DEVELOPMENT OF A SMARTPHONE-BASED BIOMIMETIC SENSOR FOR AFLATOXIN B1 DETECTION USING MOLECULARLY IMPRINTED POLYMER MEMBRANES

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Abstract

A novel smartphone-based optical biomimetic sensor based on free-standing molecularly imprinted polymer (MIP) membranes was developed for rapid and sensitive point-of-care detection of aflatoxin B1. The developed MIP membranes were capable of selective recognition of the target analyte and, at the same time, of generation of a fluorimetric sensor response, which could be registered using the camera of a smartphone and analysed using image analysis. The developed system provides a possibility of synchronous detection of aflatoxin B1 in 96 channels. UV irradiation of aflatoxin B1, selectively bound by the MIP membranes from the analysed samples, initiated fluorescence of aflatoxin B1 with intensity directly proportional to its concentration. The composition of the MIP membranes used as a recognition element was optimised taking into account data of computational modelling. Two functional monomers (2-acrylamido-2-methyl-1-propanesulfonic acid and acrylamide) were identified as optimal for the formation of aflatoxin B1-selective binding sites in the structure of the MIP membranes. Working characteristics of the smartphone-based sensor system were also estimated. The influence of pH and of buffer and NaCl concentrations on the smartphone-based sensor responses were studied. High selectivity of the developed sensor system towards aflatoxin B1 was confirmed in experiments with close structural analogues of the target analyte - aflatoxin G2, and ochratoxin A. The detection limit for aflatoxin B1 using the smartphone-based sensor systems was found to be 20 ng mL⁻¹ for the sensor based on MIP membranes synthesised with acrylamide as a functional monomer. The storage stability of the recognition elements of the

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developed sensors was estimated as one year when stored at 22°C. The possibility to detect the aflatoxin B1 in contaminated food samples was shown. The MIP-membrane-based sensor system provided a convenient point-of-care approach in food safety testing.

Key words: molecularly imprinted polymer membranes, smartphone-based sensors, fluorescent chemo-sensing, mycotoxins, aflatoxin B1.

1. Introduction

Aflatoxins are toxic mutagenic and carcinogenic substances, which are produced by microscopic fungal species *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1) is recognised as one of the most toxic representatives of this group. According to the International Agency for Research on Cancer (IARC), aflatoxin B1 is a class I carcinogenic substance [1]. Maize, millet, wheat, spices, cocoa and coffee beans, nuts as well as some fruits and vegetables are often contaminated with these mycotoxins [2]. Moreover, aflatoxins are often found in fodder, which results in significant losses mainly in the fodder industry and aviculture. Annual losses in agriculture and food industry in USA and Europe associated with aflatoxin B1 contamination comprises up to 1.5 billion USD [3]. Since contamination of food products and feeding stuffs with aflatoxin B1 comprises about 75% out of total mycotoxin contamination [4], development of a fast and reliable method of its detection is of great importance. A number of traditional instrumental analytical [5, 6] as well as immunochemical [6–9] methods were developed for aflatoxin detection. Chromatographic methods, such as TLC [10], HPLC [11] and LC-MS [7] as well as ELISA-based commercially-available test-systems, are the most widely used analytical methods for detection of aflatoxin B1. However, long analysis time along with the large amounts of raw material necessary for the extraction procedure (at least 100 g per analysis), as well as requirements of availability of large and expensive equipment, limits application of these methods for in-field analysis [12, 13]. Moreover, a large number of false-positive results are typical for the ELISA-based test-systems. There is a number of biosensors that were also developed for detection of aflatoxins [14–17]. The low storage stability (typically only up to 1 month) of these biosensors is related to the intrinsic properties of enzymes and antibodies, and this has stimulated the development of alternative approaches based on synthetic receptors and responders.

One of the most effective approaches to toxic molecules' detection is the development of chemical sensors based on synthetic mimics of enzymes or antibodies, which provide fast and selective analysis and, at the same, are much more stable as compared to biosensors based on natural

biomolecules [14, 18]. As it was previously shown, application of polymers-biomimics in the form of free-standing molecularly-imprinted polymer (MIP) membranes provides easy integration in a biosensor device as well as effective generation of the sensor response, which can be easily registered and visualised [18-20]. It was demonstrated that MIP membranes with synthetic binding sites, mimicking those of natural receptors, can be successfully used for the development of easy-to-use and reliable biosensor systems for a number of toxic molecules and metabolites [18-23].

