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Sensors

Optical sensor for the detection of mycotoxins

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Abstract. The methods of detection of mycotoxins in agricultural products have been analyzed. The advantages of using the fluorescence methods for the express diagnostics of the presence of mycotoxins in samples have been shown. The development of an optical biosensor, which allows the detection of mycotoxins in the field, has been presented. The principles of operation have been established, and the constructive solution of a sensor has been proposed. An electro-optical scheme for obtaining an information signal has been developed and tested. The particular attention has been paid to the choice of an element base of the proposed microsensor. The principles and procedure for its validation have been shown. The practical results of testing the developed technical solution have been presented. The achieved relative error in the linear approximation of the sensor conversion characteristics in the interval of concentrations 0…100 ppb at a temperature of 15…25 °C does not exceed 2%. The developed sensor can be used in agriculture for the express detection and evaluation of the mycotoxin contamination.

Keywords: biosensor, optical mycosensor, mycotoxins, fluorometry.

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1. Introduction

Mycotoxins are toxic secondary metabolic products of molds present on almost all agricultural commodities worldwide. Unlike primary metabolites (sugars, amino acids, and other substances), secondary metabolites are not essential in the normal metabolic function of the fungi. Other known secondary metabolites are phytotoxins and antibiotics.

Currently, there are about 400 mycotoxins reported. These compounds occur under natural conditions in feed, as well as in food. Some of the most common mycotoxins are as follows: aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin and ergot alkaloids. Mycotoxins are produced by different strains of fungi, and each strain can produce more than one mycotoxin [1].

Unavoidable, naturally occurring toxicants pose a unique challenge to the food safety. According to FAO, at least 25% of the world food crops are contaminated with mycotoxins at the time, when the production of agricultural commodities is barely sustaining the increasing population [2]. As a result, about 1 billion tons of food is lost over the world. Prolonged consumption of mycotoxin-containing foods by animals and humans leads to various diseases, including cancer.

Among all types of mycotoxins, the most common and regulated in different countries are:

- aflatoxin (B1, B2, G1, G2) (AFs);
- deoxynivalenol;
- fumonisin (B1, B2);
- ht 2;
- t 2;
- nivalenol;
- ochratoxin A (OTA);
- zearalenone (ZON).

To quickly collect the data on the levels of mycotoxin contamination in the fields, express methods of mycotoxin detection should be necessarily used. For the express methods for the detection of mycotoxins, an important part is the sample preparation stage. There are such sample preparation methods as the solvent extraction and solid-phase extraction.

Solvent extraction involves the extraction of the mycotoxin with a solvent. Acetonitrile or methanol mixed with water in the presence of salts can be used as solvents. The main disadvantage of the solvent extraction method is a poor selectivity of most solvents, and the final extract obtained is often colored and viscous [3].

Solid-phase extraction involves the use of a sorbent that contains an antibody. Thus, the mycotoxin acts as an

antigen, settling on the sorbent. Solid-phase extraction is considered to be significant for the sample preparation in the analysis of mycotoxins [3].

In this article, the problem of the sample preparation within the framework of express analysis will not be considered. During the last few decades, consumers have become more aware of health and food quality, consequently, the research on food safety augmented. The variety of contaminants in many food products requires the development of high-throughput, real-time, and portable detection methods. The evaluation of the various mycotoxins residues in foodstuffs became an essential factor in guaranteeing the products quality. Hence, it is essential to improve the analytical standards to detect and quantify the presence of a mycotoxin. The operation procedure should be simplified continuously for the convenience of users [4]. The article is devoted to developing a device capable of determining the number of mycotoxins in a ready-made sample using one of the express analysis methods.

Therefore, the main tasks are:

- to review the existing methods for detecting mycotoxins;
- to identify the existing solutions;
- to develop a device using one of the detection methods;
- to carry out the testing and validation of the made device.

2. Existing solutions

Mycotoxins detection methods can be divided into standard (reference methods) and express ones. In [3] the existing methods for the determination of mycotoxins are discussed in detail.

