Efficiency of Non-label Optical Biosensors for the Express Control of Toxic Agents in Food

Nickolaj F. Starodub and Nelja F. Shpirka

Abstract This chapter is devoted to the analysis of the efficiency of a different types of the immune biosensors for the control of toxic agents among of environmental objects, The main attention is paid to the non-labeled immune biosensors and, in particular, optical ones. Among them the immune biosensors based on the porous silicon (PS), surface plasmon resonance (SPR) and total reflection internal ellipsometry (TIRE) are detailed considered. In additional to, the immune biosensors based on calorimeter and thermistors as well as on the piezocristals are described. As model of toxic elements the synthetic chemicals as pesticides and nonylethoxylates as well as the nature biological substances, in particularly, a number of mycotoxins: T2, aflatoxins, patulin and others are used. It is necessary, to underline that the analysis was fulfilled with the model solutions and with real samples: some corn, vegetables and fruits. At the end of the chapter the perspectives of the developed instrumental analytical devices based on the principles of biosensorics are analyzed. Especially it draws attention to the development of multi-parameter portable biosensors based on the basis of the nano-structured porous silicon (nano-PS) from one side and on the artificial selective template surface, calyx(4)arenas and aptamers.

Keywords Toxins · Environmental objects · Control · Immune biosensors · Optical · Non-labeled

1 Introduction

Scientific-technical progress gives a lot of new possibilities to sharply increase the life of people but it brings, unfortunately, simultaneous a number of difficulties for human which should be overcome with the including of the specific measures for

N.F. Starodub (✉) · N.F. Shpirka
Laboratory of Biosensorics, National University of Life and Environmental Sciences of Ukraine, 5, Geroyiv Oborony, Kyiv 03041, Ukraine
e-mail: nikstarodub@yahoo.com

© Springer International Publishing Switzerland 2016

D.P. Nikolelis and G.-P. Nikoleli (eds.), *Biosensors for Security and Bioterrorism Applications*, Advanced Sciences and Technologies for Security Applications,
DOI 10.1007/978-3-319-28926-7_18

the adaptation of the living organisms to the new conditions. At first it means the application of very wide specters of chemical substances. According to classification from the position of ecology and sources of manufacturing the chemical substances (ChS) may be divided on: pesticides, products of industry (polychlorinated biphenyl's, hexachlorbensens) and concomitant substances (dioxins, furans and polycyclic hydrocarbons) [101]. The production of similar substances in USA only has achieved in 1997 $\approx 5 \times 10^9$ tons [52]. In spite of the fact that controlling units in all countries are recommend using biodegradable surfactants but their mineralization can not be complete and they accumulate gradually in natural water sources [18]. Acute effects of ChS are well known and the cases of their mass use in big concentrations as results of disasters are described in details [11, 24]. Chronic sub-lethal effects of ChS are associated with the worsening reproductive performance, influence on skin, immune, neural and endocrine systems [8, 15, 49]. ChS may be absorbed by phytoplankton, filtrated organisms and vegetables [25, 40, 98].

Pesticides are as the essential part of the modern agro technologies. Due to their use we have possibility to keep 50 % of the crop capacity. However, in consequence of high toxicity of pesticides there is needed a complex system to eliminate their negative effect and first of all it concerns the constant control of these substances in different classes of environmental objects.

Other aspect of the scientific-technical progress is in the intensification of the production of ChS and their application as detergents in different fields of the human activity and especially in the domestic conditions. As a rule, such ChS has not direct toxic effect but after biodegradation some substances are formed from it which may be delivered to so-called "endocrine disrupting factor". Nonylphenol ethoxylates (nPhEthO) are as one group of such factors [79, 80].

The next third of ChS belongs to biological derivatives which naturally are widespread among products and, moreover, may be used in purified form as instrument for attack of bioterrorist [23, 85]. The problem with weapons including bio- and chemical substances exists a long time. One of group of biological toxins is presented by mycotoxins which include more than 300 individual substances produced by different fungi strains [47]. T2 mycotoxin, aflatoxins, searelenone, patulin and others cause a great interest since they are widespread and characterized by high level toxicity. T2 mycotoxin has more toxic effect (in 400 times) than mustard gas and lewisite [47, 68]. It is well-known that mycotoxin T2 was packed into rockets, bombs, cisterns, some explosive cylinders, hand-grenade and they were applied in Laos and Afghanistan as yellow rain [51, 68]. This mycotoxin may be prepared by very simple way. Both circumstances (simplicity obtaining and high level of toxicity) form a very serious problems since these toxic elements may be as instrument for bioterrorists. There is necessity to mention that the use of toxins in generally among others groups of biological weapon by terrorists is the most probably since viruses and bacteria present big danger not for victims only but for executors of terrorist act too.

The list of ChS which are produced and widely used may be continued and continued but at last we would like to pay attention nano-particles. In recent

decades, it was created several thousands of varieties of nanomaterials, and therefore the possibility of exposure nanoparticles on animals, humans and the environment in whole is increased [63]. A number anthropogenic sources, such as metallurgical, cement industry; combustion of coal, polymeric compounds, oil, gas, diesel fuel, and other processes have significantly increased contents of nanoparticles in the environment [70]. It is generally recognized that changes in the physical properties of the material at the transition to form nanoparticles naturally accompanied by changes in its biological effects. In particular, a substantial their accumulation, in the lung, penetration to the tissue, overcoming skin barrier, ability to have the so-called “inflammatory potential” and to interact with different biological molecules, including nucleic acids as carrier of genetic information [27, 96]. The level of hydrophobic properties and the presence of electrical charge increase nanoparticles to binding with biomolecules and to their accumulation and in organisms since the immune system often is not able to recognize presence of such complexes [31].

Despite the dramatic increase in the use of nano sized materials, little information is available on their potential toxic effects on the environment. Their potential deleterious effects on ecological health should be identified to allow their safe use. Most current literature on the toxicity of nanoparticles come from mammalian studies that focus on respiratory exposure or from in vitro assays with mammalian cells [6]. In last time it was taken the attention to the estimation of nano-particles on the reproductive function of animals which is under effect of these substances [9, 33]. The ecotoxicological studies of nanoparticles are much more limited, with only a few reports focusing on the acute toxic effects of nanoparticles on the aquatic biotas [32].

Especially there is necessary to underline that for the an effective as well as safe use of nano-particles and different composites with their participation the detailed and comprehensive analysis should be done not only concerning general toxicity of these substances and in respect of their genotoxicity since the last effect may be reflected in form of cancerogenesis for living organisms and more significantly in arousing genetically mutations in next generations. That is why there is a very important to control of the level of genotoxicity of nanomaterials.

Taken into account the global dispersion of ChS and extraordinary dangers which they present for living organisms there arises necessity to resist imminent catastrophe. In really such situation is formed when a large scale of production and consumption of ChS is exist.

Now, there is a very important question: how to prevent non-desirable consequences, to preserve live and health of people. To counteract this undesirable situation it arises a urgent necessity in the organization of the permanent control since, as a rule, the ChS are toxic, stable, and have the ability for bioaccumulation and long term transferring in atmosphere [101]. These substances may influence on the living organisms far from places of their production and on remote territories. So, discovering chemicals in Arctic where they are never been produced and where

their concentration are increased up to level which is threatening for wild nature and humanities is confirming the global character of their dispersion [4, 54].

In all cases there is necessary to have instrumental analytical devices for the express, sensitive and simple monitoring of the contamination of the environmental objects by the concrete toxins.

Certainly, to prevent non-desirable effect and to do effective this monitoring we developed some family of biosensors for the fulfillment of control on the third levels. At first it is foreseen the determination of the general toxicity of the analyzed objects, than the revealing what is group toxins presented in the contaminated material and last the discovering of the individual chemical substance. Unfortunately, the existing conventional methods of analysis often do not satisfactorily solve these practical needs, since they are sophisticated, time-consuming, expensive and difficult to be applied under on-line regime and on-field conditions. There is thus a specific need to use a new generation of instrumental analytical devices, in particular, based on the principles of biosensorics. Nevertheless, among them, some developed variants do not meet the requirements of practice due to their complexity, high prices and others disadvantages or to lack of adaptability in the analysis of required indicators.

To overcome this gap between biosensor development and practical demand, we have started studying a number of optical and electrochemical biosensors capable to fulfill analysis under real time and on-field conditions as well as to check simultaneously a large number of samples and analytical parameters. This article is devoted to analysis of biosensors which were created by us. Among the different types of proposed biosensors, we have paid special attention to portable optical systems suitable for working under on-field conditions and obtaining results in on-line regime without the application of any additional labels, which are used, as a rule, in these cases. Recently, it has been demonstrated that achieving such goals is possible by applying nano-structured porous silicon as a transducer surface with the registration of the specific signal through measuring photoluminescence or photoconductivity. Such biosensors are very effective for screening observation. We pay attention too that the results of analysis (especially mycotoxins) depend on the conditions of the sample preparation.

2 Creation of Non-labelled Immune Biosensors for the Determination of Low Weight Toxic Synthetic Substances in Environment [79, 80]

In this section we would like to demonstrate efficiency of the immune biosensor based on the porous silicon (PS) at the determination of such low weight toxins as nonylphenol (NP) and 2,4-dichlorophenoxyacetic acid (2,4-D). Moreover, it will compared the obtained results with that which were demonstrated by the immune sensor based on the surface plasmon resonance (SPR).

