



CNCS



## Flavone-Proteine Systems Involved in Oxidative Stress Probed by Advanced Spectroscopic Methods

**Type:** YOUNG RESEARCH TEAMS - PN-II-RU-TE-2012-3

**Project Registration Code:** PN-II-RU-TE-2012-3-0055

**Contract No.:** 6 / 23. 04. 2013

**Project duration:** 01.05.2013 – 30.09.2016

**Project director:** Dr. Mariana Voicescu

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**Total budget:** 645833 lei

**Contracting Authority:** UEFISCDI

**Program:** Human Resources

## Project Summary

The oxidative stress appeared as a necessity to express the bio-unbalance regarding the ratio between the antioxidant and oxidant chemical species, one of the major causes leading to organisms being taken ill. Based on previous experience and on the obtained results connected to the pro and anti oxidant activity of some molecules of biological interest, the main goal of this project is to develop quantitative methods to study biochemical aspects of some flavone-protein systems, in different media and partnership conditions (biomimetic and nanostructured systems), using Fluorescence, Raman, FTIR and EPR spectroscopies. The used systems are chosen in order to assure superior ways of organizing biological species. One follows/thinks of a detailed study regarding the spectrophotometric properties of flavonols and isoflavones in emulsion/micellar media and in lipidic bi-layers of lecithin on nanoparticles support. Monitoring and generating free radicals in the studied systems based on luminol-hydrogen peroxide are considered. The study is relevant for clearing up the antioxidant activity of different flavones classes and flavone-protein interactions in different media and partnerships conditions especially when associated with nanoparticles. These aspects lead to new insights on the oxidative stress process in the living organism, redox regulation and consequently, a priority in biochemical and biomedical domains.

**Keywords:** Oxidative stress, flavones, spectroscopic methods

## **Project Objectives**

**The main goal of this project is to develop quantitative methods to study biochemical aspects of some flavone-protein systems, in different media and partnership conditions (biomimetic and nanostructured systems), closer to those of biological interest, using Steady-State and Time-Resolved Fluorescence, Raman and FTIR spectroscopies, Resonance Electron Spin spectroscopy, Circular Dichroism.**

**The medium conditions will be such as to assure the processes in the homogeneous conditions and than, in heterogeneous ones (eq. emulsions, lipidic bi-layers of lecithin, micells), the latest having as a result the formation of nanostructured assemblies, a superior way of organizing biological species.**

**Monitoring and generating free radicals in the studied systems based on luminol-hydrogen peroxide will be conducted as a function of pH and reaction time.**

**There is an increasing interest in understanding and controlling the interactions of nanomaterials with biological molecules hence, one follows/thinks of a detailed study regarding the spectrophotometric properties of flavones and their interaction with proteins in different medium conditions and on silver nanoparticles.**

**Silver nanoparticles can be used as a sensitive biosensor to study the electrochemistry of electron transfer proteins subsequently, new aspects regarding the antioxidant activity of different flavones classes into the living organism, the oxidative stress and redox regulation.**

**The results dissemination will be achieved by participations to the national and international conferences as well as by publishing in rated ISI journals.**

## Budget Breakdown

No.	BUDGET CHAPTER (EXPENSES)	TOTAL 2013 (lei)	TOTAL 2014 (lei)	TOTAL 2015 (lei)	TOTAL 2016 (lei)	TOTAL (lei)
1.	SALARIES	99997,93	119703,36	96821	57.184,00	373706,29
2.	OVERHEAD	21739,00	22826	29892	9783	84240
3.	MOBILITY	9606,22	21227,42	21585,66	1.366,41	53785,71
4.	INVENTORY	35322,85	11243,22	80868,34	6.666,59	134101
	TOTAL	166666	175000	229167	75000	645833

## Research Team

Crt. No	NAME	YEAR of BIRTH	DIDACTIC / SCIENTIFIC TITLE	PhD
1	VOICESCU MARIANA	1973	Senior researcher II	Yes/Since 2004
2	ANGELESCU DANIEL	1971	Senior researcher II	Yes/Since 2004
3	IONESCU SORANA	1972	Lecturer	Yes/ Since 2004
4	LATUS ALINA	1982	Assistant researcher ( <i>Postdoc stage / France</i> )	Yes/ Since 2011
5	BANDULA RODICA	1948	Researcher ( <i>Retired beginning with September 1<sup>th</sup>, 2013</i> )	No
6	GATEA FLORENTINA	1968	Senior researcher III ( <i>1.03.2014 - 1.12.2014</i> )	No
7	NEACSU GEORGIANA	1990	Assistant researcher / ( <i>Master Student - Polytechnic University of Bucharest</i> ) ( <i>1.05.2015- 30.10.2015</i> )	No

