

Recent progress in electrochemical detection of amaranth in food samples: A brief review

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Amaranth (E 123, AM) is a red synthetic azo dye authorized as a food additive in the EU. In contrast to natural colorants, AM has significant advantages such as low production cost, excellent water solubility, and high stability. AM is used to enhance the natural color, to decrease the loss of color due to processing treatments, and to provide color to colorless ultra-processed foods, making food more attractive to the consumers. AM is widely used in food industry to improve the appearance and color of different types of food and beverages, including confectionery products, jelly, candies, jams, dairy products, ice creams, sausages, wines, syrups and soft drinks. Many of these food products are manufactured directly for, and marketed directly to, children. The high amount of AM in food products can cause adverse health effects such as dizziness, allergies, and respiratory problems. Moreover, long-term studies indicated that the potential adverse effects induced by AM include genotoxicity and cytotoxicity. Therefore, the development of a precise and easy-to-handle alternative for a quick and efficient quantitative detection of AM in food products is necessary for consumer safety. Modern electroanalytical techniques have the advantages of excellent sensitivity and selectivity, instrumental simplicity and portability, providing reliable alternatives to the conventional analytical methods. In this brief review, we have summarized recent trends in the electrochemical sensor systems applied for the analysis of AM in food samples, critically evaluated the performance metrics of these sensors: sensitivity, linear range, limit of detection and stability. Finally, the current challenges and future prospects are outlined.

Keywords: electrochemical sensors; electroanalysis; azo dyes; food colorants; food analysis; food safety

INTRODUCTION

Color is one of the most important sensory attributes of food that plays an essential role in food appearance and acceptability. Color perception is the major visual factor related to product quality that influences the customer selection. Therefore, coloring agents that have been obtained by chemical synthesis, are routinely added to various food products to impart desirable sensory characteristics [1]. Synthetic colorants, especially azo-dyes, have been widely used in food industry due to their excellent water solubility, high coloring ability, good color uniformity, high chemical and photolytic stability, low microbiological contamination and low production cost [2]. However, most azo dyes have potential risks to human health because they contain azo group (-N=N-) and aromatic structures. The toxic effects of azo dyes are induced by their degradation products. Under reductive conditions, the azo groups can be cleaved leading to aromatic amines, which exhibit carcinogenic and mutagenic properties [3].

Amaranth (AM) is a widely used synthetic azo dye, normally supplied as sodium, potassium or

calcium salt. AM is soluble in water (70 g/L at 25 °C), slightly soluble in ethanol (up to 4 g/L), but insoluble in vegetable oils. The powders or granules are of a red-brown shade; in liquid form it has a bluish-red color [4].

Based on the Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommendations, acceptable daily intake of AM is between 0 and 0.5 mg/kg [5]. Recently, pediatric studies have demonstrated the relationship of AM consumption with hyperactivity and irritability in children [4]. At higher levels AM is toxic as it can cause a number of adverse health effects such as genotoxicity, cytostaticity, and cytotoxicity. Zhang and Yadi [6] performed mechanistic and conformational studies on the interaction of AM with human serum albumin (HSA). The binding of AM to HSA induced the conformational change of HSA which represents the disturbance in hydrogen bonding networks [6]. Moreover, according to the results obtained by Basu and Kumar in 2015 AM was expected to be able to induce unfolding and loss of a large part of the helical stability of hemoglobin, which significantly affects the secondary structural changes in human hemoglobin [7].

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Literature survey reveals several conventional analytical methods for determination of AM in foods and beverages including spectrophotometry [8], high performance liquid chromatography (HPLC) [9], liquid chromatography/mass spectrometry (LC-MS/MS) [10], or capillary electrophoresis [11]. However, most of these methods require specific and relatively expensive equipment, advanced technical expertise, and need of complicated, time-consuming pretreatment procedures. Therefore, it is important to develop new, straightforward, precise and easy-to-handle alternative for quick and efficient quantitative detection of AM in food products for controlling food quality and ensuring the consumers health safety. Due to high sensitivity, simplicity, and rapid sample preparation procedures with high level of automation, electrochemical techniques are the best candidates for the on-site detection of azo dyes, including AM.

In this review, we mainly introduce the research progress of electrochemical sensors in the detection of AM and its application in actual food sample detection. Because the electrode is the core part of the entire sensor device, we summarized the development process of the electrode for quantitative AM detection, and described the modification method and materials. The following part of this article will provide a brief overview on how advances in electroanalytical technology have contributed towards developing sensor devices for AM quantitative detection in food and beverage products.