2.1 The Overall Characteristics of PS Obtaining and Specific Signal Registration

To prepare PS samples the monocrystalline (MK) silicon plate (*p*-type with the specific resistance (ρ) = 10 Ohm/centimeter) was treated at first by computer controlled and in-focus beam from YAG: Nd (yttrium-aluminum garnet, alloyed Nd^{3+}) laser (wave-length $\lambda = 1.06$ µm, pulse duration $\tau = 2 \times 10^{-4}$ s and energy E = 0.3 J) in the regime of surface scanning. Then this plate was immersed in the mixture of HF: HNO_3: H_2O = 1:3:5 (in volume ration) for 3–10 min. At last the obtained plates of PS was cut into sections with the dimensions in 4 × 4 mm^2. The total scheme of PS preparation is given in Fig. 1. The dimensions of pores (diameter and depth) were controlled by atom force microscopy (AFM).

To observe visible PhL the PS plate was placed in quartz cell with the volume of 2 ml and was exposed to He-Cd laser beam (wave-length λ = 440 nm, power = 0.001 Watt. The intensity of PhL was measured with the use of the set included standard monochromator, photoelectric multiplayer (FEU-83) and personal computer (PC). The overall scheme of excitation and measurement of intensity of PS PhL is shown in Fig. 2. Before immobilization of biological components the surface of PS was washed by ethanol and several times by distilled water and then PS samples were dried at the room temperature in laminar-cleaned conditions. Immobilization of biological components was accomplished by passive sorption. For this purpose PS samples were dipped in the solution of one of immune component, which was taken in the appropriate concentration and they were incubated during some time (the concentration of solutions and time of immobilization were determined by experimentally way). After immobilization of biological components PS surface was washed by the phosphate buffer (20 mM, pH 7.3) contained NaCl (140 mmol/l) and then it was dried.

The observed depth of pores was in range 10–200 nm and their diameter (d) and width of rising (silicon crystallite) was from 50 to 400 nm. In concordance with the classification of PS on the micro- (d < 2 nm), meso- (2 nm < d < 50 nm) and macro-

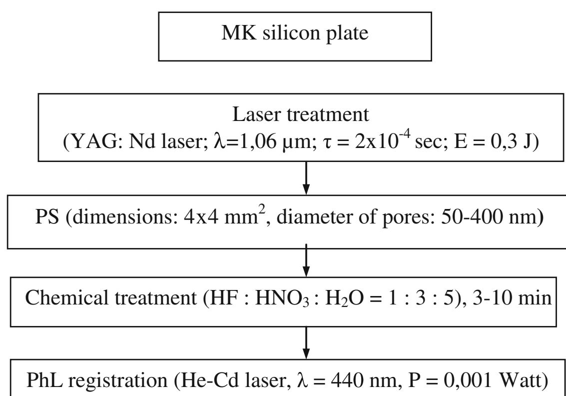

Fig. 1 The overall scheme of PS preparation

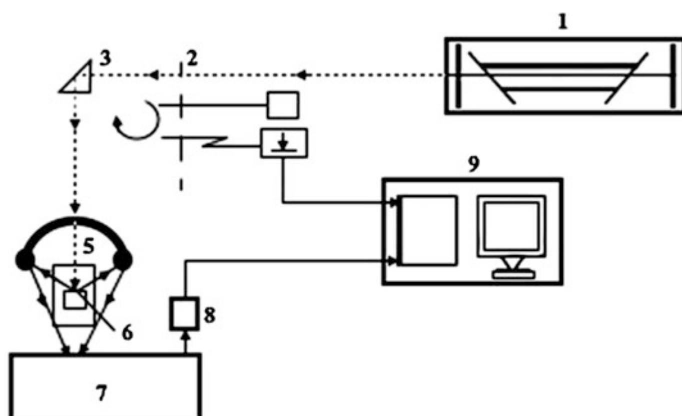

Fig. 2 Block scheme of device for PS PhL measurement. *1*—laser, *2*—modulator, *3*—prism, *4*—aluminum mirror, *5*—quartz cell, *6*—PS sample, *7*—UM-2 monochromator, *8*—FEU, 9—PC,—laser beam,—PhL quanta, —electrical connections

(d > 50 nm) porous [72] and according to our AFM analysis the obtained PS samples may be characterized as macro porous structures. PS had maximal level of fluorescence at wavelength of 650 nm with semi-band ~400 meV. The intensity of its fluorescence increased at the increasing of laser beam density from 11.5 to 33.5 J/cm^2. That is why we kept this index at the level of 33.5 J/cm^2. Exponent describes the spontaneous decreasing of PhL intensity at the temperature of 300 K and this effect is occurred during ≥200 μs. Some details of the determination of the specific immune complex formation were described early [75–78].

Three main types of analysis were compared: (a) the competitive way when the conjugate of 2,4-D or NP with bovine serum albumin (BSA) or soybean inhibitor of trypsin (STI), or ovalbumin (Ova) immobilized on the PS and free appropriate substance compete for specific antibodies (Ab) in the solution; (b) the same way as in point "a" but free 2,4-D or NP and their conjugates compete for specific Ab immobilized on the PS and (c) the direct way when appropriate specific Ab were immobilized on the PS and free 2,4-D or NP were alone in solution.

2.2 *Efficiency of the Immune Biosensor Based on the PS at the Control of Low Weight Toxic Synthetic Substances*

In case of use competitive analysis (when free and conjugated with BSA 2,4-D competed for Ab immobilized on the PS surface) we have obtained the sensitivity of 2,4-D analysis on the level of 5 μg/L and linear plot was in frame of 5–200 μg/L (Fig. 3).

If the conjugated 2,4-D with BSA was immobilized on the PS surface and free 2,4-D and specific Ab were in solution to be analysed the sensitivity analysis was

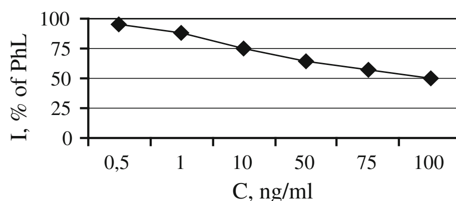

Fig. 3 Changes of intensity of PS ChL at the analysis of free 2,4-D in solution. 2,4-D-BSA was immobilized on the surface. Time of incubation 30 min

higher, approximately in 5 times (up to 1 μg/L). The optimal conditions of NP analysis were in general the same as for 2,4-D but in this case we used antiserum (not pure Ab). That is why first of all the optimal dilution of antiserum was determined. It was shown that this dilution should be about 1:7500 since in this case we can have about 50 % of maximal sensor response (Fig. 4). The sensitivity of NP analysis by competitive way and in case of the immobilisation of NP-BSA on the PS was 10 μg/L (Fig. 5). So, the developed immune sensor allows providing rapid, sensitive and selective analysis of individual pesticides.

We would like to pay attention on the possible mechanism of the work of these immune biosensors. According to our hypothesis the work of the immune sensor based on PS PhL is connected with hydrogen proton interruption from PS surface by the formed immune complex. It is accompanied by sharply decreasing intensity of ChL. The last process is directly proportional to the intensity of immune complex formation. The obtained results (Fig. 5) testify that two process, namely, interruption of hydrogen from surface and its blocking by biological molecules may effect on the PS PL.

The binding of small molecules (2,4-D and NP) with immobilised specific Ab has an influence on the PS PL at their high concentration only. At the same time conjugates of these molecules with some protein are able to change of parameters of

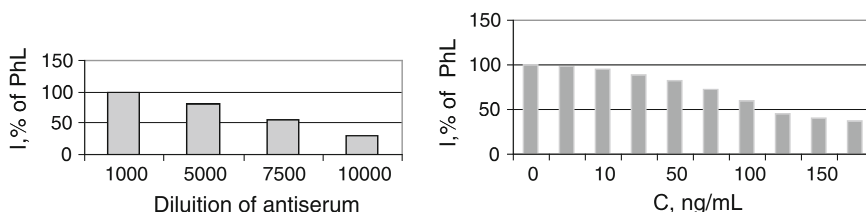

Fig. 4 *Left* The level of sensor signal at the different dilution of specific antiserum. The concentration of free NP was 100 ng/ml. The time of incubation of PS surface with mixture of antiserum and free NP was 30 min. *Right* The dependence of immune sensor signal on the concentration of NP in solution to be analyzed

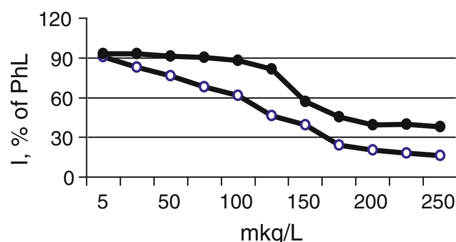

Fig. 5 The changes of intensity of PS PhL at the analysis of free 2,4-D by the competitive (o) and direct (•) analysis. Time of incubation 15 min. Concentration of 2,4-D-BSA—100 mg/L

PS PL more effective. Maybe the presence of a big mass of target molecules at the low weight substances causes the additional blocking of PS recombinant centres.

It was compare the efficiency of NP and 2,4-D analysis by the immune biosensors based on the PS and SPR. In last case the glass plate with 20 nm thickness of the evaporated gold film were as transducer. We have too analysed in detail three main variants of approaches: (a) conjugate (NP or 2,4-D with some protein—BSA, or STI, or Ova) was direct immobilised on the gold surface and free NP or 2,4-D with appropriate antiserum were in solution; (b) the specific Ab from antiserum was immobilised on the gold surface through intermediate layer from *Staphylococcal* protein A or some lectin and free NP or 2,4-D as well as NP or 2,4-D conjugated with some protein were in solution; (c) the same as in "b" but Ab immobilised and oriented toward solution reacted with free NP or 2,4-D and then with appropriate their conjugate (approach with to saturation of active binding sites on the surface). In all cases the surface was preliminary treated by polyelectrolites self assembled (PESA) as it was described early [55, 56]. The obtained results are shown in Fig. 6). It was shown that in case of the immobilisation of specific Ab from antiserum on the gold surface covered by PESA or PESA with lectins we obtained the same sensitivity of 2,4-D determination, namely: about 1 ng/ml. If we used procedure of the immobilisation of specific Ab from antiserum on the gold surface covered by PESA with protein A and fulfilled competitive analysis the sensitivity of the 2,4-D determination was increased approximately on one order. Maybe protein A is more effective intermediate agent for the directed orientation of Fab-fragments of Ab towards solution than lectins. It is connected with that Ab may have carbohydrates not at the Fc-fragment only and at the Fab-fragments also. In case of preservation of the similar immobilisation procedure but at the fulfilment of analysis with to-saturation of active binding sites on the surface some smaller concentration of 2,4-D may be registered.

