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Voltammetric determination of aflatoxin B1

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Abstract

For the first time the possibility of voltammetry used for the determination of aflatoxin B1 on a glassy carbon electrode was shown. The effect of pH of a supporting electrolyte on the analytical signal of aflatoxin B1 has been investigated and it was shown that there is a more pronounced peak with a maximum current at pH of 5.33. The most favorable supporting electrolyte for a linear range of detectable concentrations of aflatoxin B1 – 0.1 M $(NH_4)_2SO_4$ was determined. The results of research on the development of conditions of voltammetric measurement of aflatoxin B1 are presented.

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Keywords: Voltammetry, micotoxin, aflatoxin B1, method.

1. Introduction

Mycotoxins, in recent years, are one of the dominant groups of biogenic toxins contaminating food and food products. Currently, mycotoxin contamination control problems are particularly relevant and solved not only within individual states, but also at the international level, under the auspices of WHO and FAO.

The most toxic properties resulting from their reaction with nucleophilic portions of DNA, RNA and proteins have aflatoxins, produced by microscopic fungi Aspergillus, which are the cause of serious diseases of human beings and animals. The strongest and most dangerous of all hepatocarcinogens aflatoxins is an aflatoxin B1. Maximum residue limit of aflatoxin B1 in foods is 0.005 mg/kg. Literature review on analytical capabilities of physico-chemical methods for determination of mycotoxins has shown that the distribution of the number of published works on years is quite uneven. (Fig.1)

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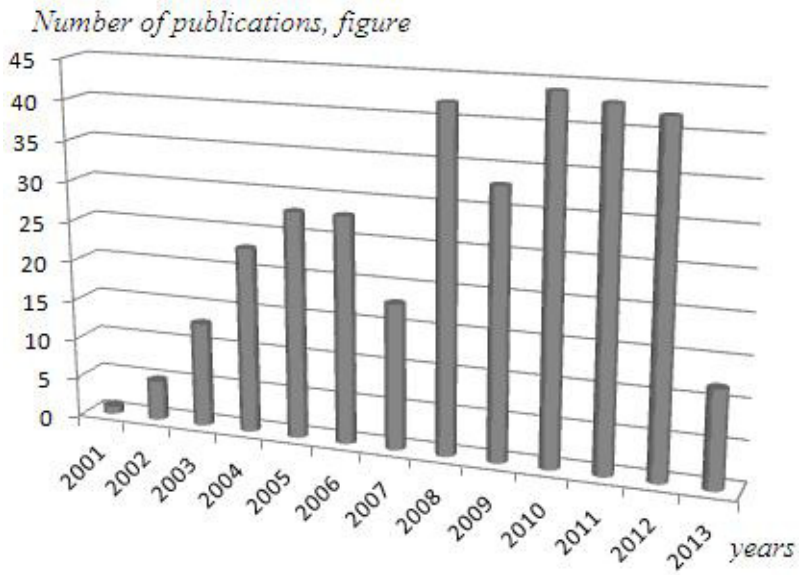

Figure.1. Distribution of the number of publications on physico-chemical methods for determination of mycotoxins (data from RJ "Chemistry").

Conducted scientometric analysis clearly indicates a sharp increase of interest in the analysis of mycotoxins in recent years. Among the published works 70% are allocated to the share of physical and chemical methods for determination of aflatoxin B1. For determination of aflatoxins and other mycotoxins HPLC (hight pressure liquid chromatography) with fluorescence detection in combination with concentration and purification by immunoaffinity columns or solid sorbents[1], HPLC with photometric detection[2], and amperometric one[3] are primarily used. In recent years, mass spectrometric detection is often used[4,5]. For determination of aflatoxins various immunochemical methods are applied: solid-phase enzyme immunodetection[6], immunoaffinity chromatography[7], biosensors[8,9], polarization fluorescence immunoassay[10]. Thin layer chromatography is also used for determination of aflatoxin B1 and its metabolites. Detection is based on their own fluorescence launched by ultraviolet radiation[11]. Purification by immunoaffinity columns allows further conducting direct fluorescent determination of aflatoxins[12]. All of the above methods are often complicated by long sample preparation, high toxicity of the solvents and the cost of equipment, which is not always suitable for serial analysis.

According to authors' opinion, promising methods for determining mycotoxins are electrochemical methods of analysis due to its high sensitivity and selectivity. Previously, the ability of organic substances on the electrochemical oxidation of various types of carbon electrodes was used to develop methods of quantitative chemical analysis of water-and fat-soluble vitamins, antibiotics, phenols and diols in pharmaceuticals, dietary supplements, cosmetics and foods.

