Highly sensitive determination of sunset yellow FCF (E110) in food products based on Chitosan/Nanoparticles/MWCNTs with modified gold electrode

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Abstract. Sunset Yellow belongs to the family of azo dyes, commonly used in food industry. High consumption of Sunset Yellow can cause health problem to human. Due to arising of the health issues, there are several analytical methods available for determination of Sunset Yellow. However, these methods are required skilled manpower, complicated procedures, time consuming and high cost. Herein, an electrochemical sensor was developed based on the combination of chitosan (CHIT), calcium oxide nanoparticles (CaONPs) and multiwall carbon nanotubes (MWCNTs) sensing film for detection of Sunset Yellow in food products. Electrochemical behavior of the modified gold electrode in the presence of Sunset Yellow was studied by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The morphological characteristics of CHIT/CaONPs/MWCNTs were observed under scanning electron microscope and transmission electron microscope. Under optimal conditions, the DPV was detected with different concentrations of Sunset Yellow in the range of 0.9 to 10 ppm, with detection limit of 0.8 ppm. The developed method has successfully applied for monitoring the presence of Sunset Yellow with different food products including candy, royal jelly, ice cream and soft drink with satisfactory results.

1. Introduction

Sunset Yellow FCF (E110) belongs to the azo group with formula of $C_{16}H_{10}N_2Na_2O_7S_2$. This color is commonly used in the production of swiss roll, apricot jam, lemon curd, sweets, beverage mix and packet soups, margarine, custard powders, packaged lemon gelatin desserts, breadcrumbs, packaged instant noodles, cheese sauce mixes and powdered marinades by food industry. Sunset Yellow commonly consisted of 2-hydroxyl-(4-sulphonatophenylazo) naphthalene-6-sulphonate and subsidiary coloring matters together with sodium chloride and sodium sulphate as the principal of non-colorant components. Sunset Yellow is synthesized by diazotizing 4-aminobenzenesulphonic acid using combinations of hydrochloric and sodium nitrite, or sulphuric acid and sodium nitrite. High consumption of Sunset Yellow may cause allergic such as contact urticaria, angioneurotic edema, asthma, contact anaphylaxis and immune suppression [1]. Besides, Sunset Yellow is able to alter the

reproductive and neurobehavioral parameters, which lead to allergies, diarrhea and other symptoms [2, 3]. Food and Drug Administration strictly fixed the maximum level of Sunset Yellow in non-alcoholic beverages is less than 100 μg mL^{-1} [4].

Several methods have been developed such as electrochemical sensor, enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), and spectrophotometry. Among these methods, electrochemical sensor has gained much attention and demonstrated the promising tool for monitoring food systems due to high sensitivity and selectivity, portability, low detection and quantification limit, faster analysis, and low cost. Sunset Yellow is electrochemical active compound that undergo reversible reaction during electrochemical process. Up-to-date, different modified electrodes are employed, for example, Ghoreishi et al. [5] have been addressed gold nanoparticles-modified carbon paste electrode (CPE) and Chen et al. [6] have introduced alumina microfibers-modified CPE. Another effort have been developed based on the multiwalled carbon nanotube (MWCNT)-modified glassy carbon electrode (GCE) [7,8], a platinum wire-coated electrode [9], a graphene layer-wrapped phosphotungstic acid (PTA) hybrid film-modified GCE [3] for determination of Sunset Yellow in food products. Carbon nanotubes (CNTs), including single wall carbon nanotubes (SWNTs) and multiwall carbon nanotubes (MWCNTs) are widely applied in electrochemical sensor due to their unique properties such as large specific surface area, good mechanical stability and high electronic conductivity [10, 11]. In this study, a novel electrochemical sensor was developed based on chitosan, calcium nanoparticles and multiwall carbon nanotubes modified gold electrode (CHIT/CaONPs/MWCNTs/AuE) for the determination of Sunset Yellow in food products. The schematic mechanism oxidation of Sunset Yellow took place a one-electron, one-proton reversible reaction on CHIT/CaONPs/MWCNTs/AuE shown in Figure 1.