Nevertheless in model experiments with the corn at the same conditions we can determine 2,4-D in concentration about 0.1 ng/ml only. The model experiments were fulfilled in two ways. (1) 2,4-D in different quantities (mass ratio) was added to corn and carefully mixed. Then corn was washed by the solution of ethanol. (2) Corn was reduced to fragments and extracted by tris-HCl buffer with pH of 7.5.

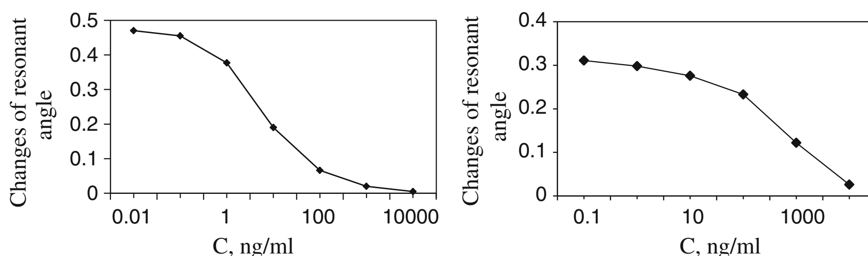

Fig. 6 SPR immune sensor response in the presence of different concentrations of 2,4-D in solution. *Left*—Conditions: Ab (antiserum) were immobilised on the surface covered by PESA and protein A. 2,4-D-BSA saturated bounds left free after interaction with 2,4-D solution. *Right*—Conditions: specific Ab (antiserum) were immobilised on the surface covered by PESA and protein A, NP-STI saturated bounds left free after interaction with NP solution

Then 2,4-D was added to extract in different concentrations. Probably in both cases some part of 2,4-D was adsorbed on the corn particles and as result of it the concentration of this substance was sharply reduced. At the immobilisation of specific Ab from antiserum on the gold surface covered by PESA with protein A and at the fulfilment of analysis with to-saturation of active binding sites on the surface the sensitivity of NP determination may be on the level of 1 ng/ml and lower.

2.3 *Efficiency of the Calorimetric Immune Biosensor at the Control of Low Weight Toxic Synthetic Substances*

This biosensor was designed in two different forms: as micro calorimeter and thermistor based devices. Efficiency of immune biosensor for the determination of low molecular substances we will demonstrate by the results obtained in the experiments with NPh [21, 22]. The portable biosensor based on the microcolorimeter and thermistors are shown in Fig. 7.

For successful development of the calorimetrical biosensor at first it was necessary to set the optimal concentration of antiserum (for example antiserum to NPh). For this purpose 150 µl antiserum in different concentrations was brought in a measuring cell and incubated during 15 min for establishment of base line (for this time a temperature in a barn was set on an optimum level). Then 50 µl solution of NPh in concentrations of 1; 5 and 10 µg/ml were brought into the cell. So, it was set that the optimum concentration of antiserum was about 5 mg of protein in 1 ml.

For the determination of NPh in solutions with the help of thermal biosensor it was necessary to build-up corresponding calibration curve. For this purpose 150 µl of antiserum (in concentration 5 µg of protein in 1 ml) was brought into a

Fig. 7 Thermal biosensor system based on the principle of the measurement of the heat transferring and the solid phase electrolyte thermistors. *1*—thermal sensitive unit; *2*—interface with the registration unit; *3*—laptop

measuring cell and then 50 μl of NPh in a range of concentration from 0.5 up to 10 μg/ml was pumped into the measuring cell. Thus, it was demonstrated an opportunity of “direct” detection of NPh by calorimetrical biosensor with the sensitivity about 1 μg/ml. The overall time of analysis is about 20–30 min.

Certainly that the sensitivity of the determination of NPh by thermal immune biosensor is much less than in case of application of SPR or TIRE biosensor but it is necessary to mention simplicity of measurement fulfilment. Maybe thermal biosensor could be used for the screening of toxic elements in environmental objects with next verification of results of analysis by optical immune biosensors.

2.4 *Conclusion*

At the optimisation of all parameters of the analysis by the proposed immune sensors it is possibility to reveal the concentrations of 2,4-D and NP on the level of practice demands, in particular, less than 0.1–1 μg/L, respectively. The total time of analysis was about 6–10 min only. The developed immune sensors allow providing rapid, sensitive and selective analysis of individual pesticides. These immune sensors may be created as stationary and portable devices. The last model of biosensors may be used for fulfilment of analysis in field conditions. The main principle of work of the immune sensor based on the SPR is common known. According to our hypothesis the work of the immune sensor based on PS PhL is connected with hydrogen proton interruption from PS surface by the formed immune complex and blocking of active silicon sites. It is accompanied by sharply decreasing intensity of ChL. The last process is directly proportional to the intensity of immune complex formation. There is necessary to underline that the immune biosensor based on the PS is more simple and more cheap than that based on SPR and it can be used for wide screening observation of environmental objects.

3 Creation of Non-labelled Immune Biosensors for the Determination of Low Weight Biological Origin Toxic Substances in Environment

In this section it will be given the main attention to the immune biosensors for control of level of mycotoxins in different environmental objects.

3.1 General Characteristics of Mycotoxins and Methods of Their Analysis

Different generations of micromycetes are able for the synthesis of a big set of mycotoxins: aflatoxins, rubratoxins, ochratoxins, fumonisines, trichothecenes and others [14]. Mycotoxins may be found in desert, in alkaline lands and in highland regions although they are more characteristic for middle latitudes [17]. These toxins are accumulated in feeds and foods at the appropriate temperature which promotes to grow of fungi's. They present a special group of low-molecular weight and non-immunogenic substances which as a rule have thermo stability. Different types of mycotoxins may cause intoxication with the destruction of organs and tissues: liver, kidney, oesophagus, bowels, brain and genitals. That is why, mycotoxins are included into list of substances which content in foods, feeds and row materials should be regulated. According to State standard of Ukraine (DSTU 3768-98) the maximal content of T2 in corn for the bread and technical grain is permitted on the level of 0.1 and 0.2 mg/kg, respectively. In Czech Republic the determination of mycotoxins is included in the system of the environment monitoring [60].

3.2 Specificity of Biosynthesis of Mycotoxins

The mechanisms of the formation of mycotoxins from the primary metabolites depend on producer type and biosynthesis conditions. Among them it is distinguished next: (1) polyketide specific for aflatoxin, ochratoxin, patulin and others; (2) terpene which is correspond to trichothecenes; (3) mediated trough cycle of tricarboxylic acids (in case of rubratoxins); (4) amino-acid when initial substances are presented by some structures (ergoalcaloids, cyclopiasonic acid and others) and (5) hybrid (composition of two or more mechanisms) which is typical for cyclopiasonic acid [83]. The main way for the biosynthesis of big group mycotoxins is polyketide mechanism. Depends on quantity of the incorporated C_2-units mycotoxins which are synthesized according to first mechanism are divided on tetraketide (patulin), pentaketide (ochratoxin), hexaketide, heptaketide, octaketide, monoketide (zearalenone) and decaketide (aflatoxins).

3.3 Chemical and Physical Abilities of Trichotecenes

They are belong to chemical substances named as sesquiterpenoids and trichothecene ring contended olefinic bond between C-9 and epoxy group in field of C-12, 13 is their speciality [16]. There are up to 150 derivates of such structure. Depends on structure of trichothecene center mycotoxins are divided on four groups: A—when substances have H or OH groups at C_8; B—in case of the presence of carboxylic group at C_8; C—micro cyclic trichothecene and D—when the second epoxy group is at C_7 [2].

Trichothecenes are low-molecular substances (250–55 Da), non volatile and bad soluble in water but they have a good solubility in acetone, ethyl acetate, chloroform, dimethyl sulfoxide, ethanol, methanol and propylene glycol [16]. Pure trichothecenes are characterized by the low level of the steam tension but they evaporate at the heating with organic solvents. High purified mycotoxins have wait color and fixed melting point [2].

Micotoxins may be preserved in solution or crystalline form at that they are stable at the light and oxygen effects. They do not inactive at the influence of 480 °C during 10 min or at 260 °C during 30 min. For chemical neutralization of T2 mycotoxin 3–5 % of solution of sodium hypo chloride is used the efficiency of which may be improve by some quantity of alkali [104].

3.4 Biotransformation of Trichotecenes

As opposite to others mycotoxines trichotecenes do not require a preliminary metabolic activation for the manifestation of their toxicity [12]. At the application on the skin or in case of use with food trichothecenes cause immediately irritation of skin or mucous of bowels. In experiments with single cells or their cultures it was shown that trichthecenes cause disintegration of polyribosome and as result of it the inhibition of protein synthesis starts (Ciegler and Bennett [14, 104]. Lipophilic character of trichotecenes gives them to sorb simple and fast on the skin and mucous of tissues. At the “per os” introduction of mycotoxin to mouse the peak of its concentration appears in the range of one hour [46, 106]. A different cells transform T2 toxin trough removal of oxygen from epoxy ring at the C-12, 13 position with the formation of binary carbonic bound and with the oxygenation of isovaleric side chains [95, 109]. Pancreas and bowels tissues are as main organs which metabolize trichotecenes. Pharmacokinetics investigations are shown that T2 mycotoxin appears in blood serum aside from way of introduction in organism. Up to 95 % of T2 mycotoxin introduced by intravenously is removed form organism through urine and excrements in ratio of 3:1 [103].

