Rapid Detection of Allura Red (E129) Based on Chitosan/Nanoparticles/MWCNTs Modified Gold Electrode in Food Products

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Abstract
Allura Red AC (E 129) is a mono azo dye that is able to form of powder or granules. It is used to make foods more attractive and appetizing for over centuries. However, excessively intake of food containing synthetic food coloring can caused toxicity and pathogenicity to human. Therefore, there is needed a simple, faster and effective method for the analysis of Allura Red in food products. Herein, an electrochemical sensor for rapid and simple detection of Allura Red was developed based on combination of nanomaterials of chitosan, nanoparticles and multiwall carbon nanotubes (MWCNTs) sensing film. Methylene blue was used as a redox indicator for increasing the electron transfer in electrochemical cell. The electrochemical behavior of modified gold electrode in the presence of Allura Red was studied by using cyclic voltammetries and differential pulse voltammetries. The morphological characteristics of modified electrode were observed under scanning electron microscope and transmission electron microscope. Under optimal conditions, this electrochemical method was found a wide linear Allura Red concentrations range from 10 to 0.5 ppm, with a detection limit are as low as 0.4 ppm. Finally, the proposed sensor method was successfully used to detect Allura Red in different sample food products.

Introduction

Synthetic colorants have been used to replace natural dyes for many years in the food industry due to high stability to light, oxygen and pH, color uniformity, low microbiological contamination and low production cost. Therefore, synthetic dyes have been widely added into foodstuffs and drinks to make them more attractive and appealing (Wang *et al*., 2010; Zhang *et al*., 2013). Allura Red AC (Figure 1), an azoic food colorant, is dark red and water-soluble powder or granules used in various applications, such as in drinks, syrups, sweets and cereals. It is contain –N=N– group, which is electrochemically active and can be reduced at the working electrodes (Wu *et al*., 2015). The excess of consuming foods contain azo groups gives rise to serious reservations concerning health which may cause pathogenic. The azo dyes can be easily decomposed by natural intestinal flora to aromatic amines which lead to headaches, asthma and allergic reaction (Kucharska & Grabka, 2010). Besides, in 1970s it was claimed that some azo coloring agents used as food additives may cause

hypersensitivity reactions (Mikkelsen *et al*., 1978). Recently, more epidemiological studies were published stating that a mixture of azo dyes, including Allura Red, may cause hyper activity in children (McCann *et al*., 2007). Consequently, Allura Red is today permitted to be used as a food additive in human food, but is not acceptable for use in animal feed because of potential genotoxic effects (EFSA, 2012). Shimadaet *et al*. (2010) reported that Comet assay study in mice and rats was presented that confirmed that Allura Red induced DNA damage in the colon of mice. The biological significance of the results from the Comet assays can be questioned in light of negative carcinogenicity studies both in mice and rats. Hence, there is an urgent need for more genotoxicity data, especially in vivo, to resolve this conflicting issue.

Nowadays, many actions were undertaken in different countries when they found the synthetic dyes suspected high potential toxicity. The use in food of many dyes was banned and positive lists containing the permitted colors together with the maxima amounts allowed in the different kinds of food and beverages were prepared (European Directive 94/35/EC). The present legislations evaluate the possible toxicity of dyes and estimate the maxima amounts allowed; however do not consider the dye inertness towards sunlight action and possible reactions with other components present in the colored food yet. The more recent alarm, supported by only few studies, concerns the behavior of dyes that are stable in aqueous solution under the action of sunlight, but can undergo degradation when present in beverages (Gosetti *et al*., 2004; Gosetti *et al*., 2005; Gosetti *et al*., 2007; Gosetti *et al*., 2008). The degradation is clearly evidenced by drink decolouration or color variations that take place in the original sealed bottles already in commerce. Thus, different electroanalytical methods using different working electrode were also reported for determination of Allura Red.

Recently, carbon nanotubes (CNTs), including single-wall carbon nanotube (SWNT) and multi-wall carbon nanotube (MWNT), have obtained wide applications in electrochemical detection owing to the distinctive properties. It have been widely used in electrochemical field due to their unique properties such as large specific surface area, good mechanical stability and high electronic conductivity (Abbaspour & Ghaffarinejad, 2010; Behzad *et al*., 2014; Virupaxi *et al*., 2014). Because of the importance of determination of Allura Red considering food safety, this work focuses on development of electrochemical sensor based on chitosan, zinc nanoparticles and multiwall carbon nanotubes modified gold electrode (CHIT/ZnONPs/MWCNTs/AuE) to investigate the voltammetric behaviours Allura Red present in food products. The sensor shows simple procedure, rapid and simultaneous determination at low concentration levels of Allura Red using differential pulse voltammetry (DPV). The proposed electrochemical sensor showed a high selectivity toward Allura Red as well as a broad linear range and a low detection limit under the optimized conditions. Satisfactory results were also obtained for the determination of Allura Red in real samples.
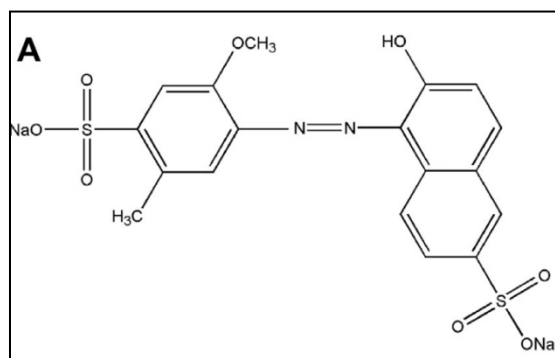
Figure 1: Chemical structure of Allura Red AC (Wu *et al*., 2015).

