

Article

Development of a Voltammetric Methodology Based on a Methacrylic Molecularly Imprinted Polymer-Modified Carbon-Paste Electrode for the Determination of Aflatoxin B1

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Abstract: Aflatoxin B1 (AFB₁) is one of the most dangerous mycotoxins found in food, necessitating the development of precise and reliable methodologies for its detection. In this study, a novel electrochemical sensor based on a molecularly imprinted polymer (MIP) integrated with a carbon-paste electrode was developed for the voltammetric determination of AFB₁. The innovative aspect of this work lies in the use of methacrylic acid (MAA) as the functional monomer, which enhances the sensor's selectivity and binding affinity. The developed electrochemical sensor exhibited a linear response range from 20.8 to 80 ng/L, with a limit of detection (LOD) of 2.31 ng/L and a sensitivity of 19.83 $\mu\text{A (ng/L)}^{-1} \text{ cm}^{-2}$. The sensor demonstrated outstanding analytical performance, with reproducibility and repeatability yielding relative standard deviations (RSDs) of 3.24% and 1.41%, respectively. To validate the sensor's practical applicability, its performance was tested in real samples of corn and wheat using the standard addition method. Samples were prepared following official Mexican standard methods. Detected AFB₁ concentrations were 0.0147 $\mu\text{g/L}$ and 0.0138 $\mu\text{g/L}$ for corn and wheat, respectively. A statistical comparison using the Student's *t*-test confirmed no significant matrix effects, underscoring the high selectivity and accuracy of the MIP-modified sensor. This work introduces a highly selective, sensitive, and reproducible methodology for AFB₁ detection, which could significantly advance food safety monitoring.

Keywords: mycotoxins detection; food safety; electroanalytical; MIP-based electrodes; electrochemical sensors



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1. Introduction

Aflatoxin B1 (AFB₁), a potent carcinogenic mycotoxin produced by certain species of *Aspergillus*, poses significant health risks in contaminated food and agricultural products [1]. Its presence in low concentrations can have severe implications for food safety and public health [2], necessitating reliable and sensitive detection methods.

In recent years, analytical methods for Aflatoxin B1 detection have involved the use of techniques such as chromatography (HPLC and GC), and immunoassays (e.g., ELISA), which are considered standard methods by several regulatory agencies [3]. While these methods show high sensitivity and selectivity, their high cost and time-consuming sample preparation and analysis are significant barriers to their widespread use in real-time

analysis and in situ analysis in farms and food-processing companies. As a result, innovative approaches utilizing modified electrochemical sensors have been developed as faster and more cost-effective solutions for detecting AFB₁ in complex food samples [4]. These advanced sensors incorporate a variety of materials, such as nanomaterials [5–8] or several types of organic and inorganic materials [9–11], to enhance analytical performance. However, achieving high selectivity towards AFB₁ in complex sample matrices remains a critical challenge. In this respect, these materials have been constantly explored in conjunction with aptamers or antibodies [12–15] in order to provide selectivity and sensitivity to aflatoxins. Nevertheless, the use of biological agents as molecular recognition sites results in difficult immobilization on the electrode surface and sensors with poor stability, which limits its practical applications. Therefore, there is an intensive quest for novel materials with specific pore sizes or recognition capabilities to be used in the design of more stable and reliable electrochemical sensors for mycotoxin detection.

Molecular imprinted polymers (MIPs) are synthetic polymers that are engineered to have specific binding sites, which are essential in the recognition of organic molecules or metallic ions [16]. Their synthesis involves a template molecule and a functional monomer, which are polymerized in the presence of a cross-linking agent and an initiator. After removing the template, the resulting polymer contains cavities that are complementary in shape and functional groups to the target analyte, allowing for precise molecular recognition [17]. The recognition mechanism of MIPs is exclusively based in a “lock and key” system to selectively bind targets in complex matrices. Since they were first reported [18], MIPs have been widely used in the design of electrochemical sensors, separation processes and gravimetric analysis. Compared with biological recognition agents, MIPs do not require specific chemical conditions to be used; hence, they can be applied at elevated temperatures and in highly acidic or alkaline mediums [19]. Moreover, MIPs can be designed for detecting any kind of small organic molecules or metallic ions using the same starting materials, whereas antibodies or aptamers, on the other hand, need to be specifically produced for the target, which is costly and complicated. Therefore, MIPs have recently attracted attention for their application in food security, especially in detecting mycotoxins in food [20,21].

On the other hand, carbon-paste electrodes (CPEs) are advantageous platforms for sensing applications due to their exceptional electrical conductivity, ease of modification of the composite mixture, and cost-effectiveness [22,23]. Their modification with certain materials modifies the specific surface area of CPEs enhancing their target-binding properties [24,25].

The modification of carbon-paste electrodes (CPEs) with molecularly imprinted polymers (MIPs) can be achieved via two methods: in situ and ex situ. The in-situ method involves the electrosynthesis of the MIP directly on the electrode surface. In contrast, the ex-situ method entails the chemical synthesis of the MIP separately, which is then combined with graphite and Nujol or mineral oil in a specific ratio to produce the modified carbon paste. Integrating molecularly imprinted polymers (MIPs) with CPEs allows for the creation of highly specific sensors that can selectively bind target analytes, improving detection accuracy and reliability [26]. Additionally, MIPs offer the benefit of high stability and reusability, further enhancing the practical application of these modified electrodes in various analytical scenarios.

