

Development of ELISA technique for detection of pesticide contaminant 3,6 dichloro-2-methoxybenzoic acid from alimentary and environmental samples

Phase I. Obtainment of immunogenic conjugate 3,6 dichloro-2-methoxybenzoic-protein and physical-chemically characterization, deadline: 10.12.2007

1. It was obtained by chemical synthesis the immunogenic conjugate 3,6 dichloro-2-methoxybenzoic-bovine serum albumin (dicamba-ASB) using dicamba and bovine serum albumin in alkaline medium at 9.6 pH.
2. In the same way like previous one it was obtained by chemical synthesis the immunoconjugate 3,6 dichloro-2-methoxybenzoic-ovalbumin (dicamba-OVA).
3. It was synthesized the immunogenic conjugate 2,4 dichlorophenoxyacetic acid-bovine serum albumin (2,4D-BSA).
4. The synthesized conjugates were purified by chromatography on a Sephadex G25 column.
5. The optical spectra of the synthesized conjugates were analyzed for the determination of the number of the pesticide residues coupled to one molecule of protein.
6. The calculated number of the pesticide residues coupled to a molecule of protein for the synthesized conjugates was of 25.4 for dicamba-BSA, 17.7 for dicamba-OVA and 33.1 for 2,4D-BSA.
7. The immunogenic conjugate 2,4 dichlorophenoxyacetic acid-bovine serum albumin (2,4D-BSA) was synthesized for the validation of the methods of obtainment and control of the dicamba-protein conjugates.

Phase II. Obtainment of anti 3,6 dichloro-2-methoxybenzoic-antibodies. Immunization. Obtaining of antisera, immunological characteristics of antisera, titre, affinity against antigen, deadline: 15.06.2008

1. The antidicamba antibodies were obtained in the laboratory by immunizing of the New Zealand rabbits with dicamba-hemocyanin as immunogenic conjugate and Freund adjuvant complete or incomplete.
2. The specific antisera obtained were prepared for the total gamma-globulin separation; the specific immunoglobulins were immunological and immunochemical characterized.
3. It was obtained the immunosorbent dicamba-bovine serum albumin-carboxymethylcellulose-cellulose and analyzed from the point of view of coupling of the antigen (dicamba-BSA) and the concentration of the antigen coupled to CM-cellulose was of 57 mg/g of cellulose.
4. The antisera obtained (antidicamba antibody) were immunological characterized: the affinity to the homologous antigen (dicamba)- the ELISA technique used as a first phase the reaction between the antigen (dicamba-BSA) from the surface of the solid phase and a solution of the purified antidicamba antibody and different known antigen concentrations (dicamba). The immune complex from the surface was reacting with the enzymatic marker anti rabbit antibody marked with alkaline phosphatase. By reaction of the enzymatic substrate p-nitrophenylphosphate with the immunosorbent was determined its enzymatic activity and after a while were done the colorimetric measurements using the plate reader ELISA. The affinity constants of the antisera were calculated from the optical absorbance correlated with the concentration of the free antigen from the mixture.

Phase III. Obtainment of enzymatic markers: 3,6 dichloro-2-methoxybenzoic-peroxidase, 3,6 dichloro-2-methoxybenzoic-alkaline phosphatase, anti 3,6 dichloro-2-methoxybenzoic antibody-peroxidase, anti 3,6 dichloro-2-methoxybenzoic antibody-alkaline phosphatase. Structural and enzymatic characteristics of the enzymatic markers, deadline: 01.01.2009

1. The enzymatic markers pesticide-enzyme: acid 3,6 dichloro-2-methoxybenzoic antibody-peroxidase (enzymatic activity: 80 U/mg) and acid 3,6 dichloro-2-methoxybenzoic antibody-alkaline phosphatase (enzymatic activity: 560 U/mg) were obtained.

2. The enzymatic markers antibody antipesticide-enzyme: antibody anti 3,6 dichloro-2-methoxybenzoic antibody-peroxidase (enzymatic activity: 60 U/mg) and antibody anti 3,6 dichloro-2-methoxybenzoic antibody-alkaline phosphatase (enzymatic activity: 230 U/mg).

3. The selected enzymatic marker dicamba-alkaline phosphatase was structural and enzymatic characterized, were determined the Michaelis constant in reaction with p-nitrophenylphosphate, $K_m=9,51$ mM, the influence of the rate reaction depending of the substrate concentration and the enzymatic stability of the marker.

Scientific communication:

Studii de cinetica a sistemului anticorp antigen pentru sistemul binar anticorp anticamba-dicamba, Livia Harangus, Ioan Dorobantu, *Ziua IFIN-HH'08, Sesiunea de comunicari a tinerilor cercetatori din IFIN-HH*, Magurele-Bucuresti, 18 decembrie 2008 (language of presentation: Romanian)

Were registered two **national patent applications** entitled:

1. „Procedure of obtainment of the enzymatic marker acid 3,6-dichloro-2-methoxy benzoil-CO-NH-(CH₂)₆-N=CH-(CH₂)₃-CH=N-alkaline phosphatase”, Dorobantu Ioan, Harangus Livia, no. OSIM A/00942 / 27.11.2008.

