

Etapa a II-a: **Obtinerea markerilor enzimatici: pesticid-enzima si anticorp antipesticid-enzima si a nanoimunisorbentilor** si cuprinde cinci activitati:

Activitate II.1.: Obtinerea markerilor enzimatici anticorp anti 2,4-D-fosfataza alcalina si anticorp anti acid 2,4 diclorofenoxiacetic-peroxidaza si caracterizarea imunochimica si enzimatica a markerilor anticorp antipesticid-enzima, **CO**

Activitate II.2.: Studii de cinetica chimica a sistemului anticorp-antigen pentru sistemul anticorp anti2,4-D-2,4-D, **P1**

Activitate II.3.: Obtinerea nanoimunisorbentilor non magnetici si magnetici antipesticid-anticorp cuplat la nanoparticule (partea I), **CO**

Activitate II.4.: Caracterizarea fizico-chimica a nanoimunisorbentilor obtinuti (partea I), **P2**

Activitate II.5.: Caracterizarea structurala a nanoimunisorbentilor anticorp antipesticid-nanoparticule (partea I), **P1**

Termen: 30.12.2013

Rezultate obtinute

In lucrarile efectuate in cadrul prezentei etape sunt prezentate procedeele de obtinere, purificare, caracterizare si testare a markerilor enzimatici acid 2,4-diclorofenoxiacetic-peroxidaza si anticorp anti-acid 2,4-diclorofenoxiacetic-fosfataza alcalina folositi in tehnica ELISA. Activitatea specifica a produsului obtinut PRH –hexametildiamina-2,4-D a fost de cca 176 u/mg in comparatie cu activitatea specifica a peroxidazei utilizate de 727 u/mg iar cea a produsului anticorp anti-acid 2,4-diclorofenoxiacetic-fosfataza alcalina a fost de cca 87 u/mg in comparatie cu activitatea specifica a fosfatazei alcaline utilizate de 840 u/mg.

De asemenea, markerii enzimatici obtinuti au fost testati in sistem ELISA determinandu-se specificitatea markerului enzimatic peroxidaza-hexametilen-2,4-D fata de anticorpul anti 2,4-D cuplat la faza solida, respectiv specificitatea markerului enzimatic anticorp anti-acid 2,4-diclorofenoxiacetic-fosfataza alcalina fata de antigen conjugatul albumina serica-2,4-D.

S-au efectuat studii de cinetica chimica a sistemului anticorp-antigen pentru sistemul anticorp anti2,4-D-2,4-D obtinandu-se valoarea constantei de echilibru a sistemului chimic studiat si anume, $K=7,61 \cdot 10^6$ l/mol.

Un alt obiectiv al prezentei etape a fost stabilirea procedurilor de obtinere a nanoimunisorbentului non magnetic SiO_2 -conjugat imunogen 2,4-D-albumina de capra si obtinerea acestui produs. A fost caracterizat fizico-chimic nanoimunisorbentul non magnetic obtinut pe baza de SiO_2 , prin determinarea gruparilor amino libere a nanoparticulelor functionalizate cu α -aminopropiltriethoxisilan marcate cu isotiocianat de fluoresceina (FITC) astfel, cuplajul FITC la gruparile $-\text{NH}_2$ de pe suprafata nanoparticulelor a fost de $8,11 \mu\text{g FITC}/\text{m}^2$ echivalent cu $1,25 \cdot 10^{12}$ molecule FITC/ cm^2 . De asemenea a fost caracterizat structural nanoimunisorbentul prin determinarea cantitatii de conjugat albumina serica-2,4-D cuplata la suprafata nanoparticulelor si anume masa de proteina (albumina serica de capra-2,4-D) cuplata la suprafata nanoparticulelor de Si functionalizate este de 2,85 mg cuplat la 15 mg de nanoparticule echivalent a $8,2 \cdot 10^{11}$ molecule de conjugat albumina serica de capra-2,4-D/ cm^2 .