## Scientific cooperation

- University of Bucharest, Department of Physical Chemistry, Bd Regina Elisabeta 4-12, Bucharest 030018, Romania
- University POLITEHNICA of Bucharest, Department of Applied Physical Chemistry and Electrochemistry, Polizu 1, 78126 Bucharest, Romania
- National Institute of R&D for Biological Sciences, Splaiul Independentei 296, 060031, Bucharest, Romania
- Polymer Department, National R&D Institute for Chemistry and Petrochemistry ICECHIM, Splaiul Independentei 202, 060021 Bucharest, Romania
- Organic Chemistry Institute of the Romanian Academy, Splaiul Independentei 202 B, 060021, Bucharest, Romania
- Institute of Atomic Physics, National Institute of Materials Physics, Magurele 077125, Romania
- Institut Universitaire de France, Laboratoire de bioélectrochimie et spectroscopie UMR 7140 - Chimie de la matière complexe, Université de Strasbourg, 1, rue Blaise Pascal, 67000 Strasbourg, France

## **Phase List / 2013 - 2016**

### **Phase I / 2013**

- O.1. The study of the structure and physico-chemical properties of flavonols and isoflavones**
- O.2. The effect of flavonols and isoflavones into an oxidation process**
- O.3. Structural aspects concerning the flavone - protein interaction on silver nanoparticles**

### **Phase II / 2014**

- O.1. Antioxidant activity of flavonols in lipidic bilayers of lecithin**
- O.2. Antioxidant activity of isoflavones in lipidic bilayers of lecithin**

### **Phase III / 2015**

- O.1. Structural aspects on flavone - protein interaction in lipidic bilayers of lecithin, on silver nanoparticles support**
- O.2. PEG - protein emulsions on silver nanoparticles support**
- O.3. PEG - protein - flavone emulsions**

### **Phase IV / 2016**

- O.1. PEG - protein - flavone emulsions on silver nanoparticles support**

# **Activity Report / 2013 - 2016**

## **Phase I / 2013**

**O.1.** Photophysical and photochemical properties of some flavones were determined in different environment (pH, solvent, micellar environment) and partnership conditions (interaction with glucidic and proteic type macromolecules), bringing thus new information regarding the use of flavones as fluorescent probes in systems of biological interest, especially in an environment characteristic for proteins.

**O.2.** Serum proteins (BSA and HSA) as well as glucidic type macromolecules (Dextran 40, Dextran 70,  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD), improve the antioxidant activity of hidroxyflavones and isoflavones by a mechanism involving the direct elimination of reactive oxygen species.

**O.3.** In the 3-HF / BSA / SNPs system, at a 3-HF: BSA = 10: 1 molar ratio, one noticed that BSA was adsorbed on SNPs surface by a mechanism that can be due to some chemical bonds or to electrostatic forces that determine a spontaneous adsoption of the protein on SNPs surface.

## **Phase II / 2014**

**O.1.** The antioxidant activity of 3-HF; 3,6-diHF and 3,7-diHF flavonols increases when these are incorporated in the lipidic bilayers of lecithin. HSA produces a different fluidity in the lipidic bilayers of lecithin, rendering difficult the HF's inclusion process and implicitly their antioxidant activity is slightly decreased.

**O.2.** In the lipidic bilayers of lecithin, isoflavones have a well ordered structure which is stabilized by H intramolecular bonds; Their antioxidant activity depends on the concentration and time.



# Activity Report / 2013 - 2016

## Phase III / 2015

**O.1.** In case of 3-HF / PC / HSA / SNPs system, no significant changes in the fluorescence emission of the two species,  $A^*$  ( $\lambda_{em} = 471 \text{ nm}$ ) and  $T^*$  ( $\lambda_{em} = 521 \text{ nm}$ ), were noticed; On SNPs support and in lipidic bi-layers of lecithin, the  $\alpha$ -helix content of BSA decreases; In the presence of 3-HF, 41.4% of the disulfide bridges, adopt a *ggg* conformation which increases when BSA is adsorbed on SNPs' surface and in PC/SNPs systems;

**O.2.** In PEG environment, the intramolecular excited state proton transfer is more rapid and depends on PEG's structure thus: Tween20  $\square$  Myrj52  $\square$  L64  $\square$  SNPs; 3,6-diHF in BSA / PEG systems reduces the phenomenon of SNPs' aggregation. PEG coated SNPs surface and Myrj52 reduces SNPs' aggregates formation when 3,6-diHF binds to BSA; Myrj52 improves the thermal stability of BSA protein and has a renaturation effect on it when the BSA adsorbs on SNPs surface;

In BSA / PEG / SNPs and BSA / PEG / 3,6-diHF / SNPs systems the content of  $\alpha$ -helix increases, resulting in BSA renaturation when PEG coated SNPs surface;

By 3,6-diHF binding to BSA, a redistribution of the disulphide bridges between *ggg* and *tgt* conformation takes place, the latter being predominant.

