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Electrochemical Analysis of Sunset Yellow Based on NiO-SWCNTs NC/IL Modified Carbon Paste Electrode in Food Samples

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ABSTRACT: In this study, a rapid and sensitive electrochemical electrode was fabricated to measure the amount of sunset yellow in food samples. This analytical sensor was mediated using a NiO-decorated single-walled carbon nanotubes (NiO-SWCNTs) nanocomposite and N-octylpyridinium hexafluorophosphate ionic liquid (IL). The morphology of the NiO-SWCNTs nanocomposite was investigated by means of X-Ray Diffraction (XRD) and Transmission electron microscopy (TEM) methods. Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) techniques were utilized to verify the appropriateness of the suggested sensor. In comparison to the bare carbon paste electrode (CPE), the electrochemical response of modified electrode had a considerable improve. According to the Chronoamperometry and scan rate analyses, diffusion index (D) and transfer coefficient (α) were obtained 1.8×10^{-5} cm^2/s and 0.52, respectively. The outcomes achieved from SWV technique demonstrated a reasonable linear dynamic range (LDR) between 0.09 and 750 μM, and the Limit of Detection (LOD) was obtained 0.05 μM. Eventually, the quantity of sunset yellow in real samples showed a recovery range between 99.2 and 99.86%, confirming the accuracy of the proposed sensor.

Keywords: *Electrochemical Sensor, Food Dyes, Ionic Liquid, Nanocomposite, Sunset Yellow.*

Introduction

Nowadays, food dyes have been classified into two basic categories, including synthetic dyes and natural dyes. Synthetic dyes can enhance natural color variations, and also, they can intensify the quality of natural colors, or they may add color to colorless foods and make them more appealing and appetizing. Moreover, food producers believe that synthetic dyes together with reducing the cost of production can make foods more durable (Khanavi *et al*., 2012).

In spite of the mentioned advantages, some of these substances pose potential health risks to consumers, especially when they are consumed excessively. In addition, some synthetic dyes have some mutagenic effects and may have potential genotoxicity dangers (Biswas & Khuda-Bukhsh, 2005; Tsuboy *et al*., 2007). They might be carcinogenic, and in some cases could cause gastric annoyance, bronchial asthma symptoms, vomiting, diarrhea, angioedema, urticarial, rhinitis, nasal congestion, itching, and headaches. Furthermore, there are a few problems stemmed from the usage of synthetic dyes which affect children, such as

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hypersensitivities, hyperactivity, negative effects on cellular immune responses, and Learning difficulties (Arnold *et al.*, 2012; Nigg *et al.*, 2012). Therefore, the application of synthetic dyes in food products is rigorously limited by different national legislation (DIRECTIVE, 1994; Medeiros *et al.*, 2012).

Sunset Yellow, also known as Orange Yellow S, Yellow 6, is an orange synthetic food dye derived from petroleum and exists in various popular food products, such as jelly beans, fruit juices, bakery products, sugar candies, powdered drinks, sports beverages, ice creams, and sugar-coated medicines (Nevado *et al.*, 1997). As the application of synthetic food dyes has been increased remarkably, various detecting procedures have been developed in order to inhibit the over usage of these substances, such as HPLC (Alves *et al.*, 2008; Culzoni *et al.*, 2009; Martin *et al.*, 2016; Tatebe *et al.*, 2012), enzyme-linked immunosorbent assay (ELISA) (Xing *et al.*, 2012), spectrophotometry (Aktaş & Ertokuş 2010; Aman & Gupta 2012; Capitán-Vallvey *et al.*, 1997), HPLC–mass spectrometry (HPLC–MS) (Gosetti *et al.*, 2005) and capillary electrophoresis (Lee *et al.*, 2008). Despite the benefits of these approaches, they have some considerable glitches, including their expensive technology, complicated sample preparation and considerable amounts of analytical resources, which can cause substantial delays when the analyses are being carried out. Thus, it is necessary to employ a method which does not have these limitations.

Currently, electrochemical technique has been utilized for the measurement of sunset yellow in some food products (Ghoreishi *et al.*, 2012; Wang *et al.*, 2014, 2015; Zhang *et al.*, 2013). This method offers plenty of advantages, namely its straightforward application, low-cost process, and brief analysis period. In comparison to the other methods, high sensitivity and noticeable precision are the other benefits offered by electrochemical method, making it an appropriate method for investigation of synthetic dyes in foods, pharmaceuticals, and cosmetics (Vladislavić *et al.*, 2018). However, the electrodes used in electrochemical analysis need to be modified, because they have some problems such as low selectivity and stability, poor sensitivity and the blockage of electron transfer (Afsharmanesh *et al.*, 2013; Bijad *et al.*, 2013; Ensafi *et al.*, 2013; Sanghavi & Srivastava, 2010).

