Rezultatele etapei

Etapa a V-a: Proprietatile cinetice si termodinamice ale sistemelor nanoimunosorbenti-analit in prezenta unui marker enzimatic (partea a II-a). Obtinerea reactivilor auxiliari pentru tehnica HnELISA si prelucrarea preliminara a probelor alimentare si de mediu (partea a II-a). Elaborarea tehnicii HnELISA pentru dozarea acidului 2,4-diclorofenoxiacetic din probe alimentare si de mediu,
Data: 31.12.2016

Activitate V.1.
Studii de cinetica pentru sistemul binar: faza solida-anticorp anti 2,4-D (nanoimunosorbent)-2,4-D in prezenta markerului enzimatic (partea a II-a), **P1**
Activitate V.2.
Proprietatile termodinamice ale sistemelor imune: anticorp-antigen in prezenta markerului enzimatic (partea a II-a), **P2**
Activitate V.3.
Obtinerea reactivilor auxiliari si a solutiilor standard (partea a II-a), **CO**
Activitate V.4.
Prelucrarea preliminara a probelor alimentare si de mediu (partea a II-a), **CO**
Activitate V.5
Elaborarea tehnicii HnELISA pentru dozarea acidului 2,4 diclorofenoxiacetic din probe alimentare si de mediu, **CO**
Activitate V.6.
Stabilirea criteriilor calitative ale tehnicii HnELISA, **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;
-Obtinerea imunosorbentilor pentru stabilirea tehnicii HnELISA;
-Validarea 2,4D prin metoda HnELISA;
-Obtinerea unui kit prototip HnELISA pentru 2,4D;
-Cerere Brevet de inventie national.

In lucrarile efectuate in cadrul prezentei etape sunt prezentate:

1. Modul de obtinere al reactivilor utilizati in tehnica ELISA in vederea efectuarii studiilor de cinetica si termodinamica pentru evaluarea caracteristicilor reactiei imune antigen-anticorp si anume imunosorbent pe baza de nanoparticule de SiO_2 la suprafata carora se cupleaza anticorpul antipesticidi anti 2,4-diclorofenoxiacetic si proteina nespecific imunologic, albumina serica de capra in patru reactii, markerul enzimatic pesticid-enzima (2,4-D-fosfataza alcalina realizat in 5 etape), antrenorul pentru nanoimunosorbentul pe baza de nanoparticule de SiO_2 functionalizate cu proteina nespecifica imunologic, albumina serica de capra si solutiile standard de 2,4-D de diferite concentratii si tehnica de lucru privind determinarea constantelor cinetice de cuplare K_{+1}, disociere K_{-1} si de echilibru, K ale complexului imun anticorp anti 2,4-D cuplat covalent la suprafata nanoimunosorbentului si antigenul 2,4-D obtinandu-se urmatoarele valori: $K_{+1} = 1,78*10^5$ l/mol*min±19% masurat la T=296 °K, constanta vitezei de disociere a acestui complex la T=296 °K, $K_{-1} = 1,15*10^{-2}$ min^{-1} si constanta de echilibru, K (la T_1=296°K=23°C)= $1,55*10^7$ l/mol si K (la T_2=277 °K=4°C)= $2,32*10^7$ 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, T_1=296 °K si T_2=277 °K, variatia energiei libere Gibbs ΔG_1 (T_1=296°K)= -29,39 kJ/mol, ΔG_2 (T_2=277°K)= -28,42 kJ/mol, variatia entalpiei de reactie ΔH^s=-14,6 kJ/mol si variatia entropiei standard ΔS^s=49,8 J/°K.
3. Modul de lucru al obtinerii reactivilor auxiliari si a solutiilor standard necesari in tehnica imunochimica de dozare ELISA a pesticidului acid 2,4 diclorofenoxiacetic si anume obtinerea antrenorului de lucru, a solutiilor standard de 2,4-D, al tamponului de lucru tampon fosfat 50 mM, pH 7,2, al tamponului carbonat de sodiu 50 mM pH 9,6 si al solventilor de extractie pentru fiecare tip de proba (faina si carne).
4. Stabilirea modului de prelucrare preliminara a probelor alimentare (faina si carne) si de mediu (apa) in vederea determinarii concentratiei pesticidului de 2,4-D prin tehnica ELISA in faza omogena si coeficientul de recuperare (regasire) a pesticidului in proba contaminata.
5. Elaborarea tehnicii HnELISA pentru dozarea acidului 2,4 diclorofenoxiacetic din probe alimentare si de mediu realizandu-se curba standard de inhibitie a anticorpilor anti 2,4-D de pe suprafata imunosorbentului si modul de obtinere a imunosorbentilor pentru stabilirea tehnicii HnELISA si stabilindu-se componenta kitului prototip HnELISA pentru 2,4-D.
6. Stabilirea criteriilor calitative ale tehnicii HnELISA: specificitate, sensibilitate, acuratete si precizie.
7. A fost depusa cererea de brevet de inventie national cu nr. OSIM A00906/25.11.2016 autori: Dorobantu Ioan, Neagu Livia, cu titlul: ”Tehnica ELISA in faza omogena pe baza de nanoimunosorbenti de SiO_2 pentru detectia de pesticide organoclorurate din produse alimentare si de mediu”.
8. Tehnica HnELISA de determinare a pesticidului 2,4-D din probele alimentare (carne si faine) a fost validata prin intercomparare cu tehnicile LC-MS (Federal Expert) si LC-MS/MS (Wessling Romania) iar in cazul probelor de mediu (apa) a fost validata prin intercomparare cu tehnicile LC-MS (Federal Expert) si HPLC-MS/MS (Wessling Romania).