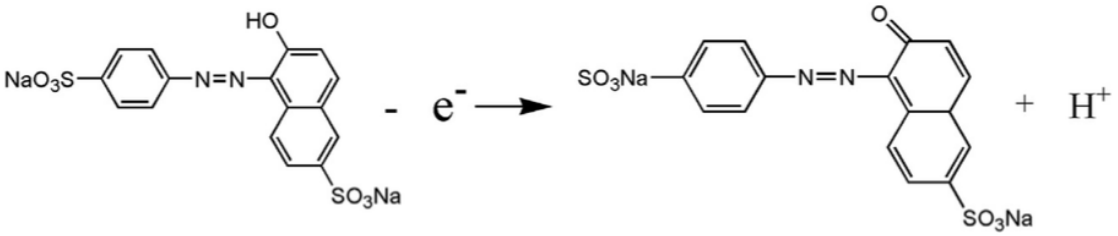

Figure 1. Oxidation mechanism for the electrochemical process of Sunset Yellow [16].

2. Experimental

2.1 Reagents and chemicals

Sunset Yellow FCF (E110), chitosan (CHIT) and multiwall carbon nanotubes (MWCNTs) were purchased from Sigma Aldrich (USA). Calcium oxide nanoparticles (CaONPs) were obtained from Biosensors and Bioelectronics Laboratory, Department of Chemistry, Faculty of Science, Universiti Putra Malaysia. Sunset Yellow (50 ppm) was dissolved into dH_2O as a stock solution and stored at 4^oC. Methylene blue (MB) (0.1 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 solutions were prepared using deionized water as the solvents and were conducted the experimental works at room temperature condition of 25 ± 0.1 ^0C.

2.2 Apparatus and equipment

All electrochemical measurements were carried out with a μ-autolab (Ecochemie, The Netherlands) voltammetric analyzer using the software package on NOVA 1.8 for CV and DPV analysis. A three-electrode systems were used in the measurement which composed of a gold electrode (d = 3 mm) as working electrode, Ag|AgCl|KCl as the reference electrode and the platinum wire as counter electrode. The morphological characteristics were observed under Hitachi S-3400N scanning electron microscope (SEM) and transmission electron microscope (TEM) (Tecnai G2 Spirit Bio TWIN, Crezh Republic).

2.3 Preparation of CHIT/CaONPs/MWCNTs

Before fabrication, the gold electrode (AuE) was pre-treatment. The AuE was polished with 3 μM aluminium slurry for 2 min and then sonicated for 2 min. Then, the AuE was rinsed with deionized water about 2 min. For modification of AuE, a 2 % of CHIT solution was prepared by dissolving the CHIT powder in 2 % acetic acid. The solution was stirred for at least 4 h at room temperature until the CHIT was fully dissolved. CaONPs were added into the 2 % CHIT solution with ratio CaONPs:CHIT was 2:5 then sonicated for 20 min. It was stirred for 8 h for highly dispersed colloidal suspension. Again 1 % of MWCNTs was added in the CHIT/CaONPs mixture and sonicated to produce a homogenous suspension. After that, MWCNTs mixtures were allowed the formation of a uniform CHIT/CaONPs/MWCNTs nanocomposite suspension.

2.4 Analysis of real samples

Candy, royal jelly, ice cream and soft drink were selected and purchased from the local super market in Kota Kinabalu, Sabah, Malaysia. The samples preparation procedures are followed previously reported by Sahraei et al. [13]. 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^{o}C). Each of the samples was filtered through a 0.45 μm membrane filter to obtain solution without precipitation for subsequent use [14]. The soft drink sample was used directly without any pretreatment. Finally, 0.1 mL sample extract was added into working buffer, and then analyzed according to the optimization electrochemical sensor protocols.

