

## **Etapa a III-a: Obținerea nanoimunisorbentilor non magnetici si magnetici antipesticid-anticorp cuplat la nanoparticule si caracterizarea fizico-chimica si structurala a acestora**

### **Activitate III.1.**

Obținerea nanoimunisorbentilor non magnetici si magnetici antipesticid-anticorp cuplat la nanoparticule (partea a II-a), **CO**

### **Activitate III.2.**

Caracterizarea fizico-chimica a nanoimunisorbentilor obtinuti (partea a II-a), **P2**

### **Activitate III.3.**

Caracterizarea structurala a nanoimunisorbentilor anticorp antipesticid-nanoparticule (partea a II-a), **P1**

### **Rezultate estimate ale prezentei etape:**

- Proceduri de sinteza si analiza structurala a nanoimunisorbentilor;
- Obținerea produsilor: nanoimunisorbenti non magnetici si superparamagnetici;
- Publicarea unui articol intr-o revista de specialitate;
- Cerere Brevet de inventie national.

Termen: 31.12.2014

In lucrarile efectuate in cadrul prezentei etape, cea de-a treia, sunt prezentate procedeele de obtinere a nanoimunisorbentilor non magnetici (nanoparticula de SiO<sub>2</sub>- albumina serica de capra(ASC)- acid 2,4-diclorofenoxiacetic(2,4-D), SiO<sub>2</sub>-anticorp anti 2,4-D) si magnetici (nanoparticule magnetice-anticorp anti 2,4-D si microparticule magnetice-anticorp anti 2,4-D) ce urmeaza a fi utilizati in tehnica HnELISA pentru detectia pesticidului acid 2,4-diclorofenoxiacetic (2,4-D). In cazul nanoimunisorbentilor non magnetici s-a stabilit cinetica de cuplare a antigenului (ASC-2,4-D) si anticorpului anti 2,4-D la nanoparticule de SiO<sub>2</sub> activate prin diferite procedee. Pentru cei patru nanoimunisorbenti obtinuti s-a calculat densitatea masica de suprafata (cantitatea de proteina (μg) cuplata la 1 mg de nanoimunisorbent) obtinandu-se urmatoarele valori:

- SiO<sub>2</sub>-ASC-2,4-D: 106 μg proteina/mg;
- SiO<sub>2</sub>-anticorp anti 2,4-D: 84 μg proteina/mg;
- Nanoparticule magnetice-anticorp anti 2,4-D: 21,3 μg proteina/mg;
- Microparticule magnetice-anticorp anti 2,4-D: 5,2 μg proteina/mg.

Rezultatele cele mai bune din punct de vedere al cantitatii de proteina cuplata pe nanoimunisorbent au fost date de nanoimunisorbentii non magnetici pe baza de SiO<sub>2</sub> si anume: SiO<sub>2</sub>-ASC-2,4-D si SiO<sub>2</sub>-anticorp anti 2,4-D ce urmeaza a fi utilizati in tehnica HnELISA.

Pentru nanoimunisorbentul SiO<sub>2</sub>-anticorp anti 2,4-D s-a studiat potentialul Zeta si variatia acestuia cu salinitatea de la -25 mV pentru pH 2,8 pana la -5 mV la pH 9,5.

In vederea evaluarii frecventei de distributie dimensionala ale nanoimunisorbentului SiO<sub>2</sub>-anticorp anti 2,4-diclorofenoxiacetic s-au efectuat studii la microscopul de forta atomica (AFM).

S-a depus Cererea de brevet de inventie national nr. OSIM A00911 /26.11.2014 cu titlul **”Procedeu de obtinere a anticorpilor anti acid 2,4-diclorofenoxiacetic (2,4D) din amestecuri complexe de proteine pe baza de nanoimunisorbenti ”**, autori: Dorobantu Ioan, Neagu Livia.

