acid 1: 1 (brown) şi 1: 0,1 (blue) molar ratio*

IV.5.1. Mass spectrometric studies of tetraglycine

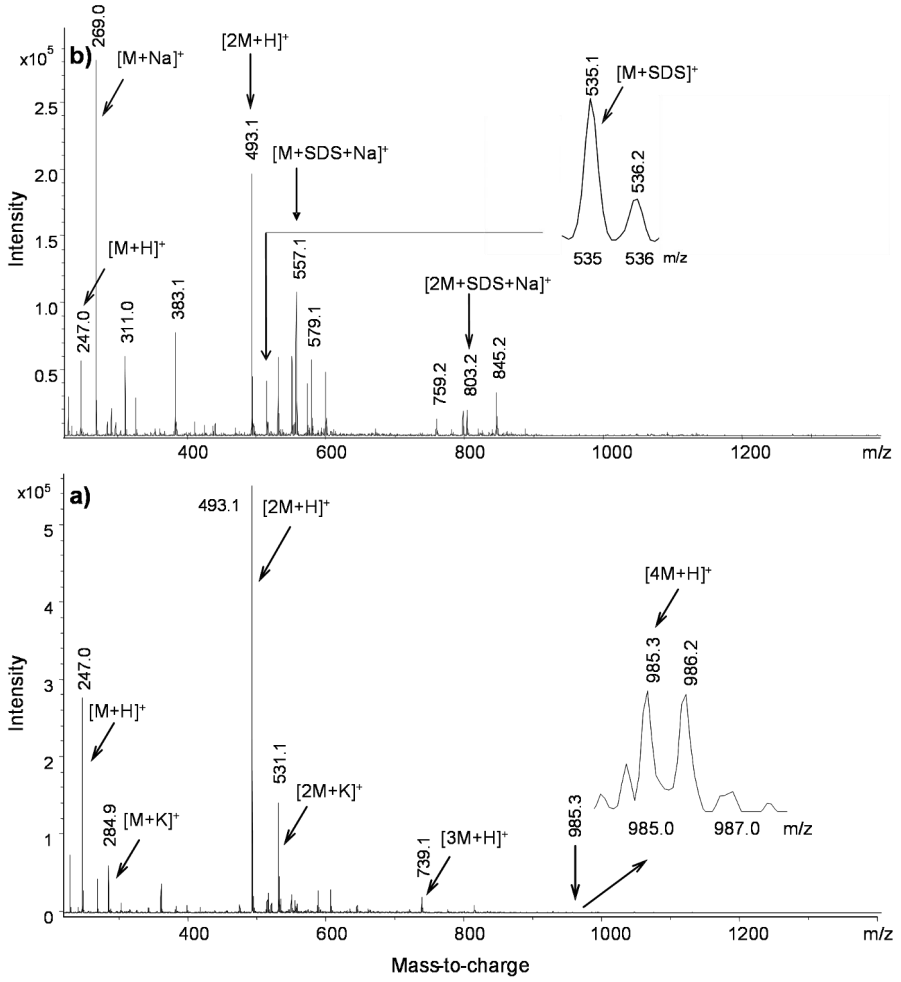

Figure IV.15. *Electrospray ionizationion trap-mass spectra of tetraglycine G_4 in the absence (a) and presence of SDS (b). The molar ratio of peptide to SDS was 1:1*