3. Results and discussion

3.1 Morphological characteristic of modified AuE

Firstly, morphology of the modified AuE surface was observed using SEM. Figure 2a, CHIT membrane was showed mesoporous surface that able to immobilize with other nanomaterials. The pure MWCNTs film clearly illustrated in Figure 2b that shows the bundles interlocked together to form netlike and highly mesoporous nanostructure due to the strong π-π electronic interactions and lack of attached oxygenated groups on the surface of MWCNTs [15]. In Figure 2c, CHIT/CaONPs revealed the well dispersed of CaONPs were adhered well to the CHIT nanocomposite membrane. The CaONPs were found well distribution that attached and reacted in the circumference of CHIT membrane, and result the surroundings of the particles were looked brighter against SEM images. Figure 2d shows CHIT/CaONPs/MWCNTs with folding structure of MWNCTs in the middle of CHIT pores. It could be seen that the surface of the CHIT/CaONPs/MWCNTs nanocomposite was relatively rough and possesses distinct interstices, which was good for intensely accumulation of Sunset Yellow and increasing the activity sites. Hence, CHIT/CaONPs/MWCNTs were provided more adsorption sites and exhibited high accumulation efficiency of Sunset Yellow. Additionally, the nanomaterials were analyzed using TEM for providing insight into the origin of enhancement effect for Sunset Yellow oxidation. Figure 2e and Figure 2f show the nanoparticles shape under 30 K magnification of TEM with nanoscale of 50 nm of CHIT/CaONPs/MWCNTs. It was clearly indicated that the CaONPs were well attached onto the nanocomposite membrane with size approximately of less than ~10 nm diameters.

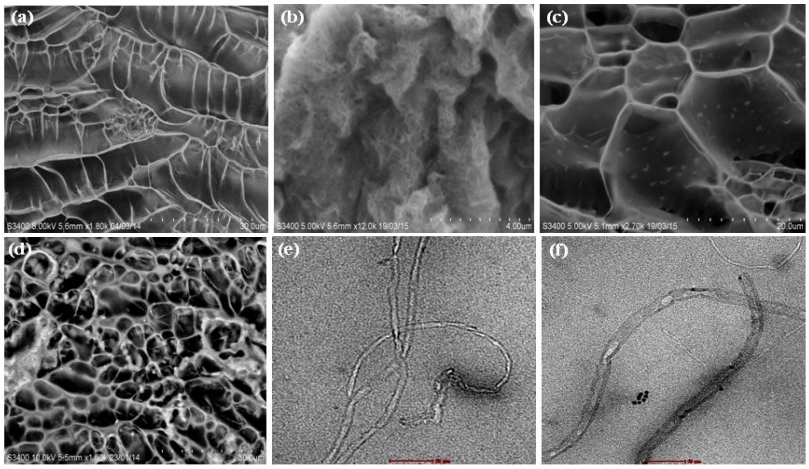

Figure 2. SEM image of the (a) CHIT, (b) MWCNTs, (c) CHIT/CaONPs, (d) CHIT/CaONPs/MWCNTs and, TEM image of (e) MWCNTs, (f) MWCNTs/CaONPs.

3.2 Optimization of the modified AuE

3.2.1 Influence of pH

The electrochemical behaviors of Sunset Yellow with different pH solutions were assayed using CV method. The highest peak current responses on the CHIT/CaONPs/MWCNTs/AuE are shown in Figure 3. The applied supporting electrolyte was measured the pH values in the range of 6.0-8.5. With the pH changing from 6.0 to 7.0, the response of oxidation current increased and reached a maximum at pH 7.0, above pH 7.0, decreasing the current response. Thus, it is indicated that the reversibility of Sunset Yellow oxidation is better at pH 7.0 which suggesting that the number of transferred proton and electron was same. Besides, at pH 7.0, it can be seen that the oxidation signals were more sensitive compare to reduction peak currents. However, the oxidation currents were then decreased obviously in the alkaline condition. The experimental results demonstrate that high acidic or alkaline surroundings have possibility damage the structure of the nanocomposite membrane and make current response decrease [6, 16]. Thus, the oxidation peak current on pH 7.0 was used as analytical signals for Sunset Yellow in subsequent experiments.

