

Fabrication of Carbon Paste Electrode Modified with Nanobentonite and Nano TiO₂ for Tartrazine Analysis by Cyclic Voltammetry

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Abstract: Tartrazine is a synthetic dye widely used in food products, and its usage needs to be strictly controlled due to potential adverse health effects. One of the methods employed to analyze tartrazine levels is cyclic voltammetry. This study modified carbon paste electrodes by incorporating bentonite and titanium dioxide (TiO₂) nanoparticles to achieve a low detection limit. This research aimed to investigate the effect of the working electrode composition consisting of carbon, bentonite nanoparticles, TiO₂ nanoparticles, and paraffin on the peak current response in the analysis of tartrazine, as well as to determine optimal measurement conditions, namely composition and pH. Bentonite nanoparticles were synthesized using the sonochemical method, while TiO₂ nanoparticles in the anatase phase were obtained commercially. Material characterization was conducted using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The electrode was optimized with a composition ratio 3:4:1:2 (carbon:nanobentonite: nano TiO₂:paraffin), resulting in a cathodic peak current of -5.43×10^{-5} A. The optimal conditions for tartrazine detection were found to be at pH 7. This study demonstrates the potential of nanomaterial-modified carbon paste electrodes for developing low-cost, sensitive, and eco-friendly electrochemical sensors, which can be applied to monitor synthetic dyes in food safety control rapidly.

Keywords: Cyclic Voltammetry; Electrode; Nano TiO₂; Nanobentonite; Tartrazine.

Introduction

Food is a basic human necessity that must be safe, nutritious, and of high quality, and it constitutes a fundamental human right [1]. Food safety is critical to prevent chemical, microbiological, and physical contamination. Government agencies conduct surveillance on both domestic and imported food products, as regulated in Article 109 of Law No. 36 of 2009 concerning Health. Continuous consumption of unsafe food can increase health risks [2]. In addition to food ingredients, colorants are also food additives that must comply with consumption safety standards. Colorants are added to maintain or enhance the visual appearance of products [3]. Synthetic dyes are increasingly used due to their more vivid and stable colors [4], but their use raises health risks and environmental pollution, particularly in water sources [5].

Tartrazine is a synthetic azo dye, yellow in color and water-soluble, widely used in food, cosmetics, and pharmaceuticals [6]. This dye is permitted in various countries such as the European Union, the United States, and Canada. In food products, tartrazine is commonly found in milk, beverages, cakes, ice cream, sauces, and jellies. Its consumption is particularly high among children, who are more attracted to bright colors. Exposure levels vary from 0.03–0.09 mg/kg body weight/day in the United States to 0.21–0.64 mg/kg body weight/day in Indonesia [7].

According to BPOM Regulation No. 11 of 2019, the maximum allowable tartrazine level in candies is 100 mg/kg (Nurfadhila, *et al.*, 2023), while BPOM RI Regulation No. 37 of 2013 sets a limit of 70 mg/kg for non-carbonated flavored drinks [8]. The WHO and JECFA recommend an

acceptable daily intake (ADI) of 0–7.5 mg/kg body weight per [7]. However, several studies have reported toxic effects of tartrazine at levels below the ADI. Tartrazine exhibits cytotoxicity toward human cells with LC50 values of 89 μ M and 78 μ M for its metabolites [9]. In zebrafish embryos (*Danio rerio*), exposure to 50 mg/L disrupts neural development [10], and even concentrations below the ADI have shown toxic effects, indicating that current toxicity thresholds may not sufficiently protect aquatic ecosystems [11].

Excessive tartrazine intake can induce various health effects. Excessive consumption of tartrazine can cause various health effects, such as thyroid cancer, asthma, eczema, migraine, genotoxicity, as well as liver and kidney disorders, and infertility. These toxic effects are exacerbated at high doses due to oxidative stress that damages cellular DNA [12]. Tartrazine is also associated with neurodevelopmental disorders in children, including hyperactivity, irritability, and sleep disturbances. A daily dose of 50 mg has been reported to trigger behavioral changes, with approximately 8% of children with ADHD showing worsened symptoms due to synthetic dyes [13]. Cytotoxic and mutagenic effects of tartrazine on human cells have also been observed [14]. Furthermore, tartrazine can cause DNA damage at concentrations of 70 μ g/mL, which are realistically found in the blood following dietary intake, as confirmed by *in silico* and *ex vivo* studies [15].