Reference methods for the quantitative and qualitative determination of mycotoxins are basically chromatographic systems with different detection systems such as HPLC-UV/DAD or LC-MS. They produce the high resolution, as well as sensitive and reproducible results. They are the accepted methods for any testing related to a dispute resolution. The disadvantages of these methods are that they are time-consuming, high in costs, sophisticated in equipment, and need the expert scientific and technical knowledge. In addition, they also require very extensive pre-analytic clean-up steps before the analysis. Instrumental methods are usually employed to confirm positive sampling results from screening methods [5].

Express methods provide the ability to quickly obtain results, ranging from 25 min to several hours. Conventional systems which are commercially available for the rapid detection of mycotoxins are:

1) enzyme-linked immunosorbent assay (ELISA);
2) lateral flow detection (LFD);
3) fluorescence polarization immunoassay (FPI);
4) in some cases, basic fluorometric measurements are used to detect and quantify mycotoxins in food or feed [6].

The use of ELISA requires laboratory conditions, so it cannot be used in the field. Lateral flow methods mainly give qualitative indicators, which is not sufficient for the forecasting. Immunoassay of polarization fluorescence is a fairly new method of detecting mycotoxins, but it also requires reagents to stain molecules or to enhance the fluorescence.

The rapid method based on the fluorescence of mycotoxins can provide a quantitative indicator of the contamination by mycotoxins, does not require laboratory conditions and a specially trained personnel. Therefore, it was chosen as a method of detection of mycotoxins.

One of the promising methods for the detection of mycotoxins involves electrochemical biosensors. Due to the widely occurring co-contamination of raw food materials by mycotoxins, Lu and Gunasekaran designed and fabricated of an electrochemical immunosensor for the simultaneous detection of two mycotoxins, fumonisin B1 (FB1) and deoxynivalenol (DON), in a single test. A dual-channel three-electrode electrochemical sensor pattern was etched on a transparent indium tin oxide (ITO)-coated glass *via* photolithography and was integrated with a capillary-driven polydimethylsiloxane (PDMS) microfluidic channel [4].

In addition, sensors are being developed based on the use of nanostructures and nanomaterials. One example of the implementation of such a sensor is considered in work [7]. However, nanoparticles- and nanostructure-based analytical devices have high sensitivity and low detection limits and can be potentially used as a portable instrumentation. The microarray technology is fast, sensitive, but not yet common because of their variability and reproducibility issues [3].

Let's consider the fluorescent method for detecting mycotoxins. Fluorometry allows the identification of low-concentration substances by the excitation with a beam of ultraviolet light, followed by the detection and measurement of the characteristic wavelength of the fluorescent light emitted [6].

The scheme of implementation of the fluorescent method for detecting mycotoxins is as follows. On one side, there should be a light source that will illuminate the test sample. From the resulting glow, mycotoxins begin to fluoresce and emit their glow. As a result, on the other hand, there must be a light sensor that detects the level of a glow. However, this method's disadvantage is that it can detect only those toxins that have fluorescence properties. These are aflatoxin B1, ochratoxin, zearalenone. It will be impossible to determine other toxins by this method. Table 1 shows the values of the wavelengths of excitation (Ex) and emission (Em) of toxins that we must provide in the device. For example, the excitation–emission matrix of aflatoxin B1 standard and extracted aflatoxin produced overlapping peaks at 340…400 nm of the excitation interval centered at 365 nm and emitting in the blue region near 450 nm [8].

Table 1. Ex/Em wavelengths for toxins in the matrix.

Group	Compound	Ex, nm	Em, nm	Matrix
Myco-toxines	AFs	365	455	Cereals
	OTA	335	460	
	ZON	275	455	

Currently, there are no devices for the detection of mycotoxins by the fluorescent method among the existing solutions. The task is to implement this method by developing an optical biosensor device. Such optical mycosensor will be able to conduct the field testing.