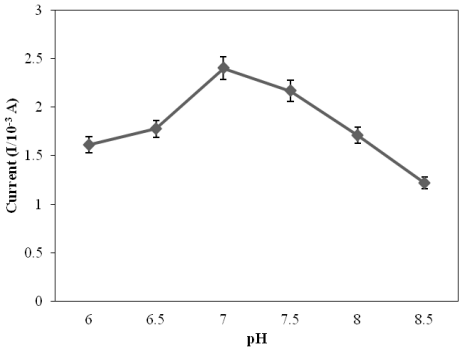

Figure 3. The relationship between the oxidation peak current and pH of Tris-HCl buffer with MB in solution containing 10 ppm of Sunset Yellow.

3.2.2 Influence of accumulation time and scan rate

For handling convenience and further improving the sensitivity, the determination of Sunset Yellow was employed with different scan rate in the range of 0.05 to 0.4 V/s. Cyclic voltammetry was used to investigate the influence of scan rate with the modified CHIT/CaONPs/MWCNTs/AuE surface. From Figure 4A, it is found that the oxidation signal of Sunset Yellow obviously increased from 0.05 to 0.3 V/s, indicating that accumulation efficiency of Sunset Yellow on the AuE surface is always higher.

After 0.3 V/s, the oxidation peak currents of Sunset Yellow were decreased. The high sensitivity and excellent oxidation currents peak was achieved at 0.3 V/s, which was selected as the optimum scan rate.

The interaction time of Sunset Yellow has influenced the performance of the CHIT/CaONPs/MWCNTs/AuE in the presence 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. Accumulation time can improve the loading amount of Sunset Yellow on the surface of CHIT/CaONPs/MWCNTs/AuE, and able to amplify the electrochemical signals. Figure 4B represents the influence of different time interaction between oxidation peak currents Sunset Yellow and CHIT/CaONPs/MWCNTs/AuE. The peak current of Sunset Yellow increased gradually and reached a plateau after 30 sec, meaning that 30 sec was sufficient to reach the saturation of Sunset Yellow on the CHIT/CaONPs/MWCNTs/AuE. Due to the electrode surface was completely attached by Sunset Yellow with the increasing accumulation time and lead to increase the peak current [16]. Consequently, 30 sec was chosen as optimum interaction time for detection.

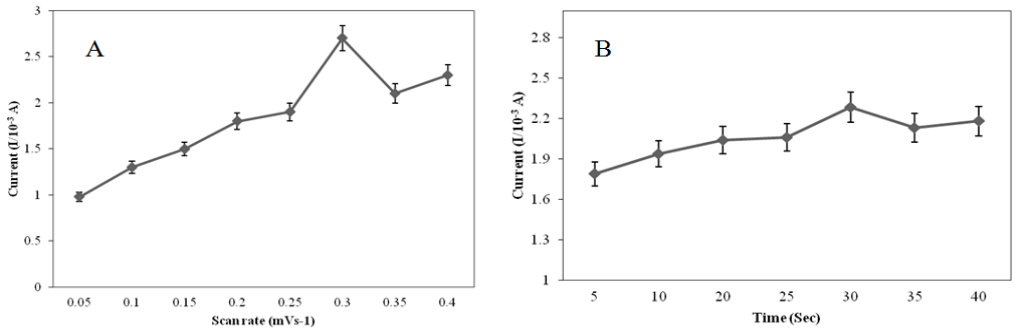

Figure 4. The relationship between the oxidation peak current (A) different scan rate and, (B) different accumulation time in solution containing 10 ppm of Sunset Yellow.

3.3 Electrochemical characterization of the modified AuE

The electrochemical behavior of the bare AuE and modified AuE were characterized by DPV using MB as electroactive indicator (Figure 5). The CHIT/CaONPs/MWCNTs/AuE (curve a) was found highest oxidation peak currents according to CHIT/CaONPs (curve b) and bare AuE (curve c). CHIT/CaONPs/MWCNTs/AuE was attributed large surface area and high accumulation efficiency of Sunset Yellow. Meanwhile, CHIT/CaONPs and bare AuE were exhibited that the oxidation activity of Sunset Yellow was very poor due to less electron and proton transferring. It is clearly indicated that the oxidation signal is more sensitive CHIT/CaONPs/MWCNTs/AuE as compare to the reduction signal.