3.5 Some Biological Effects of Trichotecenes

Mycotoxins effect on a many organs and the most toxicity was revealed for A type. Nevertheless, mycotoxins of D type have a small toxicity in spite of the presence of two epoxy groups. The reduction of double bond leads to minor decreasing of the toxicity but at the lost of epoxy ring at C_9–C_{10} stipulates sharply its reduction [62].

Semi lethal dose (LD_{50}) at the T2 inhalation is 200–5800 mg*min/m^3 [106] the similar results may be achieved for lewisite and mustard gas at the dose of 1500–1800 mg*min/m^3 [105]. At the application of T2 mycotoxin on the skin LD_{50} is on the level of 2–12 mg/kg and it is higher than in case of mustard gas (100 mg/kg) [31]. The process intoxication may be increased much more if T2 mycotoxin will be used as solution based on dimethyl sulfoxide [95]. At the low concentrations of T2 mycotoxin it is observed the inhibition of growth of young animals and the decreasing of their stability to diseases as the result of immune system destroying. In particular, experiments with birds have shown that this mycotoxin may effect on the immunocompetent organs [41]. Firstly, T2 mycotoxin was revealed at the cow toxicosis and it was stated that *Fusarium tricinctum* is as main producer. *Mycothecium, Trichoderma, Trichothecium, Cephalosporium and Stachybotrys* may provide synthesis of this toxin but in much less level [3]. It was registered the intoxication of horses in Japan and symptoms of the intoxication were revealed at the concentration of 1 mg/kg [100]. The similar disease accompanying by murrain was observed among goslings and turkey poultry [42]. The incident of the people disease as result of using of foods fabricated from moldy wheat was described [5]. During number years in Bashkiria and Orenburg district it was registered accidents of people death as result of using of foods fabricated from grain (millet, wheat and barley) which was passed the winter and effected by *Fusarium sporotrichioides* [71]. It is known a number of pathological states stipulated by the chronic intoxication: toxicosis, disease of red mould, hemorrhagic disease, alimentary toxic aleukia and others [29].

3.6 Traditional Methods of the Determination of Mycotoxins

The identification of mycotoxins in different objects of environment is not simple procedure but in spite of it the special system was developed today. The standard analytical methods include a several procedures: sampling, extraction, purification, identification, determination and, at last, verification of results. At first, after beginnings of problem with mycotoxins the biotests were proposed and for this purpose a wide set of biological objects were used: from cell cultures to the vertebrate animals [30]. Biotest in which the mortality of chick-embrio was as the criteria of toxicity may be considered as traditional. Biotests with the application of different

strains of yeasts obtained a greatest development. The limit of the sensitivity to T2 varies for individual strains and it may be in frame from 20 to 50 ng [10]. Simplified variants of such biotests gives possibility to control the contamination of corn by trichothecenes with the analyze fulfillment over the time of 24 h [7]. Biotests were used for the investigation of the dependence of toxicity on the peculiarities of mycotoxin structure [45, 69]. In what follows the methods based on the technology of the thin layer chromatography (TLCh) were elaborated. These methods provide high specificity but they have much less sensitivity (about 0.2–5 μg/ml) and demand preliminary concentration of the analyzed extract in 10–1000 times.

Implementation of idea about "bioautographic" approach combined advantages of both methods based on the microbial analysis and TLCh allow control several mycotoxins in the same sample simultaneously. Potential possibilities of biotests are not yet realized and till today a new their variants with the improved characteristics are appeared.

The application of gas liquid chromatography (GLCh) allows to increasing the sensitivity of analysis. Unfortunately, the application of this method demands multistage procedure of the sample preparation and its chemical modification [67]. The sensitivity of the T2-mycotoxin determination in serum blood by this method is on the level 30 ng/ml with the reproducibility of results upon the average of 95.5 ± 8.6 % [108]. GLCh is used as one of reliable method for the verification of results of analysis [99]. The same role may be fulfilled by the high-performance liquid chromatography (FPLC) and high pressure liquid chromatography (HPLC).

After obtaining of the specific antibodies (Ab) to some types of mycotoxins there is appeared possibility to use immune chemical approaches including the radio immune (RIA), immune chemiluminescence (IChL) and fluorescence (IFL) analyses, as well as the ELISA-method [13]. So, RIA is able to reveal T2-mycotoxin in maize and wheat at 1.0 and 2.5 μg/kg, respectively [43]. In case of others types of biological liquids the sensitivity of T2-mycotoxin analysis was from 2 to 5 ng/ml [26].

The development of the immune chemical methods was accomplished by two ways: obtaining of the specific antibodies and development of special algorithms of analysis [28]. In other case at the application of the monoclonal antibodies conjugated with horse radish peroxidase and at the realization of competitive variant of analysis it was achieved the sensitivity no less as 10 ng/ml [66]. More complicate homogenous competitive analysis with the use liposome contended some substances labeled by the fluorochrome allows to obtaining sensitivity of T2-mycotoxin determination at the concentration of 2 ng/ml [44].

Today, for the characterization, identification and confirmation of the results of the analysis the methods based on the mass spectroscopy, nuclear magnetic resonance or in combination with the GLCh, HPLC gives possibility to analyze mycotoxins at the picogram level [48, 102]. Unfortunately, in not all cases there is possible to avoid such complicate procedure of the sample preparation as process of the obtaining of derivates.

3.7 *Instrumental Analytical Approaches Based on the Principles of Biosensorics at the Determination of Some Mycotoxins*

3.7.1 Optical Immune Biosensor [57, 58, 64, 83, 84, 86–90, 92]

For the determination of some mycotoxins in the model solution it was used the immune biosensor based on the total internal reflection ellipsometry (TIRE). A typical set of Δ(λ) experimental spectra are shown on Fig. 8.

It demonstrates the spectral shift caused by consecutive adsorption of layers of polyamine hydrochloride, protein A (both as intermediate layers at the immobilization of selective structures) and specific Ab as well as by binding different concentrations of T2-mycotoxin to Ab (from 0.15 up to 300 ng/ml).

Ellipsometry data fitting allows the evaluation of thickness values of the adsorbed layer. Since the refractivity increments caused by adsorption of different biomolecues represent only 0.1–0.14 % of the refractive index, the spectral changes were associated mainly with the thickness. The resulted calibration curve for T-2 mycotoxin showed a possibility of detection of T-2 mycotoxin in concentrations down to 0.15 ng/ml (or 0.15 ppb).

The calibration curves (i.e. thickness changes vs. mycotoxin concentration) obtained from the TIRE experiments for the other two mycotoxins: zearalanone and aflatoxin are shown in Fig. 9. The response to aflatoxin appeared to be about 3 times less than that for zearalanone; and both are smaller than that for T-2 (compare with Fig. 9). This could be due to the limited concentration (or activity) of antibodies. Another explanation may be related to the hydrophobicity of the above mycotoxins and thus their abilities to form aggregates in aqueous solutions [84–87].

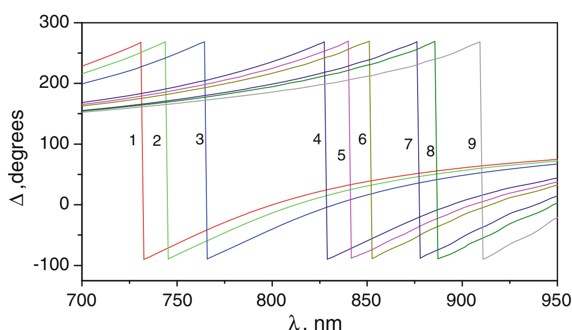

Fig. 8 Typical spectra of Δ for the bare Au surface (*1*), after consecutive adsorption of PAH (*2*), Protein A (*3*), antibodies to T-2 (*4*), and after binding T-2 mycotoxin in different concentrations of 0.15 mg/ml (*5*), 1.5 mg/ml (*6*), 7.5 mg/ml (*7*), 75 mg/ml (*8*), 300 mg/ml (*9*)

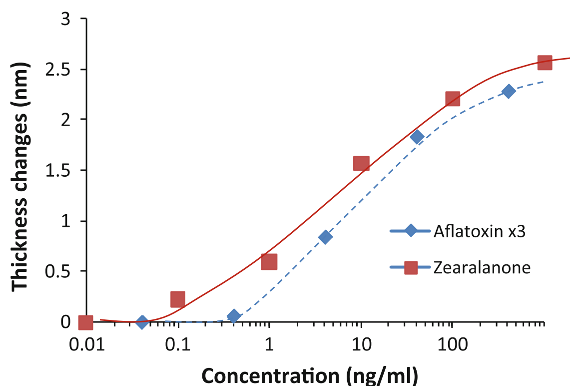

Fig. 9 TIRE calibration curves for direct immunosensors for zearalanone and aflatoxin. The thickness values for aflatoxin were three fold increased

The results showed that mould grain products contain T-2 mycotoxin in dangerously high concentrations of more than 100 ng/ml. Even stale grain products may contain up to 15 ng/ml of T-2 which is higher than the allowed amount; while fresh products do not contain any traces of T-2.

Similarly to T-2, the minimal detected concentrations for both zearalanone and aflatoxin are about 0.1–0.4 ng/ml, respectively, which is quite remarkable achievement (no other optical direct immunoassays provide such high sensitivity) [59].

The TIRE based immune biosensor was used for the control of T2 mycotoxin in real samples [84, 85]. The results of TIRE experiments (direct immunoassay) carried out on the samples of grain products are presented in Table 1. The obtained data points for fresh, stale, and mould grain product samples were added to the calibration curve for T-2 mycotoxin. The calibration was obtained earlier by TIRE direct immunoassay using commercial (from Sigma) monoclonal antibodies to T-2 mycotoxin [57, 58, 84].