Given the documented health risks associated with tartrazine consumption, analyzing its concentration in food is essential to ensure safety. Common analytical methods include HPLC, LC-MS/MS, thin-layer chromatography (TLC), capillary electrophoresis, spectrophotometry, and

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electrochemical sensors [16]. Among these, electrochemical sensors are considered the most effective due to their cost-efficiency, rapid analysis, and minimal sample preparation requirements. Electrode modification enhances sensitivity and lowers detection limits [17]. One promising electrochemical technique is cyclic voltammetry, which can detect tartrazine at low concentrations with high accuracy and provides detailed information on the redox properties of the compound [18].

Carbon paste electrodes (CPE) are commonly employed as working electrodes in voltammetry techniques due to their ease of fabrication, low cost, and versatility for modification with various materials (Setiarso & Wachid, 2017). Modification with materials such as nanobentonite and nano TiO_2 has been shown to improve electrode performance. Nanobentonite offers a high surface area and strong adsorption capacity, which enhances sensitivity [19], while TiO_2 , as a non-toxic semiconductor with electrochemical stability and a wide bandgap, contributes to improved conductivity and thermal stability of the electrode [20].

Nanobentonite and TiO_2 modified CPE development aims to increase the current response and reduce the detection limit for tartrazine analysis via cyclic voltammetry. With increasingly stringent regulations on food colorants, there is a need for analytical methods that are rapid, sensitive, and efficient. Cyclic voltammetry offers an effective alternative to conventional methods such as HPLC and spectrophotometry, providing lower costs, shorter analysis times, and environmentally friendly operation [18]. However, limited studies have focused on the combined use of nanobentonite and TiO_2 in a single carbon paste electrode formulation specifically optimized for tartrazine detection, particularly with regard to determining the ideal composition and pH conditions. This study addresses that gap by developing a modified electrode combination of nanobentonite and TiO_2 , aiming to enhance detection sensitivity and lower the detection limit.

This research aims to develop a modified carbon paste electrode with enhanced sensitivity for tartrazine detection and apply cyclic voltammetry to rapidly and accurately quantify tartrazine content in food samples. Additionally, this study aims to evaluate the performance of the modified electrode relative to other analytical techniques, including HPLC, spectrophotometry, TLC, and LC-MS/MS.

Research Methods

Materials and Tools

The materials used in this research were bentonite powder, tartrazine, 99% anatase nano TiO_2 (Merck), 96% ethanol, 5M HCl, ammonia, carbon powder, liquid paraffin, Na_2HPO_4 , NaH_2PO_4 , KCl, food product samples, distilled water, copper wire, ballpoint pen refills, and filter paper.

The tools used in this research included chemical glasses, measuring flasks, measuring cups, watch glasses, glass stirrers, Erlenmeyer flasks, mortar and pestles, porcelain dishes, droppers, micropipettes, 80-mesh and 300-mesh sieves, funnels, spatulas, magnetic stirrers, analytical balances, vacuum pumps, electric stove, oven, pH meter, Elmasonic S 30/H ultrasonic bath, 797 VA Computrace

cyclic voltmeter, Shimadzu 1800 UV-Vis spectrophotometer.