IV.7.1. Heavy metal complexes characterization by mass spectrometry

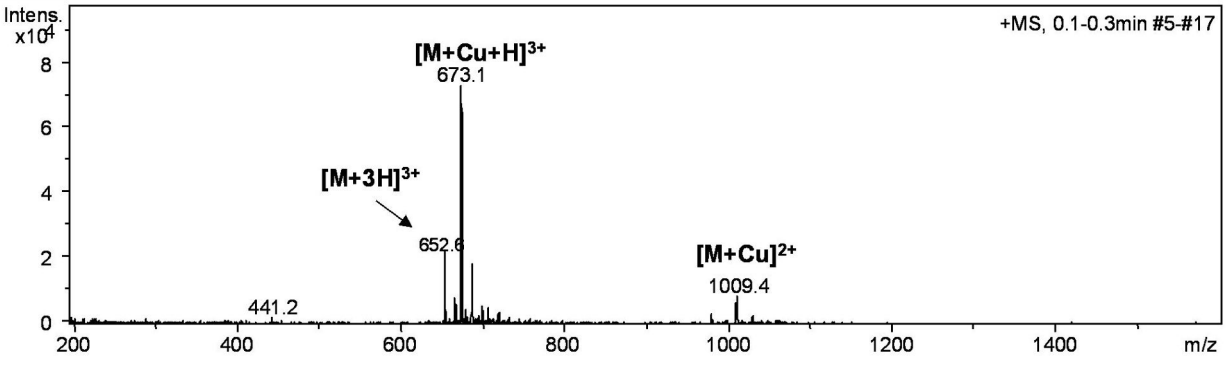

Figure IV.24. *MS spectrum of Cu^{2+}-$A\beta_{1-16}$ (2:1) complex*

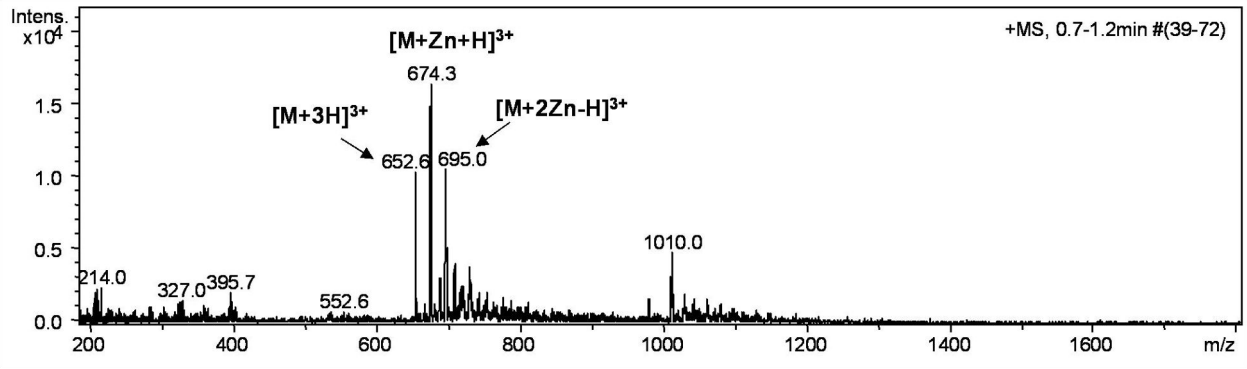

Figure IV.27. *MS spectrum of Zn^{2+}-$A\beta_{1-16}$ (2:1) complex*

IV.7.2. Heavy metal complexes caracherization by atomic force microscopy

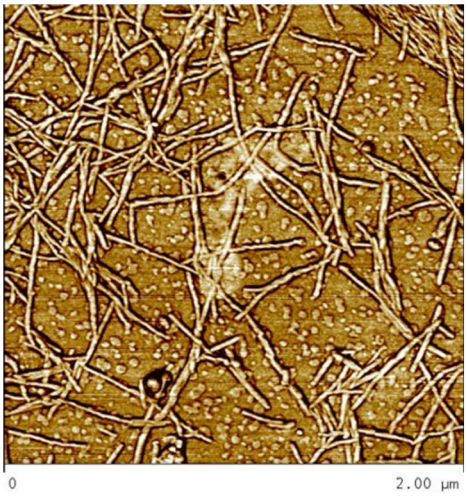

Figure IV.31. *AFM image of $A\beta_{1-16}$(F→G, H→A) peptide*

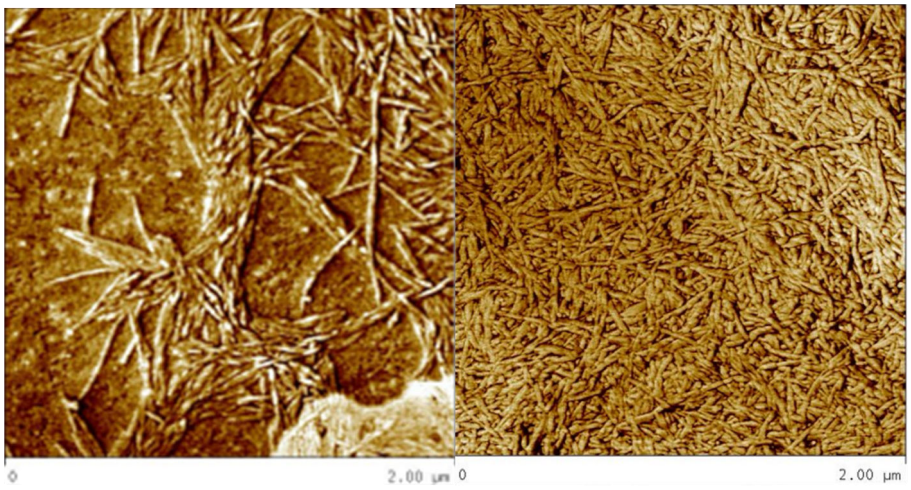

Figure IV.32. *AFM image of $A\beta_{1-16}$(F→G, H→A) peptide with Cu^{2+} şi Zn^{2+} ions*

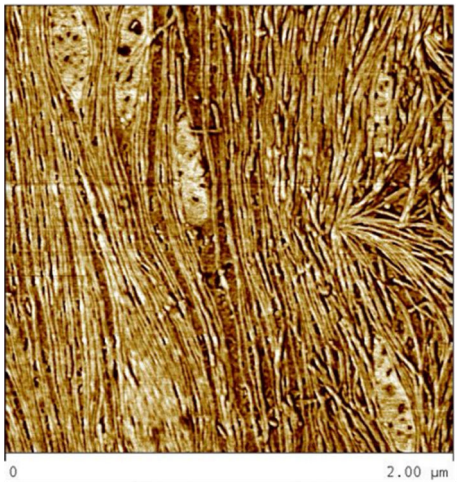

Figure IV.33. *AFM image of $A\beta_{1-16}$(F→G, H→S) peptide*

Figure IV.34. *AFM image of $A\beta_{1-16}$(F→G, H→S) peptide with Cu^{2+} şi Zn^{2+} ions*

IV.8. Conclusions

i. Fast photochemical oxidation represents an efficient method for aggregation mechanism study.

ii. Some amino acids, H13, H14, V26, V39, have presented both increases and decreases of oxidation due to their position in the structure adopted by the peptide in the aggregation process; they may be involved in the hydrophobic core formation.

iii. The data obtained using circular dichroism are consistent with the behavior of $A\beta_{1-40}$ peptide in the oxidation process;

iv. SDS adding to the Aβ solution severely changes the peptide conformation, with disappearance of the β-sheet conformers and doubling the proportion of β-turn isomers.

v. Equimolecular SDS: Gly_4 ratio was associated with a decreased proportion of Gly_4 oligomers as compared with non-SDS aqueous G_4 solutions. However, the aqueous Gly_4-SDS system may also contain Gly_4 dimers, Gly_4-Gly_4, Gly_4 oligomers, $(Gly_4)_n$, Gly_4-SDS adducts, $(Gly_4\text{-SDS})_n$, alongside with the expected monomers and their alkaline metal adducts.

vi. Peptide complexes with heavy metal ions could be formed very easily by simply mixing the metal with peptide solutions, but the complexes stability depends on the metal ion type being used and the primary and secondary structure of the peptide under investigation;

vii. ESI-MS mass spectrometry shows metal complexes with peptides and also shows that, generally, a complex peptide mixture it is being formed with one or more metal ions;

viii. Atomic force microscopy and scanning electron shows morphology differences between the peptides and metal complexes, according to the type of amino acids of the peptide sequence, and depending on the metal ions used for metal complexes formation.