STATE-OF-THE-ART IN ELECTROCHEMICAL SENSORS FOR THE ANALYSIS OF AMARANTH

It is well known that the performance of electrochemical sensors highly depends on the type of electrode materials used. The best approach to date in order to achieve high stability, sensitivity, and selectivity of sensors during electroanalysis is electrode surface modification. After modification, the electrode shows special surface effect, size effect, and catalytic activity. These specific properties enhance the electrical conductivity of the working electrode, accelerate the electron transfer in the interface, and reduce the redox overpotential of AM. In order to promote the electrochemical reaction and improve the electrode efficiency, modification of the working electrodes using inorganic nanomaterials (graphene, graphene oxide, carbon nanotubes, metal nanoparticles, metal oxide nanoparticles, quantum dots), conductive polymers, ionic liquids, and metal organic frameworks has been extensively investigated.

Graphene (GR) is a two-dimensional sheet of hexagonally arranged carbon atoms with sp^2 -hybridization. The combination of attributes such as extremely large surface area and electrical conductivity, high mechanical strength, and low cost makes GR an ideal platform for the anchoring of metal nanoparticles for electrochemical sensing applications [12]. The electrochemical detection and degradation processes of AM using gold electrodes modified with GR/TiO₂-Ag composite was investigated by Stefan-van Staden *et al.* [3]. Graphene nanosheets (GRS) prepared by ultrasonic exfoliation of graphite powder in N-methyl-2-pyrrolidone (NMP), were dispersed into N,N-dimethylformamide (DMF) and then used to modify the surface of GCE [2]. The modification of GRS obviously enhances the surface roughness of GCE and provides larger response area and higher accumulation ability for AM. As a result, the catalytic activity for AM is improved effectively on the surface of GRS and the oxidation signals enhance significantly. Based on the remarkable signal enhancement effects of NMP-exfoliated graphene, a sensitive and rapid electrochemical method has been developed for the determination of AM. The linear range is from 2.5 to 125 nM, and the detection limit is extremely low (LOD = 0.75 nM). The sensing platform was used in different drink samples (Fanta, Mirinda), and the results were found to be consistent with the values obtained by HPLC.

A new stable and interference-free voltammetric sensor for highly selective determination of AM in soft drinks was constructed [13]. Surface functionalization of GR with poly(sodium 4-styrenesulfonate) (PSS), a water-soluble polymer used as emulsifying agent, not only improves GR nanosheets dispersibility, but also allows them to be further decorated with other nanomaterials, including metal nanoparticles. Palladium nanoparticles (PdNPs), in particular, are attractive for sensing applications due to their excellent catalytic activity towards AM oxidation. In the development of electrochemical sensors Pd has been mainly used as nano-sized particles for surface modification of various electrode materials. This approach reduces the cost of catalysts, improves their electrocatalytic performance since it provides extremely high surface area and decreases the poisoning effect caused by the adsorption of intermediates and reaction products [14]. The proposed sensor platform PSS-GR-Pd/GCE was employed for measurement of AM in Fanta drink using the standard addition method with satisfying results. According to the presented results, the

proposed method has definite precision and accuracy.

As an oxidation derivative of GR, graphene oxide (GRO) is rich in oxygen-containing groups and has been demonstrated to have good catalytic activities, particularly for the oxidation of AM. Through further functionalization, the electrochemical activity of GRO could be further improved and agglomerating of GRO in aqueous solution could be effectively prevented. An electrochemical sensor was fabricated by modifying nanoporous gold (NPG)-coated glassy carbon electrode (NPG/GCE) with functionalized graphene oxide /chitosan/ionic liquid nanocomposites (fGRO/CS/IL) [15]. As a result of the synergistic effect of NPG and fGRO/CS/IL, the resulting electrode (fGRO/CS/IL/NPG/GCE) showed the highest current response signal of AM due to ultrahigh surface area, electronic conductivity, as well as the improvement of the surface structure. For practical applications, the electrode was validated for the determination of AM in three types of drinks (Fanta Drink, Mirinda Drink and Minute Maid) with satisfactory results.