3. Development of an optical mycosensor

Optical mycosensor design. Based on the diagram shown in Fig. 1, the general scheme of operation of the optical mycosensor is as follows (Fig. 1). The LED under the control over the driver shines on the sample. Mycotoxin begins to fluoresce and emits light, which is captured by the photodetector. As a result, the voltage parameters change, which can be measured and converted into values by the analog-to-digital converter (ADC).

Let's plot a schematic diagram of the placement of elements in the device. It shows the location and interaction of selected components. The diagram is shown in Fig. 2.

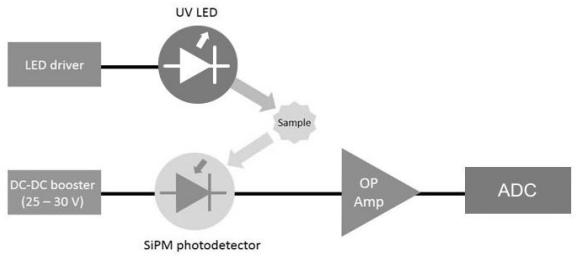

Fig. 1. Schematic diagram of the device.

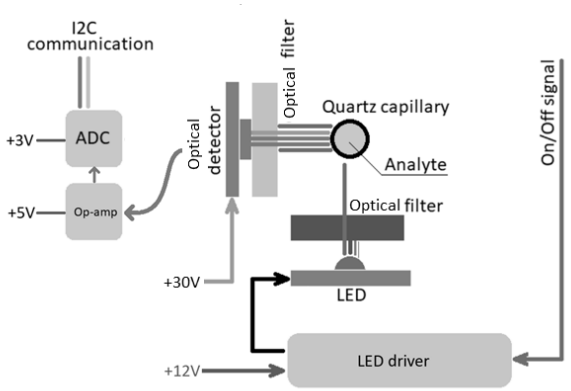

Fig. 2. Schematic diagram of the placement of components in the optical mycosensor.

When it turns on the led driver, the LED begins to irradiate the capillary. The input voltage of a driver is equal to 12 V, being the nominal value. Before entering the capillary, the light beam passes through a light filter. The capillary is a special quartz tube into which a sample with a mycotoxin enters. The mycotoxin in the irradiated sample begins to fluoresce and to emit light. A detector registers the breakdown light. Before that, the spectrum of the beam is also filtered by a light filter. A voltage of 30 V by documentation must be applied to the light detector. The value of the output voltage from the sensor is amplified through an operational amplifier (Op-amp) with a nominal voltage of 5 V and is converted to a digital value by ADC. ADC has a voltage nominal of 3 V, because it is used in Nordic nRF52840, which is used to read and to process the data from the optical mycosensor. Data re transferred by I2C communication. The Inter-Integrated Circuit (I2C) Protocol is a protocol intended to allow the multiple "peripheral" digital integrated circuits ("chips") to communicate with one or more "controller" chips. Like the serial peripheral interface (SPI), it is only intended for short distance communications within a single device. Like Asynchronous Serial Interfaces (such as RS-232 or UARTs), it only requires two signal wires to exchange information.

The optical mycosensor must have the following components and properties.

1. A light source having a wavelength close to the excitation values of mycotoxins.

2. The container for placing the analyte should be as transparent as possible, not to reflect light, and to completely transmit it.

3. A sensor that will capture light at lengths close to the emission light lengths of the toxins.

4. Controls and boards.

UV LED. A UV LED "ThorLabs" M375D4 was chosen as the light source. This Thorlabs LED on a metal-core printed circuit board (MCPCB) is designed to provide the high-power output in a compact package. The manufacturer draws attention to the fact that, for the proper operation, the LED should be installed on the appropriate radiator. For the proper thermal management, we fix MCPCB to a heat sink using two screws and use a thermal compound to provide a good thermal contact between MCPCB and the heat sink [9].