2. „Immunochemical technique of dosing the pesticide acid 3,6-dichloro-2-methoxy benzoic from the environment samples”, Dorobantu Ioan, Harangus Livia , no. OSIM A/00943 / 27.11.2008.

Phase IV: Kinetics studies of the antibody antigen system for anti 3,6 dichloro-2-methoxybenzoic antibody-3,6 dichloro-2-methoxybenzoic acid system, deadline: 15.12.2009
Results:

1. The kinetics studies of the antibody antigen system for anti dicamba antibody-dicamba system in the presence of the enzymatic marker, were determined the constant of the chemical equilibrium of the system and the constants of association and dissociation.

2. It was obtained the enzymatic marker acid 3,6-dichloro-2-methoxy benzoil -glycil- alkaline phosphatase (Dicamba-glycil-alkaline phosphatase).

It was published the **article** entitled:

Synthesis of enzymatic marker 3,6-dichloro-2-methoxy-benzoic-alkaline phosphatase and evaluation of the affinity against homologue antipesticide antibody, I. Dorobanțu, Livia Harangus, M. Radu, Romanian Journal of Biophysics, vol. 19, no. 1, p. 63–71, Bucharest, 2009

Was registered one **national patent** entitled:

Procedure of obtainment of the enzymatic marker acid 3,6-dichloro-2-methoxy benzoil -glycil-alkaline phosphatase (Dicamba-glycil-alkaline phosphatase), Dorobantu Ioan, Harangus Livia, no. OSIM A01039/11.12.2009.

Phase V. Obtainment of immunosorbents solid phase-antibody and solid phase-antigen, Physical-chemically characterization of the immunosorbent. Kinetics studies of the binary system: solid phase-anti 3,6 dichloro-2-methoxybenzoic antibody (immunosorbent)- 3,6

dichloro-2-methoxybenzoic acid in the presence of enzymatic label, Kinetics studies of the solid phase-antigen (immunosorbent)-anti 3,6 dichloro-2-methoxybenzoic antibody in the presence of enzymatic label

Deadline: 10.12.2010

Results:

The immunosorbents solid phase-antibody and solid phase-antigen were obtained the nanoimmunosorbent silicon dioxide-aminopropyltriethoxysilan-glutaraldehyd antibody anti acid 3,6-dichloro-2-methoxybenzoic and the the nanoimmunosorbent silicon dioxide-aminopropyltriethoxysilan-glutaraldehyd-bovine serum albumine- acid 3,6-dichloro-2-methoxybenzoic used in ELISA reagents necessary technique for the determination of the pesticide acid 3,6-dichloro-2-methoxybenzoic.

The obtained nanoimmunosorbents were physical-chemically characterized and the evaluation by Bradford method of the surface density of the immune components on solid phase, in the case of the immunosorbent solid phase-antibody was $\rho = 3,16 \cdot 10^{11}$ molecules/cm² and for the immunosorbent solid phase-antigen $\rho = 3,5 \cdot 10^{11}$ molecules/cm².

The kinetics studies of the binary system: solid phase-anti 3,6 dichloro-2-methoxybenzoic antibody (immunosorbent)- 3,6 dichloro-2-methoxybenzoic acid in the presence of enzymatic label determined the average value of equilibrium constant, $\overline{K} = (5,1 \pm 1,42) \cdot 10^6$ l/mol.

The kinetics studies of the solid phase-antigen (immunosorbent)-anti 3,6 dichloro-2-methoxybenzoic antibody in the presence of enzymatic label determined equilibrium constant, $K = (1,9 \cdot 10^5 \pm 0,42)$ l/mol.

Were registrated two **national patents** entitled:

1. "Procedure of obtainment of the nanoimmunosorbent silicon dioxide-aminopropyltriethoxysilan-glutaraldehyd antibody anti acid 3,6-dichloro-2-methoxybenzoic used in ELISA technique for the dosing of the pesticide acid 3,6-dichloro-2-methoxybenzoic", Dorobantu Ioan, Neagu Livia, no. OSIM A/00975/14.10.2010;
2. "Procedure of obtainment of the nanoimmunosorbent silicon dioxide-aminopropyltriethoxysilan-glutaraldehyd-bovine serum albumine- acid 3,6-dichloro-2-methoxybenzoic used in ELISA technique for the dosing of the pesticide acid 3,6-dichloro-2-methoxybenzoic", Dorobantu Ioan, Neagu Livia, no. OSIM A / 01271 / 02.12.2010.

The objective of the project is obtaining of the specific reagents used in ELISA technique: immunogenic conjugates pesticide-protein (used for the obtaining of antipesticide antibodies) antipesticide antibodies, enzymatic markers other reagents necessary for ELISA technique used in assays of this pesticide in the alimentary and environmental samples.