Publication contributions to defining aflatoxin B1 by electrochemical methods are few[13-16]. It is shown[13] that an amperometric detector with a glassy carbon electrode at a potential of 1.44 allows determining aflatoxin B at $7 \cdot 10^{-9}$ g (B2, G2), 10^{-8} g (B1, G1). Aflatoxin B1 in food is also determined by reduction voltammetry at a mercury dropping electrode[14]. Other scientists use amperometric biosensors based on planar platinum electrodes and immobilized cholinesterase in process concentration of $1 \cdot 10^{-5\ (-6)} - 1 \cdot 10^{-10\ (-11)}$ mol/l [15] for electrochemical determination of aflatoxin B1 and other mycotoxins. Cholinesterase biosensor, modified with carbon nanotubes, can shift an interval of determined mycotoxins concentrations in the direction of lower concentrations. In[16] for determining aflatoxin B1 and G1 at their joint presence a method of differential polarography is proposed. It was established that aflatoxin B1 and G1 formed peaks ($E_{1/2}$ -1.33 and – 1.25 ± 0.02V relatively to AgCl anode) on the oscillopolarogram in a background solution of 0.1 M (CH_3) 4NBr and LiCl, containing 40% of CH_3OH. The sample containing aflatoxins B1 and G1 was dissolved in 2 ml of CH_3OH, 3 ml of the background solution were added, and then, the sample was polarographed from -1.0 to -1.5 V at 25 ± 1°C. Thus, based on the literature review, it has been shown that currently a very topical question is the development of fast, economical and highly sensitive techniques for measuring mass concentration of aflatoxin B1 in various objects.

Therefore, the aim of the work was to study the ability of determining aflatoxin B1 on different types of electrodes, followed by selection of operating conditions and development of voltammetric methods of its quantification.

2. Material and methods

Voltammetric measurements were carried out on indicator carbonaceous electrodes: a graphite electrode impregnated with polyethylene with paraffin in vacuum, and a glassy carbon electrode; a saturated silver chloride electrode as a reference electrode.

Process solutions (certified mixtures) were prepared from a standard sample of aflatoxin B1 in the mixture of benzene and acetonitrile (98:2) (GSO 7936-2001) dissolving in C_2H_5OH. As a supporting electrolyte 0.1 M Na_2HPO_4 and 0.1 M (NH_4) $2SO_4$ were used, and a universal buffer mixture of Britton-Robinson.

3. Results and Discussion

In its properties aflatoxin B1 refers to a group of furocoumarins and contains lactone in the molecule, carbonyl, a methoxy group, a benzene ring and an isolated double bond. Aflatoxin B1 is converted by chemical modification to an epoxide, which is carcinogenic, and causes the development of liver cancer.

Aflatoxin B1 is readily soluble in moderately polar organic solvents (acetone, chloroform, dichloromethane, dimethyl sulfoxide, ethanol, isopropanol), but is not soluble in ether. In benzene and chloroform aflatoxin B1 is stored in the dark for several years. As a solvent ethanol, which has high polarity, market availability and low cost, has been selected.

Different types of carbon electrodes to select an indicator electrode were used: a graphite electrode impregnated with polyethylene with paraffin in vacuum, and a glassy carbon electrode. Using these electrodes is conditional on their high chemical and electrochemical stability, absence of toxic mercury, a wide range of process potentials, as well as simplicity of mechanical surface renewal.

The highest value of an analytical signal, the smallest value of a residual current and better reproducibility of signals were observed on the voltammogram on a glassy carbon electrode which had been chosen as working one.

In order to obtain an analytical signal aqueous saline supporting electrolytes, composition of which was chosen on the basis of experimental data, were researched. An analytical signal of aflatoxin B1 was obtained on the following background solutions: 0.1 M Na_2HPO_4, 0.1 M $(NH_4)_2SO_4$ and a universal buffer mixture of Britton-Robinson. Figure 2 shows a voltammogram of aflatoxin B1 obtained on the supporting electrolyte of 0.1 M $(NH_4)_2 2SO_4$.