Modul de diseminare a rezultatelor

I. Participare conferinte international si nationale

1. Livia Neagu, Ioan Dorobantu, Kynetic and thermodynamic properties of the system nanoimmunosorbent-analyte (2,4-D) in the presence of enzymatic label used in homogenous ELISA technique for detection of 2,4-D from environmental and alimentary samples, International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences IC-ANMBES 2016, 29 iunie-01 iulie 2016, Brasov, Romania, prezentare poster;
2. Irina Schiopu, Sorana Iftemi, Tudor Luchian, Probing the key metal binding residues in mutant amyloid peptides, International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences IC-ANMBES 2016, 29 iunie-01 iulie 2016, Brasov, Romania, prezentare poster.

II. Articole publicate in reviste cotate ISI

1. M. Temelie, C. Mustaciosu, M.L. Flonta, D. Savu, Cellular differentiation exacerbates radiation sensitivity in vitro in a human dopaminergic neuronal model, acceptat spre publicare

October 2016 in revista Romanian Reports in Physics, ISSN 1221-1451, factor de impact pentru anul 2015 1,367;
2. A. Asandei, Schiopu Irina, Chinappi Mauro, Seo Chang Ho, Park Yoonkyung, Luchian Tudor, Electroosmotic Trap Against the Electrophoretic Force Near a Protein Nanopore Reveals Peptide Dynamics During Capture and Translocation, ACS APPLIED MATERIALS & INTERFACES, Vol. 8, Issue 20, pp. 13166-13179, DOI: 10.1021/acsami.6b03697, 2016;
3. M. Bacalum, B. Zorila, M. Radu, Investigating the anticancer activity of some cationic antimicrobial peptides in epithelial tumor cells, Romanian Reports in Physics, vol. 68, issue 3, pp. 1159-1169, 2016;
4. B. Zorila, M. Bacalum, A.I. Popescu, M. Radu, Log-normal deconvolution of Laurdan fluorescence spectra-a tool to assess lipid membrane fluidity, Romanian Reports in Physics, vol. 68, issue 2, pp. 702-712, 2016.

III. Cerere de brevet national:
Dorobantu Ioan, Neagu Livia, Tehnica ELISA in faza omogena pe baza de nanoimunosorbenti de SiO_2 pentru detectia de pesticide organoclorurate din produse alimentare si de mediu, Cerere de brevet de inventie nr. OSIM A00906/25.11.2016.

Obiectivele prezentei faze au fost realizate integral.

Results of the Phase V

Phase V: Kinetic and thermodynamic properties of the systems nanoimmunosorbents-analyte in the presence of enzymatic label (Second part). Obtainment of the auxiliary reagents for HnELISA and preliminary preparation of alimentary and environmental samples (Second part). Elaboration of the HnELISA technique for dosing of 2,4-Dichlorophenoxyacetic acid from alimentary and environmental samples
Deadline: 31.12.2016

Activity V.1. Kinetics studies of the binary system: solid phase-anti 2,4-D antibody (nanoimmunosorbent)- 2,4-D in the presence of enzymatic label (second part), P1
Activity V.2. Thermodynamic properties of the immune systems: antibody-antigen in the presence of enzymatic label (second part), P2
Activity V.3. Obtainment of the auxiliary reagents and the standard buffers (second part), CO
Activity V.4. Preliminary preparation of alimentary and environmental samples (second part), CO
Activity V.5. Elaboration of the HnELISA technique for dosing of 2,4-Dichlorophenoxyacetic acid from alimentary and environmental samples, CO
Activity V.6. Establish the qualitative criteria of HnELISA technique, CO
Deliverables of the Phase V:

-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;
-The obtainment of the nanoimmunosorbents for establishing the HnELISA technique;
-The validation of 2,4-D by HnELISA technique;
-The obtainment of prototype HnELISA kit for 2,4-D
-National application patent.