**O.3.** Using the chemiluminescent system luminol-hydrogen-peroxide, in alkaline solution, Tris-HCl buffer, pH 8.5, one could notice that for 0.12% PEG, riboflavin antioxidant activity is improved at higher or lower values, according to their molecular structures, as follows:

Tween20 > Myrj52 > L64;

A cross-linking of PEGs to RF in the presence of BSA with maintaining the integrity of BSA, were noticed.

# Activity Report / 2013 - 2016

## Phase IV / 2016

**O.1.** Denaturation properties, under the influence of temperature, of BSA protein in RF/PEG/BSA system, were studied. A low thermal stabilization of Trp<sup>212</sup> in RF/BSA/Tween20 system, leading to a partially Tween20 self-assembly in solution, was observed; The binding energy value of the RF towards Tween20 ( $> 169.21 \text{ kJ/mol}$ ) is lower than that of the RF towards Myrj52 ( $> 157.50 \text{ kJ/mol}$ ). Thus a better stability of the RF/Tween20 system, which leads to a higher RF antioxidant activity in Tween20 than in Myrj52.

Synthesis and physicochemical characterization of RF loaded Myrj52 - silver nanoparticles (SNPs), were performed. Transmission Electron Microscopy analysis showed the formation of colloidal spherical SNPs with the average size of  $\sim 12 \text{ nm}$ . The structure, stability, dynamics and conformation of the HSA protein on SNPs, have been studied. The influence of RF on the secondary structure of HSA in the presence of SNPs showed a predominant  $\alpha$ -helix structure (61.7 %); No changes induced by RF binding to HSA.

*In vitro* cytotoxicity evaluation of RF in PEG / protein / SNPs systems was performed by Neutral Red assay and by monitoring lactate dehydrogenase release. Tween20/BSA, Myrj52/BSA as well as HSA / SNPs systems offers great promise for enhancement of the RF antioxidant activity and biocompatibility in L929 fibroblast cells culture.

## Results Dissemination / 2013 - 2016

### ➤ Publications / Cumulative IF = 23.481

1. M. Voicescu, S. Ionescu, F. Gatea,  
Photophysical Properties of some Flavones Probes in Homogeneous Media,  
*J. of Fluorescence*, DOI: 10.1007/s10895-013-1272-0, 24 (1), 75-83, 2014. (IF = 1.927)
2. M. Voicescu, S. Ionescu, F. Gatea,  
Effect of pH on the Fluorescence Characteristics of some Flavones probes,  
*Spectrochim Acta A*, DOI:10.1016/j.saa.2013.12.040, 123, 303-308, 2014. (IF = 2.353)
3. M. Voicescu, S. Ionescu,  
Fluorescence Characteristics of some Flavones Probes in Different Micellar Media,  
*J. of Fluorescence*, DOI: 10.1007/s10895-013-1346-z, 24 (3), 735-743, 2014. (IF = 1.927)
4. M. Voicescu, S. Ionescu,  
3-Hydroxyflavone - Bovine Serum Albumin Interaction in Dextran Medium,  
*J. Serb. Chem. Soc.*, doi: 10.2298/JSC140425075V, 80 (4), 517-520, 2015. (IF = 0.970)
5. M. Voicescu, Cristina L. Nistor, A. Meghea,  
Insights into the Antioxidant Activity of some Flavones on Silver Nanoparticles using the Chemiluminescence Method,  
*J. of Luminescence*, DOI: 10.1016/j.jlumin.2014.08.058, 157, 243-248, 2015. (IF = 2.693)
6. M. Voicescu, R. Bandula,  
3, 6-diHydroxyflavone / Bovine Serum Albumin Interaction in Cyclodextrin Medium: Absorption and Emission Monitoring,  
*Spectrochim. Acta A*, DOI:10.1016/j.saa.2014.11.093, 138, 628-636, 2015. (IF = 2.653)
7. M. Voicescu, O. Craciunescu, L. Moldovan, M. Anastasescu, D.G. Angelescu, V. S. Teodorescu,  
Physicochemical Characterization and *in vitro* Cytotoxic Effect of 3-Hydroxyflavone in a Silver Nanoparticles Complex,  
*J. of Fluorescence*, dx.doi.org/ 10.1007/s10895-015-1608-z, 25, 1215-1223, 2015. (IF = 1.601)
8. M. Voicescu, P. Hellwig, A. Meghea,  
Antioxidant Activity of Phytoestrogens types Isoflavones in Biomimetic Environments,  
*New J. Chem.*, DOI: 10.1039/c5nj01568e, 40, 606-612, 2016. (IF = 3.277)
9. M. Voicescu, S. Ionescu, C. L. Nistor,  
Spectroscopic study on 3-Hydroxyflavone - protein interaction in lipidic bi-layers immobilized on silver nanoparticles,  
*Spectrochim. Acta A*, doi:10.1016/j.saa.2016.06.039, 170, 1-8, 2017. (IF=2.653)
10. M. Voicescu, G. Neacsu, A. Beteringhe, O. Craciunescu, R. Tatia, L. Moldovan,  
Antioxidant and cytotoxic properties of Riboflavin in PEG/BSA Systems,  
*Chemical Papers*, under review – MS No. 0310-16. (IF = 1.326)
11. M. Voicescu, S. Ionescu, C. L. Nistor,  
Physicochemical characterization of 3,6-diHydroxyflavone binding BSA immobilized on PEG-coated Silver Nanoparticles,  
*J. Nanopart. Res.*, under review – MS No. NANO-D-16-01028. (IF = 2.101)