## **Modul de diseminare a rezultatelor**

**I.** Au fost publicate sau inaintate spre publicare in reviste de specialitate cotate ISI urmatoarele **articole stiintifice:**

1. Loredana Mereuta, Mahua Roy, Alina Asandei, Jong Kook Lee, Yoonkyung Park, Ioan Andricioaei, Tudor Luchian, Slowing down single-molecule trafficking through a protein nanopore reveals

intermediates for peptide translocation, *SCIENTIFIC REPORTS*, vol. 4, article number 3885, pp. 1-11, DOI: 10.1038/srep03885, 2014;

2. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Probing of Various Physiologically Relevant Metals: Amyloid- $\beta$  Peptide Interactions with a Lipid Membrane-Immobilized Protein Nanopore, *JOURNAL OF MEMBRANE BIOLOGY*, vol. 247(6), pp. 523-530, 2014, DOI 10.1007/s00232-014-9662-z;

3. Loredana Mereuta, Alina Asandei, Chang Ho Seo, Yoonkyung Park, Tudor Luchian, Quantitative Understanding of pH- and Salt-Mediated Conformational Folding of Histidine-Containing,  $\beta$ -Hairpin-like Peptides, through Single-Molecule Probing with Protein Nanopores, *ACS Applied Materials & Interfaces*, vol. 6:15, pp. 13242–13256, dx.doi.org/10.1021/am5031177, 2014;

4. B. Zorila, Mihaela Bacalum, A. I. Popescu, M. Radu, Log-normal deconvolution of Laurdan fluorescence spectra - A tool to assess lipid membrane fluidity, *Romanian Reports in Physics*, articol acceptat spre publicare.

**II. S-a depus Cererea de brevet de inventie national** nr. OSIM A00911 /26.11.2014 cu titlul "Procedeu de obtinere a anticorpilor anti acid 2,4-diclorofenoxiacetic (2,4D) din amestecuri complexe de proteine pe baza de nanoimunisorbenti", autori Dorobantu Ioan, Neagu Livia.

### **III. Participari la Conferinte Internationale**

1. Neagu Livia, Dorobantu Ioan, Nanoimmunisorbents based on functionalized silicon dioxide nanoparticles and their use in homogenous ELISA technique for detection of 2,4-D from environmental samples, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, "IC-ANMBES 2014", Brasov, Romania, June 13th-15th, 2014, prezentare poster;

2. Loredana Mereuta, Alina Asandei, Daniela Ciumac, Tudor Luchian, Different Steps in Translocation of Peptides through a Protein Nanopore, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania, prezentare poster –premiat Best poster;

3. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Investigating the Affinity of Various Physiologically Relevant Metals to Human A $\beta$ (1-16) Peptides via Nanopore Sensing Techniques, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania prezentare poster;

4. Tudor Luchian, Loredana Mereuta, Irina Schiopu, Alina Asandei, Sorana Iftemi, Aurelia Apetrei, Nanoscopic Interrogation of Molecular Interactions with Protein Nanopores, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania, invited keynote presentation.

## Obtained results

### Phase III: Obtainment of non magnetic and magnetic nanoimmunosorbents antipesticide-antibody coupled to nanoparticles and their physico-chemical and structural characterization

#### Activity III.1.

Obtainment of non magnetic and magnetic nanoimmunosorbents antipesticide-antibody coupled to nanoparticles (2nd part), **CO**

#### Activity III.2.

Physico-chemical characterization of the obtained nanoimmunosorbents (2nd part), **P2**

#### Activity III.3.

Structural characterization of antipesticide antibody-nanoparticles nanoimmunosorbent (2nd part), **P1**

#### Estimated results:

- Procedures of obtainment and structural analysis of the nanoimmunosorbents;
- Obtainment of the products: non magnetic and superparamagnetic nanoimmunosorbents;
- Publishing of one paper in a scientific journal;
- National patent application form.

Deadline: 31.12.2014

In the current phase of the project, the third one, are presented the procedures of obtaining of non magnetic nanoimmunosorbents ( $\text{SiO}_2$  nanoparticle-goat serum albumine(GSA)-2,4-dichlorophenoxyacetic acid(2,4-D),  $\text{SiO}_2$ -anti 2,4-D antibody) and magnetic nanoimmunosorbents (magnetic nanoparticles-anti 2,4-D antibody and magnetic microparticles-anti 2,4-D antibody) that are going to be used in HnELISA technique for dosing of the pesticide 2,4-dichlorophenoxyacetic acid. In the case of non magnetic nanoimmunosorbents was established the coupling kinetics of the antigen (GSA-2,4-D) and of the anti 2,4-D antibody to the activated  $\text{SiO}_2$  nanoparticles by different procedures. For the obtained nanoimmunosorbents was calculated the surface mass density (quantity of coupled protein ( $\mu\text{g}$ ) to 1 mg of nanoimmunosorbent) thus:

- $\text{SiO}_2$ -GSA-2,4-D: 106  $\mu\text{g}$  protein/mg;
- $\text{SiO}_2$ - anti 2,4-D antibody: 84  $\mu\text{g}$  protein/mg;
- Magnetic nanoparticles - anti 2,4-D antibody: 21,3  $\mu\text{g}$  protein/mg;
- Magnetic microparticles- anti 2,4-D antibody: 5,2  $\mu\text{g}$  protein/mg.

