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Advancement in Electrochemical DNA Biosensors for GMO Detection: A Review Study

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ABSTRACT

Genetically modified organisms (GMOs) are plants or animals whose genetic composition has been transformed using recombinant DNA technology. This technology has various new features, such as resistance to herbicides, viruses, and insects. Recently, genetic modification of food products has increased in order to reduce poverty and hunger across the world and increase food production. However, the impact of GMOs on human health is a growing concern worldwide. Due to the increased global production of GMOs, the presence of these agents in food products needs to be monitored, which has recently attracted the attention of many researchers in order to develop rapid, simple, accurate, and sensitive detection methods for these products. Electrochemical DNA biosensors are among the quickest methods that have been extensively studied due to their high sensitivity, cost-efficiency, rapid reaction, and applicability in aqueous solutions. The present study aimed to review the studies focused on the detection of GMO based on electrochemical biosensors.

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Introduction

Genetic modification of food products has recently increased in order to reduce world poverty and hunger and enhance food production (1). Genetically modified organisms (GMOs) are plants or animals whose genetic composition has been transformed using recombinant DNA technology. This technology has various new features, such as resistance to herbicides, viruses, and insects (2, 3). Despite their benefits, these modified organisms have been reported to adversely affect human health and cause environmental hazards and economic burdens (3).

Due to the increased global production of GMOs worldwide, as well as the need to monitor the presence of these agents in food products, extensive research has been focused on finding cost-efficient, rapid, accurate, and sensitive detection methods for GMOs (2, 4). Considering the need to distinguish GMO and non-GMO food products, numerous countries (European Union, the United Kingdom, Japan, Australia, Brazil, South Korea, China, and New Zealand) have been required to develop food labeling regulations (5). Therefore, new methods are constantly proposed for the accurate and rapid detection of transgenic products in the market (6), including analytical methods such as polymerase chain reaction (PCR) (7), real-time PCR (8), digital PCR (9), next-generation

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sequencing (10), ELISA (11), surface Plasmon resonance biosensors (12), quartz crystal microbalance (QCM) biosensor (13), lateral flow strip biosensors (14), and electrochemical methods (15).

Real-time PCR (RT-PCR) is the most common method for the measurement of GMOs and is considered to be an accurate technique for the identification of recombinant DNA sequences (16). However, RT-PCR is costly and requires expert personnel. Alternatively, DNA-hybridization detection techniques have been considered for these purposes owing to their cost-efficiency, high sensitivity, and no need for expert technicians for the detection of recombinant DNA (2). This field is a significant trajectory for chemistry research. Furthermore, electrochemical DNA biosensors are considered to be an appropriate alternative for the detection of GMOs (16).

The present study aimed to review the studies focused on the utilization of electrochemical genosensors for the analysis and diagnosis of GMO crops, foodstuff, and feed.

Electrochemical Biosensors

Electrochemical biosensors have been extensively studied owing to their high sensitivity, cost-efficiency, rapid reaction, and applicability in aqueous solutions (17-22) Various electrochemical methods are used for the detection of GMOs, including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), square wave voltammetry (SWV), and anodic stripping voltammetry (ASV) (23). In the current review, we have assessed several studies regarding the detection of GMOs using electrochemical sensors (Table 1).

Table 1. Summary of Studies Regarding Detection of GMOs Using Electrochemical Sensors

Method	Organism	Target Sequence/Gene	Template Sensor	Linearity Range	Ref.
DPASV[1]	Agrobacterium tumefaciens	NOS Terminator	Gold Electrode	8.0×10^{-12}-4.0×10^{-9} mol/L	(19)
DPASV and DPV[2]	Cauliflower	CaMV 35S	Gold Electrode	1.2×10^{-11}-4.8×10^{-8} mol/L	(20)
LSV[3]	Maize	CBH 351	Disposable Electrochemical Printed (DEP) Chip	20 mM	(21)
CV[4]		35S Promoter	Ag/AgCl Wire as Reference Electrode and Platinum Coil as Counter Electrode	5-200 nM	(22)
QCM[5]	Soybean	CaMV 35S			(13)
DPV	Soybean	A2704-12 Gene	Carbon Ionic Liquid Electrode	1.0×10^{-12}-1.0×10^{-6} mol/L	(23)
DPV	Soybean	Taxon (Lectin) and Event-specific (RR)	Disposable Carbon Electrode	2-250 pM for Both Targets	(24)