Methodology

Reagents and chemicals

Allura Red (E129), Chitosan (CHIT) and multiwall carbon nanotubes (MWCNTs) were purchased from Sigma Aldrich (USA). ZnONPs was obtained from Biosensors and Bioelectronics Laboratory, Department of Chemistry, Faculty of Science, Universiti Putra Malaysia. The MB (1 mM) solution was prepared in 50 mM Tris-HCl, 20 mM NaCl buffer solutions (pH 7.0) and stored at room temperature. Other chemicals were of analytical reagent grade and used as received. All aqueous solutions were prepared with deionized water and were carried out at room temperature condition of $25 \pm 0.1\ ^0$C.

Apparatus and equipment

Electrochemical measurements were performed with a μ-autolab (Ecochemie, The Netherlands) voltammetric analyser using the software package on NOVA 1.8 for cyclic voltammetry (CV) and differential pulse voltammetry (DPV) analysis. A standard three-electrode systems were used in the measurement which composed of a bare gold electrode (d = 3 mm) as working electrode, Ag|AgCl (3 M KCl) as the reference electrode and the platinum wire as counter electrode. All solvent pH measurements were performed with pH-2700 Eutech Instruments. The morphological characteristics were observed under Hitachi S-3400N scanning electron microscope (SEM) and transmission electron microscope (TEM).

Preparation of CHIT/ZnONPs/MWCNTs

Before modification, pre-treatment of AuE was performed to ensure the AuE clean. Firstly, the AuE was polished with 3 μM aluminium slurry for 2 min. After that, the AuE was sonicated for 2 min and rinsed with deionized water about 2 min. For immobilization of modified AuE, a 2 % of CHIT solution was prepared by dissolving the CHIT powder in 1 % acetic acid. After that, this solution was stirred for at least 4 h at room temperature until the CHIT was fully dissolved. Later, ZnONPs were added into the 2 % CHIT solution then sonicated for 20 min. Then it was stirred for 8 h for highly

dispersed colloidal suspension. The mass ratio of the ZnONPs:CHIT was 2:5. Then, 1 % of MWCNTs was added in the CHIT/ZnONPs mixture and again sonicated to produce a homogenous suspension. After that, MWCNTs mixtures were allowed for the formation of a uniform CHIT/ZnONPs/MWCNTs nanocomposite suspension.

Analysis of real sample

Candy, royal jelly, ice cream and soft drink were selected as detection samples in this work based on the published work with some modification (Sahraei *et al*., 2013; Tripathi *et al*., 2004). These four samples were purchased from the local super market in Kota Kinabalu, Sabah, Malaysia. Firstly, 5.7199 g candy, 10.0742 g royal jelly and 30.0512 g ice cream were respectively dissolved in 100 mL hot pure water (~45°C). Then, the each of the samples was filtered through a 0.45 µm membrane filter to obtain solution without precipitation for subsequent use (Shawish *et al*., 2013). The soft drink sample was used directly without any pretreatment. When detecting Allura Red using the developed electrochemical colorants sensor, a 0.1 mL sample extract was added into working buffer, and then analyzed according to the optimize analytical procedure.

