

## Rezultate etapa IV/2015

Denumirea etapa: **Proprietatile cinetice si termodinamice ale sistemelor nanoimunisorbenti-analit in prezenta unui marker enzimatic** (partea I). **Obtinerea reactivilor auxiliari pentru tehnica HnELISA si prelucrarea preliminara a probelor alimentare si de mediu** (partea I)

### Activitate IV.1.

Studii de cinetica pentru sistemul binar: faza solida-anticorp anti 2,4-D (nanoimunisorbent)-2,4-D in prezenta markerului enzimatic (partea I), **P1**

### Activitate IV.2.

Proprietatile termodinamice ale sistemelor imune: anticorp-antigen in prezenta markerului enzimatic (partea I), **P2**

**Activitate IV.3.** Obtinerea reactivilor auxiliari si a solutiilor standard (partea I), **CO**

**Activitate IV.4.** Prelucrarea preliminara a probelor alimentare si de mediu (partea I), **CO**

### Rezultate estimate ale prezentei etape:

-Studii de cinetica si termodinamica pentru evaluarea caracteristicilor reactiei imune antigen-anticorp;

-Procedura de obtinere a reactivilor auxiliari;

-Obtinerea solutiilor standard 2,4D;

-Cerere Brevet de inventie national.

Termen de predare: 31.12.2015

## Rezumatul etapei

In lucrarile efectuate in cadrul prezentei etape sunt prezentate:

1.Studii de cinetica pentru sistemul binar: faza solida-anticorp anti 2,4-D (nanoimunisorbent)-2,4-D in prezenta markerului enzimatic si metodologia experimentală de obtinere a acestor date, astfel s-au obtinut urmatoarele valori ale constantei vitezei de formare a complexului imun (reactie directa)  $K_{+1} = 2,11 \cdot 10^5 \text{ l/mol} \cdot \text{min} \pm 12,32\%$ , constanta vitezei de disociere a acestui complex,  $K_{-1} = 1,42 \cdot 10^{-2} \text{ min}^{-1}$  si constanta de echilibru,  $K$  (la  $T_1=296^\circ\text{K}=23^\circ\text{C}$ )=  $1,47 \cdot 10^7 \text{ l/mol}$  si  $K$  (la  $T_2=277^\circ\text{K}=4^\circ\text{C}$ )=  $2,88 \cdot 10^7 \text{ l/mol}$ .

2.S-au determinat proprietatile termodinamice ale sistemelor imune: anticorp-antigen in prezenta markerului enzimatic si anume:

- valoarea constantelor de echilibru la cele doua temperaturi,

$$K_1=0,147 \cdot 10^8 \text{ mol}^{-1} \text{ (} T_1=296^\circ\text{K)} \text{ si}$$

$$K_2=0,29 \cdot 10^8 \text{ mol}^{-1} \text{ (} T_2=277^\circ\text{K)},$$

-variatia energiei libere Gibbs

$$- \Delta G_1 \text{ (} T_1=296^\circ\text{K)} = -40,11 \text{ kJ/mol},$$

$$- \Delta G_2 \text{ (} T_2=277^\circ\text{K)} = -39,09 \text{ kJ/mol},$$

-variatia entalpiei de reactie  $\Delta H^S = -24,16 \text{ kJ/mol}$

-variatia entropiei standard  $\Delta S^S = 53,87 \text{ J/}^\circ\text{K}$ .

3.S-a stabilit modul de lucru al obtinerii reactivilor auxiliari si a solutiilor standard necesari in tehnica imunochimica de dozare a pesticidului acid 2,4 diclorofenoxiacetic si anume obtinerea tamponului de lucru, tamponului carbonat de sodiu 50 mM pH 9,6, substratului enzimatic pentru enzima fosfataza alcalina, solutiei de antigen marcat enzimatic acid 2,4 diclorofenoxiacetic-fosfataza alcalina si a solutiilor standard de 2,4 D.

4.S-a stabilit modul de prelucrare preliminara a probelor alimentare in vederea determinarii coeficientului de regasire a pesticidului in solvent, valoarea experimentală obtinuta in cazul probei de carne (muschi de vita) a fost  $\alpha = 63,4 \%$  iar in cazul probei de faina de grau integral a fost  $\alpha = 75,8 \%$ .

### **Modul de diseminare a rezultatelor**

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

1. Alina Asandei, Mauro Chinappi, Jong-kook Lee, Chang Ho Seo, Loredana Mereuta, Yoonkyung Park, Tudor Luchian, Placement of oppositely charged aminoacids at a polypeptide termini determines the voltage controlled braking of polymer transport through nanometer-scale pores, SCIENTIFIC REPORTS, vol. 5, pg. 1-13, 2015; DOI 10.1038/srep10419; factor de impact pentru anul 2014 5,578;
2. Alina Asandei, Mauro Chinappi, Hee-Kyoung Kang, Chang Ho Seo, Loredana Mereuta, Yoonkyung Park, Tudor Luchian, Acidity-Mediated, Electrostatic Tuning of Asymmetrically Charged Peptides Interactions with Protein Nanopores, ACS APPLIED MATERIALS & INTERFACES, vol. 7, issue 30, pp. 16706 – 16714, 2015, DOI 10.1021/acsami.5b04406; factor de impact pentru anul 2014 6,723;
3. Mihaela Bacalum, Bogdan Zorila, Mihai Radu, Investigating the anticancer activity of some cationic antimicrobial peptides in epithelial tumor cells, Romanian Reports in Physics, Acceptat spre publicare; factor de impact pentru anul 2014 1.52;