The patulin and T-2 mycotoxin were determined in model solution by immune biosensor based on the SPR [57, 58, 64, 84, 86, 87]. There were realized a number of algorithms of analysis, namely: (1) “direct” way when the immobilized specific antibodies interact with the analyte in solution, (2) “competitive” way when the

Table 1 The evaluation of T-2 mycotoxin contents in the grain products

Sample	δd (nm)	Estimated concentration of T-2 mycotoxin (ng/ml)
Mouldy fodder	4.900	>600
Mouldy bread	4.391	>600
Mouldy maize	4.369	>600
Mouldy buckwheat	5.274	>600
Stale bread	0.031	<1.5
Stale muesli	0.298	<1.5
Stale buckwheat	1.199	7.5–15
Fresh muesli	0.000	None

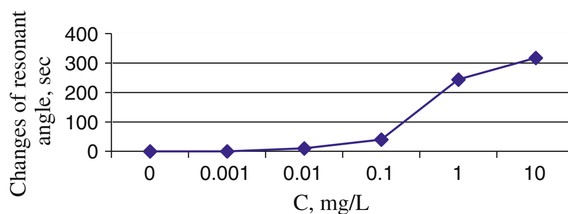

Fig. 10 Calibration curve for the patulin determination by SPR immune biosensor at "direct" way

immobilized conjugate of analyte with some protein compete with free analyte for the specific antibodies in solution. In last case it is possible to realize third variant when the specific antibodies are immobilized on the transducer surface and the appropriate conjugate and free analyte compete for the free binding centers. Next variant of analysis is "to saturate" one when the immobilized specific antibodies interact at first with the analyte and than with the appropriate conjugate. So, it was shown that the "direct" analysis by SPR immune biosensor allows to us revealing patulin at the concentration of 1 mg/L (Fig. 10). It is not practically significant level. The "competitive" way (variant 3) was much more sensitive (up to 2 orders). It can provide the determination of patulin at the concentration in the range from 0.01 to 10 mg/L (Fig. 11). The similar results were obtained at the realization of "to saturate" way at the patulin determination.

To provide optimal regime of the immune biosensor analysis we have examined a different approaches for the antibody immobilization of the transducer surface [81]. There was necessary to achieve high density of the immobilized selective structures and to expose maximally binding centers towards solution. For this purpose we used direct immobilization of the antibodies on the gold surface or through intermediate layers from dodecanthiol, or such polyelectrolyte as polyalylamine hydrochloride. I was shown that the formation of the above mentioned intermediate layer increase the analysis sensitivity and the application of polyelectrolyte and dodecanthiol demonstrates the advantages of first before second one. Mainly it is connected with the stability of results between measurements.

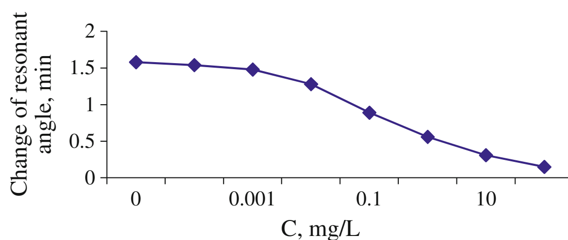

Fig. 11 Calibration curve for the patulin determination by SPR immune biosensor at "competitive" analysis

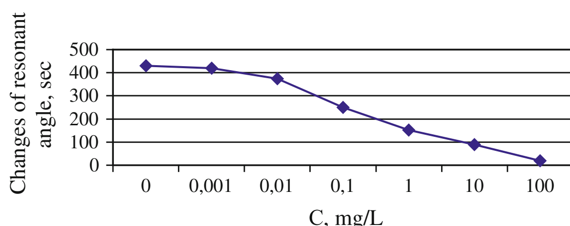

Fig. 12 Calibration curve for the T-2 mycotoxin determination by SPR immune biosensor at "to saturate" way of analysis

The same algorithms of analysis as in respect of patulin were fulfilled with T-2 mycotoxin [57, 58, 64, 84, 86, 87]. There is necessary to underline that it was revealed the similar regularities including the sensitivity. The minimal quantity which can be determined by the "direct" way of analysis is 100 ng/ml. At the "competitive" and "to saturate" ways the sensitivity are on the level of 10 ng/ml (Fig. 12. At the same time the similar results were obtained in case of the use of mono- and polyclonal antibodies.

SPR based immune biosensor was developed for the determination of aflatoxin too. It was examined in two algorithms of analysis; "competitive" way (variant 2) and "to saturate" one [19]. In both cases the polyclonal antibodies were used. The linear field of the biosensor responses were in the frame of 12–25000 ng/ml and 3–98 ng/ml, respectively for the "competitive" and "to saturate" ways. The specificity of the analysis depended on the specificity of antibodies. To fulfillment of the repeated analysis the transducer surface was treated by the solution of 1 M ethanolamine with 20 % of acetonitrile (pH 12). Authors confirmed our results that the total time of the above mentioned analysis is taken about 20–30 min. This time may be shortened at the "direct" way of analysis (up to 10 min) but its sensitivity will be 50 ng/ml only.

To determine deoxynivalenol the "competitive" analysis was developed when the mycotoxin conjugated with casein was immobilized on the SPR surface and the specific monoclonal antibodies and free analyte were in solution [53]. The optimal controlled concentrations were in the frame of 2.5–30 ng/ml. For the fulfillment of the repeated analysis (up to 500 times) the transducer surface was treated by 6 M guanidine hydrochloride.

The results of patulin determination in real samples (tomato juice) by "competitive" way are presented in Fig. 13. It can see that we have possibility to reveal patulin at the concentration starting from 0.05 mg/L. The linear diapason is in range of 0.05–10 mg/L. We think that the difference which is observed between standard solution and real sample is stipulated by some non-reversible sorption of patulin in organic phase.

At the subsistence farming of the National university of Life and Environmental Sciences of Ukraine the sample of maize and bran affected by fungi's were chosen. With the help of the ELISA-method it was stated that the concentration of T-2

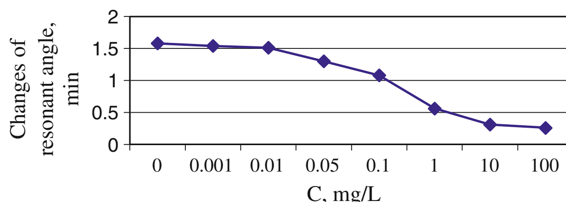

Fig. 13 Determination of patulin in tomato juice by SPR immune biosensor at “competitive” analysis

mycotoxin in these samples was in frame 220 and 180 ng/g, respectively. At the same time in case of the use of the “competitive” analysis by the immune biosensor it was revealed the T-2 mycotoxin concentration about 230 and 188 ng/g. There is necessary to mention that the maximal permitted concentration of this mycotoxin in food is 100 ng/g [39].

Now we start to develop the biosensors based on the nanostructured silicon which attracts a special attention. Such type of the immune biosensors can registrate the formation of the specific complex by the photoluminescence or light activated electro conductivity [35, 36, 73, 91]. The overall scheme of such immune biosensor with the registration of the photoconductivity is presented in Fig. 14. The similar situation is in case of the photoluminescence registration. The detailed information about obtaining of nano-structured porous silicon and some concrete devices for both types of signal registration are presented in [93].

The changes of the biosensor signals at the both methods of the specific immune complex formation are shown in Figs. 15 and 16. It can see that at the formation of the specific immune on the nano-structured porous silicon the intensity of photoluminescence decreases and photocurrent increases in dependence on the concentration of T-2 mycotoxin in solution to be analyzed. The main advantages of such approaches for practice are their simplicity, possibility to fulfill “direct” analysis

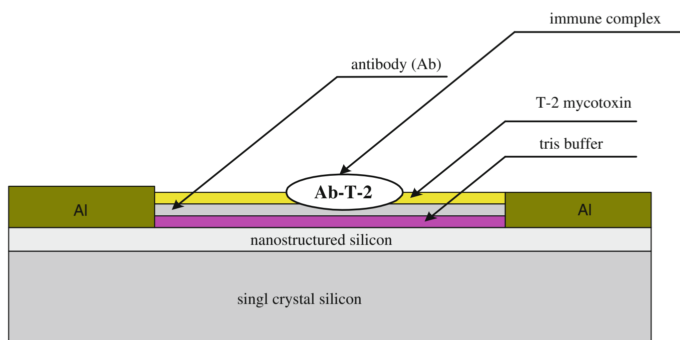

Fig. 14 Schematic scene of the photo resistor design on base of the nanostructured silicon for the measurements formation of Ab-T-2 mycotoxin complex

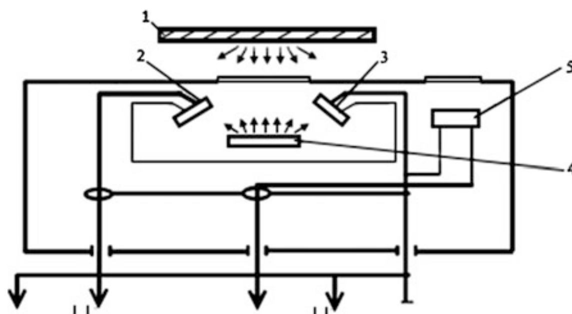

Fig. 15 PhL biosensor, where: *1*—the source of the ultraviolet (UV) radiation with the wavelength of 350 nm, *2* and *3*—two s based on the mono crystalline silicon; *4*—photo diode; *5*—photodiode intended for the determination of the incident UV

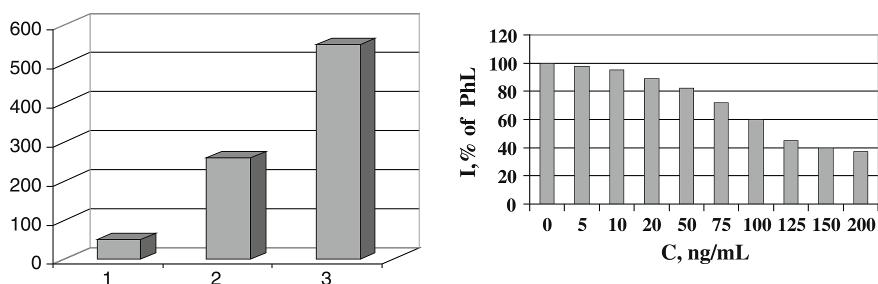

Fig. 16 *Left* Dependence of photocurrent on the surface state: *1*—bare; *2*—with antibody and *3*—with the specific antibody and T-2mycotoxin. *Right* Dependence of immune sensor signal (intensity of porouse silicon photoluminescence) on the concentration of T2-mycotoxin in the solution to be analysed

and not necessary to have a complicate electronic devices. Certainly, the sensitivity of these immune biosensors should be increased and according to our opinion it may be done through some biochemical aspects.