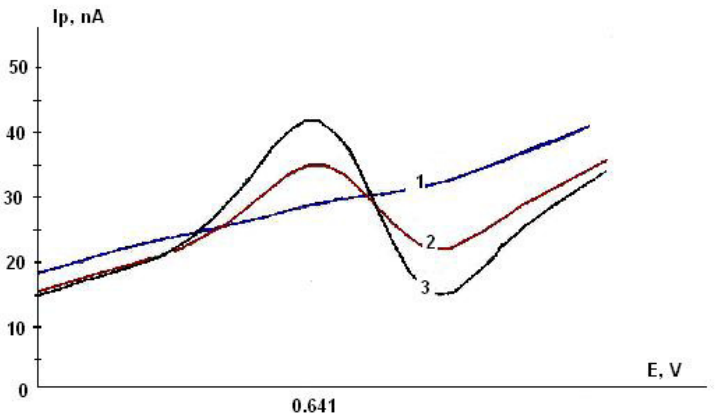

Figure.2.Voltammogramm of electrooxidation of aflatoxin B1:
(IE - TMN, supporting electrolyte, 0.1 M $(NH_4)_2SO_4$):
1 – background solution; 2 - sample C_{afxDB1} = 0.005 mg/dm^3;
3 - additive C_{afxB1} = 0.01 mg/dm^3.

As shown in Figure 2 on the voltammogram (Fig. 2, Curve 2), at a potential of E = 0.641 V, there is a well defined peak, which when an additive of certified aflatoxin B1 is introduced into increases in proportion to the entered concentration (Fig. 2, Curve 3) that indicates the possibility of quantification.

Furthermore, Table 1 shows the values of the peak capacity of Aflatoxin B1 in the supporting electrolyte. The presented data show that the most lightweight electrochemical process of aflatoxin B1 on TMN occurs in 0.1 M $(NH_4)_2SO_4$ of pH 3 - 4.

Table 1. Peak Potentials of aflatoxin B1 on different supporting electrolytes.

Peak potential, E_{pB1}, V	0.64±0.03	0.59±0.01	0.41±0.02
Supporting electrolyte	0.1 M $(NH_4)_2SO_4$ (pH 3 – 4)	0.1 M Na_2HPO_4 (pH 4 – 5)	buffer mixture of Britton-Robinson (pH 5.33)

Each of these supporting electrolytes can be used for determination of aflatoxin B1. However, the best supporting electrolyte is 0.1 M $(NH_4)_2SO_4$, at which well-reproducible electrooxidation peaks are recorded and linear dependence of calibration curves is stored in a wide range of concentrations (Fig. 3).

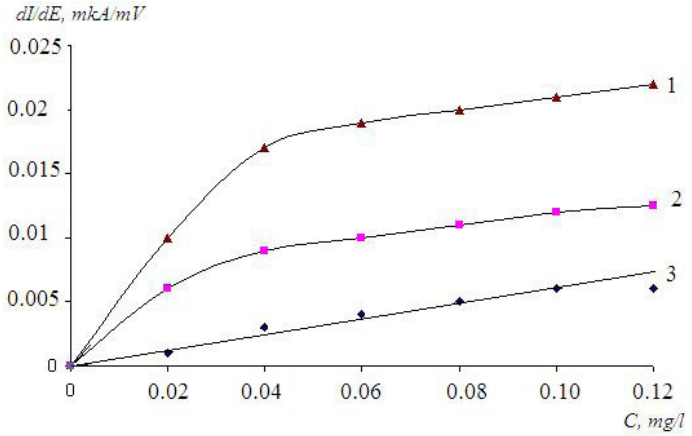

Figure.3.Calibration dependences of electrooxidation of aflatoxin B1 on different supporting electrolytes ont GCE:
1 - 0,1 M Na_2HPO_4;
2 - Britton-Robinson universal buffer mixture (pH 5.33);
3 - 0.1M $(NH_4)_2SO_4$; w = 30 mV/s.

However, a more sensitive analytical signal was obtained for 0.1 M Na_2HPO_4 (Fig. 3). Therefore, in the range of low concentrations (up to 0,015 mg/dm^3) it is promising to use 0.1 M Na_2HPO_4 (Fig. 3, Curve 1) as a supporting electrolyte or Britton-Robinson universal buffer mixture (pH 5.33) (Fig. 3, Curve 2) for a maximum current value of the analytical signal of aflatoxin B1 in this concentration range.

An important factor in voltammetric detection influencing electrooxidation of aflatoxin B1 is pH of the medium.