Recently, a wide range of electro-active compounds have been utilized in the modification of surface of electrochemical sensors (Ebrahimi *et al.*, 2020; Keyvanfard *et al.*, 2014; Tavana *et al.*, 2012; Zabihpour *et al.*, 2020a). For instance, nanomaterials (e.g. Carbon Nanotubes) are potential electrode-modifying compounds (Chandran *et al.*, 2014; Wang *et al.*, 2010; Zhu *et al.*, 2014). In addition, ionic liquids are employed as new types of modifiers. Since they have many useful characteristics such as high ionic conductivity, non-flammability, wide electrochemical window, non-volatility and excellent solubility (Ansari *et al.*, 2013; Beitollah *et al.*, 2012; Tavana *et al.*, 2012), they have been identified as a valuable non-aqueous medium for numerous electrochemical processes (Ensafi *et al.*, 2011; Salmanpour *et al.*, 2012; Vahedi *et al.*, 2013). The interaction of ionic liquid with hydrophilic molecules like sunset yellow improves the recognition capacity of the modified sensor and makes it a potential mediating material in the sensor used for the determination of this food dye (Zhu *et al.*, 2014).

There are some scientific works struggling to investigate the quantity of sunset yellow in food samples. Motahharinia *et al.* (2020) conducted a research offering an electrochemical sensor modified with ZnO-Pd and ionic liquid for the detection of sunset yellow. Eventually, their sensor determined sunset yellow in the concentration range of 0.001-280 µM and in the limit of detection of 0.4 nM. Moreover, Wang *et al*. (2015) carried out a study investigating the electrochemical behavior of sunset yellow, using a gold nanoparticles/graphene electrode. The LDR obtained by this was between 0.002 µM and 109.14 µM and the LOD was 2 nm.

This invistigation, as its main aim, suggests a modified electrochemical sensor to analyze the electrochemical behavior of sunset yellow, which can determine the amount of this food dye in food samples. The NiO-SWCNTs nanocomposite and N-octylpyridinium hexafluorophosphate ionic liquid were used to modify the carbon paste electrode. Also, CV and SWV methods were employed to measure the amount of sunset yellow in orange jelly and orange juice.

Materials and Methods

- *Reagents and instruments*

The characteristics of the chemicals used in this study are shown in Table 1. Double distilled water with the conductivity of 0.2–0.7 µS was used for the preparation of solutions. A vacuum oven model Vaciotem-T was used in the drying process of fabricated nanocomposite and a pH meter model 766 Calimatic was utilized in preparing the used buffers. A centrifuge manufactured by Hettich model EBA200 was used in the preparation of real samples. Also, the XRD pattern investigating the structure of the synthesized nanocomposite was recorded using an XRD apparatus model X' Pert Pro, and TEM analysis was carried out using a JEOL JEM-2100 instrument. All parts of the electrochemical analysis were carried out using a µ-Autolab, potentiostat/galvanostat (FRA2, TYPE III) device connected to a PC (Pentium IV). A Metrohm electrochemical cell was used as a container to hold electrodes, electrolyte and analyte. The employed electrodes were a Platinum wire as Auxiliary electrode (Azar Electrode), an Ag/AgCl (KClsat) electrode as reference electrode (Azar Electrode) and a modified Carbon paste electrode as working electrode.

- *Preparation of NiO/SWCNTs nanocomposite*

Firstly, the 1.5 g SWCNTs-COOH bought from Sigma-Aldrich Company, was added into 75 mL solution including 1 M Nickel (II) nitrate hexahydrate and stirred for 2.5 hours. Secondly, 75 mL of 1.0 M sodium hydroxide was dispersed into the obtained blend, and it was shaken for 40 min. Eventually, the sedimentation of the fabricated mixture was filtered and after a drying process in a vacuum oven for 20 hours at 80°C, the obtained compound was calcined at 400°C for 3 hours.