Therefore, inspired by previous reports, we have developed a novel electrochemical sensor for AFB₁ detection. This sensor involves the ex-situ modification of carbon-paste electrodes with a molecularly imprinted polymer (MIP) based on methacrylic acid, specifically imprinted for AFB₁. The carbon paste provides a conducive matrix for electron transfer [27], while the MIPs introduce molecular recognition capabilities, allowing for the selective binding of AFB₁ [28]. This synergistic combination aims to enhance the sensor's sensitivity and specificity, making it suitable for detecting trace amounts of AFB₁ in complex samples such as foodstuffs and agricultural products. The performance of the MIP-modified CPE sensor was evaluated in terms of its analytical characteristics, including sensitivity, selectivity, and reproducibility. By leveraging the unique properties of MIPs

and the favorable electrochemical characteristics of carbon paste, this approach seeks to advance the development of reliable, efficient, and user-friendly sensors for the detection of Aflatoxin B₁.

2. Materials and Methods

2.1. Reagents

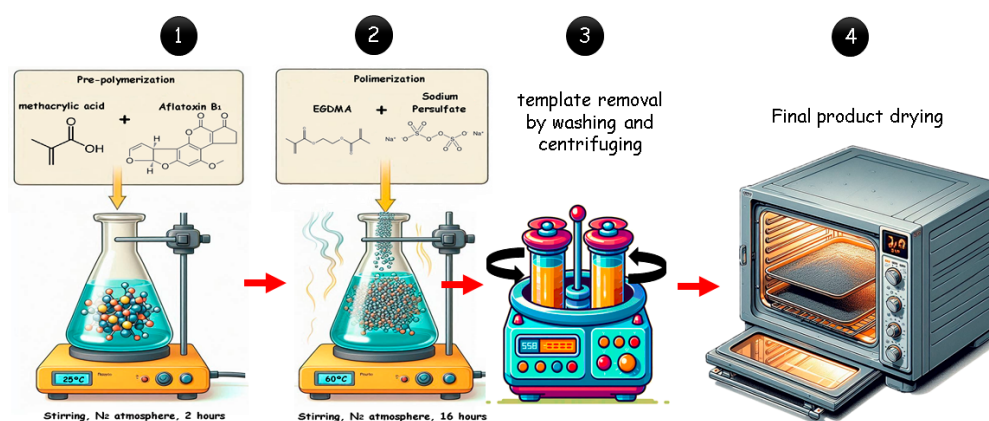
Methacrylic acid (C₄H₆O₂, 99% purity), ethylene glycol dimethyl acrylate (C₉H₁₄O₄, 98% purity), sodium persulphate (Na₂S₂O₈, 98% purity), graphite powder (45 µm, 99.9% purity), mineral oil, Aflatoxin B₁ (98% purity) and an Aflatoxin B₁-certified reference material were acquired from Sigma-Aldrich and used without further purification. High-purity water, used to prepare all standard solutions, was obtained with a Milli-Q water purification system.

2.2. Instrumentation

FT-IR spectra were acquired in a Perkin-Elmer Spectrum 100 device. Scanning electron microscopy (SEM) images were obtained using an EmCrafts Cube II SEM. X-ray photoelectron spectroscopy (XPS) analyses were performed in an ultra-high vacuum (UHV) system scanning XPS microprobe—a PHI 5000 VersaProbe II—with a monochromatic Al Kα X-ray source ($h\nu = 1486.6$ eV) and a 200 µm beam diameter, and an MCD analyzer.

2.3. Synthesis of the MAA-MIP

The molecularly imprinted polymer based on methacrylic acid was synthesized in a two-step procedure in an N₂ atmosphere (Scheme 1). Firstly, a pre-polymerization stage was performed by reacting 6 mol of methacrylic acid and 2.5×10^{-8} mol of AFB₁ in 20 mL of methanol for two hours at room temperature. Subsequently, the polymerization stage was started by adding 20 mol of ethylene glycol dimethyl acrylate and 4.2×10^{-3} mol of sodium persulfate. The resulting mixture was heated at 60 °C for 16 h with constant stirring. The obtained white precipitate was centrifuged, rinsed with methanol, and dried at 80 °C for 12 h. The final product was characterized by FT-IR, XPS, and SEM. A non-imprinted polymer (MMA-NIP) was also obtained with the same procedure but without adding AFB₁ in the pre-polymerization stage.



Scheme 1. General synthesis procedure for the MAA-MIP.

2.4. Construction of the Modified Carbon-Paste Electrodes

The carbon-paste electrodes were prepared using composite mixtures with different proportions of the MIP: 5%, 10%, and 15% by dry weight (Scheme 2). For each composite, the respective amount of MIP was mixed with graphite powder to total 100% by weight. To each mixture, five drops of mineral oil (used as binder agent) were added for every 0.3 g of the composite. The resulting composite mixtures were then packed into 4 cm supports made from insulin syringe tubes. A 2 mm banana plug connector was placed at the end of the electrode and used as electrical contact with the galvanostat/potentiostat.



Scheme 2. Modified carbon-paste elaboration procedure.