**II.** S-a depus **Cererea de brevet de inventie national** nr. OSIM A00885/25.11.2015 cu titlul " Procedeu de obtinere a markerului acid 2,4-diclorofenoxi-albumina serica de caprina-biotina utilizat in tehnica imunochimica in faza omogena pentru dozarea acidului 2,4-diclorofenoxiacetic (2,4-D) in probe biologice si de mediu", autori Dorobantu Ioan, Neagu Livia.

**III. Participare la Conferinte Nationale:**

1. Ioan Dorobantu, Livia Neagu, Mihaela Bacalum, Cristina Ionescu, Cristian Ion, Antoniu Moldovan, Obtaining of nanoimmunisorbent SiO<sub>2</sub>-2,4dichlorophenoxyacetic acid and its physico-chemical characterization, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015, Prezentare poster;
2. Mihaela Bacalum, Florina Zorila, Therapeutic potential of de novo synthesized antimicrobial peptides, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015, Prezentare orală 10 min;

3. Bogdan Zorila, Mihaela Bacalum, Aurel Popescu, Study of the interaction between antimicrobial peptides and model cell membranes by time resolved fluorescence, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015, Prezentare poster;
4. Alina Asandei, Loredana Mereuta, Tudor Luchian, Braking of peptide passage across nanopores with oppositely charged aminoacids at the peptide termini, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015, Lucrarea a obtinut Premiul III (Best Poster); prezentare poster
5. Schiopu Irina, Iftemi Sorana, Apetrei Aurelia, Luchian Tudor, Nanopore-based assay for chiral recognition by metals, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015, Prezentare orală 10 min;
6. Sorana Iftemi, Marta de Zotti, Fernando Formaggio, Claudio Toniolo, Lorenzo Stella, Tudor Luchian, Electrophysiology studies of Trichogin GA IV activity in reconstituted planar lipid membranes, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 Iunie 2015; prezentare poster;

#### **IV. Participare la conferințe internaționale**

1. Alina Asandei, Loredana Mereuta, Tudor Luchian, Single-molecule investigation of peptide conformational changes with a protein nanopore, Gordon Research Conferences frontiers of science - Membrane Protein Folding, 21-26 iunie 2015, Bentley University, Boston, USA, prezentare poster;

Obiectivele prezentei faze au fost realizate integral.

## Results of the phase IV/2015

**Phase IV: Kynetic and thermodynamic properties of the systems nanoimmunosorbents-analyte in the presence of enzymatic label (first part). Obtainment of the auxiliary reagents for HnELISA and preliminary preparation of alimentary and environmental sample (first part)**

### Activity IV.1.

**Kinetics studies of the binary system: solid phase-anti 2,4-D antibody (nanoimmunosorbent)- 2,4-D in the presence of enzymatic label (first part), P1**

### Activity IV.2.

**Thermodynamics properties of the immune systems: antibody-antigen in the presence of enzymatic label (first part), P2**

**Activity IV.3. Obtainment of the auxiliary reagents and the standards buffers (first part), CO**

**Activity IV.4. Preliminary preparation of alimentary and environmental sample (first part), CO**

### Deliverables of the Phase IV:

- Kinetic and thermodynamic studies concerning evaluation of characteristics of immune reaction antigen-antibody;
- Procedure of obtainment of auxiliary reagents;
- The obtainment of 2,4D standard solutions;
- National application patent.

Deadline: 31.12.2015

### Obtained results

In the current phase of the project are presented:

1. Kinetics studies of the binary system: solid phase-anti 2,4-D antibody (nanoimmunosorbent)- 2,4-D in the presence of enzymatic label and the experimental methodology of the obtainment of these data, thus were obtained the following values of immune complex forming rate constant (direct reaction)  $K_{+1} = 2,11 \cdot 10^5 \text{ l/mol} \cdot \text{min} \pm 12,32\%$ , dissociation of the complex rate constant,  $K_{-1} = 1,42 \cdot 10^{-2} \text{ min}^{-1}$  and equilibrium constant  $K$  (for  $T_1 = 296^\circ\text{K} = 23^\circ\text{C}$ ) =  $1,47 \cdot 10^7 \text{ l/mol}$  and  $K$  (for  $T_2 = 277^\circ\text{K} = 4^\circ\text{C}$ ) =  $2,88 \cdot 10^7 \text{ l/mol}$ .