Result and Discussion

Morphological characteristic of modified AuE

The surface morphological analysis of the CHIT, MWCNTs, CHIT/ZnONPs and CHIT/ZnONPs/MWCNTs were also observed by SEM and TEM shown in Figure 2. Figure 2a shows the mesoporous surface of CHIT membrane. Based on the results, CHIT/ZnONPs was uniform granular porous morphology. The ZnONPs were found well distributed which attached and reacted in the circumference of CHIT membrane, and resulting the surroundings of the particles look brighter against SEM images. No aggregation of the nanoparticles on the surface was found (Figure 2c). The ZnONPs were found brighter images and well-distributed in CHIT network that able to increase the specific surface area. Figure 2d SEM observation of CHIT/ZnONPs/MWCNTs shows the folding structure of MWNCTs in the middle of chitosan pores. Besides, the image clearly illustrates that ZnONPs are distributed individually with legible boundary lines. After that, nanomaterials are observed by using TEM for analysing their particle size and shape. The image of pure MWCNTs shows a typically smooth surface and looks like gel-like substance in Figure 2e. Figure 2f shows the nanoparticles shape under 30 K magnification of TEM with nanoscale of 50 nm of CHIT/ZnONPs/MWCNTs. It can be seen that the zinc nanoparticles are well attached onto the CHIT with size of ZnONPs approximately of 4–5 nm diameter. The ZnONPs were spherical in shape.
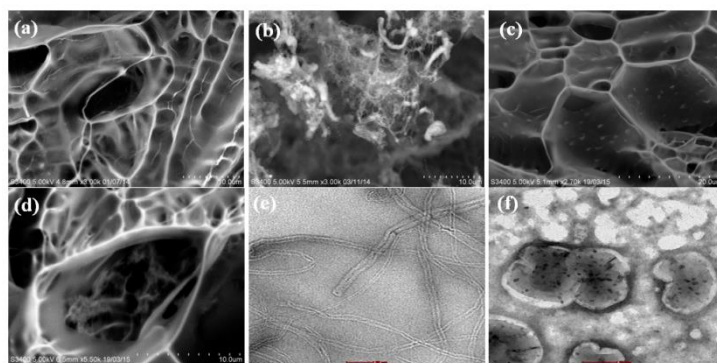
Figure 2: SEM image of the (a) CHIT, (b) MWCNTs, (c) CHIT/ZnONPs,
(d) CHIT/ZnONPs/MWCNTs and, TEM image of (e) MWCNTs, (f) CHIT/ZnONPs

Optimization of modified AuE

i.   Influence of pH

The pH value of the electrolyte solution is important for the performance of the electrochemical sensor. Therefore, the pH effect of the working electrolyte solution on the electrochemical behavior of Allura Red was studied by using CV. With the pH changing from 6.0 to 8.5, the response current increased and reached a maximum at pH 7.0, and then decreased obviously shown in Figure 3. Therefore, pH 7.0 was selected as the optimized value for the electrochemical oxidation of Allura red. The anodic peak potential is strongly dependent on solution pH. A linear negative shift was observed in the anodic peak potential of allura red by increasing ph of the electrolyte solution. This is a consequence of the deprotonation involved in the oxidation process, which is facilitated at higher pH values. According to the Nernst equation, the slope indicated that the numbers of electrons and protons involved in the charge transfer was the same.
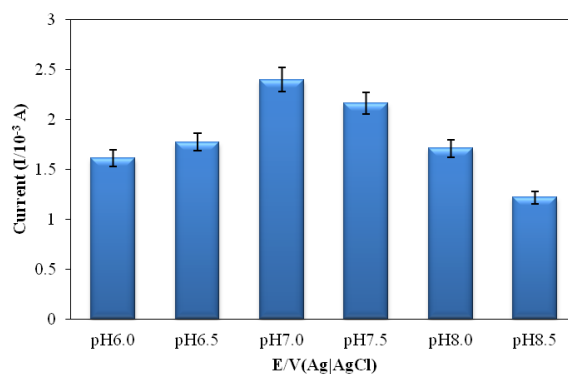
Figure 3: The relationship between the oxidation peak current and pH of Tris-HCl buffer with MB in solution containing 10 ppm of Allura Red.

ii.   Influence of scan rate and time interaction

The interaction time of Allura Red has influenced the performance of the AuE in the present of MB. In the same solution as above, the interaction time changed from 5 to 40 s when the scan rate fixed of

0.30 V/s. The maximum current response was obtained at 30 s, implying that the accumulation of Allura Red is very rapid to each of saturation at the surface of AuE. The peak current value was increased slowly with the time, thus 30 s was choosen as optimum interaction time for the rapid detection. Useful information involving electrochemical reaction can usually be obtained from the relationship between the peak current and scan rate. The kinetics of the AuE reaction was investigated by studying the effects of scan rate on the peak currents. Therefore, the influence of scan rate on oxidation of Allura Red at the surface AuE was investigated by CV. The MB was used in Tris-HCl buffer solution (pH 7.0) containing 10 ppm of Allura Red by CV, varying the scan rate was used from 0.05 to 0.4 V/s, with 5 s. The peak current increased with increasing scan rate and reached the highest at 0.3 V/s. Therefore, 0.3 V/s was selected as the optimum scan rate.

