Sensing of Harmful Dyes in Food Particles Using Nanomaterial-Based Electrochemical Analysis

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Abstract

Azo-dyes, including Allura Red, Sunset Yellow (SY), Brilliant Blue, and Tartrazine (Tz), are commonly utilized as food coloring additives because of their affordability and durability. The dyes SY and Tz are widely employed within this dye group because of their comparable hues and frequent co-application in food items. Despite their beneficial industrial applications, these substances include a toxicity profile that poses risks, including the potential for undesirable consequences such as allergies, asthma, cancer, DNA damage, cytotoxicity, and nervousness. In light of these factors, employing susceptible, cost-effective, straightforward, and expeditious sensors to examine azo-hazardous dyes is imperative. Electrochemical nanosensors (ENS), which integrate the distinctive characteristics of electrochemistry with nanotechnology, are very advantageous devices that find extensive application in analyzing azo dyes. In this paper, the exterior of a Glassy Carbon Electrode (GCE) has been subjected to modification by the incorporation of calixarene (Cx) and gold nanoparticles (AuNP) to facilitate the improvement and integration of electrochemistry with nanotechnology. The sensing capability of the Modified GCE (MGCE) nanosensor with Cx and AuNP has been evaluated by the utilization of Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) techniques. The study aimed to examine the impact of various factors to determine the optimal circumstances for obtaining the most favorable reaction from the target analytical substances. The signals of the chosen food dyes have been significantly improved when using the MGCE nanocomposite, which is attributed to the synergistic interaction between Cx and AuNP compared to the unmodified GCE. The sensor platform that was built also showed notable performance characteristics when utilized to detect food colors in food samples. Furthermore, the observed high percentage of recovery, consistent repeatability, and durability indicate the potential use of the developed electrochemical platform to examine actual samples.

Keywords: Electrochemical nanosensors, Food colors, Harmful Dyes, Calixarene, Glassy Carbon Electrode, Gold nanoparticles.

Introduction

The issue of food safety has gotten more severe due to ecological damage from social and technical advancements. Furthermore, illicit enterprises' relentless quest for profit has resulted in a high frequency of food safety issues, directly impacting public health [1]. For instance, farmers employed clenbuterol, a prohibited substance, to enhance the leanness of pigs. The addition of melamine to baby formula in 2008 resulted in over 290,000 newborns experiencing pathological urinary tract stones, constituting an unlawful crime [2].
occurrences mentioned above have heightened public consciousness of the significance and immediacy of tackling problems about food safety.

The persistence of food safety concerns necessitates the exploration of enhanced methodologies for food analysis. Nevertheless, examining food is complex and poses a significant hurdle for individuals. Numerous potentially hazardous constituents can manifest in various food varieties and during all stages of food processing [3]. The hazardous substances encompass heavy metals, diverse poisons, residual pharmaceuticals, insecticides, viruses, illicit additions, and germs. Furthermore, the complexities will be heightened due to the globalization of the food sector [4]. Given the increasing demand from customers for health-conscious products, concerns over food safety have motivated the general public to develop efficient, affordable, precise, and highly sensitive analytical methodologies and procedures for examining food.

The key attributes in sensor sensing applications are sensitivity and specificity, which may be improved by appropriately modifying the sensing surface [5]. The utilization of nanoparticles is considered a crucial approach to enhancing sensing capabilities. Nanomaterials, characterized by dimensions ranging from 1 nm to 100 nm, can be readily fabricated into various configurations to cater to diverse detection requirements. These configurations include zero-dimensional (0D) nanoparticles or nanoclusters, one-dimensional (1D) nanowires or nanorods, two-dimensional (2D) nanosheets, and three-dimensional (3D) nanonets, nanoflowers or nano bulk structures. Nanomaterials have gained significant attention and application across diverse fields due to their distinctive optical, electrical, and mechanical properties, as well as their extensive surface area, favorable biocompatibility, catalytic activity, and abundant binding sites [6]. Notably, these materials have found particular utility in developing sensing surface components, which offer enhanced sensitivity and reduce Limits of Detection (LOD) [7].