At the same time, new trends in modern biosensors technology include wide application of smartphones either as detectors or analysers, which can significantly simplify the detection procedure and reduce its price [24-26] as well as simplify commercialisation of this type of devices. Smartphone-based biosensors were already reported in different areas, including food safety [27], healthcare [28], and environmental monitoring [29]. An advantage of this approach is the possibility of application of smartphone-based sensors as point-of-care devices and in *in-field* conditions. The present paper is aimed at a combination of MIP membranes capable of recognition of small organic molecules and generation of optical sensor response with smartphones, which can be used as detectors of an optical sensor response.

Our research is focused on the development and analytical performance of an easy-to-use fluorescent smartphone-based biomimetic sensor system based on free-standing MIP membranes for rapid detection of a widespread food toxin - aflatoxin B1, optimisation of the composition of the MIP membranes used as recognition elements of the biomimetic sensor systems, as well as their application for analysis of both model and real samples.

2. Materials and methods

2.1. Materials. Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), and aflatoxin G2 (AFG2), ochratoxin A (OTA), acrylamide (AA), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), allylamine, 2-(diethylamino)ethylmethacrylate (DEAEM), *N,N'*-methylenebisacrylamide (MBAA), polyethyleneglycol MM 20,000 (PEG 20,000) and tri(ethyleneglycol)dimethacrylate were purchased from Sigma-Aldrich (St. Louis, USA). Ethyl-2-oxocyclopentanecarboxylate was purchased from (Acros, UK). Oligourethaneacrylate (OUA) was synthesised as described earlier [30]. All the other reagents of analytical grade were obtained from Sigma-Aldrich (USA) and used without additional purification. The standard check sample of maize flour “Romer Labs Check Sample Survey Aflatoxins in corn (CSSMY013 – M17411A)” contaminated with AFB1 was kindly provided by Dr.P.V.Futernyk (Romer labs, Kyiv, Ukraine). Concentration of AFB1 in the sample was estimated as 7 $\mu\text{g kg}^{-1}$ according to the results of HPLC and ELISA analysis. As an alternative samples the

2.3. Synthesis of molecularly imprinted polymeric membranes. Molecularly imprinted polymer membranes were synthesized *in situ* using the technique of molecular imprinting combined with the method of synthesis of semi-interpenetrating polymer networks (semi-IPN). The mixture of monomers contained ethyl-2-oxocyclopentanecarboxylate as a “dummy” template (5wt% of total weight of the monomers). Acrylamide, MBAA, AMPSA, DEAEM, and allylamine were used as functional monomers, with a 1:2 molar ratio of template : functional monomer. The monomeric mixture also contained dimethylformamide and polyethyleneglycol Mr 20,000 as a porogens and a mixture of triethyleneglycoldimethacrylate and oligourethaneacrylate (Mr 2,600) as the main components of the polymeric network. As an initiator of the polymerisation 2,2'-dimethoxy-2-phenylacetone was used. The compositions of the MIP and corresponding blank membranes are presented in Table 1.

Table 1. Compositions of the AFB1-selective MIP and corresponding Blank membranes.

[illegible]

PEG	60	60	60	60	60	60	60	60	60	60
2,2'-dimethoxy-2-phenylacetone	2	2	2	2	2	2	2	2	2	2

**TGDMA/OUA - a mixture of triethyleneglycoldimethacrylate and oligourethaneacrylate (85/15)*

The monomeric mixtures were polymerised between two glass slides. The thickness of the membrane was determined by 60- μm polytetrafluoroethylene spacers. The polymerisation was initiated using UV light ($\lambda=365\text{ nm}$, intensity 3.4 W m^{-2}) and performed for 30 min. The polymerisation was followed by 8-h Soxhlet extraction procedure in ethanol for removal of the template and monomers which were not included in the polymeric network. That was followed by the 8-h extraction of the linear component of the semi-interpenetrating polymer network (PEG 20,000) by keeping the membranes in water at $80\text{ }^{\circ}\text{C}$.