CHAPTER V

Obtained results:

Electrophoretic studies of zein polypeptide compounds

V.1. Electrophoretic characterization of zein extract

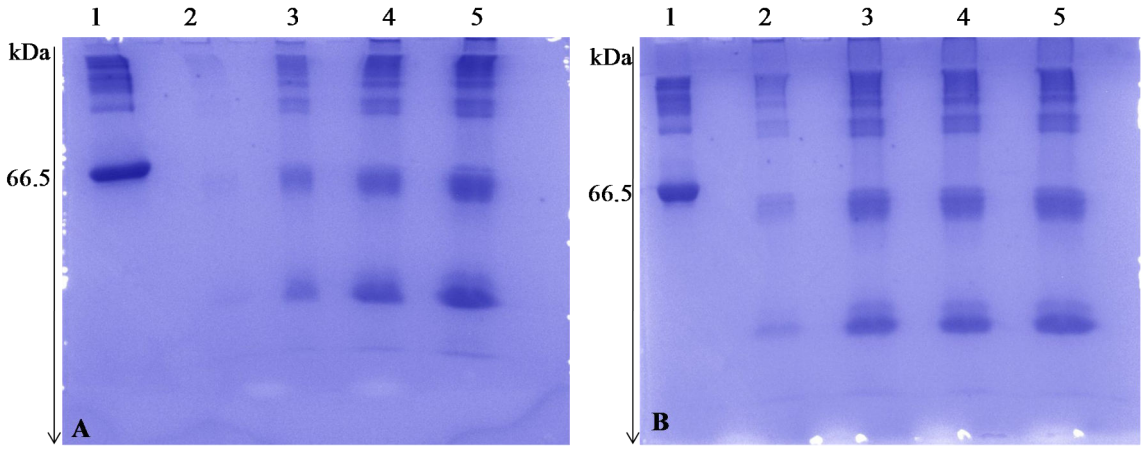

Figure V.2. *The gels obtained using sonication as extraction method.* ***A****-sample flour with a particle size of 250 micrometres and,* ***B****- sample flour with a particle size of 500 micrometres*

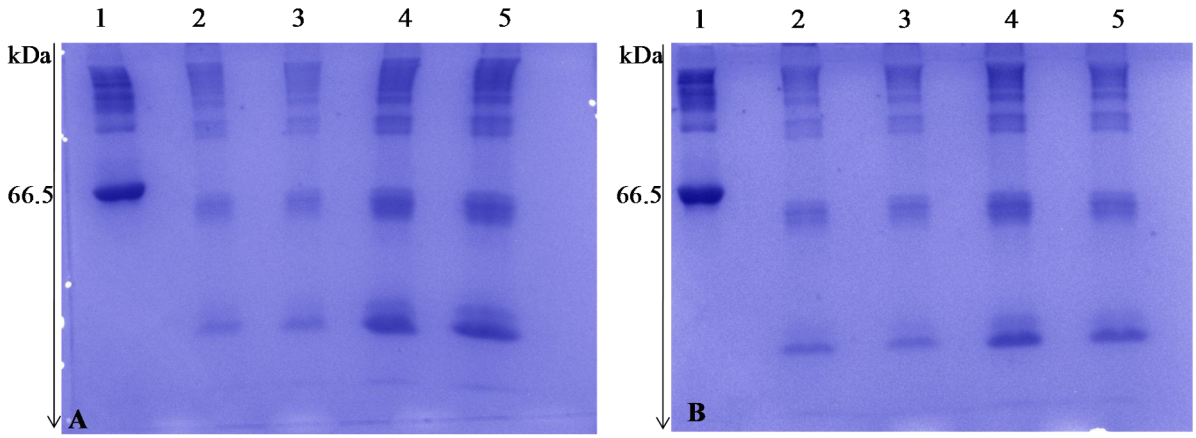

Figure V.3. *The gels obtained using stirring as extraction method. sample flour with a particle size of 250 micrometres and,* ***B****- sample flour with a particle size of 500 micrometres*