- *Fabrication of the modified electrode*

The NiO-SWCNTs nanocomposite/IL modified carbon paste electrochemical sensor was fabricated by blending 0.10 g of ionic liquid (N-octylpyridinium

Table 1. The characteristics of chemicals used in this study

Substance	Purity (%)	Cas Number	Manufacturer
Sunset Yellow	90	2783-94-0	Sigma-Aldrich
Graphite Powder	99	7782-42-5	Merck
Paraffin oil	99	8002-74-2	Merck
Diethyl ether	99.5	60-29-7	Merck
Sodium hydroxide	97	1310-73-2	Merck
Phosphoric acid	99	7664-38-2	Merck
N-octylpyridinium hexafluorophosphate	99	304680-36-2	Alfa-Chemistry
SWCNTs-COOH	90	308068-56-6	Sigma- Aldrich
Nickel (II) nitrate hexahydrate	99.9	13478-00-7	Sigma- Aldrich

hexafluorophosphate), 0.90 g of the liquid paraffin, 0.1 g of NiO-SWCNTs nanocomposite and 0.9 g of graphite powder. The mentioned blend was mixed for 40 min until achieving a soft moist paste. A glass tube was used as electrode frame; Therefore, the obtained paste was filled into the glass tube. In order to increase the accuracy of the test, the electrode was polished on the surface of a paper in every stage.

- *Real sample preparation*

Orange jelly powder (Farmand) and orange juice (Sun Star) were purchased from the local markets in Iran. In order to prepare the orange jelly sample, 1.5 g of orange jelly purchased from Farmand was dissolved into 50 mL hot distilled water, and the obtained blend was stirred with a glass rod stirrer. The mixture was then cooled at room temperature. After cooling, using distilled water, the solution was diluted to 100 mL in a volumetric flask. The obtained solution was used in the real sample test.

In order to prepare the purchased orange juice for the real sample analysis, a proper amount of the juice was poured at the centrifuge tube and was centrifuged at 5000 RPM for 15 minutes. The separated liquid phase from the centrifuge tube was employed in the real sample examination. Eventually, in two separate stages, 5 mL of the orange jelly and orange juice were transferred to the electrochemical cell, and the amount of sunset yellow used in these food products was detected using SWV under the optimized conditions.

- *Electrochemical analysis*

All the analyses were carried out at the temperature of 24 ± 1 °C. Phosphate buffer (0.1 M, pH 6.0) was used as an electrolyte solution and all the electrochemical analyses of sunset yellow were conducted in its presence. In doing this, firstly, Phosphate buffers from pH 2 to 7 were prepared by means of a pH meter, and the optimized pH was obtained using CV method, therefore the pH indicating the highest current in the cyclic voltammogram of NiO-SWCNTs/IL/CPE was chosen as the optimized one. Secondly, the chronoamperometry method and scan rate test were employed to obtain the diffusion index (D) and transfer coefficient (α), respectively. The SWV method was carried out to obtain the LDR and LOD of proposed sensor (the potential range was from 650 to 950 mV (vs Ag.AgCl)). Eventually, the real sample test was carried out to examine the ability of the suggested sensor in the determination of sunset yellow in real food samples.

Results and Discussion

- *Characterization*

TEM and XRD methods were used to study the morphology of the NiO-SWCNTs nanocomposite. The X-ray diffraction pattern and TEM image of NiO-SWCNTs nanocomposite are shown in Figure 1, A and B, respectively. As it can be seen, the synthesis of NiO-SWCNTs nanocomposite was confirmed by the results. The X-ray diffraction pattern (Figure 1 A) of the prepared NiO-SWCNTs illustrates its crystalline structure, and it indicates the absorption of the diffraction peaks at 2θ values. This pattern has a wide plane that is correlated to the carbon phase of SWCNTs with a Miller index of [002], and also, 5 other planes indicated using [111], [200], [220], [311] and [222] Miller indexes are correspond to NiO (Miraki *et al*, 2019). These planes dramatically resemble the X-ray diffraction patterns of NiO/SWCNTs obtained by other scientific works (Afshar *et al*., 2020; Salmanpour *et al*., 2018). Furtheremore, TEM image showed in Figure 1 B proved that the spherical NiO nanoparticle are decorated at the surface of SWCNTs (single wall carbon nanotubes).