2.5. Fluorescent sensor system for the selective detection of AFB1. Ability of both MIP and blank polymeric membranes to generate fluorescent sensor responses as a result of selective binding of target mycotoxin was estimated after 1-hour incubation of $1\text{ cm} \times 2\text{ cm}$ membrane samples in $5\text{-}700\text{ ng mL}^{-1}$ solutions of AFB1, AFG2, and OTA, containing 10% of acetonitrile. The sensor responses were registered after UV-irradiation of the membrane samples fixed in a holder for membranes and polymers using Perkin-Elmer LS 55 (UK) spectrofluorimeter (excitation wavelength 365 nm , and emission wavelength of 435 nm (AFB1), 450 nm (AFG2 and OTA) directly at the membranes' surface. The influence of pH of the analysed sample on the intensity of the sensor responses was investigated using 20 mM Na-acetate buffer (pH 3.0, 4.0 and 5.0), 20 mM Na-phosphate buffer (pH 6.0 and 7.0), 20 mM Tris HCl buffer (pH 8.0 and 9.0). The influence of NaCl concentration on the values of the sensor response of the fluorescent sensor system was evaluated at optimal pH value (in 20 mM Na-phosphate buffer, pH 6.0 containing $5\text{-}150\text{ mM}$ NaCl).

2.6. Smartphone-based sensor system for AFB1 detection. After the adsorption procedure the MIP and blank membranes were dried at room temperature and placed on a plain black cotton fabric. Fluorescence of AFB1 adsorbed on the surface of molecularly imprinted polymer membranes was visualised after excitation with UV-irradiation using TMW-20 Transilluminator equipped with UV Wood glass (UVP, CA, USA). Intensity of fluorescence of AFB1 adsorbed on the surface of the MIP and blank membranes reached maximum after 1 min excitation with UV-irradiation. After 1 min excitation the images of the polymeric membranes samples were taken by Meizu U 10 smartphone camera (10 MP, aperture F/2.0). Smartphone camera was fixed using photographic tripod at a distance of 25 cm in front of the polymeric membranes samples strip and an angle of 90 degrees with respect to the surface normal. The MIP membrane samples could be photographed after initiation of the natural fluorescence of aflatoxin B1 for 5 minutes with the same intensity measurement. After 5

minutes excitation with UV-irradiation intensity of fluorescence was declined, while the time of the fluorescence decay comprised more than 40 min. It was shown that distance between samples and camera (25-60 cm) doesn't influence intensity measurement. The images of the MIP and blank membranes were captured with automatic camera settings. All images were saved in JPG format with a resolution of 4160 x 3120 pixels and further were analysed using a commercially-available application for a smartphone Spotxel® Reader (Sicasys Software GmbH, Germany) for Android 6.0+. The smartphone application transforms the intensity of staining in arbitrary units and gives a possibility to get dependencies of fluorescence intensity on toxin concentration in the sample within real time.

2.7. Extraction of AFB1 from maize samples. AFB1 was extracted from ground maize samples according to a previously described protocol [31]. One gram of ground maize sample was shaken for 3 min in 10 mL of acetonitrile : H₂O solution (80:20 v/v) using a Vortex laboratory shaker (Fisher Scientific, Germany). The extract was collected in a filtration procedure using filter paper, vortexed with 7 mL of acetonitrile : H₂O solution (80:20 v/v) for 3 min and filtered through the filter paper. The procedure was repeated. All the extracts were collected and centrifuged for 10 min at 10,000 g. The supernatant fluid was analysed for AFB1 presence.

2.8. Determination of AFB1 in real samples. AFB1 content in the real maize flour samples was calculated according FDA and SOP standards (UCM612334) according to [32] as follows:

1) The concentration of AFB1 in the extract was estimated in using the calibration plot of the smartphone-based sensor system (C, ng mL⁻¹).

2) The AFB1 content in the sample (in µg kg⁻¹) was calculated using formula (1):

$$C V_1/k V_2 m, \quad (1)$$

where V₁ - volume of the obtained extract, V₂ - volume of the sample, m - mass of the sample, k - coefficient of AFB1 extraction in the organic solvent (k=20.6 for acetonitrile).

3. Results and discussion

According to the results of computational modeling [19], six potential functional monomers capable of formation of strong complexes with aflatoxin B1 were selected and used in present research for synthesis of recognition elements of the smartphone-based sensors devices. Molecularly-imprinted polymer membranes were synthesised using ethyl-2-oxocyclopentanecarboxylate as a “dummy” template instead of aflatoxin B1, while allylamine, AA, DEAEM, MBAA, and AMPSA were selected *in silico* as the functional monomers that possessed natural affinity towards AFB1. The

replacement of the toxin molecule with its structural analogue allowed a decrease in the price of the toxin-selective MIP membranes, and also an increase in the sensitivity of the biomimetic sensors due to minimisation of background fluorescence. The molar ratio of dummy template : functional monomer in the monomer mixtures was between 1:2 to 1:6. Blank membranes were synthesised from the identical mixtures, which were made without any template. Structural formulae of the monomers used for the MIP membrane synthesis as well as the structures of the target analyte and the dummy template are shown in Fig. 1.