Modul de diseminare a rezultatelor

I. In perioada 13.06.2013 – 16.06.2013 au participat in calitate de coautori la 12-th National Conference on Biophysics “CNB 2013”-Biophysics for Health, with International participation, Iasi, Romania, June 13-16, 2013 organizata de catre Universitatea Alexandru Ioan Cuza din Iasi,

Universitatea de Medicina si Farmacie Grigore T. Popa din Iasi si Romanian Society of Pure and Applied Biophysics urmatoarele persoane:

1. Neagu Livia, lucrarea "Homogenous ELISA technique based on silicon nanoparticles for assay of 2,4-dichlorophenoxyacetic acid from environmental samples", autori Ioan Dorobantu, Livia Neagu, identificata cu numarul O18 in cadrul sesiunii "Biocompatibility and Biomaterials", prezentare orala cu durata de 15 minute;
2. Loredana Mereuta, Alina Asandei, Tudor Luchian, lucrarea "Analysis of copper ion induced peptide folding through a nanopore sensing technique", autori Irina Schiopu, Loredana Mereuta, Alina Asandei, Tudor Luchian identificata cu numarul O14 in cadrul sesiunii "Physiological Measurement Techniques", prezentare orala cu durata de 15 minute;
3. Loredana Mereuta, Tudor Luchian, lucrarea "Physicochemical characteristics of the medium which guide peptide translocation through protein pores", autori Daniela Ciurac, Loredana Mereuta, Aurelia Apetrei, Tudor Luchian, prezentare poster identificata cu numarul P4 in cadrul sesiunii "Biophysics of excitable cells and tissues", lucrarea a primit Premiul I.
4. Adrian Pirnau, lucrarea "Inclusion complex studies of procaine hydrochloride and β - cyclodextrin by ^1H NMR and Isothermal titration calorimetry (ITC)", autori Adrian Pirnau, Mihaela Mic, Mircea Bogdan, Ioan Turcu, prezentare poster identificata cu numarul P28 in cadrul sesiunii "Biocompatibility and Biomaterials";
5. Mihaela Mic, lucrarea "Calorimetric and structural investigation of the interaction of local anesthetic with β - cyclodextrin", autori Mihaela Mic, Adrian Pirnau, Ioan Turcu, Mircea Bogdan, prezentare poster identificata cu numarul P49 in cadrul sesiunii "Health Physics";
6. Silvia Neamtu, lucrarea "Is stavudine binding to human serum albumin? Fluorescence spectroscopy and ITC study", autori Silvia Neamtu, Mihaela Mic, Mircea Bogdan, Ioan Turcu, prezentare poster identificata cu numarul P19 in cadrul sesiunii "Physiological Measurement Techniques".

Rezumatele lucrarilor prezentate la conferinta au fost publicate in revista The Medical-Surgical Journal of Society of Physicians and Naturalist Iasi-Romania, Vol. 117, No.1, Supl.1, 2013, ISSN 0048-7848 indexata de Index Medicus, Medline and other International Database (IDB+).

II. In data de 26.11.2013 dr. Dorobantu Ioan a prezentat sub forma de comunicare orala cu durata de (15+5) minute in cadrul Seminarului Stiintific al Departamentului de Fizica Vietii si Mediului (DFVM) din cadrul Institutului National de Cercetare-Dezvoltare pentru Fizica si Inginerie Nucleara "Horia Hulubei" Magurele (IFIN-HH) Raportul stiintific al prezentei etape.

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

1. Buimaga-Iarinca Luiza, Calborean Adrian, Electronic structure of the LL-cysteine dimers adsorbed on Au(111): a density functional theory study, Physica Scripta, volume 86, issue 3, article number 035707, DOI: 10.1088/0031-8949/86/03/035707, 2012;
2. Alina Asandei, Irina Schiopu, Sorana Iftemi, Loredana Mereuta, Tudor Luchian, Investigation of Cu^{2+} binding to human and rat amyloid fragments A β (1-16) with a protein nanopore, Langmuir, vol. 29, issue 50, pp. 15634-15642, 2013;
3. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Microscopic investigation of the human A β 1-16 peptide - metals interactions with a protein nanopore, Sensors and Actuators B: Chemical, in evaluare;
4. L. Mereuta, M. Roy, A. Asandei, J. K. Lee, Y.Park, I. Andricioaei, T. Luchian, Slowing down single-molecule trafficking through a protein nanopore reveals intermediates for peptide translocation, Scientific Reports (publishers of Nature), acceptat spre publicare.