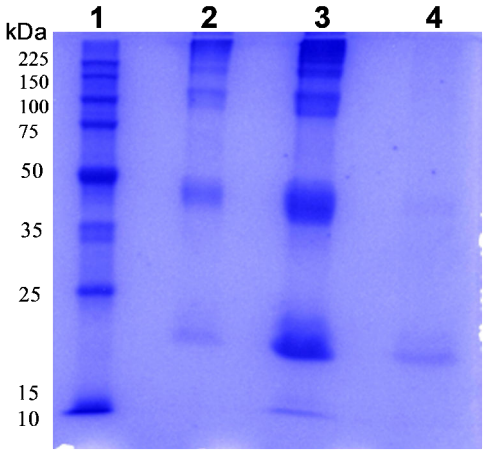

Figure V.4. *1D electrophoresis separation. (1) marker; (2) zein extracted with 70 % ethanol and washed with water, grain size 250 μm; (3) zein extracted with 70 % ethanol; (4) commercial α - zein*

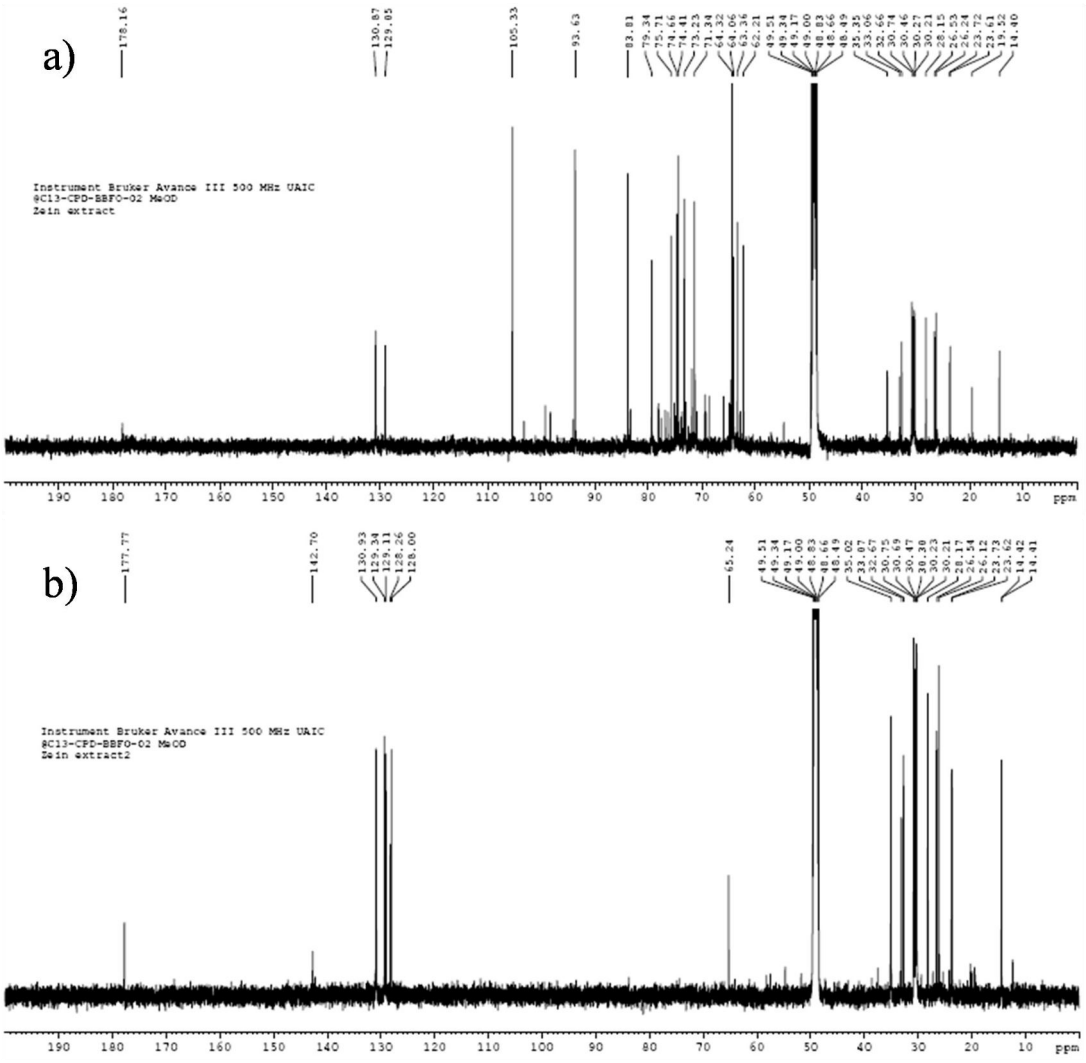

Figure V.5. *^{13}C-NMR spectrum of zein extracted with 70 % ethanol (a) and washed with water (b)*