- ***Electrochemical behavior of sunset yellow***

Figure 2 demonstrates electrochemical behavior of 700 μM sunset yellow at the CPE (curve a), NiO-SWCNTs/CPE (curve b), IL/CPE (curve c) and NiO-SWCNTs/IL/CPE (curve d). As expected, when we used both of the mediators in the structure of carbon paste, there was a strong anodic peak for sunset yellow, (curve d). According to the recently conducted electrochemical research in food sample analyses, it can be claimed that when the modified electrode indicates a higher Current, it has a more excellent ability in the characterization of the analyte (Ebrahimi *et al*., 2020; Karimi-Maleh *et al*., 2014; Zabihpour *et al*., 2020b). Thus, the high oxidation peak acquired for sunset yellow at the NiO-SWCNTs/IL/CPE confirms the excellent electronic conductivity of synthesized nanocomposite and used ionic liquid.

- ***Effect of pH values***

In this stage, the investigation of the electrochemical behavior of sunset yellow was conducted at the surface of NiO-SWCNTs/IL/CPE with different pH values (from 2.0 to 7.0) using cyclic voltammetry method (Figure 3 inset), and eventually, pH 6.0 was chosen as an optimum pH, because it had the maximum level of current. According to Figure 3, a good linear relationship between the maximum potentials of anodic peaks and pH values can be observed, which due to the slope of 58 mV/pH, it can be said that it is completely proportional to the Nernst equation (Azab *et al*., 2020; Wang *et al*., 2019; Zheng *et al*., 2020).

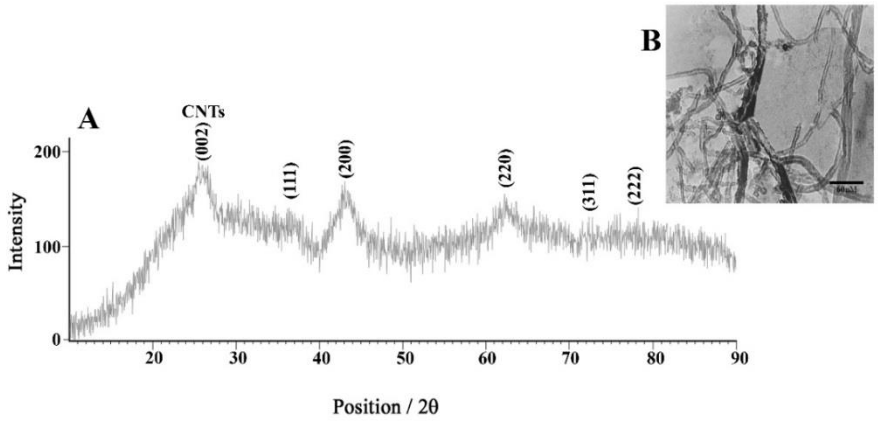

Fig. 1. A: X-ray diffraction pattern of NiO-SWCNTs nanocomposite **B:** TEM image of NiO-SWCNTs nanocomposite.

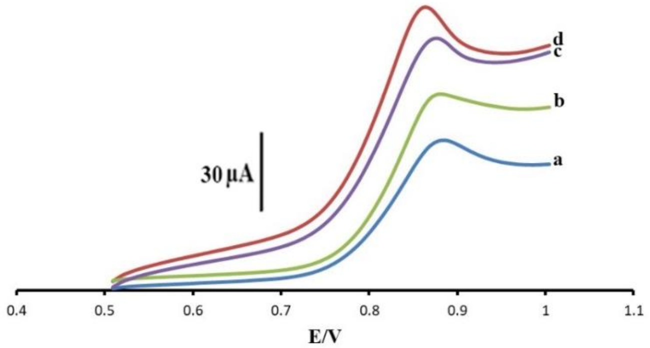

Fig. 2. Cyclic voltammograms of (a) CPE; (b) NiO-SWCNTs/CPE; (c) IL/CPE and (d) NiO-SWCNTs/IL/CPE (in the presence of 700 μM sunset yellow and 10 mL Phosphate buffer solution (0.1 M, pH 6.0)).