2.5. Electrochemical Measurements

Electrochemical evaluations were performed in triplicate using an Autolab-PGSTAT302N galvanostat/potentiostat, configured with a three-electrode system. In this setup, an Ag/AgCl (saturated KCl, 3M) electrode was used as the reference electrode, a 6 mm diameter graphite rod served as the counter electrode, and the modified electrode functioned as the working electrode. A Britton–Robinson buffer was utilized as the supporting electrolyte.

3. Results and Discussion

3.1. Characterization of the MIP and the Modified Electrode

The infrared (IR) characterization of the molecularly imprinted polymer (MIP) was performed and compared with the non-imprinted polymer (NIP) to elucidate the differences in their chemical structures. The IR spectra of both MIP and NIP, as depicted in Figures S1–S3, reveal common functional groups inherent to their polymeric structures.

A prominent characteristic stretching band for the hydroxyl group (--OH) is observed at around 3500 cm^{-1} . This peak is indicative of hydroxyl groups, which are typically present in methacrylic acid-based polymers, suggesting there were hydrogen bonding interactions within the polymer matrix. The spectra also show typical stretching vibrations corresponding to the C–H bonds in the structural alkenes of the polymer. These appear at around $2950\text{--}2980\text{ cm}^{-1}$, signifying the presence of aliphatic C–H stretches. Complementary bending vibration bands are observed at around $1380\text{--}1450\text{ cm}^{-1}$, further confirming the alkane characteristics within the polymer backbone.

The presence of a characteristic carbonyl group (--C=O) band is observed in all spectra at 1720 cm^{-1} , and it is associated with free carboxylic acids present in terminal parts and ramification within the structure of polymers based on methacrylic acid [29,30]. Additionally, the bending bands corresponding to the C–O bond suggest the presence of esters and ethers within the polymeric matrix. These bands are notably more intense in the NIP compared to the MIP. This disparity indicates a potential interaction between the MIP and the template molecule, which may have led to a different structural configuration in the MIP. It is possible that the imprinting process created specific cavities within the MIP, resulting in fewer exposed C–O groups due to the occupancy or influence of the template compared with the NIP.

Furthermore, bending bands of the alkenyl group --C=C-- in the structure are confirmed by bands appearing between $600\text{ and }870\text{ cm}^{-1}$. Notably, only the unwashed MIP exhibits weak bands around $2000\text{--}2100\text{ cm}^{-1}$, which is related to the presence of AFB_1 .

XPS analysis was used to confirm the changes in the elemental composition of the carbon-paste (CP) composite after its modification with the MIP. As seen in Figure 1, the spectrum of the CP only presented a dominant peak at 286 eV, corresponding to the graphite C–C bond, which is characteristic of graphite [31]. On the other hand, the XPS spectra of the modified CP showed not only the presence of the C–C peak but also a smaller peak at 292 eV,

attributed to C=O groups (Figure 1b), which confirms the presence of carbonyl groups in the MIP [32]. Moreover, an additional peak at 529 eV (Figure 1c), indicative of oxygen (O 1s), was observed, whereas the CP presented a slight and broad peak. These results demonstrate the successful incorporation of the MIP into the carbon-paste composite.

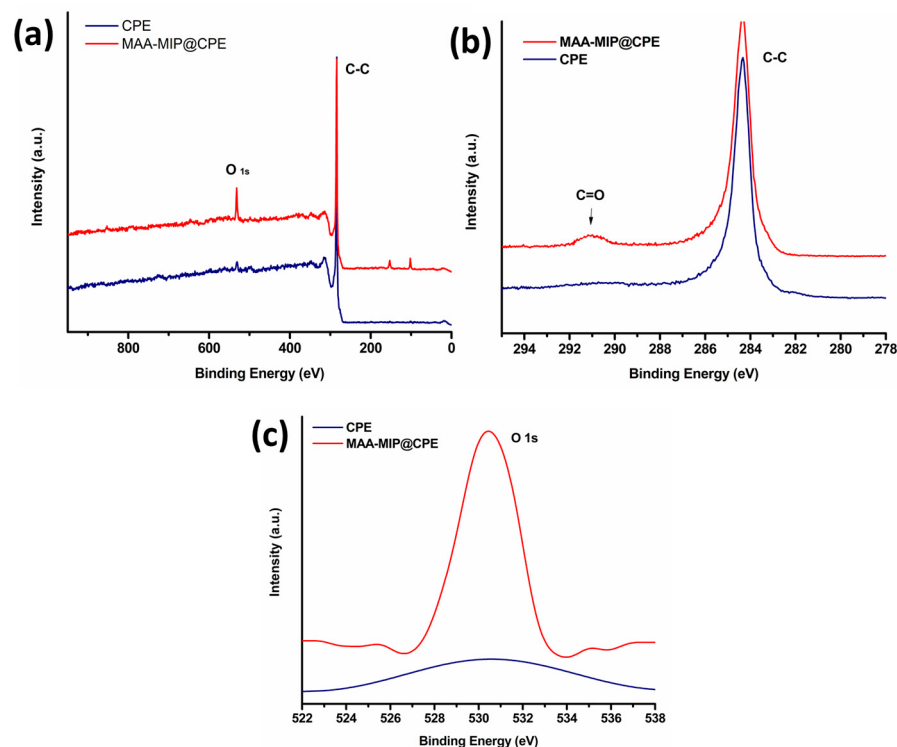


Figure 1. (a) Overall XPS survey spectra of CPE and modified electrode, and XPS spectra centered on (b) C–C and C=O groups and (c) O 1s.