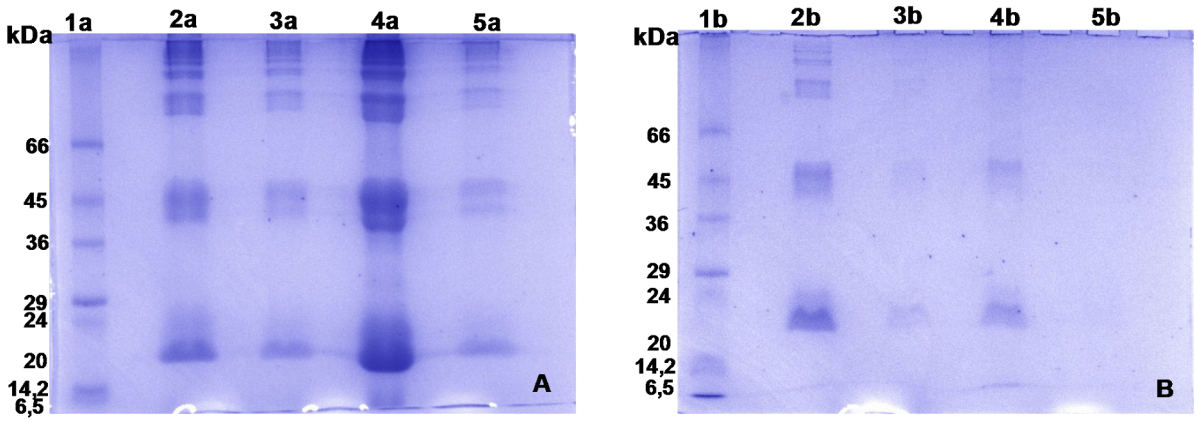

Figure V.9. *Separation of zein alcoholic extract by 1D electrophoresis* ***(A)*** *– 65% ethanol and* ***(B)*** *– 95% ethanol: proteins standard (1a/1b); KWS 3871, particle size 250 µm (2a/2b), KWS 3871, particle size 710 µm (3a/3b); DKC 4717, particle size 250 µm (4a/4b); DKC 4717, particle size 710 µm (5a/5b)*