According to the stated characteristics, the wavelength is close to the wavelength of the excitation of aflatoxin. However, it is longer than the corresponding lengths for ochratoxin and zearalenone.

In order to be able to work with ochratoxin, for example, a light filter should be added. Its task is to filter out the unnecessary wavelengths. A light filter "Hoya U-340" was chosen.

However, based on the spectrum of LED, it is evident that the light intensity is quite low at lengths suitable to measure ochratoxin. The signal may be amplified.

Analyte capacity. The container for an analyte must be a tube with a diameter of not less than 3 mm. It should also be as transparent as possible and not react with aggressive environments such as alcohol, because alcohol is used to extract and wash out mycotoxins from samples. According to these requirements, the tube made of quartz glass is used in the device.

SIPM sensor. A silicon photomultiplier (SiPM) MicroFC-30035-SMT was chosen as a sensor for analyzing the signal of mycotoxin emission light.

The C-series low-light sensors from ON semiconductor feature an industry-leading low dark-count rate combined with a high PDE. For ultrafast timing applications, C-series sensors have a fast output that can have a rise time of 300 ps and a pulse width of 600 ps. The C-series is available with different sensor sizes (1, 3, and 6 mm) and packaged in a 4-side tileable surface mount (SMT) package that is compatible with the industry standard, lead-free, reflow soldering processes.

The C-series silicon photomultipliers form a series of high gain, single-photon sensitive, UV-to-visible light sensors. They have performance characteristics similar to a conventional PMT, while benefiting from the practical advantages of the solid-state technology: low operating voltage, excellent temperature stability, robustness, compactness, output uniformity, and low cost [10].

SiPM is formed of a large number (hundreds or thousands) of microcells. Each microcell is an avalanche photodiode with its own quench resistor and a capacitively coupled fast output. These microcells are arranged in a close-packed array with all of the like terminals (*e.g.*, all of the anodes) summed together. The array of microcells can thus be considered as a single photodiode sensor with three terminals: anode, cathode, and fast output [10].

According to the manufacturer parameters, the sensor has a performance that meets our needs for measuring the fluorescence of mycotoxins. The highest photon detection level occurs at wavelengths in the interval 400…450 nm. It is almost correlated with the light emission region of mycotoxins (455…460 nm).

It should be not forgotten that not only mycotoxins can glow. The analyte can contain a lot of impurities that can illuminate. In addition, the liquid itself can scatter light. With this in mind, a light filter should be installed in front of the detector, which will filter the light at the required wavelengths. So, a light filter "Hoya G530" was chosen for this purpose.

It should be noted that the luminescence spectrum of mycotoxins is 450…460 nm, and this is the peak of the luminescence intensity. However, this glow also occurs in other spectral regions, including the 500-nm one, but with a lower intensity. At the same time, the UV LED has a luminescence intensity peak in the 375-nm region. Moreover, it was revealed that it gives light with lower intensity in the 400…500 nm spectrum interval. Based on these data, the filtered light at 460 nm will give the mycotoxin emission and a sizeable parasitic emission from LED, which will give us an additional error. Therefore, a compromise decision was made to take a filter "Hoya G530" for the 530-nm region, which will cut-off the parasitic light of LED, but will transmit the light in the 460-nm region with a lower intensity. Thus, we deliberately reduce the glow intensity in the interval of the mycotoxin luminescence to remove the parasitic effect of the diode and to increase the determination accuracy.

Controls and boards. LED control can be provided by a LDD-1000L LED driver [11]. The optical mycosensor consists of three separate PCBs:

- Main mycosensor board.
- LED driver board.
- SiPM board.

The main mycosensor board and LED driver board interconnect *via* PLS connectors in a sandwich-like assembly (J6 to H2, J5 to H1, J4 to H4, J3 to H3). The SiPM board connects with wires to the main mycosensor board *via* J7 (+30V), J8 (SiPM signal), and J11 (ground) tabs. The power line for SiPM must be adjusted to +30 V on the main PCB.