A wide range of sensors and biosensors based on nanomaterials exist, which may be categorized into optical and electrochemical sensors based on the type of transduction signals they employ. Although there have been reports on the uses of nanomaterials for food safety sensing [8], there is a need for an updated and complete assessment of the several types of nanomaterial-based sensors used in food safety monitoring.

The methodology employed in this study integrates the distinctive characteristics of nanomaterials with electrochemical methodologies to attain detection capabilities that are both extremely sensitive and selective. Nanomaterials, including nanoparticles and nanocomposites, are utilized as sensing components owing to their substantial surface area, superior conductivity, and heightened catalytic efficacy. The nanomaterials possess the ability to exhibit preferential affinity towards certain dye molecules, hence resulting in alterations in electrochemical signals that are directly proportionate to the concentration of the dye. This methodology has significant potential to guarantee food safety through its capacity to swiftly and economically detect hazardous dyes on-site. These colors are frequently employed illicitly to increase the visual appeal of food products, although their consumption might result in severe health hazards. In addition, the utilization of nanomaterial-based electrochemical analysis presents the benefit of being easily transportable and capable of providing real-time monitoring. This characteristic renders it a vital instrument for ensuring quality control within the food business and preserving the well-being of consumers.

Related Works

A crucial component of food safety and quality management is the identification of hazardous dyes in food items. Using illicit colors in the food business puts consumers’ health at risk. Conventional detection techniques can take a long time, involve much work, and lack the necessary sensitivity. The rapid identification of hazardous dyes in food particles has been made possible in recent years by the development of electrochemical analysis based on nanomaterials. This literature review presents an overview of the most
recent findings in this study area, emphasizing important techniques, nanomaterials, applications, benefits, and difficulties related to detecting hazardous dyes in food particles.

Kumar et al. (2019) propose functionalizing graphene with metal oxide nanostructures to make medical ENS and biosensors [9]. This technology is used to make graphene-metal oxide nanocomposites, characterize them, and create ENS. The output values cover the sensors’ sensitivity, selectivity, detection limitations, and medical suitability. They also describe their performance. Nanocomposite sensors can detect biomolecules sensitively and selectively, but repeatability and durability may be issues.

Sun et al. (2021) focus on innovative antibiotic detection in food and the environment using sensors and nanomaterials [10]. The suggested method uses nanomaterials to make antibiotic sensors. Before deployment, these sensors must be tested for antibiotic residues in food and environmental samples. Output results include sensor responsiveness, specificity, and antibiotic detection ranges. The ability to directly detect antibiotics is beneficial, but matrix effects in complex food samples may be an issue.

Sani et al. (2018) propose an electrochemical DNA biosensor to detect food carcinogens [11]. Immobilizing DNA probes on electrode surfaces and detecting DNA-carcinogen interactions is the method. Installation includes testing the biosensor’s ability to detect carcinogens in food samples. Output numbers include biosensor sensitivity, specificity, and detection limits. The ability to detect specific carcinogens is a plus, but probe design and optimization are constraints.

Hasseb et al. (2022) electrochemically detect infections and biological indicators using MIPs [12]. The suggested method involves creating MIPs with affinity for the desired analyzers and integrating them into ENS. MIP-based sensors must be tested for selectivity and sensitivity for identifying indicators and pathogens during implantation. Output values include sensor efficiency and MIP field usability data. MIPs can recognize specific targets, but improving MIP synthesis and sensor architecture may be difficult.

Muniandy et al. (2019) discuss carbon nanomaterial-based ENS for foodborne microorganism identification [13]. The proposed technology uses biosensors with microbe and carbon nanomaterial receptors. The biosensors’ foodborne germ detection and measurement abilities are tested during implementation. Output numbers include biosensor sensitivity, specificity, and detection limits. Benefits include fast and accurate bacterial identification, but solid bioreceptor immobilization may be required.