V.3. Mass spectrometric characterization of zein extract

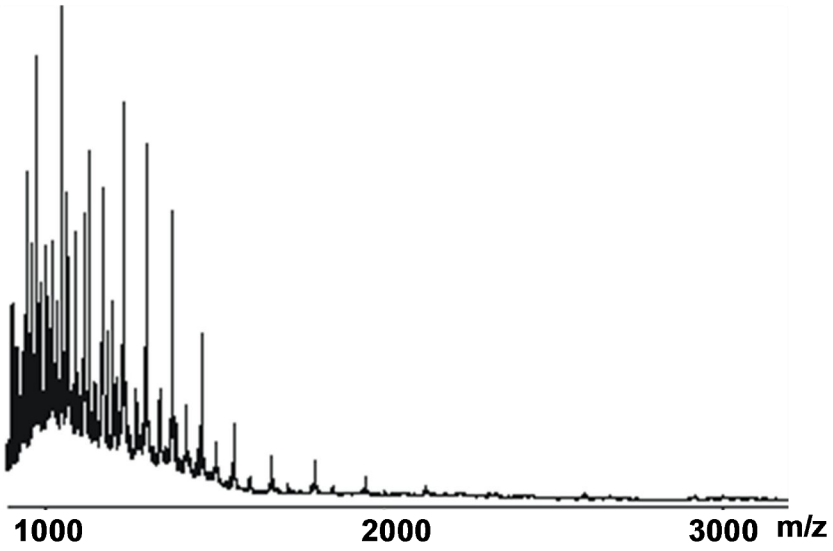

Figure V.14. *NanoESI mass spectrum of A1 sample (250 µm, 65% ethanol)*

Table V.4. *MS spectrum of molecular ions present in A165 % sample*

m/z	z	MW
2124.83	11.00	23362.13
1947.68	12.00	23360.16
1797.98	13.00	23360.74
1669.58	14.00	23360.12
1558.32	15.00	23359.80
1461.00	16.00	23360.00
1375.07	17.00	23359.19
1298.81	18.00	23360.58
1230.29	19.00	23356.51
1168.99	20.00	23359.80
1113.36	21.00	23359.56
1062.77	22.00	23358.94
1016.68	23.00	23360.64
Average		23359.86
Standard deviation		1.26
1503.00	16.00	24032.00
1414.63	17.00	24031.71
1336.06	18.00	24031.08
1265.81	19.00	24031.39
1201.02	20.00	24000.40
1143.57	21.00	23993.97
1092.00	22.00	24002.00
Average		24017.51
Standard deviation		17.68

V.3. Conclusions

i. Several samples of corn have been studied by electrophoretic and mass spectrometric methods.

ii. It was noticed that the extraction method, ultrasound bath and thermomixer, did not influence the extracted zein type;

iii. 1D electrophoresis of hybrids maize shows the differences, from quantity point of view, of extracted zein with different alcohol concentration. This is due to different solubility of zeins;

iv. It was noticed the disappearance of bands in gel which were assigned of α-zein dimers by using a disulfide bridges reducing agent, however it is still possible to

achieve dimerization also by non-covalent bonds;

v. Nuclear magnetic resonance spectra confirmed, alongside zeins, the extraction of a complex mixture;

vi. Most samples, $A_1$65, $A_1$95, $A_2$65, $A_2$95, $C_1$65 and $C_2$65 respectively, showed protein ion signals with more than one series of ions, indicating that the samples contained complex mixtures.

Conclusions

Based on the experimental studies on peptide compounds, we can draw the following conclusions:

I. **By using solid phase peptide synthesis by Fmoc strategy, a number of six peptides with physiological importance, of which three sequences are unknown in the literature, were synthesized:**

Aβ$_{1-40}$ ^{1}DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV^{40}-$CONH_2$

Aβ$_{25-35}$ ^{25}GSNKGAIIGLM^{35}-$CONH_2$

Aβ$_{1-16}$ ^{1}DAEFRHDSGYEVHHQK^{16}-$CONH_2$

Aβ$_{1-16(F\rightarrow G)}$ ^{1}DAE**G**RHDSGYEVHHQK^{16}-$CONH_2$ - unknown in literature

Aβ$_{1-16(F\rightarrow G,\ H\rightarrow A)}$ ^{1}DAE**G**R**A**DSGYEV**AA**QK^{16}-$CONH_2$ - unknown in literature

Aβ$_{1-16(F\rightarrow G,\ H\rightarrow S)}$ ^{1}DAE**G**RSD**S**GYEV**SS**QK^{16}-$CONH_2$ - unknown in literature

- The obtained peptides were separated and purified by high performance liquid chromatography using C4 and C8 columns (stationary phase) and acetonitrile: water (0.1% TFA) (mobile phase).
- Peptide purity was confirmed by MALDI ToF and electrospray mass spectrometry. Synthesized peptides experimental molecular weights were similar to their theoretical masses;
- Histidine residue modification in the peptide sequence leads to significant changes in peptide character.

II. **Synthesized peptides conformational and aggregation studies were performed by mass spectrometry, circular dichroism, fluorescence and atomic force microscopy. The relevant experimental results are the following:**

- Fast photochemical oxidation represents an efficient method for aggregation

mechanism study.