[1]Differential pulse anodic stripping voltammetry
[2]Differential pulse voltammetry
[3]Linear sweep voltammetry
[4]Cyclic voltammetry
[5]Quartz crystal microbalance

DNA-based biosensors require the DNA probe sequence to be immobilized on the surface of a transducer element in order to recognize the target DNA or complementary sequence through the hybridization reaction. An electrochemical signal could be detected by differential pulse voltammetry. The sample containing the target copy numbers could be estimated based on the signal size in the voltammogram and calibration curve (24-26). Figure 3 depicts the potential response of DNA-based biosensors based on differential-pulse voltammetry. As can be seen, the probe single strain DNA sequence is immobilized on the surface of a screen printed electrode, which is covered by nano-gold, and the response resulted from the hybridization of the target DNA is attached to electrochemical labels.

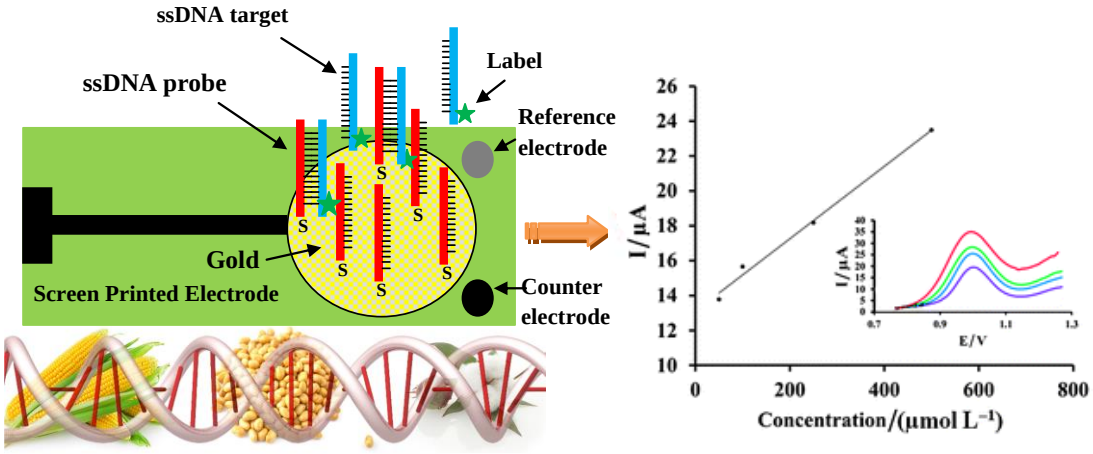

Figure 1. Design Protocol of GMO DNA Genosensor with Labelled Probe

In a study, Sun et al. used an electrochemical DNA biosensor based on cadmium sulfide (CdS) nanoparticles to identify the GMO-specific sequence samples and diagnose the nopaline synthase (NOS) terminator gene sequence (5′-PO4-AC GGA CGA GGT CGT CCG TCC-3′). The mercaptoacetic acid-modified CdS nanoparticle was covalently linked to the NH_2-modified NOS oligonucleotide probe sequences (5′-NH2-GGA CGG ACG ACC TCG TCC GT-3). Afterwards, the target ssDNA sequence was fixed on the mercaptoethanol self-assembled gold electrode, and the CdS nanoparticle was hybridized with the target ssDNA on the surface of the electrode. The detection results had a linear correlation with concentration of the target ssDNA within the range of 8.0×10^{-12}-4.0×10^{-9} mol/L^{-1} (27).

In another research conducted by Sun et al., lead sulfide (PbS) nanoparticles were used as oligonucleotide labels to identify the sequences of cauliflower mosaic virus (*35S* gene). PbS nanoparticles were linked to the oligonucleotide probe after correction, followed by the hybridization of the DNA probe with the DNA target on mercaptoacetic acid on a gold electrode. In the mentioned research, the suitable concentration range of the target ssDNA sequence in the electrochemical DNA biosensor based on PbS nanoparticles was determined to be 1.2×10^{-11}-4.8×10^{-8} mol/L. In addition, the electrochemical DNA biosensor was reported to have good capability in detecting the *CaMV 35S* sequences from GMOs (28).