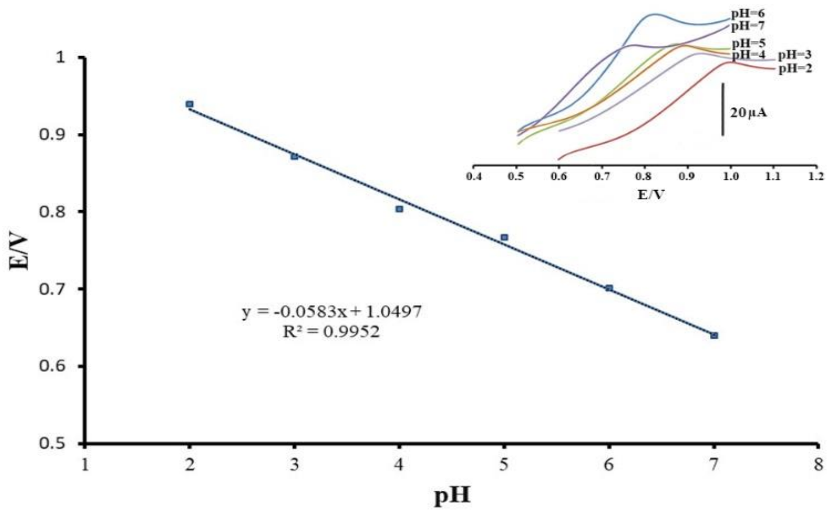

Fig. 3. E_p vs. pH Plot for electro-oxidation of sunset yellow at the surface of NiO-SWCNTs/IL/CPE. **Inset**: Cyclic voltammograms of NiO-SWCNTs/IL/CPE at Phosphate buffer pHs.

- ***Scan rate effect***

The Scan rate effect was investigated to obtain the transfer coefficient (α) at the surface of NiO-SWCNTs/IL/CPE in the range of 10–150 mV and in the presence of 700.0 μM sunset yellow. Figure 4 illustrates the cyclic voltammograms of NiO-SWCNTs/IL/CPE in various scan rates (10.0, 70.0, 100.0 and 150.0 mV $s^{-}1$) (inset) and the relationship between I_p (oxidation peak current) and $v^{1/2}$. According to the calculated R^2 (0.9981), the I_p was directly proportional to the $v^{1/2}$. Therefore, we concluded that there was a diffusion-controlled process at the surface of NiO-SWCNTs/IL/CPE for sunset yellow.

We used Tafel plot (E_p vs. I_p log) for obtaining the electron transfer coefficient (α) of sunset yellow at the surface of NiO-SWCNTs/IL/CPE with the scan rate of 100 mV s^{-1} (Figure 5). Eventually, electron transfer coefficient (α) was obtained 0.52 using Tafel equation (Eq. 1) (Khaleghi *et al.*, 2016), confirming the electro-oxidation of sunset yellow at the surface of NiO-SWCNTs/IL/CPE.

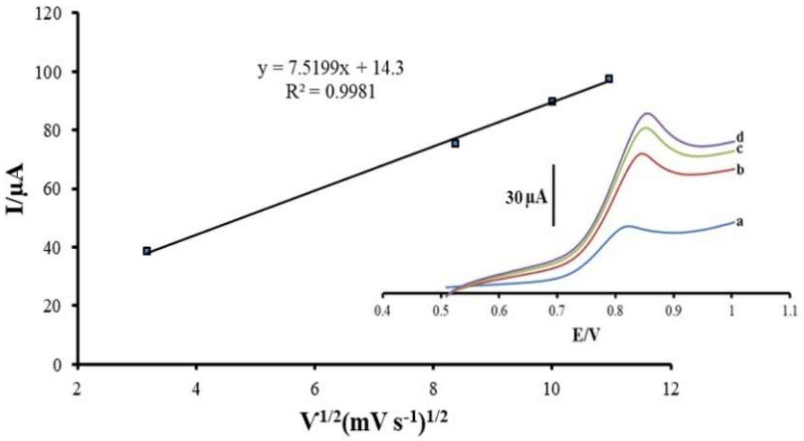

Fig. 4. I_p vs. $v^{1/2}$ Plot for maximum anodic peaks of electro-oxidation of 700 μM sunset yellow. **Inset:** Cyclic voltammograms of NiO-SWCNTs/IL/CPE at various scan rates (a–d are 10.0, 70.0, 100.0 and 150.0 mV s^{-1}, respectively) (in the presence of 700 μM Sunset yellow and 10 mL Phosphate buffer solution (0.1 M, pH 6.0)).

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$$E=\frac{2.3RT}{(n-1)\alpha F \log I} \qquad (1)$$

Where α is electron transfer coefficient, E is oxidation peak potential in V, I is current in µA, n is molecular mass, T is temperature in K (298K), F is Faraday constant (96,485) in C mol^{-1}, and R is gas constant (8.314) in J mol^{-1} K^{-1}. The α was measured after calculating the slope of I_p vs. $\nu^{1/2}$ Plot. The obtained slope for this plot is equal to E=1/log I.