- Aminoacids such as phenylanine, histidine, lysine or methionine have presented the most intense oxidation process.
- Some amino acids, H13, H14, V26, V39, have presented both increases and decreases of oxidation due to their position in the structure adopted by the peptide in the aggregation process; they may be involved in the hydrophobic core formation.
- The data obtained using circular dichroism are consistent with the behavior of $A\beta_{1-40}$ peptide in the oxidation process;
- SDS adding to the Aβ solution severely changes the peptide conformation, with disappearance of the β-sheet conformers and doubling the proportion of β-turn isomers.
- Computer simulation proved to be a tool complementary to experimental methods, such as CD and FTIR measurements; it augmented the experimental information by providing an atomic picture of the molecules under investigation.
- By using electrospray ionization mass spectrometry, the formation of non-covalent oligomers of tetraglycine (Gly_4) in the presence of SDS was studied in detail.
- Equimolecular SDS: Gly_4 ratio was associated with a decreased proportion of Gly_4 oligomers as compared with non-SDS aqueous G_4 solutions. However, the aqueous Gly_4-SDS system may also contain Gly_4 dimers, Gly_4-Gly_4, Gly_4 oligomers, $(Gly_4)_n$, Gly_4-SDS adducts, $(Gly_4\text{-SDS})_n$, alongside with the expected monomers and their alkaline metal adducts.
- The mechanism by which SDS is splitting G_4 dimers seems to involve $–O–SO_2^–Na^+$ group interaction with $–COO^-$ groups of each G_4 molecule, whereas the affinity of sodium ion for negative charged groups seems to be the key step of the dissociation process.

- The fluorescence results confirmed the circular dichroism and fast photochemical oxidation results; the Aβ peptide fluorescence decreases with oxidation process.
- NAP peptide, due to its neuroprotective role, prevents Aβ aggregation and the fluorescence remains relatively constant throughout the measurement;
- Peptide complexes with heavy metal ions could be formed very easily by simply mixing the metal with peptide solutions, but the complexes stability depends on the metal ion type being used and the primary and secondary structure of the peptide under investigation;
- ESI-MS mass spectrometry shows metal complexes with peptides and also shows that, generally, a complex peptide mixture it is being formed with one or more metal ions;
- Atomic force microscopy and scanning electron shows morphology differences between the peptides and metal complexes, according to the type of amino acids of the peptide sequence, and depending on the metal ions used for metal complexes formation.

III. Polipeptide compounds such as zein, has been characterized by electrophoresis, mass spectrometry and nuclear magnetic resonance:

- Several samples of corn have been studied by electrophoretic and mass spectrometric methods.
- Due to the different zeins solubilities at various alcohol concentrations, their extraction has been studied by using ultrasonic bath and thermomixer;
- It was noticed that the extraction method, ultrasound bath and thermomixer, did not influence the extracted zein type;
- 1D electrophoresis of hybrids maize shows the differences, from quantity point of view, of extracted zein with different alcohol concentration. This is due to different solubility of zeins;

- It was noticed the disappearance of bands in gel which were assigned of α-zein dimers by using a disulfide bridges reducing agent, however it is still possible to achieve dimerization also by non-covalent bonds;
- Nuclear magnetic resonance spectra confirmed, alongside zeins, the extraction of a complex mixture of sugar and aminoacids;
- Most samples, $A_1$65, $A_1$95, $A_2$65, $A_2$95, $C_1$65 and $C_2$65 respectively, showed protein ion signals with more than one series of ions;
- The molecular masses that were determined experimentally were on average roughly 23500 Da +/- 300 and 24000 Da +/- 300 for the samples major compounds, corresponding to α-zeins based on UNIPROT database;
- Based on nanoESI-MS analyses, it seems that most proteins have derived from truncated α-zein.

The peptide compounds synthesized and studied in the actual work are having biological, physiological and biomedical - both structural and functional - significance. The obtained results and the synthesized peptides or isolated proteins can be further investigated, giving continuity to new research in the field.

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