The SEM images of the MIP-based electrode (MAA-MIP@CPE) and a bare CPE were acquired to compare structural changes on the surface of both electrodes. As observed in Figure 2a, the modified electrode presented a rugous surface composed of a spherical-like structure, which is related to the presence of the MIP in the carbon-paste composite, whereas the unmodified CPE (Figure 2b) only presented a smooth surface composed of flake-like structures, which are characteristic of graphite. These significant changes confirm the successful homogeneous incorporation of the MIP on the electrode surface.

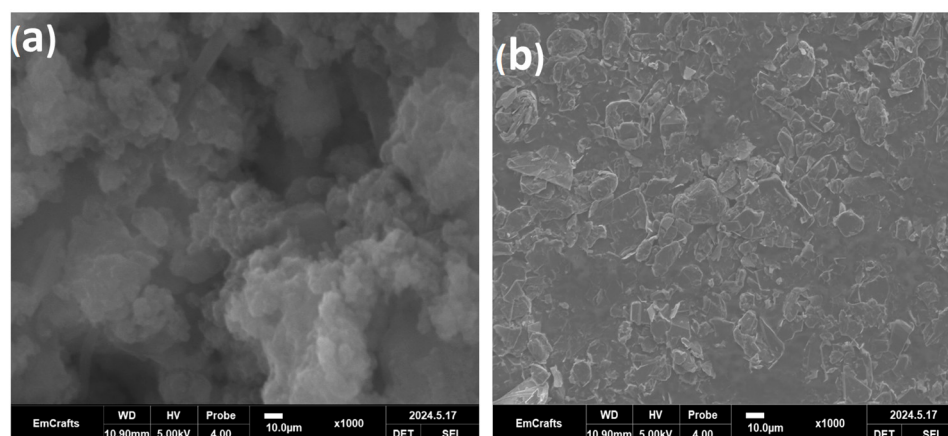


Figure 2. (a) SEM image of MAA-MIP@CPE. (b) SEM image of bare CPE.

3.2. Electrochemical Behavior in the Absence and Presence of AFB₁

To determine the activity of MAA-MIP and MAA-NIP in the absence and presence of AFB₁, a cyclic voltammetry (CV) evaluation was performed in a 0.1 M phosphate buffer solution (pH 8) using a scan rate of 0.1 V/s in cathodic direction in an N₂ atmosphere. Before the measurement, a pre-concentration was performed by placing the modified electrodes in a phosphate buffer solution containing 1 µg/L of AFB₁ for 10 min, under constant stirring at 100 rpm. After this time, the electrode was immersed in the supporting electrolyte, and the corresponding voltammogram was recorded. The results were compared using a bare CPE in the same experimental conditions.

As observed in Figure 3, the bare CPE did not show changes in its electrochemical response in the presence of AFB₁, indicating the null activity of the analyte.

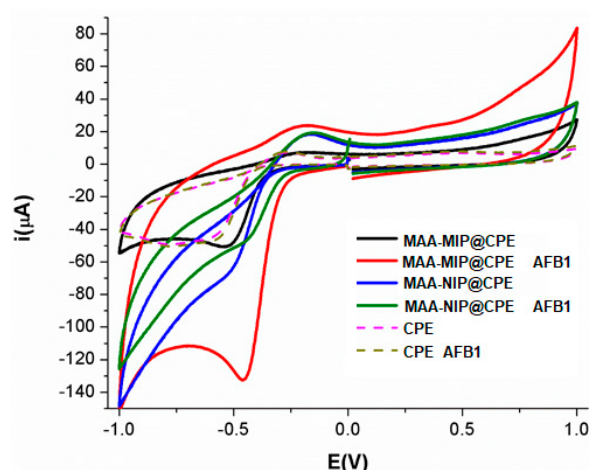
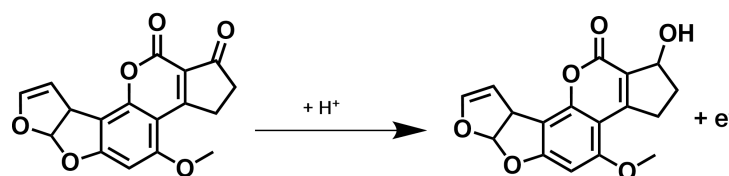


Figure 3. CV studies to achieve the electrochemical performance of the MAA-MIP@CPE, MAA-NIP@CPE, and CPE in the absence and presence of 1 µg/L AFB₁.

On the contrary, in the presence of AFB₁, the MAA-MIP@CPE displayed a drastic modification in the cathodic peak current intensity, with an increase of about 43% compared with the current intensity in the absence of AFB₁. This behavior indicates a remarkable activity of MIP to AFB₁ as a result of the accurate formation of templates in the polymeric structure, which favors specific interactions between the molecularly imprinted polymer (MIP) and AFB₁. In this regard, the proposed MIP is designed to possess specific binding sites that are complementary in shape and size to the AFB₁ molecule. When AFB₁ is present in the solution, it selectively binds to these tailored sites within the MIP, facilitating its concentration on the electrode surface and the reduction process. Posteriorly, during the cyclic voltammetry (CV) experiment, an increase in the cathodic peak current is observed as a consequence of the reduction of concentrated AFB₁ to aflatoxicol, leading to a more pronounced cathodic peak around −500 mV [33]. This reduction process involves the transfer of one proton and one electron and is summarized in Scheme 3.