Fig. 1. Structural formulae of the compounds used for the MIP membrane synthesis.

All synthesised membranes were tested for their ability to bind AFB1 from diluted aqueous solutions selectively and generate a fluorescent sensor response, which could be registered optically. The highest values of differential sensor responses were revealed for the MIP membranes synthesised using AA and AMPSA as functional monomers (Fig. 2).

Fig. 2. Dependence of differential sensor responses* on the type of functional monomer used for the membranes' synthesis following the addition of 100 ng mL^{-1} AFB1. The ratio of dummy template : functional monomer in the monomer mixture for the MIP membranes synthesis was 1:2. Aqueous solutions of AFB1 containing 10% acetonitrile were used. *Differential sensor responses were calculated as differences between sensor responses generated by the MIP and blank polymeric membranes.

These data are in accordance with the earlier-obtained results of computational modelling and solid-phase extraction on porous MIP membranes [19]. The lowest values of differential sensor response were observed for the MIP membranes synthesised using allylamine, DEAE, and MBAA. It could be explained by the fact that these functional monomers interacted with the ketolactone portion of the AFB1 molecule according to the data of molecular dynamics obtained earlier [19], while the selective site in the MIP membranes' structure was formed using template analogue, which mimicked the difurane portion of the AFB1 molecule.

To optimise the membrane composition and select the best functional monomer, the specificity of all synthesised MIP membranes was tested. It was important to ensure the ability of the synthetic binding sites to discriminate between AFB1 and potential interfering compounds which may be present in the analysed samples (i.e. very close structural analogues of AFB1 - aflatoxin G2 and

another widespread mycotoxin OTA). In order to do this, the differential fluorescent sensor signals, generated with the MIP membranes in response to addition 100 ng mL^{-1} AFB1, AFG2 and OTA, were evaluated.

It was clearly demonstrated that MIP membranes synthesised using AA and AMPSA as functional monomers demonstrated the best selectivity of their synthetic binding sites (Fig. 3).

Fig. 3. Cross-reactivity of differential sensor responses* of fluorescent sensor system based on AFB1-selective MIP membranes, synthesised using AA, AMPSA, DEAE, allylamine, and MBAA as functional monomers. Differential sensor responses after addition 100 ng mL^{-1} AFB1, AFG2 and OTA were estimated. The ratio of template : functional monomer in the monomer mixture for the MIP membranes synthesis was 1:2. Aqueous solutions of AFB1 containing 10% acetonitrile were used. *Differential sensor responses were calculated as differences between sensor responses generated by the MIP and Blank polymeric membranes.

It was found that the presence of AFB1 generated the highest values of the differential sensor response. At the same time only insignificant binding of AFG2 was observed which could potentially be explained by non-specific hydrophobic interactions. Either negligible or very low sensor responses were observed in response to OTA addition for the sensor systems based on acrylamide- and AMPSA-containing MIP membranes. The MIP membranes, which were synthesised using DEAE, allylamine, and MBAA, demonstrated significantly higher levels of cross-reactivity of their receptor sites in response to addition AFG2 and OTA as compared to addition of AFB1. Since these sensors systems were characterised as having significantly lower selectivity and relatively low values of the

fluorescent sensor responses, they were excluded from further experiments. However, since these membranes have demonstrated a high binding affinity towards AFG2, they can potentially be used for development of group-selective sensors, capable of detection of all mycotoxins affiliated to aflatoxins.

Since AA- and AMPSA-based MIP membranes were characterised as possessing a high selectivity of their binding sites for recognition of AFB1 as compared to potential interfering compounds and also by ability to generate high fluorescent sensor responses, they were used for the further development of smartphone-based sensors.

Typical calibration plots for the fluorescent sensor systems based on acrylamide- and AMPSA-containing MIP-membranes are presented in the Fig. 4.

Fig. 4. Typical calibration plots of the MIP-membrane-based fluorescent sensor systems for AFB1 detection. Fluorescence of MIP and Blank polymeric membranes synthesised using AMPSA (a) and AA (b) as functional monomers after incubation in aqueous AFB1 solutions, containing 10% acetonitrile.