The main mycosensor board populated with Op-amp circuit (1st stage transimpedance amplifier TIA, 2nd stage – non-inverting amplifier), voltage inverter circuit for Op-amp offset, ADC circuit, and precision voltage reference circuit for the LED driver brightness setting. The LED driver board populated with a LED driver (Meanwell LDD-1000L) and a driving MOSFET circuit. High logic level on UVLED_en line turns LED driver ON and low logic level on UVLED_en – OFF.

ADC ADS1115 is set to its default I^2C address (0×48). Op-amp output line is connected to AI2 input of ADC. The programmable gain amplifier (PGA) of ADC must be set with caution not to drive the Op-amp output voltage level higher than the internal voltage reference of ADC at the given PGA value. Incorrect PGA setting will irreversibly kill ADC.

The result of the design and development is the optical mycosensor, which can detect the concentration of fluorescent mycotoxins in the sample. The model of the developed device is shown in Figs 3 and 4.

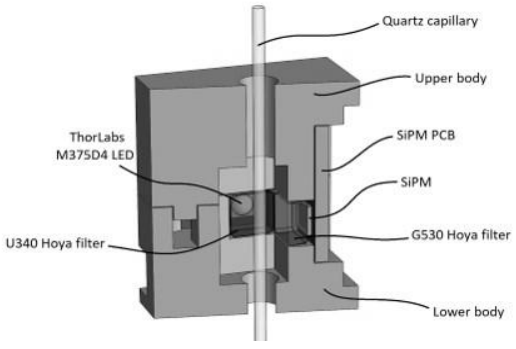

Fig. 3. Optical mycosensor model, bottom view.

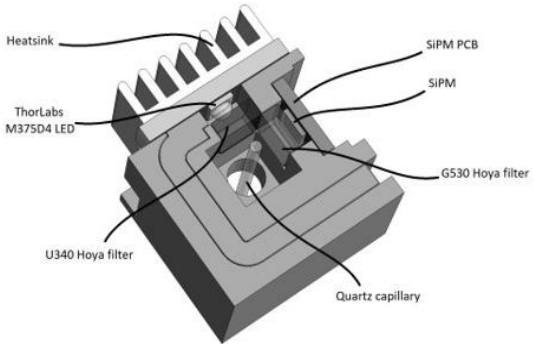

Fig. 4. Optical mycosensor model, side view.

Fig. 3 shows the device, bottom view. It can be seen that a special capillary runs along the entire length in the central part of the body. When the sample liquid fills the capillary, the test can be started. The block body itself is made of aluminum. It must ensure that the measuring area is protected from the external light, since the latter will give an additional error, by illuminating the sensor. The device measuring zone is the zone between the light emitter and the sensor, through which the capillary with the sample passes. LED and sensor are at 90 degrees to each other. This is done so that the LED radiation does not directly hit the detector. In front of the sensor and LED, the special square areas are made to locate the filters.

Fig. 4 shows the device, side view. In addition to the previous picture, it can be seen that a cooling heatsink is installed on top of the LED. This is done following the manufacturer operating requirements. The sensor control board (SiPM PCB) is located in a specially designated place on top of it.

4. Validation

Validation parameters

Accuracy (closeness of a measured value to the "true" value), defined as the degree of coincidence of a result with the known concentration in the spiked sample.

Precision as an indicator of the approximation of the consistency of the independent test results indicated in the form of the standard deviation (SD), relative standard deviation (RSD), or standard deviation of the mean (SDM) of a series of repeated tests.

Bias is the difference between the test results and the accepted reference value. Ideally, the analyzed sample is CRM (certified standard sample, reference material) and is similar to the working sample in terms of its shape, matrix composition and the analyte concentration. To calculate the percent bias, we use C_0 (mean value of measurements using the validating method) and C_R (certified standard sample value).

Sensitivity – the resolution of the method as the level of a change in the response of the measuring instrument, depending on the change in the concentration. The method allows one to distinguish close concentrations.