Synthesis of Nanobentonite

Bentonite synthesis was carried out using the sonochemical method [21]. A total of 20 grams of bentonite powder was first sieved using an 80-mesh sieve, then mixed with 100 mL of 5 M hydrochloric acid (HCl). The mixture was heated and stirred using a magnetic stirrer at 70°C and 400 rpm for 4 hours to activate the bentonite. Afterward, the mixture was left to stand at room temperature for 24 hours. The solution was then separated into filtrate and residue. The residue was washed repeatedly with hot distilled water until a neutral pH was reached. Once neutral, the bentonite was dried in an oven at 200°C for 2 hours. The dried bentonite was ground using a mortar and sieved through a 300-mesh sieve. The resulting powder was then mixed with 50 mL of 96% ethanol. This mixture was subjected to sonication in an ultrasonic bath for 4 hours at 30°C with an ultrasonic frequency of 37 kHz. After the sonication process, the mixture was oven-dried at 100°C for 30 minutes. The dried bentonite was then ground again using a mortar and further dried in an oven at 100°C for 5 hours. The resulting nanobentonite was characterized using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). As for TiO_2 , no nanoparticle synthesis was carried out, as the TiO_2 obtained was already in nanoparticle form with 99% anatase phase purity.

Preparation of Modified Carbon Paste Working Electrode

The working electrode is made from a 15 cm long copper wire, with the top and bottom ends stripped 0.5 cm each and the other end 1.5 cm for the alligator clip. Copper wire was chosen for its high conductivity and electrochemical stability. The surface of the wire is polished clean to ensure optimal conductivity. The lower part of the wire is fitted with a 1 cm long insulator as a container for the modified carbon paste. The electrode paste consists of a mixture of carbon, paraffin, nanobentonite, and nano TiO_2 . Carbon acts as a conductor and adsorbent, while nanobentonite and nano TiO_2 enhance adsorption capacity due to their small particle size and large surface area. Paraffin is used as a binder. The compositions used are 3:3:2:2; 3:2:3:2; 3:1:4:2; and 3:4:1:2 (w/v). All materials are weighed, mixed, and homogenized. The paste is then inserted and compacted into the pre-weighed electrode body to avoid voids that could interfere with electron transfer.

Determination of Optimum Electrode Composition

Electrodes with various compositions were tested using cyclic voltammetry in a 25 ppm tartrazine solution dissolved in 10 mL of 2500 ppm KCl and a pH 7 phosphate buffer solution. Measurements were taken at a potential range of -1 V to 1 V with a deposition time of 40 seconds and a scan rate of 0.05 V/s. The voltammograms from each modified electrode measurement were compared to determine the highest current peak.

the Debye-Scherrer equation, which is formulated as follows.

$$D = \frac{K \lambda}{\beta \cos \theta}$$

D = crystal size
K = shape factor of the crystal (0.89)
 λ = wavelength of X-ray (1.5406Å)
 β = value of Full Width at Half Maximum (FWHM) (rad)
 θ = angle of diffraction (degree)

Table 1. XRD diffraction peaks list of nanobentonite with particle size

Pos, [°2Th,]	FWHM Left [°2Th,]	d-spacing [Å]	Particle Size (nm)
19.806	0.3995	4.48084	20.19
20.825	0.153	4.26595	52.79
21.906	0.274	4.06325	29.53
22.733	0.180	3.90507	45.02
23.611	0.208	3.77004	39.02
24.343	0.168	3.64971	48.38
26.573	0.174	3.35135	46.91
27.715	0.145	3.21761	56.43
27.949	0.098	3.18860	83.54
28.378	0.118	3.14186	69.44

Based on calculations using the Debye-Scherrer equation, the average crystallite size was found to be 49.13 nm, confirming that the material is on the nanometer scale.

Determination of Optimum Nanobentonite and Nano TiO₂ Modified Carbon Paste Electrode Composition

The determination of the optimal composition of the modified carbon paste electrode with nanobentonite and nano TiO₂ was performed in 10 mL of 25 ppm tartrazine solution in 10 mL of 2500 ppm KCl solution and 5 mL of phosphate buffer at pH 7. Measurements were performed using cyclic voltammetry over a potential range of -1 V to 1 V with a deposition time of 40 seconds and a scan rate of 0.05 V/s. The following is the voltammogram of the optimum electrode composition.