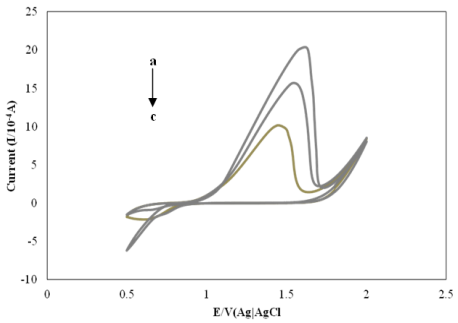

Figure 5. DPV measured different electrode surface in the presence in pH 7.0, Tris-HCl buffer: a) CHIT/CaONPs/MWCNTs/AuE, b) CHIT/CaONPs/AuE and c) bare AuE with 10 ppm of Sunset Yellow.

3.4 Determination of Sunset Yellow

Differential pulse voltammetry (DPV) was performed to investigate the relationship between the peak currents and different concentration of Sunset Yellow. Figure 6 shows the DPV responses with different concentrations of Sunset Yellow using MB as a redox indicator. Under the optimum conditions, DPV was detected with different concentration of Sunset Yellow in the range of 0.9-10 ppm, with detection limit of 0.8 ppm (n = 5). At potential volt -0.18, a linear regression equation was expressed as I (10^{-6} A) = 0.6006 (ppm) + 0.7071, with correlation value of r^2 = 0.9931. These results indicated a very good analytical performance of the developed electrochemical sensor in food products analysis.

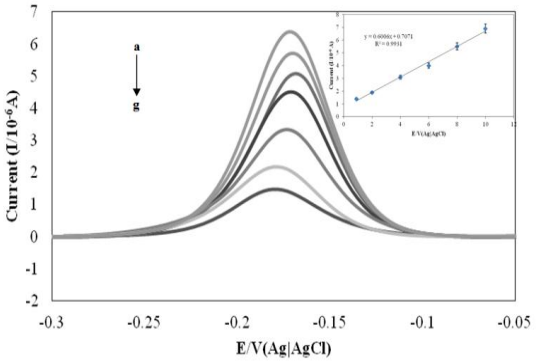

Figure 6. (A) DPV method was measured with different concentrations of Sunset Yellow in analytical buffer (Tris-HCl, pH 7.0) (a-g: 10, 8, 6, 4, 2, 0.9, and 0 ppm). Inset: calibration plots of the oxidation peak currents as a function of Sunset Yellow.

3.5 Analytical application in food products

In order to validate and verify the applicability of the developed method, the CHIT/CaONPs/MWCNTs/AuE was applied for determination of Sunset Yellow at varying food samples purchased from local market, Kota Kinabalu, Sabah. Four different samples were analyzed including candy, royal jelly, ice cream and soft drink. The accuracy of the modified AuE was analyzed by performing a recovery test after spiking the samples. Based on the results obtained, the recovery rates were calculated to be 91.8 to 97.5%, showing the high accuracy and feasible. Three replications were conducted per experiment, and the relative standard deviation was found lower than 1%, revealing that the results obtained by the developed method are acceptable and good precision (Table 1). Thus, the developed method can be successfully applied for detection of Sunset Yellow in food products.

Table 1. Recovery studies of food products.

Samples	Recovery	RSD (%)
Candy	95.2	0.27
Royal jelly	91.8	0.31
Ice cream	92.3	0.62
Soft drink	97.5	0.38

4. Conclusion

A simple and rapid electrochemical sensor has developed based on the modified gold electrode (CHIT/CaONPs/MWCNTs/AuE) using MB as a redox indicator. The developed method has exhibited high sensitivity and stability for detection of Sunset Yellow. The peak currents have increased with the increasing Sunset Yellow concentrations from 0.9-10 ppm. The detection limit is calculated of 0.8 ppm, which is much lower than traditional methods, with linear coefficiency of 0.9931. The recovery rates of food beverages products are 91.8-97.5 %. The developed method is successfully applied for determination of Sunset Yellow level in food products. The developed electrochemical sensor offers a simple, fast, high selectivity and sensitivity, wide detection range and convenient method for use in food research laboratories.

5. References

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Acknowledgments

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