3.7.2 Other Types of Immune Biosensors for the Determination of Mycotoxins

Two other types immune biosensors intended for the control of T-2 mycotoxin were developed by us. One of them based on the application of the piezocrystalls [57, 58] and second one—on the thermistors [85, 88, 89]. First immune biosensor shown a very high sensitivity at the analysis mycotoxin in model solution by the “direct”

way (up to 0.15 ng/ml). Unfortunately the sensitivity of the immune biosensor based on the thermistors had a low sensitivity (about 1 μg/ml). In this case we used thermistors with the sensitivity 10^{-5} °C.

Flow injection immune analysis with the fluorescent detection is very interested in the analyzed aspect. It allows revealing fumonisin B_1 and B_2 in maize at the sensitivity about 1 mg/kg [61].

3.7.3 Peculiarities of Sample Preparation for the Immune Biosensor Analysis

This aspect was considered in detail by us in the special article [82]. At the revealing and the quantitative determination of mycotoxins there is necessary to provide objectivity of analysis, its reproducibility and adequacy to the concrete situation. In view of this European Commission has discussed in 1995 the possibility of standardization of all steps of analysis [20, 107]. The most appropriate procedure for the sample preparation in case of the mycotoxin determination is extraction with including of chloroform, methanol, acetonitrile and ethers or hexane for the defeating of the obtained products. At the use of immune analysis the application of acetonitrile dissolved in five or three times is more appropriate for the extraction of B_1 and B_2 fumonisines, deoxynivalenol and nivalenol in comparison with methanol [97]. We have demonstrated [65] a high efficiency of acetonitrile at the extraction of patulin and T-2 mycotoxin from corn and potato juice. In last time a special approaches based on the immune extraction and immune filtration were appeared [1, 38, 50]. Unfortunately it is additional procedures which are complicated and prevented to have express analysis. To avoid this disadvantage the application of some imprinting variants when polymer models specific sites is proposed [34]. There is necessary to underline that the above mention approach is very perspective for the replacement of biological selective material on the artificial chemical one. According to our data in case of the multisensory analysis the application of the artificial sites allows increasing specific signal against the background of nonspecific ones [85, 88, 89].

3.7.4 Conclusion

The described above data testify that mycotoxins are much dispersed and they are very dangerous for all living organisms. The concentration of these toxic substances should be controlled. For this purpose a number of traditional analytical approaches are exist. Unfortunately they are laborious, time consumable, expensive and do not allow fulfilling analysis in regimes on line and of line. All demands of practice may be fulfilled by the development of the new generation of these approaches, in particular, based on the principles of biosensorics. We have analyzed the efficiency of the immune biosensors based on the TIRE, SPR, piezocrystals, thermisters and others. Made a first steps in the field of the application of such devices confirm their perspective to achieve high sensitivity, selectivity, providing a chip and express analysis.

4 Perspectives of the Development of the Biosensors for the Express Control of Toxic Substances

At last time, we have realised possibility to create such prototype as lab-on a chip with the application of nano-structured porous silicon (nSPS) as transducers. This prototype is able to provide not only simultaneous analysis of several samples and number of biochemical quantities. The manufacturing of such lab-on a chip biosensor is fully compatible with silicon planar technology which is realized at the production of semiconductor devices. In this case the registration of specific sensor signal in form of a change of such parameters as photoluminescence or photocurrent of nSPS surface may be done with the help of simple and portable device.

The model of new type of lab-on a chip immune biosensor based on the nSPS is presented on Fig. 17.

From other side we try to change biological selective and sensitive structures in form of specific Ab on artificial sites on the basis of template programmed surface [74, 94], calix [4] arenas [37] and aptamers (Fig. 18).

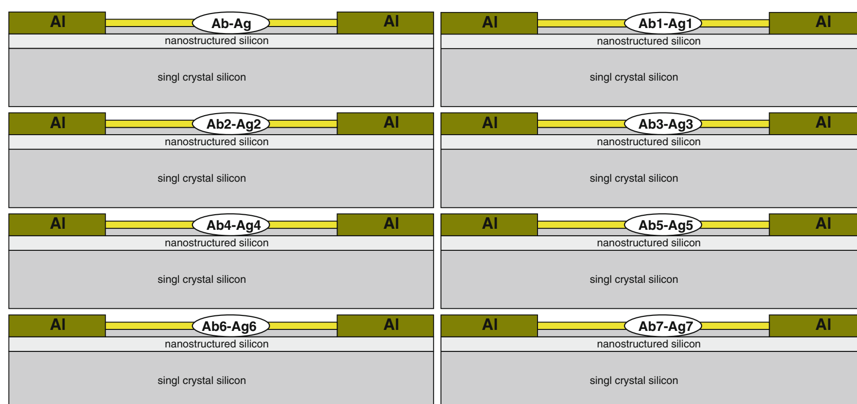

Fig. 17 General view of lab on a chip prototype based on nSPS

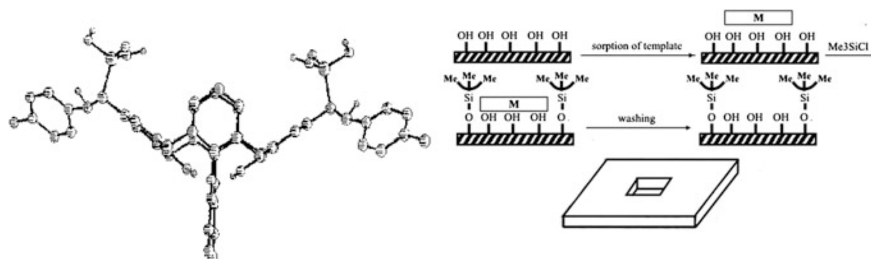

Fig. 18 Structural view of calyx [4] arene (*left*) and surface modification for obtaining "template", as sensitive element for low weight substances (*right*)

Acknowledgments This work was partially supported by EU funded BIOSENSORS-AGRICULT project (Development of nanotechnology based biosensors for agriculture FP7-PEOPLE-2012-IRSES, contract N. 318520) and NATO multi-year Science for Peace Project NUKR.SFPP 984637—"Development of Optical Bio-Sensors for Detection of Bio-Toxins" as well as National University of Life and Environmental Sciences of Ukraine, projects "Development of new generation of the instrumental analytical approaches based on the principles of nano-biotechnology and biosensorics for providing systems of biosafety" and "Creation of effective methods for the construction of artificial selective sites for the instrumental analytical devices of new generation based on the principles of biosensorics", N 110/476-Appl. and № 110/71-fund., appropriate.

References

1. Banza R, Rois A, Gomes-Hens A, Valcarcel M (2004) Supercritical fluid immunoextraction: a new approach for immunoassay automation. Anal Chem Acta 518:151–156
2. Bamburg JR (1976) Mycotoxins and other fungal related food problems. Washington, D.C., pp 144–162
3. Bamdurg JR, Riggs NV, Strong FM (1968) The structure of toxins from two strains of Fusarium tricinctum. Tetrahedrom 24:3329–333
4. Barrie LA, Gregor D, Hargrave B, Lake R et al (1992) Arctic contaminants: sources, occurrence and patways. Total Environ 122:1–74
5. Bhat VR, Ramakrishna J, Sashidhar RB (1989) Outbreak of mycotoxicosis in Kashmir Valley. Nutr News India 10(1):1–3
6. Blum JL, Xiong JQ, Hoffman C, Zelikoff JT (2012) Cadmium associated with inhaled cadmium 32 oxide nanoparticles impacts fetal and neonatal development and growth. Toxicol Sci 126(2):478–486
7. Binder J (1999) A yeast bioassay for trichothecenes. Nat Toxins 7(N6):401–406
8. Birnbaum LS (1995) Workshop on potential exposure to dioxin-like compounds:V. immunologic aspects. Environ Health Persp 103:157–159
9. Boisen AM, Shipley T, Jackson P et al (2012) NanoTiO(2) (UVTitan) does not induce ESTR mutations in the germline of prenatally exposed female mice. Part Fibre Toxicol 9:9–19
10. Boltjanskaja EV, Kuvajeva IB, Krojakova EA (1988) Screening of yeast strains which are sensitive to T2-mycotoxin. Probl Nutr N 6:67–68
11. Brouwer A, Morse DC, Lans MC, Schuur AG et al (1998) Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health 14:59–84
12. Busby WF Jr, Wogan GN (1980) Trichothecenes. In: Shank RC (ed) Mycotoxins and N-Nitroso compounds: environmental risks, vol 2. Fleering Center: CRC Pressm, Boca Raton, pp 29–41
13. Chu FS, Grossman S, Wei RD, Microcha CJ (1979) Production of antibody against T-2 toxin. Appl Environ Microbiol 37:104–108
14. Ciegler A, Bennett JW (2014) Mycotoxins and mycotoxicoses. Bioscience 30(N8):512–515
15. Colborn T (1991) J. Epidemiology of Great Lakes bald eagles. Tococol Environ Health 33:395–453
16. Cole RJ, Cox RH (1981) The trichothecenes. In: Cole RJ, Cox RH Handbook of toxic fungal metabolites. Academic Press, New York, pp 152–263
17. Committee (1983) Committee on Protection Against Mycotoxins, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, National Research Council. Protection Against Trichothecene Mycotoxins. National Academy Press, Washington, DC