The voltammetry results show that tartrazine produces only one current peak, namely the reduction peak (I_{pc}), without the appearance of an oxidation peak (I_{pa}) in the reverse direction of the scan. This indicates that the electrochemical process that occurs is irreversible. In an irreversible system, only a one-way reaction occurs; tartrazine undergoes reduction but does not reoxidize. This phenomenon may be caused by the instability of the reduction product, the occurrence of further chemical reactions, or the adsorption of the reduction product on the electrode surface, rendering it unavailable for oxidation. Structurally, tartrazine contains an azo group (-N=N-), which is known to undergo irreversible reduction in electrochemical media [27].

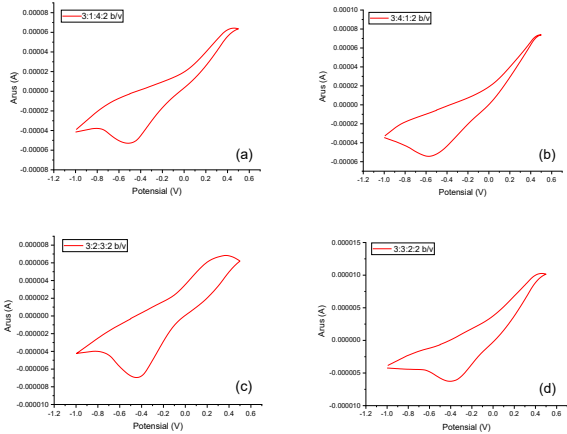


Figure 3. Voltamogram of Carbon Paste Electrode Composition (Carbon:Nanobentonite: Nano TiO₂:Paraffin) with (a) 3:1:4:2; (b) 3:4:1:2; (c) 3:2:3:2; (d) 3:3:2:2

The highest cathodic current peak was obtained at the composition variation of 3:4:1:2 w/v (carbon:nanobentonite:nano TiO₂:paraffin), compared to other compositions such as 3:1:4:2; 3:2:3:2; and 3:3:2:2 w/v. Nanobentonite, with its layered structure and high surface area, possesses excellent adsorption capacity and cation exchange ability, thereby enhancing tartrazine accumulation on the electrode surface. Meanwhile, nano TiO₂ acts as an electrocatalytically and photocatalytically active semiconductor, accelerating electron transfer in the electrochemical process [28]. The 3:4:1:2 composition combination creates synergy between the adsorption capacity of nanobentonite and the electrochemical activity of nano TiO₂, resulting in the best electrode performance for tartrazine detection.

Table 2. Peak current of nanobentonite and nano TiO₂ modified carbon paste electrode composition

Composition	I _{pc} (A)
3:1:4:2	-5,29 × 10 ⁻⁵
3:4:1:2	-5,43 × 10 ⁻⁵
3:2:3:2	-6,95 × 10 ⁻⁶
3:3:2:2	-6,26 × 10 ⁻⁶

Modification of carbon paste electrodes with a combination of nanobentonite and nano TiO₂ has a significant effect on electrode sensitivity in tartrazine analysis. Compared to conventional carbon paste electrodes or those modified with only one material, the combination of both produces a higher peak current. A high current value indicates improved electron transfer efficiency, thereby enhancing the electrode's ability to detect and measure tartrazine concentration in samples. The linear relationship between current and concentration supports the sensitivity of this method, as demonstrated in the following voltammogram.

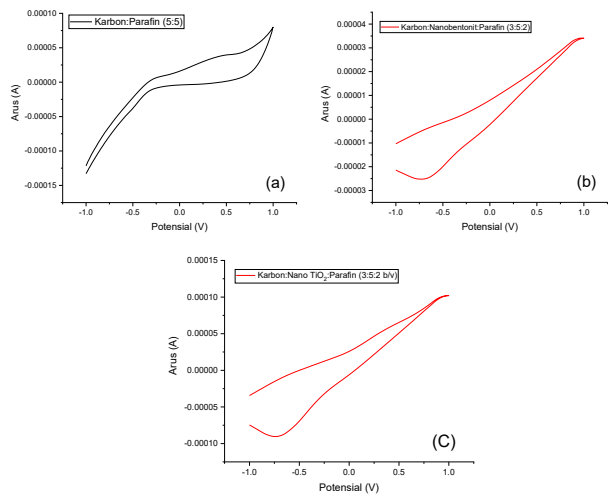


Figure 4. Voltammogram of Carbon Paste Electrode Composition (a) Carbon: Paraffin (5:5), (b) Carbon:Nanobentonite: Paraffin (3:5:2), (c) Carbon: Nano TiO₂:Paraffin (3:5:2)