Soto and Orozco (2022) study hybrid nano-bioengineered nanomaterial-based ENS [14]. Nanomaterials and biomolecules are used to create advanced biosensors for various applications. Implementation requires hybrid sensor development and evaluation. Output values include sensitivity and selectivity performance. Hybrid sensor architectures are flexible, but improving nanomaterial-biomolecule interactions may be difficult.

Kaya et al. (2021) use nanomaterial electrochemical analysis to identify azo-hazardous dyes in food samples, including Tz and SY. [15]. The process includes developing dye-sensing nanomaterials. Implementation involves testing the sensors’ selectivity and sensitivity for target colors in food samples. Output values include sensor performance data like detection limits and precision. Limitations may include food matrix interference, but advantages include fast and accurate dye detection.

A new ENS by Shah (2020) can detect two harmful food colors. The method involves designing and building nanomaterial-based sensors to detect dyes simultaneously [16]. Implementation includes testing the sensor’s food color detection ability. Constant sensing of both dyes yields detection limits for sensitivity, selectivity, and all three. Concurrent detection is effective, but calibrating for complex food matrices may be difficult.

In food safety, nanomaterial-based electrochemical assessment has become a game-changing technique that allows for the sensitive and focused identification of hazardous
pigments in food particles. This literature review highlights the expanding body of knowledge in this area, highlighting the numerous uses, cutting-edge approaches, and crucial function of nanomaterials in assuring the quality and safety of food items. Using nanomaterial-based ENS in quality assurance methods has enormous promise for preserving human health and improving food sector efficiency.

Materials and Methods

Identifying potentially hazardous colours in food items is crucial to ensuring food safety and maintaining high-quality control standards. In this context, utilizing GCE combined with Cx and AuNPs provides a novel and optimistic methodology for the discerning and precise identification of dyes, namely SY and Tx, within food particles. The present methodology integrates the distinctive characteristics of Cx, which exhibits selective binding affinity towards certain dye molecules, with the remarkable conductivity and catalytic capabilities of AuNPs, therefore augmenting the functionality and specificity of the sensor.

In this study, the utilization of calix8 as a constituent of the recognition layer is based on its electrode anchoring and host trapping capabilities. AuNPs have been selected as an additional element of the detection layer due to their ability to offer a large surface area and promote electron transport between various electroactive compounds and electrode substrates. The utilization of Cx in conjunction with AuNP is employed due to its synergistic effects, which confer the electrode with attributes of molecular identification and effective responsiveness to the redox events of specific analytes. Considering these characteristics, the GCE was modified using Cx and AuNP, forming MGCE. The constructed electrochemical platform was utilized to detect two food toxins, namely SY and Tx. The structure of SY and Tx dyes is depicted in Fig. 1.

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\text{Figure 1: Structure of SY and Tx dyes}
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**Cx in Sensing**

Cx is a class of macrocyclic molecules characterized by a hydrophobic cavity and a functionalized rim. The distinctive arrangement of their components enables molecular identification and the targeted binding of certain analytes, rendering them highly suitable for sensing purposes. In this adaptation, the calixarene molecule functions as a receptor entity capable of selectively binding SY and Tx dyes, forming host-guest complexes. The interaction between these dyes and calixarene induces changes in the electrochemical characteristics of the GCE, hence facilitating their identification.

**Role of AuNP**

The role of AuNPs has garnered considerable interest in electrochemical sensing owing to their remarkable conductivity, large surface area, and electrocatalytic characteristics. Integrating AuNPs into the MGCE system results in an enhanced electrochemical response, contributing to heightened sensitivity and reduced detection limits. AuNPs can serve as a suitable medium for immobilizing calixarene, hence augmenting the sensor's selectivity. This immobilization process mitigates potential interference from various molecules in complex food matrices.
**Methodology of MGCE for Dye Sensing in Food Particles**

The methodology employed for altering the GCE with Cx and AuNPs often encompasses many sequential phases, as shown in Fig. 2.