It is known that in aqueous solutions organic compounds can exist in different forms (cationic, anionic or neutral). We have investigated the effect of pH on the analytical signal of aflatoxin B1, as mechanism and flow rate of the electrode process in voltammetric determination of aflatoxin B1 is strongly dependent on pH. Dependence of peak potential of electrooxidation of aflatoxin B1 is shown in Figure 4.

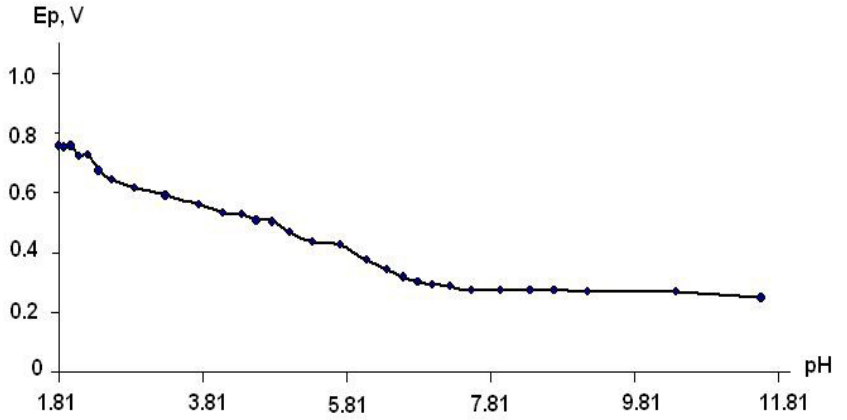

Figure.4. Dependence of peak potential electrooxidation of aflatoxin B1 on pH at TMN, C_{afxB1} = 0.005 mg/dm^3, τ_e = 30 s; w = 30mV/s; background solution - universal buffer mixture Britton-Robinson.

Figure 4 shows that with increasing concentration of hydroxide ions there is a shift of a peak potential in a cathode area that relieves the process of electrooxidation of aflatoxin B1, which is associated with a stepped reaction of deprotonation of various forms of aflatoxin B1. Deprotonation stage may precede the stage of electron transmission from a depolarizer molecule to the electrode or run concurrently with it.

With increasing supporting electrolyte pH the shape of finding the substance in a solution, determined by protolytic equilibria, changes. As electron isolation from a neutral particle requires less energy than that of the cation, the peak potential of aflatoxin B1 in a neutral medium where neutral molecules are predominant, is lower than in acidic one. pH values at the points of intersection of the extensions of straight sections of experimental dependence of the peak potential on pH correspond to the effective dissociation constants pK_1 = 2.21 and pK_2 = 7.0.

Thus, based on these results process conditions for voltammetric measurements of aflatoxin B1 have been suggested and shown in Table 2..

Table 2. Process conditions for voltammetric determination of aflatoxin B1.

Parameters of measurement	Parameter value
Background electrolyte	0.1 M $(NH_4)_2SO_4$
The system used	
electrodes: • Working • Reference • Auxiliary	 GCE SCE SCE
The range of potentials, V	0.0… +1.1
The rate of linear variation of the potential, mV s	30
Peak potential, V	0.64 ± 0.03
Polarazing voltage for electroconcentration, V	0.0

Based on these studies a technique of aflatoxin B1 measurement performance was proposed and by the example of model solutions validation of the technique was performed by a standard addition method. The model solution consisted of distilled water with a various addition of standard solution of aflatoxin B1. Validation results are shown in Table 3.

Table 3. Validation of standard addition method.

Sample	Concent of aflatoxin B_1, mg/dm^3		
	Test	Added	Obtained
Model solution №1	0.019±0.002	0.02	0.039±0.003
Model solution №2	0.015±0.004	0.04	0.055±0.004

4. Conclusion

Thus, on the basis of the research the possibility of determining aflatoxin B1 on GC electrode for the first time was demonstrated. Of a series of supporting electrolytes the most favorable for the linear range of detectable concentrations of aflatoxin B1 - 0.1 M of $(NH_4)_2SO_4$ were identified.

The effect of supporting electrolyte pH on the analytical signal of aflatoxin B1 was studied. It has been shown that the signal at pH 5.33 has a more pronounced peak with a maximum value of current and there is a shift in the peak potential to the cathode area with pH transition to the acidic one.

On the basis of the research process conditions measuring the analytical signal of aflatoxin B1 were selected and an algorithm for its voltammetric determination was suggested. At the moment the authors have been developing methods of quantitative chemical analysis of aflatoxin B1 in foods, feeds and feed additives.

Acknowledgments

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