The limit of detection – this is the minimum analyte concentration that can be detected with statistical certainty. It can be determined by re-analyzing an empty test portion or a test portion containing only a small amount of the analyte.

The limit of quantitation is the lowest concentration of the analyte that can be determined. It shall be installed using the appropriate measurement standard or sample. This is the lowest concentration at which the standard deviation of the mean is ≤ 10%.

Materials:

- Mycotoxins-free corn CRM (mycotoxins content below the LC/MS detection level), particle size ≈0.3 mm w/o flour.
- AFs-contaminated corn CRM (in the 5…100-ppb interval), particle size ≈0.3 mm w/o flour.
- OTA-contaminated corn CRM (in the 5…100-ppb interval), particle size ≈0.3 mm w/o flour.
- AFs standard 10 ppm stock solution in methanol.
- OTA standard 10 ppm stock solution in methanol.

Procedure validation

The main goal of tests was the evaluation of the sensitivity and linearity of an optical mycosensor. The procedure has the following steps.

1. Take CRM and add the appropriate amount of the standard solution.
2. Perform the manual sample preparation by the standard method.
3. Inject the sample into the capillary of the optical mycosensor.
4. Start the measurement by protocol.

Protocol: The linearity of the detector response at different concentrations of OTA is assessed using a 6-point calibration curve obtained by the sequential introduction into the detector standard OTA solutions, two measuring points for each concentration. After constructing the calibration curve, we evaluate the correlation coefficient *R*-squared.

Preparation of standard solutions of OTA:

1. 0 ppb – 950 µl EtOH + 50 µl H_2O.
2. 5 ppb – 945 µl EtOH + 50 µl H_2O + 5 µl St OTA.
3. 10 ppb – 940 µl EtOH + 50 µl H_2O + 10 µl St OTA.
4. 25 ppb – 925 µl EtOH + 50 µl H_2O + 25 µl St OTA.
5. 50 ppb – 900 µl EtOH + 50 µl H_2O + 50 µl St OTA.
6. 100 ppb – 850 µl EtOH + 50 µl H_2O + 100 µl St OTA.

After testing the device in the temperature interval 15…25 °C, we obtained the results presented in Table 2. The least-squares method was used to calculate the linear formula. The linear correlation formula of the dependence of the fluorescence intensity on the real concentration x is $27.2x + 11398$. *R*-squared is 0.98 in the interval 0…100 ppb. This means that the maximum deviation of points from the linear curve does not exceed 2% when approximating a function linearly. In other words, the device relative error is less than 2% at high levels. The obtained linearity of the results confirms the ability of the device to determine the concentration of mycotoxins, but the device cannot accurately determine the concentration at low levels. Accordingly, the accuracy at low concentrations will be reduced. The linearity graph is shown in Fig. 5.

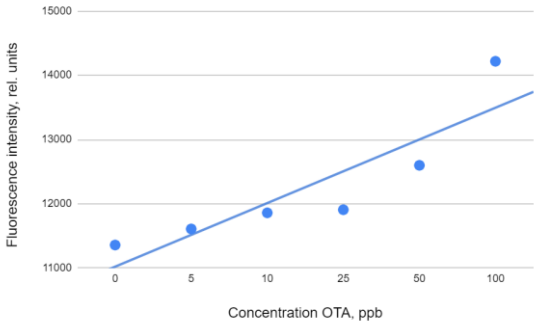

Fig. 5. Graph of the linearity of the fluorescence intensity determined by the optical mycosensor versus the concentration.