In the current phase of project are presented:
1. The procedure of obtainment of the reagents used in ELISA in order to carrie out kinetic and thermodynamic studies for evaluation of antigen-antibody immune reaction characteristics, namely immunosorbent based on SiO_2 nanoparticles on the surface which bound the antipesticide antibody anti 2,4-dichlorophenoxyacetic and the immunologically non-specific protein, serum albumin goat obtained in four reactions, enzymatic marker pesticide-enzyme (2,4-D-alkaline phosphatase - obtained in 5 stages), carrier for the SiO_2 nanoparticles based nanoimmunosorbent functionalized with protein nonspecific immunological, serum albumin goat and standard solutions of different concentrations of 2,4-D and procedure of determining the kinetic constant: coupling K_{+1}, dissociation K_{-1} and equilibrium, K of the immune complex anti 2,4-D antibody covalently coupled to the surface of nanoimmunosorbent and 2,4-D antigen resulting the following values: $K_{+1}=1,78*10^5$ l/mol*min ± 19% measured at T=296°K, the rate constant for dissociation of the complex at T = 296 K, $K_{-1} = 1,15*10^{-2}$ min^{-1} and the equilibrium constant, K (at T_1=296°K=23°C)=1,55 x 10^7 l/mole and K (T_2=277°K = 4°C)= 2,32x10^7 l/mole.
2.Were determined the thermodynamic properties of the immune system: antibody-antigen in the presence of enzymatic marker namely equilibrium constants at two temperatures, T_1= 296°K and T_2=277°K, the variation of Gibbs free energy ΔG_1 (T_1=296°K)=-29,39 kJ/mol, ΔG_2 (T_2= 277°K)=-28,42 kJ/mol, variation of the reaction enthalpy ΔH^s=-14,6 kJ/mol and variation of the standard entropy variation ΔS^s=49,8 J/°K.

3.The procedure of obtaining auxiliary reagents and standard solutions required in ELISA immunochemical technique for dosage of the pesticide 2,4-dichlorophenoxiacetic namely obtaining of the carrier, standard solutions of 2,4-D, the 50 mM, pH 7,2 phosphate buffer, 50 mM pH 9,6 sodium carbonate buffer and solvent extraction for each sample type (flour and meat).
4.Was established the procedure of the preliminary preparation of alimentary samples (flour and meat) and environmental (water) in order to determine the concentration of the pesticide 2,4-D by homogeneous ELISA technique and recovery coefficient (retrieval) of the pesticide in the contaminated sample.
5. Develop the HnELISA technique for dosing 2,4 dichlorophenoxiacetic in alimentary and environmental samples being carried out the standard curve inhibition of anti 2,4-D antibodies on the surface of the immunosorbent and the procedure of obtainment of the immunosorbents in order to establish the HnELISA technique and settling the components of HnELISA prototype kit for 2,4-D.
6. Establishing qualitative criteria of HnELISA technique: specificity, sensitivity, accuracy and precision.
7. National patent application no. OSIM A00906/25.11.2016, „Homogenous ELISA technique based on SiO_2 nanoimmunosorbents for detection of organochlorinated pesticides from alimentary and environmental samples", Dorobantu Ioan, Neagu Livia.
8. HnELISA technique for determining the pesticide 2,4-D was validated by intercomparison with LC-MS technique (Federal Expert) and LC-MS/MS technique (Wessling Romania) in food samples (meat and flour) and for environmental samples (water) has been validated by intercomparison with LC-MS technique (Federal Expert) and HPLC-MS/MS technique (Wessling Romania).

Mode to disseminate the results

I.National and International conferences
1.Livia Neagu, Ioan Dorobantu, Kynetic and thermodynamic properties of the system nanoimmunosorbent-analyte (2,4-D) in the presence of enzymatic label used in homogenous ELISA technique for detection of 2,4-D from environmental and alimentary samples, International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences IC-ANMBES 2016, 29 June-01 July 2016, Brasov, Romania, poster presentation;
2. Irina Schiopu, Sorana Iftemi, Tudor Luchian, Probing the key metal binding residues in mutant amyloid peptides, International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences IC-ANMBES 2016, 29 June-01 July 2016, Brasov, Romania, poster presentation.

II. Published articles in ISI ranked scientific journals
1. M. Temelie, C. Mustaciosu, M.L. Flonta, D. Savu, Cellular differentiation exacerbates radiation sensitivity in vitro in a human dopaminergic neuronal model, accepted for publication October 2016, Romanian Reports in Physics, ISSN 1221-1451, impact factor 2015: 1,367;
2. A. Asandei, Schiopu Irina, Chinappi Mauro, Seo Chang Ho, Park Yoonkyung, Luchian Tudor, Electroosmotic Trap Against the Electrophoretic Force Near a Protein Nanopore Reveals Peptide Dynamics During Capture and Translocation, ACS APPLIED MATERIALS & INTERFACES, Vol. 8, Issue 20, pp. 13166-13179, DOI: 10.1021/acsami.6b03697, 2016;

3. M. Bacalum, B. Zorila, M. Radu, Investigating the anticancer activity of some cationic antimicrobial peptides in epithelial tumor cells, Romanian Reports in Physics, vol. 68, issue 3, pp. 1159-1169, 2016;
4. B. Zorila, M. Bacalum, A.I. Popescu, M. Radu, Log-normal deconvolution of Laurdan fluorescence spectra-a tool to assess lipid membrane fluidity, Romanian Reports in Physics, vol. 68, issue 2, pp. 702-712, 2016.

III. National patent application:

Dorobantu Ioan, Neagu Livia, National patent application no. OSIM A00906/25.11.2016, ”Homogenous ELISA technique based on SiO_2 nanoimmunosorbents for detection of organochlorinated pesticides from alimentary and environmental samples”.

The objectives of this phase were fully achieved.