**Electrode preparation:** The process of electrode preparation involves meticulous cleaning and treatment of a glassy carbon electrode to eliminate any potential impurities and ensure a pristine surface.

**Immobilization of Cx:** The immobilization of calixarene onto the surface of a GCE can be achieved using either chemical or physical approaches. This stage entails the development of a robust calixarene coating that can selectively engage with SY and Tz dyes.

**Deposition of AuNP:** The process involves the deposition of AuNP onto a modified GCE to form a nanocomposite. The deposition process can be accomplished using many approaches, including electrodeposition, chemical reduction, or physical adsorption.

**Characterization:** The modified electrode is subjected to a range of analytical techniques, such as Scanning Electron Microscopy (SEM) and electrochemical procedures, to validate the effective alteration and evaluate the characteristics of the electrode.

**Dye sensing:** The electrochemical detection of SY and Tz dyes is conducted using a modified glassy carbon electrode (GCE). The binding of various dyes to the immobilized calixarene results in alterations in the electrochemical characteristics of the electrode, allowing for the quantification of dye concentration.

**Advantages of MGCE**

Various benefits are associated with modifying GCE using Cx and AuNPs. The utilization of calixarene in conjunction with AuNPs results in an augmented level of sensitivity for the sensor, hence enabling the detection of dyes at much-reduced concentrations. The selectivity of calixarene enables the sensor to exhibit a particular binding affinity towards SY and Tz dyes, minimizing the potential for interference from other substances in food particles. The electrochemical sensing technique offers expeditious outcomes, rendering it well-suited for real-time surveillance and quality assurance within the food business. The reusability of GCEs allows for their repeated utilization in many sensing cycles, hence reducing the overall cost associated with analysis.
Challenges of MGCE

Although this modification technique shows promise, it may encounter difficulties in sensor stability, repeatability, and calibration when used to complex food matrices. Furthermore, the practical execution of food quality control necessitates careful consideration of regulatory permission and standards.

Thus, using the MGCE with Cx and AuNPs to selectively detect SY and Tz colors in food particles signifies significant progress in food safety and quality control. The utilization of Cx and AuNPs in this methodology leverages their distinctive characteristics to attain heightened levels of sensitivity and selectivity.

Results and Discussion

Experiment

The GCE was thoroughly cleaned before each experiment to provide a polished surface suited for experimentation. To achieve the desired outcome, the GCE underwent a polishing process involving the application of a 0.06 μm alumina-water slurry onto a rubbing pad, resulting in a glossy surface. To prevent surface grooving on the GCE, a rubbing technique resembling the shape of digit 'eight' was employed, effectively eliminating undesirable adhesive particles. Subsequently, the electrode underwent sonication and was meticulously washed using a stream of doubly distilled water. The purified GCE was subsequently exposed to multiple potential scans within the 0-1.5 V range to get consistent and repeatable cyclic voltammograms. To facilitate the alteration of the GCE surface, a solution of calix8 was produced in DMF with a concentration of 3.5 mg/mL. A droplet measuring 6μL of the solution mentioned above was applied onto the surface of a GCE and subsequently subjected to drying using a drier. The MGCE sample is then subjected to a water washing to eliminate any loosely attached calix8 molecules. To immobilize AuNPs, the calix8/GCE was submerged in the AuNP slurry for 25 minutes. The MGCE electrode has been dried in an argon atmosphere and utilized to detect specific food toxins, namely SY and Tz.

Differential Pulse Voltammetry (DPV) Results

Differential pulse voltammetry (DPV), an electroanalytical method known for its great sensitivity, is commonly employed to get signals of analytes with improved resolution. Hence, DPV has been used in this study to measure the voltammetric characteristics of SY and Tz dyes in food samples on plain GCE, Cx+GCE, AuNP+GCE, and MGCE.