2. Were determined the thermodynamics properties of the immune systems: antibody-antigen in the presence of enzymatic label, thus:

-the values of the equilibrium constants at the two temperatures:

$$K_1 = 0,147 \cdot 10^8 \text{ mol}^{-1} \quad (T_1 = 296^\circ\text{K}),$$

$$K_2 = 0,29 \cdot 10^8 \text{ mol}^{-1} \quad (T_2 = 277^\circ\text{K}),$$

-variation of the Gibbs energy:

$$-\Delta G_1 \quad (T_1 = 296^\circ\text{K}) = -40,11 \text{ kJ/mol},$$

$$-\Delta G_2 \quad (T_2 = 277^\circ\text{K}) = -39,09 \text{ kJ/mol},$$

-variation of the reaction enthalpy  $\Delta H^s = -24,16 \text{ kJ/mol}$

-variation of the standard entropy  $\Delta S^s = 53,87 \text{ J/}^\circ\text{K}$ .

3. Were established the procedures of obtainment of the auxiliary reagents and of the standard solutions necessary in immunochemical technique for dosing of the pesticide 2,4-dichlorophenoxyacetic acid: phosphate buffer saline Tween20, sodium carbonate buffer 50 mM pH 9,6, enzymatic substrate for alkaline phosphatase enzyme, solution of the enzymatic marked antigen 2,4-dichlorophenoxyacetic acid-alkaline phosphatase and the obtainment of the standard solutions of 2,4 D.

4. Was established the procedure of the preliminary preparation of alimentary samples in order to determine the extraction recovery coefficient of the pesticide in solvent, thus the obtained data for the meat sample (beef) was  $\alpha=63,4\%$  and for the whole wheat flour was  $\alpha=75,8\%$ .

### Mode to disseminate the results

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

1. Alina Asandei, Mauro Chinappi, Jong-kook Lee, Chang Ho Seo, Loredana Mereuta, Yoonkyung Park, Tudor Luchian, Placement of oppositely charged aminoacids at a polypeptide termini determines the voltage controlled braking of polymer transport through nanometer-scale pores, SCIENTIFIC REPORTS, vol. 5, pg. 1-13, 2015; DOI 10.1038/srep10419; Impact factor for 2014: 5,578;

2. Alina Asandei, Mauro Chinappi, Hee-Kyoung Kang, Chang Ho Seo, Loredana Mereuta, Yoonkyung Park, Tudor Luchian, Acidity-Mediated, Electrostatic Tuning of Asymmetrically Charged Peptides Interactions with Protein Nanopores, ACS APPLIED MATERIALS & INTERFACES, vol. 7, issue 30, pp. 16706 – 16714, 2015, DOI 10.1021/acsami.5b04406; Impact factor for 2014: 6,723;

3. Mihaela Bacalum, Bogdan Zorila, Mihai Radu, Investigating the anticancer activity of some cationic antimicrobial peptides in epithelial tumor cells, Romanian Reports in Physics, Accepted for publication (in press); Impact factor for 2014: 1.52;

II. It was registered one **national patent application** no. OSIM A00885/25.11.2015 entitled: "Procedure of obtainment of the marker 2,4-dichlorophenoxyacetic acid-goat serum albumine-biotin used in the immunochemical technique in homogenous phase for dosing of 2,4 dichlorophenoxyacetic acid (2,4-D) from biological and environmental samples", authors Dorobantu Ioan, Neagu Livia.

#### III. National Conferences

7. Ioan Dorobantu, Livia Neagu, Mihaela Bacalum, Cristina Ionescu, Cristian Ion, Antoniu Moldovan, Obtaining of nanoimunosorbent  $\text{SiO}_2$ -2,4dichlorophenoxyacetic acid and its physico-chemical characterization, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015, poster presentation;
8. Mihaela Bacalum, Florina Zorila, Therapeutic potential of de novo synthesized antimicrobial peptides, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015, Oral presentation 10 min;

9. Bogdan Zorila, Mihaela Bacalum, Aurel Popescu, Study of the interaction between antimicrobial peptides and model cell membranes by time resolved fluorescence, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015, poster presentation;
10. Alina Asandei, Loredana Mereuta, Tudor Luchian, Braking of peptide passage across nanopores with oppositely charged aminoacids at the peptide termini, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015, Awarded with III-rd Award (Best Poster); poster presentation
11. Schiopu Irina, Iftemi Sorana, Apetrei Aurelia, Luchian Tudor, Nanopore-based assay for chiral recognition by metals, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015, oral presentation 10 min;
12. Sorana Iftemi, Marta de Zotti, Fernando Formaggio, Claudio Toniolo, Lorenzo Stella, Tudor Luchian, Electrophysiology studies of Trichogin GA IV activity in reconstituted planar lipid membranes, 13th National Conference on Biophysics, Timișoara, Romania, 4-6 June 2015; poster presentation;

#### **IV. International conferences**

1. Alina Asandei, Loredana Mereuta, Tudor Luchian, Single-molecule investigation of peptide conformational changes with a protein nanopore, Gordon Research Conferences frontiers of science - Membrane Protein Folding, 21-26 June 2015, Bentley University, Boston, USA, poster presentation;

The objectives of this phase were fully achieved.