Scheme 3. Electro-reduction of AFB₁ to aflatoxicol.

The electrochemical response presented by MAA-NIP@CPE, on the other hand, showed a slight increase in the cathodic current intensity. Although this current increase could be related to a possible interaction between the analyte and the functional groups in NIP, the electrochemical signal obtained is not as pronounced or specific as that observed with the

MIP. This suggests that the MIP provides a more selective and stronger binding affinity towards AFB₁, leading to a higher sensitivity and better analytical performance in detecting the target analyte compared to the non-imprinted polymer.

3.3. Effect of pH on the Electrochemical Response

To investigate the influence of the pH of the supporting electrolyte on the cathodic peak intensity associated with the reduction of AFB₁, and to identify the optimal electrochemical response, cyclic voltammetry was conducted in the presence of 1 µg/L of AFB₁, considering a pH range from 8 to 11 and using 0.1 V/s as the scan rate, a preconcentration time of 10 min, and stirring at 100 rpm. As observed in Figure 4, the CV evaluation displayed a gradual modification in the cathodic peak intensity, which decreased from −32.9 µA (pH 8) to −38.4 µA (pH 10), whereas at a higher pH (pH 11), the current intensity becomes less negative. This behavior is attributed to changes in the conformation of the polymeric matrix upon pH alteration and the deprotonation of terminal carboxylic acids in the polymer, which modifies the cavities within the structure; consequently, these modifications affect the polymer's ability to effectively capture and interact with the target analyte [34]. As a result, pH 10 showed a superior response compared with other values. Based on this finding, pH 10 was selected as the optimal condition for subsequent evaluations.

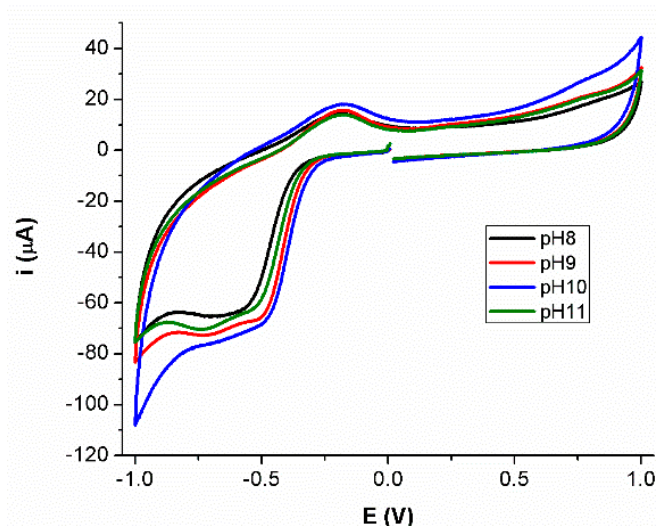


Figure 4. CV curves at different pH in the presence of 1 µg/L AFB₁ using the MAA-MIP@CPE.

3.4. Effect of Stirring Rate and Time in the Pre-Concentration Stage

During the preconcentration stage, the time and stirring frequency are critical parameters for optimizing the sensitivity and selectivity of the MIP-based CPE. The preconcentration time directly influences the amount of analyte accumulated on the MIP-modified electrode, thereby affecting the sensor's sensitivity. Also, the stirring frequency governs the efficiency of the analyte interaction with the MIP at the electrode surface. To determine the optimal parameters for the preconcentration stage, CV evaluations were conducted considering a concentration of 1 µg/L AFB₁ at pH 10. As observed in Figure 5a, the modification of the stirring rate directly influences the behavior of the cathodic peak intensity related to the analyte. The lowest value studied (50 rpm) presented the best current intensity (−62.6 µA), which increases gradually to −38.4 µA at 300 rpm. Moreover, the preconcentration time also exerted a significant effect on the peak intensity. In this regard, loading times over 10 min showed no significant difference in the electrochemical signal, resulting in a maximum current intensity (−57.3 µA). Shorter loading times (5 min) drastically modify the cathodic peak intensity, displaying a minimum current intensity of −37 µA. In this context, the rotational frequency and the time involved in the loading process of AFB₁ into the MIP are shown to be crucial parameters that enable the proper diffusion of the analyte

into the polymer cavities. Therefore, a stirring rate of 50 rpm and a loading time of 10 min were selected as the optimal values.

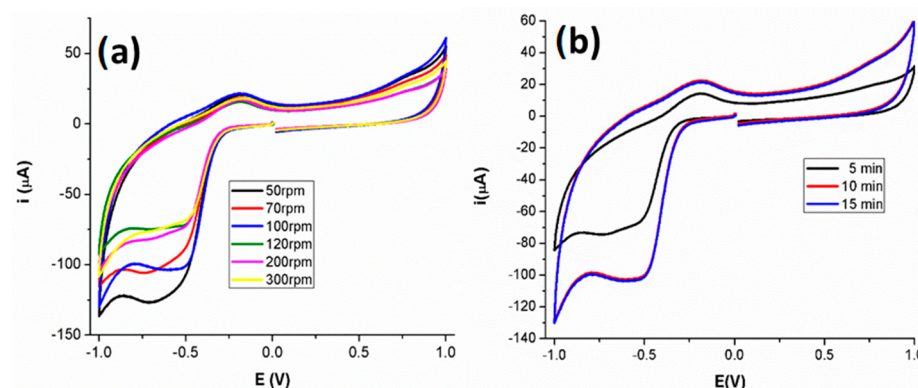


Figure 5. CVs obtained using the MAA-MIP@CPE in the presence of 1 $\mu\text{g/L}$ AFB₁ at pH 10 after (a) 10 min pre-concentration and different stirring rates, and (b) different pre-concentration times using a stirring rate of 50 rpm.