The best results in terms of quantity of protein coupled on the nanoimmunosorbent were obtained by the non magnetic nanoimmunosorbents based on  $\text{SiO}_2$ :  $\text{SiO}_2$ -GSA-2,4-D and  $\text{SiO}_2$ -anti 2,4-D antibody that are going to be used in HnELISA technique.

For the nanoimmunosorbent  $\text{SiO}_2$ -anti 2,4-D antibody was study the Zeta potential and its variation with salinity from -25 mV for pH 2,8 to -5 mV for pH 9,5.

Studies were done by Atomic Force Microscopy (AFM) in order to evaluate the dimensional distribution frequency of the obtained nanoimmunosorbent  $\text{SiO}_2$ -anti 2,4-dichlorophenoxyacetic acid antibody.

It was registrated one **national patent application** no. OSIM A 00911 /26.11.2014 entitled "Procedure of obtainment of anti acid 2,4-dichlorophenoxyacetic (2,4D) antibodies from protein mixture complex based on nanoimmunosorbents ", authors Dorobantu Ioan, Neagu Livia.

## Mode to disseminate the results

I. Were published or submitted in ISI ranked scientific journals next articles:

1. Loredana Mereuta, Mahua Roy, Alina Asandei, Jong Kook Lee, Yoonkyung Park, Ioan Andricioaei, Tudor Luchian, Slowing down single-molecule trafficking through a protein nanopore reveals

intermediates for peptide translocation, *SCIENTIFIC REPORTS*, vol. 4, article number 3885, pp. 1-11, DOI: 10.1038/srep03885, 2014;

2. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Probing of Various Physiologically Relevant Metals: Amyloid- $\beta$  Peptide Interactions with a Lipid Membrane-Immobilized Protein Nanopore, *JOURNAL OF MEMBRANE BIOLOGY*, vol. 247(6), pp. 523-530, 2014, DOI 10.1007/s00232-014-9662-z;

3. Loredana Mereuta, Alina Asandei, Chang Ho Seo, Yoonkyung Park, Tudor Luchian, Quantitative Understanding of pH- and Salt-Mediated Conformational Folding of Histidine-Containing,  $\beta$ -Hairpin-like Peptides, through Single-Molecule Probing with Protein Nanopores, *ACS Applied Materials & Interfaces*, vol. 6:15, pp. 13242–13256, dx.doi.org/10.1021/am5031177, 2014;

4. B. Zorila, Mihaela Bacalum, A. I. Popescu, M. Radu, Log-normal deconvolution of Laurdan fluorescence spectra - A tool to assess lipid membrane fluidity, *Romanian Reports in Physics*, accepted.

**II.** It was registered one **national patent application** no. OSIM A 00911 /26.11.2014 entitled "Procedure of obtainment of anti acid 2,4-dichlorophenoxyacetic (2,4D) antibodies from protein mixture complex based on nanoimmunosorbents ", authors Dorobantu Ioan, Neagu Livia.

### **III. International Conferences**

1. Neagu Livia, Dorobantu Ioan, Nanoimmunosorbents based on functionalized silicon dioxide nanoparticles and their use in homogenous ELISA technique for detection of 2,4-D from environmental samples, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, "IC-ANMBES 2014", Brasov, Romania, June 13th-15th, 2014, poster presentation;

2. Loredana Mereuta, Alina Asandei, Daniela Ciumac, Tudor Luchian, Different Steps in Translocation of Peptides through a Protein Nanopore, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania, poster presentation –Awarded Best Poster of the Conference;

3. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Investigating the Affinity of Various Physiologically Relevant Metals to Human A $\beta$ (1-16) Peptides via Nanopore Sensing Techniques, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania, poster presentation;

4. Tudor Luchian, Loredana Mereuta, Irina Schiopu, Alina Asandei, Sorana Iftemi, Aurelia Apetrei, Nanoscopic Interrogation of Molecular Interactions with Protein Nanopores, The Third International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences, IC-ANMBES 2014, June 13th-15th, 2014, Brasov, Romania, invited keynote presentation.