Dependence of the intensity of the MIP-membranes' fluorescence on AFB1 concentration in aqueous sample was analysed within the range of 5-700 ng mL⁻¹. It can be seen that predominant binding of AFB1 was observed for both MIP membranes which were used as a basis of the fluorescent sensors. The MIP membranes generated significantly higher fluorescent sensor responses as compared to blank ones. Moreover, AA-based membranes demonstrated twice lower levels of non-specific binding as compared to AMPSA-based ones, which was estimated by the level of fluorescence of Blank membranes (the imprinting factors comprised 1.5 and 2.7 for AMPSA- and AA-based membranes, correspondingly).

The detection limits for both the sensor systems were calculated using the IUPAC criteria [33]. The detection limit (3 σ) of AFB1 detection for both the fluorescent sensors was found to be 15 ng mL⁻¹. The linear dynamic range for the sensors based on AA- and AMPSA-containing MIP membranes was between 15-500 ng mL⁻¹. Taking into account that AA-based MIP membranes were characterised by better mechanical stability as compared to AMPSA-based membranes, they were used as recognition elements of the smartphone-based sensor systems for AFB1 detection.

The principle of the sensor signal registration in the case of smartphone-based sensor is the following. Blue fluorescence of AFB1 selectively adsorbed by the receptor sites in the MIP membrane was excited using UV-irradiation ($\lambda=365$ nm) and registered by the digital camera of a smartphone running an Android 6.0+ system. The intensity of blue fluorescence in the digital image of the fluorescent MIP membranes was evaluated using standard software for digital image analysis Spotxel® Reader (Sicasys Software GmbH, Germany). The calibration plot of the smartphone-based sensor system was presented as dependence between differential sensor responses and AFB1 concentration, which was based on the difference in the intensity of fluorescence of the MIP and corresponding blank membranes. The Spotxel® Reader application provided a possibility of automatic generation of the calibration plot for the AFB1 detection in real time.

Accordingly to the typical calibration plot, the limit of aflatoxin B1 detection was estimated as 20 ng mL⁻¹, while the linear dynamic range of aflatoxin B1 detection was between 20-100 ng mL⁻¹ (Fig. 5).

Fig 5. Typical calibration plot of the MIP-membrane-based smartphone sensor system for AFB1 detection: dependence of the smartphone sensor differential response on AFB1 concentration generated by Spotxel® Reader smartphone application (Sicasys Software GmbH, Germany).^{*} An inset shows photo of fluorescence of the MIP membranes after incubation in solutions, containing 0 ng/ml and 100 ng/ml of aflatoxin B1.

^{*}Differential sensor responses were calculated as differences between sensor responses generated by the MIP and Blank polymeric membranes.

For the MIP membranes synthesised using AA as a functional monomer, the content of AA in the initial monomer mixture was optimised (Fig. 6).

It was shown that the MIP membranes synthesised from mixtures where the molar ratio of template : functional monomer was 1:2 demonstrated the highest values of AFB1 binding, and, consequently, the highest values of the fluorescent sensor responses. A further increase in concentration of the functional monomer in the monomer mixture (mixtures with molar ratios of template : functional monomer 1:3, 1:4 and 1:5) resulted in a significant increase in levels of non-specific binding of AFB1 with the surface of blank membranes, and, as a result, to the decrease in values of differential sensor responses upon addition of AFB1. The MIP membranes synthesised from mixtures where the molar ratio of template : AA was 1:2 were used for all further investigations.

Fig. 6. Dependence between differential sensor responses* of the MIP-membrane-based fluorescent sensor system in response to addition 100 ng mL^{-1} AFB1 and a ratio template : AA in the mixture used for the MIP membranes' synthesis. Aqueous solutions of AFB1 containing 10% acetonitrile were used. *Differential sensor responses were calculated as differences between sensor responses generated by the MIP and Blank polymeric membranes

The influence of the composition of the analysed sample on values of differential sensor responses of the developed sensor system was also evaluated. The influence of pH (Fig. 7a), buffer concentration (Fig. 7b), and concentration of NaCl (Fig. 7c) in the analysed sample were also investigated.