3.5. Optimization of the MIP Ratio in the Composite Mixture

The effect of the MIP ratio ($w/w\%$) on the electrochemical response to AFB₁ in the modified composite was conducted by CV considering the previously determined optimal parameters. As illustrated in Figure 6, by varying the MIP ratio from 5% to 15%, we observed a modification of the cathodic peak intensity, which ranges from -6.5 to $-73.1 \mu\text{A}$. However, when the MIP ratio increases to 16% w/w , we observed a 23% decrease in the cathodic current intensity. Moreover, MIP ratios above 16% did not present a favorable response to AFB₁; this is attributed to the low conductivity of the MIP, which increases the electrode resistance and consequently causes a significant reduction in the electroanalytical signal [35,36]. Based on these findings, a 15% MIP ratio was selected as the optimal proportion for the design of modified electrodes.

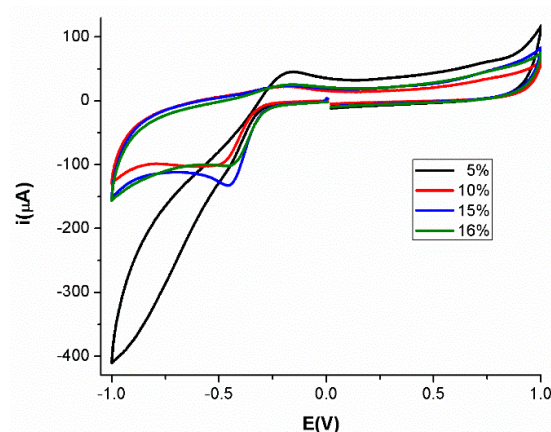


Figure 6. CVs obtained in a 1 $\mu\text{g/L}$ solution of AFB₁ considering different proportions of the MIP in the MAA-MIP@CPE.

3.6. Electroactive Surface Area Determination

The electroactive surface areas of a bare CPE and the MAA-MIP@CPE were obtained by CV at different scan rates using a 0.1 M solution of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 0.1 M of KCl, according to the literature [37]. The proper statistical analysis of the plot of the cathodic peak intensity vs the square root of the scan rate (Figure 7) was used to calculate the electroactive area considering the Randles–Sevcik equation [38–41]:

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2} \quad (1)$$

where A represents the electroactive area (cm^2), D is the $\text{K}_3[\text{Fe}(\text{CN})_6]$ diffusion coefficient ($6.70 \pm 0.02 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), n is the number of electrons involved in the redox reaction for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($n = 1$), and C is the $\text{K}_3[\text{Fe}(\text{CN})_6]$ concentration. The obtained electroactive areas were 0.056 cm^2 for the unmodified CPE and 0.0226 cm^2 for the MAA-MIP@CPE, as a consequence of the low conductivity of MIP, which reduces the available conductive area of the electrode [42,43].

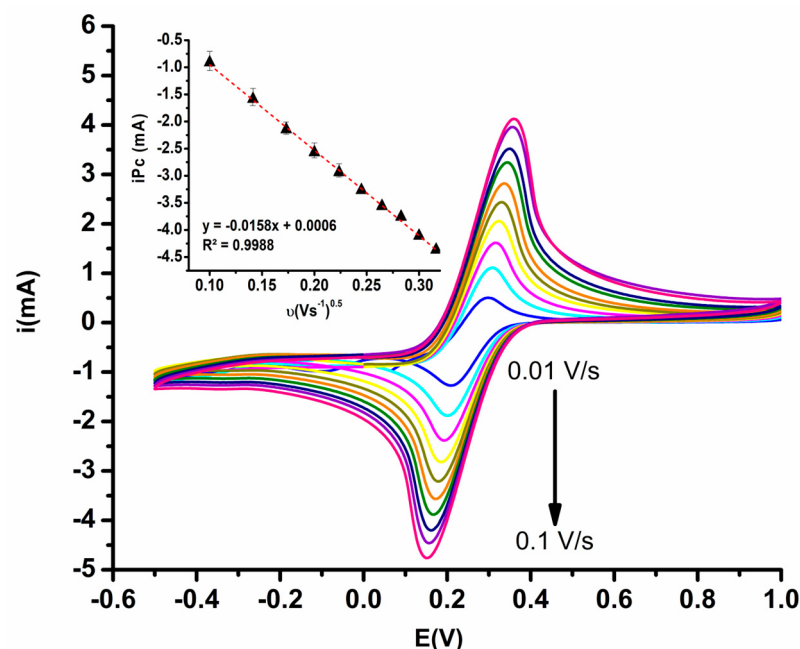


Figure 7. Plot of cathodic peak intensity as a function of the square root of scan rate, the different voltammograms analyzed can be observed in different colors.