18. Council Directive 82/242 EEC of March 31, 1982 on the approximation of the laws of the Member States relating to methods of testing degradability of non-ionic surfactants on amending Directive 73/404/EEC
19. Daly SJ, Keating GJ, Dillon PP et al (2000) Development of surface plasmon resonance-based immunoassay for aflatoxin B_1. J Agric Food Chem 48(N11):5097–5104
20. De Koe WJ (1995) Natural toxins. Chairman, Panel discussion., No 3, p 280
21. Demchenko AV, Mel'nik VG, Starodub NF (2007) Thermal biosensor for detecting nonylphenol in the environment. Ukr Biochem J 79(5):173–176
22. Demchenko AV, Starodub NF (2007) Modification of immune SPR biosensor surface at nonylphenol analysis. Biopolym Cell 23(2):143–147
23. Ember LR (1984) Yellow rain. Chem Eng News 62:8–34
24. Eriksson P (1997) Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology 18:719–726
25. Eriksson G, Jensen S, Kylin H, Strachan W (1989) The pine needle as a monitor of atmospheric pollution. Nature 341:42–44
26. Fan TSL, Zhang GS, Chu FS (1984) An indirect enzyme-linked immunosorbent assay for T-2 toxin in biological fluids. J Food Protect 47(N12):964–967
27. Gomes SI, Soares AM, Scott-Fordsmand JJ, Amorim MJ (2013) Mechanisms of response to silver nanoparticles on Enchytraeus albidus (Oligochaeta): survival, reproduction and gene expression profile. Hazard Mater 15:254–255
28. Gendloff EH, Pestka JJ, Swanson SP, Hart L. P. (1984) Detection of T-2 toxin in *Fusarium sporotrichioides*—infected corn by enzyme-linked immunosorbent assay. Appl Environ Microbiol 1161–1163
29. Hassanin N, Gabal MA (1990) Biological and chemical characterization of metabolites of Fusarium moniliforme isolates. Vet Hum Toxicol 32(N6):536–540
30. Hayes MA, Schiefer HB (1990) A method for accurate measurement of cutaneous irritancy of trichothecenesJ. Environ Pathol Toxicol Oncol 10(3):103–105
31. Hooper HL, Jurkschat K, Morgan AJ et al (2011) Comparative chronic toxicity of nanoparticulate and ionic zinc to the earthworm Eisenia 6 veneta in a soil matrix. Environ Int 37(6):1111–1117
32. Houk VS, Waters MD (1996) Genetic toxicology and risk assessment of complex environmental mixtures. Drug Chem Toxicol 19:187–219
33. Hsu PC, O' Callaghan M, Al-Salim N, Hurst MR (2012) Quantum dot nanoparticles affect the reproductive system of Caenorhabditis elegans. Environ Toxicol Chem 31(10): 2366–2374
34. Jodldauer J, Maier NM, Linder W (2002) To wards ochratoxin A selective molecularly imprinted polymers for solidphase extraction. J Chromatogr 945A:45–63
35. Kachinskaya TS, Melnichenko MM, Starodub MF, Shmyryeva OM (2008a) Nanobiosensors for the determination of the concentration of mycotoxins. In Proceedings of the Electronic and connection. Issue: “Problems of Electronics”, part 1. “medical devices and systems”, pp 153–159
36. Kachinskaya TS, Melnichenko MM, Starodub MF et al (2008b) Production of biosensor controls on the basis of nanostructured silicon. In: Abstract of international symposium on surface science and nanotechnology, 9–13 Nov 2008, International Conference Center, Waseda University, Tokyo, Japan, p 550
37. Kalchenko OI, Solovyov AV, Cherenok SA et al (2003) Complexation of Calix are nephosphonous acids with 2,4-dichlorophenoxyacetic acid and atrazine in water. Incl Phenom Microcyclic Chem 46:19–25
38. Kataoka H (2002) Automated sample preparation using in#tube solidphase microextraction and its application. Rev Anal Bioanal Chem 373:31–45
39. Kononenko GP, Burkin AA, Soboleva NA, Zotova EV (1999) Enzyme immunoassy for determination of T-2 toxin in contaminated grain. Appl Biochem Microbiol 35:457–462
40. Kipopoulou AM, Manoli E, Samara C (1999) Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environ Pollut 106:369–380

41. Kotik AN (1999) Micotoxicosis of avian. Publ. “Donets”, Borki, 267 pp
42. Kotik AN, Truchanova VA (1997) Events of micotoxicosis among farming birds in Ukraine in 1974–96 years. In: Breeding, interdepartmental proceedings (Borki, Kharkiv destrict), B. 47, pp 92–100
43. Lee S, Chu FS (1981) Radioimmunoassay of T-2 toxin in corn and wheat. J Assoc Anal Chem 64(1):156–161
44. Ligler FS, Bredehorst R, Talebian A et al (1987) A homogeneous immunoassay for the mycotoxin T-2 utilizing liposomes, monoclonal antibodies, and complement. Anal Biochem 163(2):369–375
45. Madhyastha MS, Marquardt RR, Abramson D (1994) Structure-activity relationships and interactions among trichothecene mycotoxins as assessed by yeast bioassay. Toxicon 32 (9):1147–1152
46. Matsumoto H, Ito T, Ueno Y (1978) Toxicological approaches to the metabolites of fusaria, XII: Fate and distribution of T-2 toxin in mice. Jpn J Exp Med 5(N48):393–399
47. Mirocha CJ, Pawlosky RA, Chatterjee K, Watson S, Hayes W (1983) Analysis for Fusarium toxins in various samples implicated in biological warfare in Southeast Asia. J Assoc Anal Chem 66(6):1485–1499
48. Mirocha CJ, Panthre SV, Pawlosky RJ, Hewetson DW (1986) Mass spectra of selected trichothecenes. In: Cole RJ (ed) Modern methods in the analysis and structure elucidation of mycotoxins. Academic Press, New York, pp 353–392
49. Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL Jr (1996) Change in sex ration with exposure to dioxin. Lancet 348:409
50. Morais S, Maquieeira A, Puchades R (1999) Immunofiltration: A new methodology for preconcentration and determination of organic pollutants. Anal Chem 71:1905–1909
51. Morris BA, Clifford MN (eds) (1985) Immunoassays for Food Analysis. Elsevier Applied Science, London
52. Morse PM (1999) Soaps and detergents. C&EN, February 1:35–48
53. Mullett W, Lai EPC, Yeung JM (1998) Immunoassay of fumonisins by a surface plasmon resonance biosensor. Anal Biochem 258:161–167
54. Mulvad G, Pederson HS, Hansen JC, Dewailly E et al (1996) Exposure of Greenlandic Inuit to organochlorines and heavy metals through the marine food-chain: an international study. Tot Environ 186:137–139
55. Nabok AV, Starodub NF, Ray AK, Hassan AK (2000a) Registration of immunoglobuline AB/AG reaction with planar polarisation interferometer. Biochemical and biomolecular sensing. In: Liberman RA (ed) Proceedings SPIE 4200, pp 1–9
56. Nabok AV, Starodub NF, Ray AK, Hassan AK (2000b) Enzyme/indicator optrodes for detection of heavy metal ions and pesticides. Biochemical and biomolecular sensing. In: Liberman RA (ed) Proceedings SPIE 4200, pp 32–41
57. Nabok AV, Tsargorodskaya A, Holloway A et al (2007a) Registration of T2 mycotoxin with total internal reflection ellipsometry and QCM impedance methods. Biosens Bioelectron 22:885–890
58. Nabok A, Tsargorodskaya A, Holloway A et al (2007b) Specific binding of large aggregates of amphiphilic molecules to the respective antibodies. Langmuir 23:8485–8490
59. Nabok A., Tsargorodskaya A., Mustafa M.K., et al (2009) Detection of low molecular weight toxins using optical phase detection techniques. Proc. of the Eurosensors XXIII conference. Procedia Chem www.elsevier.com/locate/procedia, pp 1–4
60. Ostrŷ V (1999) Micromycetes, mycotoxins and human health. Cas Lek Cesk 138(17): 515–521
61. Paepens C, De Saeger S, Sibanda L et al (2004) A flow-through enzyme immunoassay for the screening of fumonisins in maize. Anal Chem Acta 523:229–235
62. Peng S, Dong J, Yang J (1996) Studies on relationship between toxicity of trichothecene toxin T-2 and its structure. Chung Hua Yu Fang I Hsueh Tsa Chih 30(N 3):141–143
63. Powers KW, Brown SC (2006) Characterization of nanoscale particles for toxicological evaluation. Toxicol Sci 90(2):296–303