The presented implementation of the fluorescent method for determining the presence of mycotoxins works and can be used in the field. However, it is worth to make some remarks. First, before the analysis, the sample preparation should be performed. It can be done manually without laboratory conditions. However, it is better to create a device with the automated sample preparation such as a device developed by "BIOSENS". So, our optical mycosensor can be combined with such device [12]. Second, although the device has a high linearity coefficient, the parameter *b* equal to 11398 is too high. Given a maximum detection of 100 ppb that equals 14260 conventional units of fluorescence intensity, this figure should not exceed 10% of this value of conventional units, which is equal to 1426. This is of importance, because, at low concentrations, there is a minimal difference in conventional units. Therefore, it is almost impossible to accurately determine the concentration at low levels. It is also worth noting that the device is calibrated under certain climatic conditions. Changes lead to changes in results, which negatively affects the reproducibility. This leads to the need to develop at least the thermal stabilization. Thus, the optical mycosensor still needs to be refined, and after this, the device can be used as a part of the data collection system aimed at the prediction of the contamination by mycotoxins, which was discussed in [13].

Table 2. Results of optical mycosensor tests.

C, ppb	Signal	Average	SD	RSD
0	11402	11360	59.39696962	0.5228606481
	11318			
5	11504	11610	149.9066376	1.291185509
	11716			
10	11866	11860.5	7.778174593	0.06558049486
	11855			
25	11994	11908.5	120.9152596	1.015369355
	11823			
50	12716	12599	165.4629868	1.313302538
	12482			
100	14342	14216.5	177.4838021	1.248435283

C – concentration

4. Conclusions

There are available the reference and rapid methods for the detection of mycotoxins. The reference methods are used in laboratories and require a highly skilled personnel and sophisticated expensive equipment.

Rapid on-site analysis requires the use of rapid methods such as enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD), fluorescence polarization immunoassay (FPI), or basic fluorometric measurements. The method of fluorescence of mycotoxins can show a quantitative value of the concentration, does not require a specially trained personnel to be used, and does not require the organization of special conditions.

The developed optical mycosensor implements the method of mycotoxin fluorescence. Constructively, it consists of a quartz tube, aluminum body, LED, sensor, filters, control boards, Op-amp, ADC, and cooling heatsink. LED and the sensor are positioned at 90 degrees to each other.

"ThorLabs" M375D4 was chosen as LED, which gives a luminescence in the region required for the fluorescence. "Hoya U-340" and "Hoya G530" were selected to filter the light spectra of LED and mycotoxins, respectively. Silicon Photomultipliers MicroFC-30035-SMT was chosen as a mycotoxin glow detection sensor. Control boards for block elements have been developed.

When validating the device, 2 g of a sample were tested at the concentrations: 0, 5, 10, 25, 50, and 100 ppb, two measuring points for each concentration. The sample was prepared by a manual sample preparation according to the standard method.

As a result of the testing, the satisfactory linearity (with a relative error less than 2% at high levels) of the correlation between the concentration of mycotoxins in the interval 0…100 ppb and the fluorescence intensity in relative units was obtained. The developed sensor can be used in agriculture for the express detection and the evaluation of the mycotoxin contamination. However, it requires improvements associated with a reduction of the parameter *b* in the linear formula.

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Оптичний сенсор для виявлення мікотоксинів

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Анотація. Проаналізовано методи виявлення мікотоксинів у сільськогосподарській продукції. Показано переваги використання флюоресцентних методів для експрес-діагностики наявності мікотоксинів у зразках. Представлено розробку оптичного біосенсора, який дозволяє у польових умовах виявляти мікотоксини. Показано принципи функціонування та запропоновано конструктивне рішення датчика. Розроблено та апробовано електрооптичну схему отримання інформаційного сигналу. Особливу увагу приділено вибору елементної бази запропонованого мікосенсора. Показано принципи і процедура його валідації. Наведено практичні результати тестування розробленого технічного рішення. Досягнута відносна похибка при лінійній апроксимації характеристики перетворення сенсора у діапазоні концентрацій 0…100 ppb при температурі 15…25 °C не перевищує 2%. Розроблений сенсор можна використовувати в галузі сільського господарства для швидкого визначення присутності мікотоксинів і оцінки рівня такого забруднення продукції.

Ключові слова: біосенсори, оптичний сенсор, мікотоксини, флуорометрія.