- *Chronoamperometry*

The Chronoamperometry method was employed to determine the diffusion index (D) of sunset yellow, at the potential step of 600 mV (Figure 6). In doing so, the Cottrell equation (Eq. 2) was used to calculate the diffusion index (D) of different concentrations of sunset yellow (100, 300 and 500 µM) (Granström *et al.*, 1995; Khaleghi *et al.*, 2016). Finally, by means of the slopes in figure 6 (inset) and the applied potential step, the mean value of obtained diffusion indexes was calculated as 1.8×10^{-5} cm^2/s.

$$I=\frac{nFAD^{1/2}}{\pi^{1/2}t^{1/2}} \qquad (2)$$

Where D is diffusion index in cm^2/s, t is time in s, n is the number of exchanged electrons, I is current in µA, F is Faraday constant (96,485) in C mol^{-1}, A is the area of the electrode surface in Cm^2.

- *Determination of sunset yellow*

The SWV method was employed to determine the amount of sunset yellow using the NiO-SWCNTs/IL/CPE (Figure 7 inset). According to the I_p vs. concentration plot and $LOD=3*S_b/m$ equation, the limit of detection (LDR) of proposed sensor was obtained 0.05 µM. Also, the range of concentration in which the sensor was capable of detecting the analyte (LDR) was between 0.09 and 750 µM.

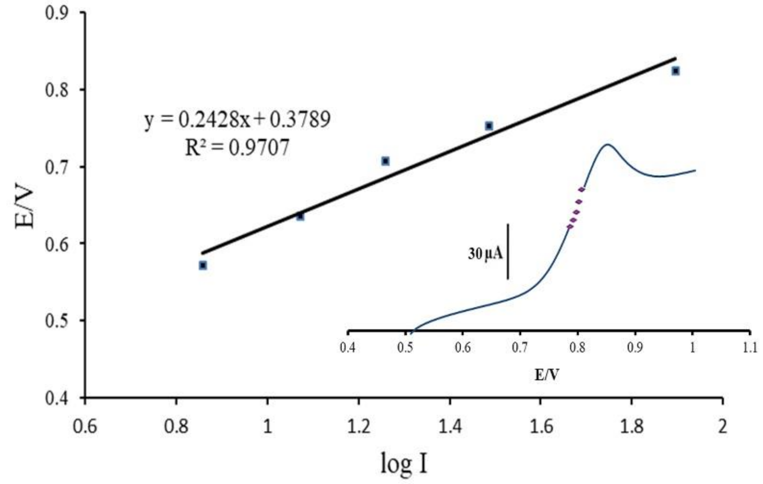

Fig. 5. Tafel plot (E_p vs. I_p log) for NiO-SWCNTs/IL/CPE at the scan rate of 100 mV s^{-1} (in the presence of 700 µM sunset yellow and 10 mL Phosphate buffer solution (0.1 M, pH 6.0)).

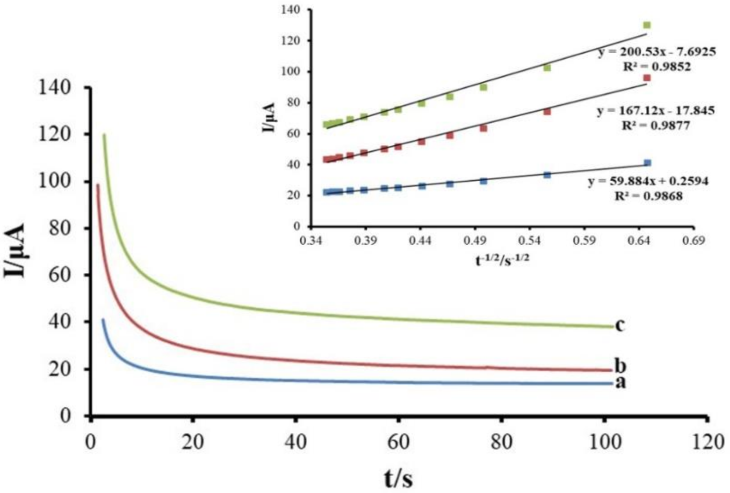

Fig. 6. Chronoamperograms obtained at the NiO-SWCNTs/IL/CPE in the presence of (a) 100, (b) 300 and (c) 500 µM sunset yellow at pH 6.0. **Inset:** Plots of I_p vs. $t^{-1/2}$ obtained from Chronoamperometry.