3.7. Analytical Performance of the Proposed Methodology for the Determination of AFB_1

The effect of AFB_1 concentration on the electrode was assessed using cyclic voltammetry under previously optimized conditions of pH, preconcentration time, and MIP proportion in the modified electrode. As depicted in Figure 8, there is a notable absolute increase in the cathodic peak current as a function of AFB_1 concentration, spanning a range of 7.01–80 ng/L; at higher concentrations, the current intensity did not present relevant changes, probably because of the saturation of the electrode surface, which limits an accurate measurement.

Through the proper statistical analysis using least-squares regression ($R^2 = 0.9957$) and considering the recommendations of the IUPAC [44], the limit of detection (LOD) and sensitivity were calculated considering the following equations:

$$\text{LOD} = 3.3 \sigma / S \quad (2)$$

$$\text{LOQ} = 10 \sigma / S \quad (3)$$

$$\text{Sensitivity} = S / A \quad (4)$$

where σ represents the typical error, S is the slope of the calibration curve, and A is the electrochemical surface area. The evaluation results in a value of 2.31 ng/L for the LOD, with a linear range from 20 to 80 ng/L and a sensitivity of $19.83 \mu\text{A} (\text{ng/L})^{-1} \text{ cm}^{-2}$. A comparison of the analytical parameters of the proposed electrode with other voltammetric methodologies (Table 1) demonstrates a competitive LOD and the sensitivity for real sample analysis.

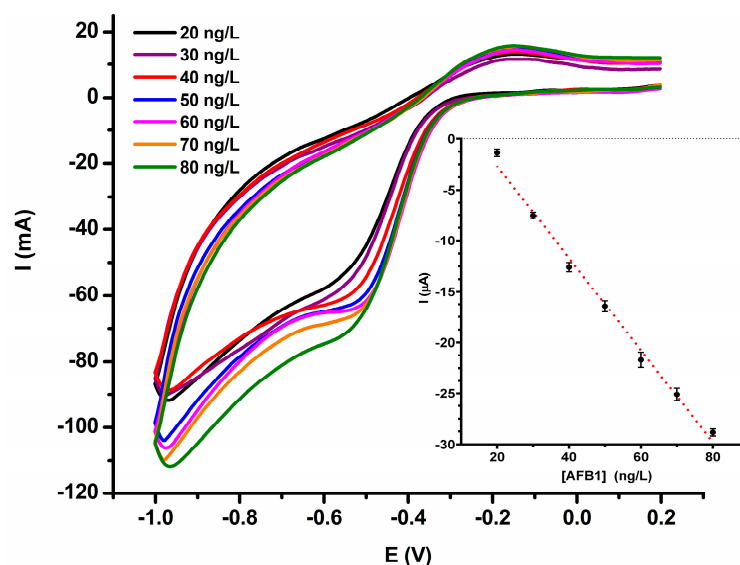


Figure 8. Calibration curve obtained using the MAA-MIP@CPE electrode and different AFB1 concentrations.

Table 1. Summary of analytical parameters reported for different voltametric methodologies.

Electrode	LOD (ng/L)	Sensitivity	Ref.
PANI-MIP@ITO	0.313	$0.07 \mu\text{A (ng/L)}^{-1} \text{ cm}^{-2}$	[45]
Bi film	11.2	$0.0132 \mu\text{A (ng/L)}^{-1}$	[46]
Au/Deep Eutectic Solvent	50	$111.8 \mu\text{A (ng/L)}^{-1}$	[47]
Hg drop	150	$6.22 \mu\text{A (ng/L)}^{-1}$	[48]
Screen Printed CE	7	$0.004 \mu\text{A (ng/L)}^{-1}$	[49]
MAA-MIP@CPE	2.31	$19.83 \mu\text{A (ng/L)}^{-1} \text{ cm}^{-2}$ $0.45 \mu\text{A (ng/L)}^{-1}$	This work

3.8. Reproducibility and Repeatability

The reproducibility of the electrode fabrication process was evaluated by constructing five different electrodes under identical conditions. Each electrode was used to measure a 50 ng/L solution of Aflatoxin B1 (AFB1) in triplicate (Figure 9a). The cathodic current intensities obtained were then compared using a one-way ANOVA (Table 2). The analysis revealed no statistically significant difference ($p > 0.05$) in the responses of the electrodes, indicating no variability in electrode performance.

Table 2. One-way ANOVA results of the five evaluated electrodes.

Source of Variation	SS	df	SM	F	p
Between Groups	0.012227	4	0.003057	1.287921	0.338115
Within Groups	0.023733	10	0.002373		
Total	0.035960	14			

On the other hand, the repeatability of the MAA-MIP@CPE electrode was evaluated by CV using a 50 ng/L solution of AFB₁ under the previously optimized conditions, with five replicates. As depicted in Figure 9b, the measurements show no significant variations in the cathodic peak intensity, despite renewing the electrode surface before each measurement. The obtained relative standard deviation (RSD) was 1.41%, indicating exceptional reproducibility in the analysis of AFB₁. The overall consistency in electrochemical responses across the different electrodes supports the reliability and robustness of the sensor, demonstrating its precision in AFB₁ evaluation.