In another study in this regard, Ahmed et al. (2009) introduced an accurate, cost-efficient, and rapid diagnostic method based on an electrochemical printed chip for the detection of CBH 351 maize GMO using linear sweep voltammetry. In the mentioned research, the Hoechst 33258 [20-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2, 50-bi (1H-benzimidazole), H33258] label was used without the required immobilization probe on the electrode surface. The biosensor showed a working range of 10-50 μM for H33258 with the detection limit of 20 μM for the optimization of the desired DNA binder. Moreover, the findings indicated that this electrochemical biosensor could eliminate cross-contamination and be applied as an effective sensor for environmental protection since it required no probe immobilization (29).

In a research, Berti et al. developed a new electrochemical genosensor based on multi-walled carbon nanotube (MWCNT) thin films for the detection of recombinant DNA in GMO products. This analysis was performed using non-labeled and enzyme-labeled methods. In the non-labeled method, the linear response was estimated at 0.5 millimeter and 10 micrometer, while in the enzyme-labeled method, the linear response was observed at the concentrations of 5-200 nanometers (30).

In another study, Lien et al. used a DNA biosensor based on MWCNT-doped polypyrrole (PPy) for GMO detection (herbicide-resistant RR soybeans) using QCM and EIS. In the mentioned study, GMO detection (label-free DNA) was based on the C-PPy-ODN system, and with the improved performance of C-PPy-ODN composite

material, the range of *CaMV 35S* target concentration was observed to reduce. Therefore, it could be inferred that within the range of low *CaMV 35S* target concentration (25-80 pM), the EIS data were well fitted with the Randles model (13).

According to the literature review, Sun et al. introduced an electrochemical DNA sensor based on reduced graphene oxide (RGO)-modified carbon electrodes. The sensor was used for the sensitive detection of the target ssDNA sequence in the transgenic soybean A2704-12 sequence. Moreover, 1-butylpyridinium hexafluorophosphate was applied as a binder for developing a carbon ionic liquid electrode. The sensor functioned within the concentration range of 1.0×10^{-12}-1.0×10^{-6} mol/L with the detection limit of 2.9×10^{-13} mol/L (3σ). Considering the reasonable findings of the mentioned research, it is suggested that this electrochemical DNA biosensor be applied to detect the PCR products of transgenic soybean (31).

Manzanares-Palenzuela CL. et al. introduced the electrochemical genosensor based on multiplex electrochemical DNA platform for the femtomolar-level quantification of specific GMO events in food products. The immobilization, hybridization, and labeling of both sequences (one targeting an event-specific sequence of RR soybean, and the other targeting the endogenous lectin gene) were simultaneously performed in a single tube. In the mentioned study, the labeled probes were used for the hybridization of sandwich signaling using fluorescein isothiocyanate (FITC) for RR soybean or digoxigenin (Dig) for lectin, and one reporter macromolecule (horseradish peroxidase enzyme) was applied for binding via anti-FITC or anti-Dig conjugation. In both systems, the optimization of the number of PCR cycles (30 and 35 amplification cycles for lectin and RR soybean products, respectively) resulted in linearity within the ranges of 53-4425 DNA copies for RR soybean and 1093-88496 DNA copies for the lectin sequences. With the limit of detection (LoD) of 53 copies of RR soybean DNA (relative LoD: 0.06%), electrochemical magnetoassay coupled to PCR as a sensitivity approach, which is comparable with the one reported in the RT-PCR assay using the same primers (32).

Conclusion

With the advancement of genetic engineering in food production and the associated socioeconomic and environmental implications, special attention has been paid to the detection and traceability of food products. Electrochemical genosensors could be used as appropriate devices for the in-field analysis of GMOs owing to their cost-efficiency, high sensitivity, simplicity, and portability. These approaches take advantage of the interactions between the solid electrode surface, recognition probe, and analyte DNA.

To enhance the performance of GMO DNA biosensors, a combination of various methods and immobilization matrix are considered essential since they may affect DNA probe immobilization. On the other hand, using nanomaterials (e.g., nanoparticles) could result in a larger surface area, which allows more DNA probes to be immobilized on the matrix, thereby improving the performance of the biosensor.

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