64. Pylypenko LN, Egorova AV, Pylypenko IV et al (2007) Investigation of toxic effect of patulin with the help of biosensorics systems. Food Sci Technol N1:35–38
65. Pylypenko IV, Pylypenko LN, Starodub NF (2010) Biosensors at the determination of mycoyoxins. In: Proccedingsof 4th International schentific-technical conference "Sensor electronics and microsystem technologies" (SMEST-4), Ukraine, Odessa, 28 June–02 July, p 227
66. Ramakrishna N, Lacey J, Candlish AA et al (1990) Monoclonal antibody-based enzyme linked immunosorbent assay of aflatoxin B1, T-2 toxin, and ochratoxin A in barley. J. Assoc Anal Chem 73(1):71–76
67. Romer TR (1984) Chromatographic techniques for mycotoxins. In: Lawrence JF (ed) Food constituents and food residues: their chromatographic determination. Marcel Dekker, New York, pp 393–415
68. Rosen RT, Rosen JD (1982) Presence of four Fusarium mycotoxins and synthetic material in yellow rain. Evidence for the use of chemical weapons in Laos. Biomed Mass Spectrom 9 (10):443–450
69. Rotter BA, Thompson BK, Prelusky DB, Trenholm HL (1991) Evaluation of potential interactions involving trichothecene mycotoxins using the chick embryotoxicity bioassay. Arch Environ Contam Toxicol 21(4):621–624
70. Sahoo SK, Parveen S, Panda JJ (2007) The present and future of nanotechnology in human health care. Nanomedicine: Nanotechnol Hum Healthc 3:20–31
71. Sarkisov A (1954) Ch. Mycotoxicoses. M., Publ. SIAL, 216 pp
72. Schoening MJ, Ronkel F, Crott M et al (1997) Miniaturization of potentiometric sensors using porous silicon microtechnology. Electrochim Acta 42:3185–3193
73. Solodkij MV, Melnichenko MM, Starodub MF, Shmyryeva OM (2008) Biosensor for the determination of the concentration of toxic substances with the help of nanostructured silicon. In: Proceedings of the of VI International scientific-technical conference, 21–23 Oct 2008, pp 160–163
74. Starodub NF, El'skaya AV, Piletsky SA, Lavryk NV (1993) Template sensors for low weight organic molecules based on SiO2 surfaces. Sens Actuat 708–710
75. Starodub NF, Fedorenko LL, Starodub VM, Dikij SP, Svechnicov SV (1996) Sens Actuat 35:44–47
76. Starodub VM, Fedorenko LL, Starodub NF (1998) Control of a myoglobin level in solution by the bioaffinic sensor based on the photoluminescence of porous silicon. Eurosensors XII. In: Proceedings of the European conference on solid-state transducers and 9-th UK Conference on sensors and their applications, Southampton, UK, 13–16 September, vol 2, pp 817–820
77. Starodub VM, Starodub NF (1999) Optical immune sensors for the monitoring protein substances in the air. Eurosensor XII. In: The 13th European conference on solid-state transducers, 12–15 Sept 1999, The Hague, The Netherlands, pp 181–184
78. Starodub VM, Fedorenko LL, Sisetskiy AP, Starodub NF (1999) Control of mioglobin level in an immune sensor based on the photoluminescence of porous silicon. Sens Actuat B 58:409–414
79. Starodub NF, Starodub VM, Demchenko AV et al (2004a) Creation of optical immune sensors for the determination of low weight toxic substances in environment. In: Proceedings of the International trade fair for optical and microtechnology products with conference, 25–27 May 2004. Nuremberg Exhibition Center, Germany, pp 335–340
80. Starodub NF, Demchenko A, Starodub VM et al (2004b) Optical immune sensors for the determination of low weight toxic substances in environment. Sens Electron Microsyst Technol Odessa 2004:150–151
81. Starodub NF, Pirogova LV, Starodub VM, Demchenko A (2005) Antibody immobilization on the metal and silicon surface. The use of self-assembled layer and specific receptors. Bioelectrochemistry 66:111–115
82. Starodub NF, Pilipenko LN, Egorova AV et al (2007) Analysis of mycotoxins: selection and preparation of samples. Biotechnology 1(1):106–115

83. Starodub NF, Nabok AV (2008) Immune biosensors for the express determination of some endocrine disrupting factors in environment. In: Book of Abstracts: Europtrode IX, Ninth European conference on optical chemical sensors and biosensors, Dublin, March 30–April 2, p 241
84. Starodub NF, Nabok AV, Tsargorodskaya A et al (2006) Control of T2 mycotoxin in solutions and foods by biosensors based on SPR and TIRE. In Proceedings of the Sensor + Test 2006, Nurenberg, pp 87–92
85. Starodub NF (2008) Biosensor express measurement of chemicals regularly used in agriculture and as terrorist means in the environment to prevent dangerous consequences. In: Jiaguo Q, Evered KT (eds) NATO science for peace and security series: environmental security Proceedings of the Environmental Problems of Central Asia and their Economic, Social and Security Impacts, pp 109–133
86. Starodub NF, Kanjuk MI, Ivashkevich SP et al (2008a) Patulin toxicity and determination of this toxin in environmental objects by optical biosensor systems. In: Proceedings of 3th International scientific-technical conference “Sensor electronics and microsystem technologies” (SMEST-3), Ukraine, Odessa, 2–6 June, pp 237–238
87. Starodub NF, Pilipeko LN, Pilipenko IV, Egorova AV (2008b) Mycotoxins and other low weight toxins as instrument of bioterrorists: express instrumental control and some ways to decontaminate polluted environmental objects. Timisoara Med J 58(1–2):9–18
88. Starodub NF (2009a) Biosensors in a system of instrumental tools to prevent effects of bioterrorism and automotive control of water process purification. In: Jones JAA, Vardanian TG, Hakopian Ch (eds) Proceedings: NATO Science for peace and security, Ser. C: Environmental security. Threats to global water security. Springer Sci. + Business Media B.V., pp 59–71
89. Starodub NF (2009b) Biosensors in the system of express control of chemicals regularly used as terrorist means to prevent non desirable consequences. In: The role of ecological chemical in pollution research and sustainable development. Springer Sci + Business Media BV, pp. 275–384
90. Starodub NF, Kanjuk MI, Ivashkevich SP et al (2009a) Determination of patulin toxicity and its control in environment by optical biosensor system. In: Proceedings of OPTO 2009&IRS^2, Nurenberg, Germany, 26–28 May 2009, pp 151–156
91. Starodub NF, Mel’nichenko NN, Shmireva AN (2009b) Biosensors based on the nanostructured silicon: development and practical application. In: Abstract book of Advanced materials and technologies for micro/nano-devices, sensors and actuators”, NATO ARW, NanoDSA’ 2009, St. Petersburg, Russia, June 29–July 02, p 20
92. Starodub NF, Pylypenko IV, Pylypenko LN et al (2010) Biosensors for the determination of Mycoyoxins: development, efficiency at the analysis of model samples and in case of practical applications. Lect Notes ICB 86:81–101
93. Starodub NF, Sitnik JA, Melnichenko MM, Shmyryeva OM (2011) Optical immune biosensors based on the nanostructured silicon and intended the diagnostics of retroviral bovine leucosis. The SENSOR + TEST 2011. AMA Service GmBH, Nurenberg, pp 127–132
94. Starodub NF (2013) New ways to develop biosensors towards addressing practical problems. Biophotonics—Riga 2013, edited by Janis Spigulis, Ilona Kuzmina. Proc SPIE 9032, 90320Y © 2013 SPIE CCC code: 1605–7422/13/$18. doi:10.1117/12.2044651
95. Swanson SP, Helaszek C, Buck WB, Rood HDJ, Haschek WM (1988) The role of intestinal microflora in the metabolism of trichothecene mycotoxins. Food Chem Toxicol 26(10): 823–830
96. Takeda K, Shinkai Y, Suzuki K et al (2011) Health effects of nanomaterials on next generation. Yakugaku Zasshi 131(2):229–236
97. Tanaka T, Yoneda A, Inoue S et al (2000) Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatog raphymass spectrometry. J Chromatogr 882(1–2):23–28

98. Thomann RV, Connolly JP, Parkerton TF (1992) An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ Toxicol Chem 11:615–629
99. Tuteljan VA, Eller KS, Sobolev VS (1984) Methodical recommendation for revealing, identification and determination of T2 content in foods and bread grain. pp 1–9
100. Ueno Y (1985) The toxicity of mycotoxins. CRC Crit Rev Toxicol 14(2):99–132
101. Vallack HW, Bakker DJ, Brandt I, Brostrom-Lunden E et al (1998) Controlling persistent organic pollutants-what next? Env Toxicol Pharmacol 6:143–175
102. Vesonder RF, Rohwedder WK (1986) Gas chromatographic-mass spectrometric analysis of mycotoxins. In: Cole RJ (ed) Modern methods in the analysis and structure elucidation of mycotoxins. Academic Press, New York, pp 335–352
103. Wannemacher RW Jr, Pace JG (1987) Medical defence against biological warfare: Exploratory immunotherapy studies on toxins of potential BW threat. In: US Army Medical Research Institute of Infectious Diseases Annual Report 1987. Fort Detrick, Frederick, Md: USAMRIID, pp 129–135
104. Wannemacher RW Jr, Bunner DL, Dinterman RE (1989) Inactivation of low molecular weight agents of biological origin. In: Proceedings for the symposium on agents of biological origins, 1989. US Army Chemical Research Development and Engineering Center, Aberdeen Proving Ground
105. Wannemacher RW Jr, Bunner DL, Neufeld HA (1991) Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS (eds) Mycotoxins and animal foods. CRC Press, Boca Raton, pp 499–552
106. Westlake K, Mackie RI, Dutton MF (1987) T-2 toxin metabolism by ruminal bacteria and its effect on their growth. Appl Environ Microbiol 53(3):587–592
107. Wilkes JG, Sutherland JB (1998) Sample preparation and high resolution separation of mycotoxins possessing carboxyl groups. J Chromatogr 717B:135–156
108. Yagen B, Bialer M, Sintov A (1985) Gas chromatographic assay with pharmacokinetic pplications for monitoring T-2 and HT-2 toxins in plasma. J Chromatogr 343(1):67
109. Yoshizawa T, Sakamoto T, Okamkoto K (1984) In vitro formation of 3’ -hydroxy T-2 and 3′ -hydroxy HT-2 toxins from T-2 toxin by liver homogenates from mice and monkeys. Appl Environ Microbiol 47(1):130–134