IV. A fost depusa Cererea de brevet de inventie national nr. OSIM A/00936/28.11.2013 cu titlul "Procedeu de obtinere a produsului acid 2,4-diclorofenoxi-amidopropilen-amido biotina", autori: Dorobantu Ioan, Neagu Livia.

Phase II: Obtainment of enzymatic markers: pesticide-enzyme and antibody antipesticide-enzyme and of nanoimmunosorbents

Activity II.1.: Obtainment of enzymatic markers anti 2,4-D antibody- alkaline phosphatase and anti 2,4-dichlorophenoxyacetic acid antibody-peroxidase and immunochemical and enzymatic characterization of the markers antipesticide antibody-enzyme, CO-IFIN-HH;

Activity II.2. Kinetics studies of the antibody-antigen system for anti 2,4-D antibody-2,4-D system, P1-UAIC

Activity II.3.: Obtainment of non magnetic and magnetic nanoimmunosorbent antipesticide-antibody linked to nanoparticles (part I), CO-IFIN-HH

Activity II.4.: Physico-chemical characterization of the obtained nanoimmunosorbents (part I), P2-INCDTIM

Activity II.5.: Structural characterization of nanoimmunosorbent antipesticide antibody-nanoparticles (part I), P1-UAIC

Deadline: 30.12.2013

Obtained results

In the current phase of the project are presented the procedures of obtaining, purifying, characterization and testing of the enzymatic markers 2,4-dichlorophenoxyacetic acid-peroxidase and antibody anti-2,4-dichlorophenoxyacetic acid-alkaline phosphatase used in ELISA technique. The specific activity of the obtained product PRH-hexamethylendiamine-2,4-D was 176 u/mg compared with the specific activity of the peroxidase used was 727 u/mg and those of the product antibody anti-2,4-dichlorophenoxyacetic acid-alkaline phosphatase was 87 u/mg compared with specific activity of the used alkaline phosphatase was 840 u/mg.

Also, the obtained enzymatic markers were tested in ELISA system determining the specificity of the enzymatic marker peroxidase-hexamethylen-2,4-D antibody anti 2,4-D linked to the solid phase, respectively the specificity of the enzymatic marker antibody anti-2,4-dichlorophenoxyacetic acid-alkaline phosphatase towards antigen, the conjugate serum albumine-2,4-D.

Kinetics studies were performed for antibody-antigen system anti2,4-D antibody-2,4D for which the equilibrium constant of the studied system was $K=7,61 \cdot 10^6$ l/mol.

Another objective of the present phase was to establish the obtaining procedures of the non magnetic nanoimmunosorbent SiO₂-immunogenic conjugate 2,4-D-goat albumine and its obtainment. The obtained nanoimmunosorbent was physico-chemical characterized by determining the free amino groups of the functionalized nanoparticles with α -aminopropyltriethoxysilane labeled with fluorescein isothiocyanate (FITC), thus coupling of FITC to amino groups from the surface of the nanoparticles was 8,11 $\mu\text{g FITC}/\text{m}^2$ equivalent with $1,25 \cdot 10^{12}$ molecules FITC/cm². Also the nanoimmunosorbent was structurally characterized by determining the quantity of serum albumine-2,4-D conjugate coupled on the surface of the nanoparticles and protein mass (goat albumine-2,4-D) coupled on the surface of the functionalized Si nanoparticles was 2,85 mg coupled to 15 mg of nanoparticles equivalent of $8,2 \cdot 10^{11}$ molecules of goat albumine-2,4-D conjugate/cm².