Reduced graphene oxide (rGRO) is the functionalized product of GRO which has enormous advantages such as better conductivity than that of GRO because of reinstated sp^2 -carbon network. A new SnO_2 /rGRO nanocomposite has been engineered to be utilized as chemically modified sensor for the low-level quantification of AM in soft drinks and water samples [16]. The combinations of rGRO improved the properties of SnO_2 nanoparticles and effectively reduced the degree of restacking of graphene nanosheets, as well as increased the active adsorption sites of rGRO. The developed sensor SnO_2 /rGRO/Nafion/GCE showed excellent anti-interference behavior which highlights the selectivity profile of the sensor for targeted analysis. The effectiveness of SnO_2 /rGRO/Nafion/GCE was investigated for AM analysis in Sprite, 7-Up, and Sting. The proposed method exhibited acceptable recovery values in the real matrix that proves its reliability to be utilized as a sensitive sensor for AM monitoring at commercial level in different soft drinks.

Researchers have used various types of ionic liquids (ILs) in electrode fabrication with the intention of developing novel electrochemical sensor platforms or as unique electrocatalytic substances, as they possess sophisticated conductivity, low volatility and high chemical stability [12]. Due to wide electrochemical windows and high electrical conductivity, ILs were suggested for modification of electrochemical sensors in

voltammetric analysis. Sheikhsaie *et al.* 2017 reported coupling of ILs and nanomaterials for fabrication of a highly sensitive sensor for AM [17]. RuO_2 nano-road, synthesized by sol-gel method, was used for modification of carbon paste electrode in the presence of 1,3-dipropylimidazolium bromide as a conductive binder. The developed electrode CPE/ RuO_2 /NR/DPIBr showed high performance ability for quantification of AM at nanomolar levels. A linear calibration was achieved in range of 0.008–550 μM (LOD = 3 nM). This device was successfully applied for the detection and analysis of AM in soft drinks, orange and apple juices. CPE/ RuO_2 /NR/DPIBr exhibited advantages including improved electrocatalytic ability and good stability. Authors reported a relative standard deviation (RSD) of 2.2% of the electrode signal when CPE/ RuO_2 /NR/DPIBr was stored for 35 days in laboratory that confirms stability of the novel sensor for analysis of AM.

The electrochemical field has witnessed the enormous use of screen-printed carbon electrodes (SPCEs) mainly because of their unique properties compared to other conventional electrodes, including wide potential window, standard background current, reproducibility and high repeatability, commercial availability, cost-effectiveness, ability to connect to portable devices, need for low power, linear output, and possibility of miniaturization. It is therefore considered a potential tool for on-site applications. Sensor systems for AM were developed using screen-printing technique [18–22]. rGRO-methionine [18], double-stranded copper(I) helicate and SWCNTs [19], Pd/GRO nanocomposite [20], Ni–Mo–MOF [21], and V_2O_5 -nanoplates [22] were employed as electrode modifying materials. Tajik *et al.* have fabricated metal–organic framework (MOF) – modified screen printed electrode for sensitive and rapid detection of AM [21]. Conductive metal–organic frameworks (MOFs) are a class of porous materials consisting of organic nodes connected by organic linkers. These attractive materials have many advantages, such as adjustable structure, extraordinarily high surface areas, simple synthesis protocol, and catalytic capacity, making them helpful in effective coating materials for electrocatalytic electrodes that are used in sensing applications. Additionally, the high porosity of MOFs makes them easy to encapsulate metal nanoparticles (MNPs) within their porous channels/cavities, resulting in the formation of MNPs/MOF composites that show high conductivity, excellent sensing and catalytic applications because of synergistic interactions between components [23]. The proposed novel Ni–

Mo-MOF-modified screen printed electrode (Ni-Mo-MOF/SPCE) was applied for quantitative detection of AM in real samples [21]. According to the proposed protocol, appropriate recoveries were found to detect the AM in fruit juices and tap water. Authors stated that the hybrid bimetallic MOF

possesses increased stability and improved catalytic properties for the electrooxidation of AM.

Table 1 summarizes the electrochemical sensors applicable to the quantitative analysis of AM in foods and beverages.

Table 1. Comparison of the analytical parameters of electrochemical sensors for the determination of AM in foods and beverages