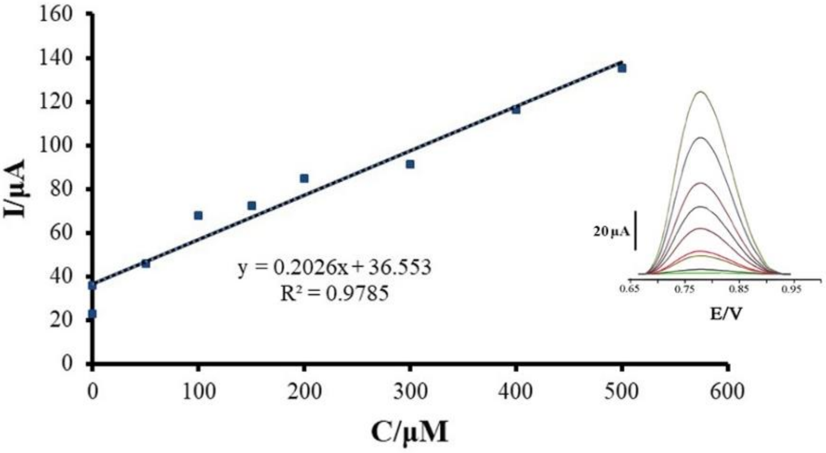

Fig. 7. The I_p vs. concentration plot of sunset yellow concentration at the NiO-SWCNTs/IL/CPE. **Inset:** the SWVs of NiO-SWCNTs/IL/CPE in 0.1 M Phosphate buffer solution (pH 6.0) containing different concentrations of sunset yellow.

- *Evaluation of reproducibility and stability of NiO-SWCNTs NC/IL*

In order to be assured about the reproducibility and Stability of the fabricated sensor, these factors were evaluated using SWV method with 200 µM sunset yellow in 10 mL phosphate buffer. The relative standard deviation for 9 consecutive tests was 2.67%. When various NiO-SWCNTs NC/IL electrodes fabricated by the same procedure were used, the relative standard deviation for 5 measurements was 3.56%. The stability of NiO-SWCNTs NC/IL electrode was assessed after 14 and 30 days and the sensor maintained 97.2% and 94.36% of its primary response, respectively.

- *Determination of sunset yellow in real samples*

NiO-SWCNTs/IL/CPE was used in the analysis of sunset yellow in some real samples. The sunset yellow content of some food samples was measured using the standard addition method. Initially, we measured the quantity of sunset yellow in the chosen samples. In the second stage, spike samples were made by adding a specific amount of sunset yellow to the samples. The outcomes are present in Table 2. The recovery of the fabricated sensor was calculated using a proportion between the amount of sunset yellow found in the spike sample and the amount of sunset yellow expected to be found, showing the accuracy of fabricated sensor. The analysis of recovery in the range of 99.2-99.86% confirms the capability of NiO-SWCNTs/IL/CPE in the determination of sunset yellow in food samples.

Conclusion

NiO-SWCNTs/IL/CPE fabricated in this work was modified using NiO-SWCNTs nano composite and N-octylpyridinium hexafluorophosphate ionic liquid and it showed a limit of detection (LOD) of 0.05 µM and a reasonable linear dynamic range (LDR) between 0.09 and 750 µM which means that this sensor can determine the amount of sunset yellow in this range. The quantities of sunset yellow in the orange juice and orange jelly were 4.93±0.16 and 2.88±0.36 ppm, respectively. Moreover, the recovery of the sensor after preparing spike samples was tested and the obtained range (99.2-99.86%) confirms the accuracy of the proposed sensor. The stability of NiO-SWCNTs NC/IL electrode in the range of 97.2% and 94.36% proves its reliability in the determination of sunset yellow. Accordingly, the suggested sensor could be considered as a useful tool for determining the amount of sunset yellow, and it can be claimed that this sensor could be used as a rapid and sensitive tool for the detection of sunset yellow in food samples.

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Table 2. Determination of sunset yellow in real samples (n=6)

Sample	Added amount of Sunset yellow (ppm)	Expected amount of sunset yellow (ppm)	Amount of sunset yellow Found using the proposed sensor (ppm)	Recovery%
Orange juice	---	---	4.93 ± 0.16	---
	10.00	14.93	14.82±0.56	99.2
Orange jelly	---	---	2.88 ± 0.36	---
	10.00	12.88	12.09 ± 0.27	93.86

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