Electrochemical characterization of modified AuE

The electrochemical behaviour of the bare AuE and modified AuE were characterized by DPV using MB as electroactive indicator was shown in Figure 4. The CHIT/ZnONPs showed highest oxidation peak current when compared to bare AuE that shown in Figure 4 curve b. From this result, it suggested that the CHIT/ZnONPs/MWCNTs/AuE (Figure 4 curve c) had a great electrochemical activity for Allura Red detection when compared to bare AuE.
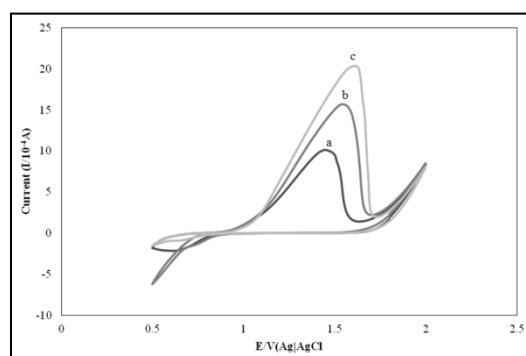

Figure 4: DPV of bare AuE (a), CHIT/ZnONPs/AuE (b) and CHIT/ZnONPs/MWCNTs/AuE in 0.1 M Tris-HCl (pH 7.0) with presence of 10 ppm of Allura Red.

Electrochemical determination of Allura Red

In this study, DPV was employed for the quantitative determination of Allura Red, which is relatively sensitive compared to the CV analysis. Figure 5 shows the DPV responses of different concentration of Allura Red in 50 mM Tris-HCl 20 mM NaCl (pH 7.0) with 1 mM MB as the redox indicator. Under the optimized experimental conditions, the increase of anodic peak current was directly proportional to Allura Red concentration in the range of 10 ppm to 0.5 ppm, with detection limit was 0.4 ppm (n = 5). At potential volt -0.17, the linear regression equation was expressed as y = 0.4947x + 1.6721 with correlation value of r = 0.9627. These results indicated a very good analytical performance of the current chemical sensor.
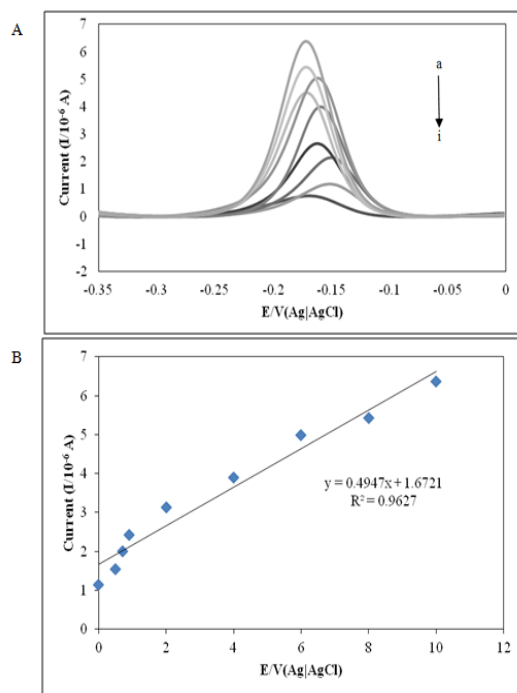
Figure 5: (A) DPV of different concentration Allura Red in analytical buffer (Tris-HCl, pH 7.0) (a-i: the concentration of Allura Red is 10, 8, 6, 4, 2, 0.9, 0.7, 0.5 and 0 ppm). (B) Linear relationship between the oxidation peak currents obtained by DPV and the logarithm of Allura Red concentrations.

Analysis of real sample

In order to validate and verify the applicability of the proposed electrochemical method, the detection of Allura Red in some food sample was studied. The electrochemical method was applied four different samples which are candy, royal jelly, ice cream and soft drink to investigate the applicability. The recovery rate was calculated to be 90.8 to 96.7 %, showing the accuracy of the developed method. The recovery rate of the proposed methods range showed good relative standard deviation (RSD) which was less than 1 % (Table 1).

Table 1: Recovery studies of food products

Samples	Recovery	RSD (%)
Candy	90.8	0.31
Royal jelly	94.1	0.46
Ice cream	96.7	0.35
Soft drink	92.3	0.23

Conclusion

In conclusion, this study has successfully developed a simple and rapid electrochemical technique to detect Allura Red by using CHIT/ZnONPs/MWCNTs modified gold electrode with MB as redox

indicator. The developed electrochemical sensor has a good sensitivity and stability for the detection of Allura Red. The peak currents increased with the increasing Allura Red concentration from 10 ppm to 0.5 ppm. The detection limit is lower than that of traditional methods which is of 0.4 ppm, with linear coefficient of 0.9627 and the recovery rate for food beverages products was 90.8 to 96.7 %. Finally, the developed method is successfully applied for the determination of Allura Red level in food products. These results suggested that the developed electrochemical biosensor offers a simple, fast response, good selectivity, high sensitivity, wide detection range and convenient method for use in food research laboratories.

Acknowledgements

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