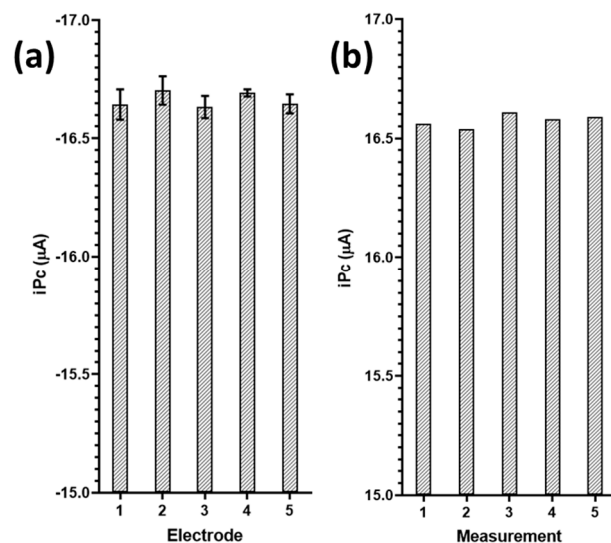


Figure 9. (a) Reproducibility of the analytical response to AFB₁ using different MAA-MIP@CPE electrodes. (b) Repeatability of the analytical response to AFB₁ using a single MAA@CPE electrode.

3.9. Analysis of Real Samples

To evaluate the sensor's capability for the determination of AFB₁ in real samples, these were pretreated according to the Mexican standard procedure described in the NOM-247-SSA1-2008: 5 mg of ground and sieved samples were homogenized in 50 mL of 80% methanol to extract the aflatoxins; then, the mixture was filtered and spiked with different AFB₁ concentrations using an AFB₁ certified reference material. The resulting solutions were analyzed by cyclic voltammetry at a scan rate of 0.1 V/considering the optimum conditions previously evaluated. Table 2 summarizes the AFB₁ content in the analyzed samples: AFB₁ content was 0.0147 μg/L in corn, while the wheat sample contained 0.0138 μg/L.

To discard any matrix effects on the proposed electrode, the analysis of real samples (without evidence of AFB₁) spiked with a known concentration of aflatoxin was performed using both the calibration method and the standard addition method and compared by using the Student's *t*-test for two different treatments. In this respect, the standard addition method effectively eliminates matrix effects. Therefore, if the results obtained using the calibration method are statistically equivalent to those obtained using the standard addition method, it confirms that the proposed electrode is selective and not influenced by other molecules present in the samples. From the experimental data collected during the evaluation of the two analysis methods, the test statistics *t_o* were calculated, yielding values of 1.51 and 2.02 for corn and wheat samples, respectively (Table 3). The results indicate that there are no significant differences between the two methods, reinforcing the conclusion that the electrode is not influenced by the matrix components of the samples. Therefore, the analysis using both quantitative methodologies demonstrates that the incorporation of the molecularly imprinted polymer offers accurate selectivity when analyzing real samples.

Table 3. Statistical comparison of calibration and standard addition methods for the determination of AFB₁ in different samples.

Sample	Results Comparison		Statistical Comparison	
	Standard Addition n = 5	Calibration Curve Analysis n = 5	T _o Vs T _{α/2}	
Corn	41.1 ng/L	41.7 ng/L	1.51	2.30
Wheat	42.8 ng/L	43.6 ng/L	2.02	2.30

4. Conclusions

This study developed an innovative electrochemical sensor based on a molecularly imprinted polymer (MIP) integrated with a carbon-paste electrode (CPE) for the voltammetric determination of Aflatoxin B1 (AFB₁). The sensor's novel aspect lies in the exclusive use of methacrylic acid (MAA) as the functional monomer, enhancing selectivity and binding affinity. The sensor exhibited remarkable analytical performance, with a linear response range from 20.8 to 80 ng/L, a limit of detection (LOD) of 2.31 ng/L, a sensitivity of 19.83 $\mu\text{A (ng/L)}^{-1} \text{ cm}^{-2}$, and a relative standard deviation (RSD) for repeatability of 1.41%.

The optimal response to AFB₁ was observed considering a MIP proportion of 15%, a pH of 10, a preconcentration time of 10 min, and constant stirring at 50 rpm to ensure efficient analyte binding. The electroactive area of the sensor was calculated to be 0.0226 cm².

The practical applicability of the sensor was validated through tests on real samples of corn and wheat, using the standard addition method. The detected concentrations of AFB₁ were 0.0147 $\mu\text{g/L}$ in corn and 0.0138 $\mu\text{g/L}$ in wheat. Statistical comparison with the calibration curve using the Student's *t*-test confirmed no significant matrix effects, underscoring the high selectivity and accuracy of the MIP-modified sensor.

Biological-based electrodes have led the way in the development of alternative electrochemical methodologies for the analysis of AFB₁, although they present disadvantages such as instability, sensitivity to temperature and pH changes, and high costs due to the purification of the biological element. This work represents a highly selective, sensitive, and reproducible non-biological methodology for the detection of AFB₁, which could significantly advance food safety monitoring. By leveraging the unique properties of MIPs and the favorable electrochemical characteristics of carbon paste, the non-biological methodology sensor offers a reliable, efficient, and user-friendly solution for detecting trace amounts of AFB₁ in complex food matrices. Future research could focus on further enhancing the sensor's performance and exploring its application in detecting other mycotoxins and contaminants in food and agricultural products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations11080246/s1>, Figure S1: IR spectra of MIP; Figure S2: IR spectra of NIP; Figure S3: IR spectra of MIP loaded with AFB₁.

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