# Results Dissemination / 2013 - 2016

## ➤ International Conferences

1. M. Voicescu, F. Gatea, A. Meghea, *Antioxidant Activity of some Flavones Evidenced by Chemiluminescence Method*, 8-th International Conference of the Chemical Societies of the South-East European Countries, Belgrade, Serbia, June 27-29, 2013.
2. M. Voicescu, S. Ionescu, D. G. Angelescu, M. Anastasescu, V. S. Teodorescu, *Flavonoid-Protein Interaction at the Silver Nanoparticle Surface*, 13th Conference on Methods and Applications of Fluorescence, 8-11 September, Genoa, Italy, 2013.
3. M. Voicescu, S. Ionescu, F. Gatea, *Photophysical and Photochemical Studies of some Flavones Probes*, International Conference of Physical Chemistry - ROMPHYSICHEM 15th, September 11-13, Bucharest, Romania, 2013.
4. M. Voicescu, S. Ionescu, F. Gatea, R. Bandula, *Steady-State and Time-Resolved Fluorescence Analysis of 3,6 diHydroxyflavone*, International Conference of Physical Chemistry- ROMPHYSICHEM 15th, September 11-13, Bucharest, Romania, 2013.
5. M. Voicescu, F. Gatea, A. Meghea, *Glucidic type macromolecules on the antioxidant activity of isoflavone genistein*, The Annual International Conference of the Romanian Society of Biochemistry and Molecular Biology, Baile Felix, Oradea, Romania, 5-7 June, 2014.
6. M. Voicescu, S. Ionescu, M. Anastasescu, O. Craciunescu, R. Tatia, L. Moldovan, D. G. Angelescu, V. S. Teodorescu, *Biological Evaluation of 3-Hydroxyflavone in a Silver Nanoparticles Complex*, FEBS-EMBO International Conference, Paris, Franta, 30 Aug.-4 Sept, 2014.
7. M. Voicescu, S. Ionescu, A. Meghea, *Flavone - Protein Interaction in Lipidic Bilayers of Lecithin*, IC<sup>3</sup>EM - 1<sup>st</sup> International Caparica Conference on Chromogenic and Emissive Materials, Caparica / Lisabona, Portugalia, 8-10 September, 2014.
8. M. Voicescu, S. Ionescu, *3-Hydroxyflavone - Bovine Serum Albumin interaction on silver nanoparticles. A fluorescence and Raman study*, 13-th National Conference of Biophysics, June 4-6, Timisoara, Romania, 2015.
9. M. Voicescu, Z. Boubegtiten, P. Hellwig, *Spectroscopic studies on the structural changes in Human Serum Albumin upon 3-Hydroxyflavone binding immobilized on Silver Nanoparticles*, The 40<sup>th</sup> FEBS Congress, The Biochemical Basis of Life, Berlin, Germany, July 4 - 9, 2015.
10. M. Voicescu, S. Ionescu, A. Meghea, *Flavone - protein interaction in lipidic bilayers of lecithin and on silver nanoparticles support*, International Conference on Chemical and Biochemical Engineering, Paris, France, 20-22 July, 2015.
11. M. Voicescu, S. Ionescu, C. L. Nistor, M. Maganu, *Spectroscopic analysis of 3,6-diHF / Bovine Serum Albumin / PEG emulsions on silver nanoparticles*, 14-th Conference on Methods and Applications in Fluorescence (MAF-14), Würzburg, Germany, 13-16 September, 2015.
12. M. Voicescu, G. Neacsu, *Antioxidant activity of riboflavin in Polyethylene Glycol – Bovine Serum Albumin systems*, RSBMB Anniversary Conference 25 years of promoting molecular life sciences, Bucharest, Romania, 17-18 September, 2015.
13. M. Voicescu, S. Ionescu, C.L. Nistor, *Fluorescence characteristics of 3,6-dihydroxyflavone on PEG-coated silver nanoparticles*, The International Conference CHIMIA 2016 "New Trends in Applied Chemistry", Constanta, Romania, 26-28 May 2016.