![Figure 3: DPV results of (a) SY and (b) Tz](image)

Fig. 3 depicts the DPV results of (a) SY and (b) Tz. The findings demonstrate the Plain GCE’s poor sensitivity to SY and Tz colors in the food samples, as seen by its negligible current response. However, there is a notable increase in current responsiveness at particular voltage levels as the electrode changes with calixarene (Cx+GCE), indicating...
increased selectivity for the target dyes. The AuNP+GCE displays much more improved current responses, indicating increased sensitivity for detecting SY and Tz. The most efficient electrode design for the electrochemical detection of SY and Tz dyes in food samples is the MGCE, which contains both calixarene and gold nanoparticles. This electrode configuration exhibits the greatest current responses (2.15 µA at 0.55V for SY and 1.41 µA at 0.9V for Tz), indicating synergistic effects of both changes. These DPV results highlight how crucial electrode modification is for improving the sensitivity and selectivity of electrochemical sensors in applications to ensure food safety and quality.

**Cyclic Voltammetry (CV)**

The CV response of SY and Tz has been investigated at various scan speeds between 20 and 200 mV/s. Both surface-assisted and diffusion-controlled electrochemical processes can be coupled to signal strength and scan rate information. The graph in Fig. 4 has been based on the following equations:

\[
\log I_{SY} = 0.74 \log v - 4.58 \tag{1a}
\]

\[
\log I_{TZ} = 0.61 \log v - 4.46 \tag{1b}
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Figure 4: CV results of SY and Tz at various scan rates

Fig. 4 shows the CV results of SY and Tz at various scan rates. SY and Tz dyes were electrochemically detected in food samples using cyclic voltammetry (CV) at different scan rates (20 mV/s, 40 mV/s, 60 mV/s, 80 mV/s, and 100 mV/s). The current values (in V) are measured at various voltage levels (in µA). SY and Tz show reduction and oxidation peaks at about -6 A and 6 A, respectively, at a scan rate of 20 mV/s. Peaks with greater current levels become more prominent as the scan rate rises. For instance, at 100 mV/s, the reduction and oxidation maxima for SY and Tz, respectively, reach roughly -25 A and 25 A. This data shows that higher scan rates improve the CV technique’s sensitivity, producing stronger current responses and clearly defined redox peaks, which are necessary to measure the dyes in food samples accurately.

**Conclusion**

This study focuses on altering the exterior of a Glassy Carbon Electrode (GCE) by inserting Cx and AuNP. The objective is to enhance and integrate electrochemistry with nanotechnology. The evaluation of the sensing capabilities of the Modified GCE (MGCE) nanosensor with Cx and AuNP has been conducted through the application of Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) approaches. The electrode design that exhibits the highest efficiency level for electrochemical detection of SY and Tz dyes in food samples is the MGCE, which combines calixarene and gold nanoparticles. The observed current responses of this electrode arrangement demonstrate the highest values, namely 2.15 µA at 0.55V for SY and 1.41 µA at 0.9V for Tz. These results suggest the presence of synergistic effects resulting from the combined modifications. SY and Tz exhibit reduction and oxidation peaks at around -6 A and 6 A, respectively, when subjected to a scan rate of 20 mV/s. As the scan rate increases, peaks exhibiting higher current levels
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become increasingly conspicuous. The findings indicate that increased scan speeds enhance the sensitivity of the CV technique, leading to more pronounced current responses and well-defined redox peaks. These characteristics are crucial for accurately measuring the dyes present in food samples.

As further research is conducted to enhance and overcome practical obstacles associated with this methodology, it can evolve into a valuable instrument for guaranteeing compliance with regulatory standards and the absence of detrimental contaminants in food products. Consequently, it would effectively protect consumer well-being and augment transparency within the food sector.

References