Modified electrode	Method	Linear range	LOD (nM)	Analytical application	Ref.
GS/GCE	DPV	2.5–125 nM	0.75	soft drink	2
Au/GTA	LSV	0.3–100 μM	100	–	3
PSS-GR-Pd/GCE	DPV	0.1–9 μM	7	Fanta	13
fGRO/CS/IL/NPG/GCE	SWV	8–1200 nM	23	Fanta, Mirinda, Minute Maid	15
SnO ₂ /rGRO/Nafion/GCE	DPV	1–800 nM 1–60 μM	0.68 2.7	Sprite, 7-Up, Sting, river water	16
CPE/RuO ₂ /NR/DPIBr	SWV	0.008–550 μM	3	soft drink, orange and apple juices	17
rGRO-methionine/SPCE	DPV	1–10 μM 10–100 μM	57	SPY red wine cooler, Sprite	18
H-SWCNT/SPCE	SWV	0.057–1.4 μM	30	synthetic orange juice, soft drink	19
Pd/GRO/SPE	DPV	0.08–360 μM	30	orange and apple juices, tap water	20
Ni–Mo-MOF/SPCE	DPV	0.15–500 μM	50	orange and apple juices, tap water	21
V ₂ O ₅ - NPs/SPE	DPV	0.1–270 μM	40	apple juice, tap water	22
AC-Co ₃ O ₄ -CPE	DPV	0.1–215 μM	10	grape soda, lemon soda	24
N-rGRO/GCE	DPV	0.1–600 μM	30	orange and apple juices, tap water	25
PLA-ERGO/GCE	DPV	0.75–75 μM	250	soft drink	26
1-M-3-BIBr/CuO/SWCNTs/CPE	SWV	0.004–750 μM	1	fruit juices; sausage	27

Poly(L-arginine)–electrochemically reduced graphene modified glassy carbon electrode (PLA-ERGO/GCE) was fabricated by cyclic voltammetry method and the electrochemical behavior of AM and carmine on this electrode was studied [26]. The experimental results indicated that the oxidation peaks of carmine and AM were separated at 121 mV in phosphate buffer solution at pH=2.5. The linear range for the simultaneous determination of carmine and AM were 7.5×10^{-7} – 7.5×10^{-5} M and the detection limits were 7×10^{-8} M for carmine and 2.5×10^{-7} M for AM, respectively. The method has been applied to the simultaneous determination of carmine and AM in commercial soft drink with satisfactory results.

Karimi-Maleh *et al.* [27] designed a highly sensitive electrochemical sensor for the simultaneous detection of AM and nitrite in foodstuffs, based on carbon paste electrode modified

with 1-methyl-3-butylimidazolium bromide and CuO decorated single-wall carbon nanotubes (1-M-3-BIBr/CuO/SWCNTs/CPE). Synergistic effect between CuO/SWCNTs and 1-M-3-BIBr helps in increasing the ability of proposed sensor for nanomolar determination of AM in food samples. Also, 1-M-3-BIBr/CuO/SWCNTs/CPE resolved the overlapping signal of AM and nitrite for simultaneous analysis of these compounds in food samples. The square-wave voltammograms of AM and nitrite showed two well-separated signals corresponding to the oxidation of AM and nitrite that is sufficient for simultaneous determination of amaranth and nitrite at a surface of 1-M-3-BIBr/CuO/SWCNTs/CPE. The developed electrode achieved dynamic ranges 0.004–750 μM for AM and 1.0–10000 μM for nitrite. The detection LODs of AM and nitrite were 1.0 nM and 0.5 μM, respectively. The same electrode was successfully

applied to the analysis of AM and nitrite in foodstuffs (fruit juice and sausage) by standard addition method.

Based on above discussion, we can conclude that the electrochemical determination of AM is a reliable, simple and cheap method, but further improvement is needed in terms of anti-interference ability, reproducibility, and long-term stability. Therefore, future research on electrochemical detection of AM could be based on these aspects.

CONCLUSION, REMARKS AND OUTLOOK

This paper summarizes recent electrochemical sensors designed to detect AM in foodstuffs. Despite the drastic advancements, some challenges are yet to be tackled in order to commercialize electrochemical sensing technologies and apply to food quality control. Addressing the below-mentioned issues is necessary for the application of electrochemical sensors, particularly for AM analysis. Factors including accuracy and affordability should be seriously considered with novel strategies to overcome the limitations in these aspects.

- Considering the high matrix complexity of food/beverage samples, effective approaches should be developed to suppress the non-specific adsorption of interfering species on the electrode surface. Biofouling is a persistent problem that results in a loss of catalytic activity and negatively affects electrochemical sensor's performance in real samples.

- Development of low-cost fabrication methods for mass production of sensor devices while maintaining the accuracy and precision achieved within a laboratory environment. Additionally, the preparation process should be improved by adopting standardized and controllable manufacturing process ensuring the repeatability between sensor devices.

- Fabrication of inexpensive and durable portable electrochemical sensors.

- Integrated smartphone-based electrochemical sensors are important to accomplish the practical application and commercialization of electrochemical sensors for food safety analysis.

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