Mode to disseminate the results

I. Between 13.06.2013 – 16.06.2013 next persons has participated as co-authors to 12-th National Conference on Biophysics “CNB 2013”-Biophysics for Health, with International participation, Iasi, Romania, June 13-16, 2013 organized by Alexandru Ioan Cuza University of Iasi, Grigore T. Popa University of Medicine and Pharmacy Iasi and Romanian Society of Pure and Applied Biophysics:

1. Neagu Livia, "Homogenous ELISA technique based on silicon nanoparticles for assay of 2,4-dichlorophenoxyacetic acid from environmental samples", Ioan Dorobantu, Livia Neagu, oral communication, number O18, session "Biocompatibility and Biomaterials", duration 15 minutes;
2. Loredana Mereuta, Alina Asandei, Tudor Luchian, "Analysis of copper ion induced peptide folding through a nanopore sensing technique", Irina Schiopu, Loredana Mereuta, Alina Asandei, Tudor Luchian, oral presentation, number O14, session "Physiological Measurement Techniques", duration 15 minutes;
3. Loredana Mereuta, Tudor Luchian, "Physicochemical characteristics of the medium which guide peptide translocation through protein pores", Daniela Ciumac, Loredana Mereuta, Aurelia Apetrei, Tudor Luchian, poster presentation, number P4, session "Biophysics of excitable cells and tissues", the presentation was awarded with first Prize.
4. Adrian Pirnau, "Inclusion complex studies of procaine hydrochloride and β - cyclodextrin by ^1H NMR and Isothermal titration calorimetry (ITC)", Adrian Pirnau, Mihaela Mic, Mircea Bogdan, Ioan Turcu, poster presentation, number P28, session "Biocompatibility and Biomaterials";
5. Mihaela Mic, "Calorimetric and structural investigation of the interaction of local anesthetic with β - cyclodextrin", Mihaela Mic, Adrian Pirnau, Ioan Turcu, Mircea Bogdan, poster presentation, number P49 session "Health Physics";
6. Silvia Neamtu, "Is stavudine binding to human serum albumin? Fluorescence spectroscopy and ITC study", Silvia Neamtu, Mihaela Mic, Mircea Bogdan, Ioan Turcu, poster presentation, number P19 session "Physiological Measurement Techniques".

The abstracts of the presented papers at the conference were published in journal The Medical-Surgical Journal of Society of Physicians and Naturalist Iasi-Romania, Vol. 117, No.1, Supl.1, 2013, ISSN 0048-7848 indexed by Index Medicus, Medline and other International Database (IDB+).

II. On 26.11.2013 dr. Dorobantu Ioan presented the Scientific Report of the present phase as an oral communication for (15+5) minutes in Scientific Seminary of the Department of Life and Environmental Physics from Horia Hulubei National Institute of R&D for Physics and Nuclear Engineering Magurele (IFIN-HH).

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

1. Buimaga-Iarinca Luiza, Calborean Adrian, Electronic structure of the LL-cysteine dimers adsorbed on Au(111): a density functional theory study, Physica Scripta, volume 86, issue 3, article number 035707, DOI: 10.1088/0031-8949/86/03/035707, 2012;
2. Alina Asandei, Irina Schiopu, Sorana Iftemi, Loredana Mereuta, Tudor Luchian, Investigation of Cu^{2+} binding to human and rat amyloid fragments $\text{A}\beta$ (1-16) with a protein nanopore, Langmuir, vol. 29, issue 50, pp. 15634-15642, 2013;
3. Alina Asandei, Sorana Iftemi, Loredana Mereuta, Irina Schiopu, Tudor Luchian, Microscopic investigation of the human $\text{A}\beta$ 1-16 peptide - metals interactions with a protein nanopore, Sensors and Actuators B: Chemical, in evaluation;
4. L. Mereuta, M. Roy, A. Asandei, J. K. Lee, Y.Park, I. Andricioaei, T. Luchian, Slowing down single-molecule trafficking through a protein nanopore reveals intermediates for peptide translocation, Scientific Reports (publishers of Nature), accepted for publication.

IV. It was registered one national patent application no. OSIM A / 00936 / 28.11.2013, entitled „Procedure of obtainment of the product 2,4-dichlorophenoxy acid-amidopropylene-amido biotin”, authors: Dorobantu Ioan, Neagu Livia.