Fig. 7. Dependencies of values of differential fluorescent sensor responses* on pH (a), concentration of buffer (b) and NaCl (c) in the analysed sample. Measurements were carried out in 20 mM buffer solutions, pH 3-9 (a), 5-50 mM Na-phosphate buffer, pH 6.0 (b) and 20 mM Na-phosphate buffer, pH 6.0, containing 5-150 mM NaCl. All buffer solutions contained 10% acetonitrile. *Differential sensor responses were calculated as differences between sensor responses generated by the MIP and Blank polymeric membranes.

It was shown that neither an increase in buffer nor in NaCl concentration of the analysed sample significantly influenced the values of differential sensor responses. At the same time, pH dependence of the sensor response had a typical bell-like shape, while the highest values of differential sensor responses were observed at neutral pH values (pH 6-7). Both an increase and a decrease in pH values

resulted in a significant decrease in the ability of the MIP membranes to bind to AFB1 selectively, and in the values of differential sensor responses.

The developed smartphone-based sensor system was used for AFB1 analysis in both model and real samples. The possibility of successful application of the sensor system for the analysis of AFB1-contaminated maize samples was demonstrated. Two samples of maize flour contaminated with AFB1 were used in the investigation (Table 2). The first (sample No.1), was naturally contaminated with aflatoxin B1 sample, was provided by Romer Labs (Kiev, Ukraine), i.e. “Romer Labs Check Sample Survey Aflatoxins in corn (CSSMY013 – M17411A)”. The concentration of AFB1 in this sample was declared by the manufacturer as $7 \mu\text{g kg}^{-1}$ accordingly to the results of the estimation using standard analytical methods, such as HPLC and ELISA. As an alternative, the samples of AFB1-free wheat flour produced by three different manufacturers (“Zernovita”, Ozeryanka, Zhytomyr region (sample No.2), Ukraine, “Aro”, Kiev, Ukraine (sample No.3), “KyivMlyn”, Kiev, Ukraine) (sample No.4), and the AFB1-free maize flour (“Dobrodiya Foods”, Kiev, Ukraine) purchased in the local supermarket were used in the investigation (sample No.5).

The extracts of the aflatoxin B1-free wheat and maize flour, obtained using the standard procedure, were spiked with different amount of AFB1 (10 ng mL^{-1} , 50 ng mL^{-1} , 80 ng mL^{-1} , and 100 ng mL^{-1}) and analysed using the developed method. The number of replicates for the each sample $n=5$. It was found that quantitative detection of AFB1 was demonstrated in all the analysed samples (Table 2), therefore a possibility of the smartphone-based sensor detection for AFB1 detection in real wheat and maize samples was clearly demonstrated.

Table 2. Results of aflatoxin B1 detection in real wheat and maize flour samples*.

Sample No.	Amount of AFB1 in the sample confirmed by the traditional method	Amount of AFB1 in the sample confirmed by the MIP-based sensor method	Recovery, %
1	$7 \mu\text{g kg}^{-1}$	$6.1 \pm 0.52 \mu\text{g kg}^{-1}$	87 ± 7
2	10 ng mL^{-1}	$9.6 \pm 1.31 \text{ ng mL}^{-1}$	96 ± 13
3	50 ng mL^{-1}	$48.7 \pm 1.21 \text{ ng mL}^{-1}$	97.4 ± 2
4	80 ng mL^{-1}	$76.8 \pm 14.69 \text{ ng mL}^{-1}$	96 ± 18
5	100 ng mL^{-1}	$96.3 \pm 8.17 \text{ ng mL}^{-1}$	96 ± 8

* $n=5$ in all the experiments

4. Conclusions

Smartphone-based fluorescent sensor systems for AFB1 detection were developed on the basis of free-standing molecularly-imprinted polymer membranes. The sensor system was shown to be highly-selective towards the target analyte with only negligible sensor responses observed for structurally similar and different mycotoxins which could be considered as potential interfering compounds. The composition of the MIP membranes used as recognition elements of the sensor system was optimised taking into account the type and concentration of the functional monomer, as well as the molar ratio between the template and functional monomers used for the MIP membrane synthesis. It was demonstrated that the highest sensor responses were observed at neutral pH values, while both buffer and NaCl concentration did not influence values of the sensor responses significantly. A possibility of the sensor system application for analysis of AFB1-contaminated maize samples was demonstrated. It is possible to conclude that the protocols described in the paper are generic and could be applied for the development of the smartphone-based sensors for other important food contaminants possessing fluorescent properties.

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