Carbon paste electrodes with paraffin as a binder exhibit very low cathodic peak current (I_{pc}), indicating suboptimal electrochemical activity. Modification with nanobentonite or nano TiO₂ separately can increase the peak current due to their ability to enhance tartrazine adsorption capacity and support electron transfer processes. However, the simultaneous use of both in the electrode composition results in a more significant improvement in electrochemical performance. The synergy between the adsorption capacity of nanobentonite and the semiconductor properties of nano TiO₂ collectively accelerates electron transfer, resulting in a higher peak current and enhanced analytical sensitivity.

Table 3. Peak current of the modified and unmodified working electrode

Composition	I_{pC} (A)
5:5	-8.63×10^{-7}
3:5:2 Nanobentonite	-9.05×10^{-7}
3:5:2 Nano TiO ₂	-9.64×10^{-7}
3:4:1:2	-2.70×10^{-5}

A significant increase in cathodic peak current (I_{pc}) was observed in carbon paste electrodes modified with a combination of nanobentonite and nano TiO₂, compared to unmodified electrodes. The use of either nano material alone also showed improved electrochemical performance compared to conventional electrodes. This indicates that electrode surface modification can produce new electrochemical properties, such as enhanced adsorption capacity for analytes and improved electron transfer efficiency, thereby making the electrode more sensitive and effective in analysis.

Determination of Optimum pH

The optimum pH is determined as the condition at which the analyte produces the highest current peak in the

voltammetric measurement. The analysis is performed in a voltammetric cell containing 10 mL of 25 ppm tartrazine solution, 10 mL of 2500 ppm KCl solution (100 times the analyte concentration), and 5 mL of pH 5 phosphate buffer. Measurements were performed over a potential range of -1 V to 1 V, with a deposition time of 40 seconds and a scan rate of 0.05 V/s. The same procedure was applied to other pH variations (6, 7, and 8). The following are the voltammogram results from the optimal pH measurement:

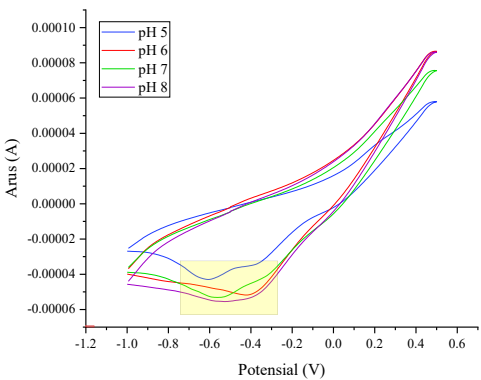


Figure 5. Voltammogram of pH variation

Table 4. Peak current of pH variation

pH	I_{pC} (A)
5	$-4,41 \times 10^{-5}$
6	$-4,62 \times 10^{-5}$
7	$-5,25 \times 10^{-5}$
8	$-4,77 \times 10^{-5}$

Based on the voltammogram, although pH variations did not show significant differences in peak current, pH still influenced the increase in peak current (I_{pC}). Buffers play an important role in maintaining ionic strength, increasing conductivity, and preventing ion migration in an electric field [29]. The voltammetry results indicate that pH 7 produces the highest reduction current compared to pH 5, 6, and 8, indicating optimal conditions for electron transfer between tartrazine and the electrode. This neutral condition also maintains electrode surface stability, so pH 7 can be concluded as the optimal pH for tartrazine analysis via voltammetry.

Conclusion

Carbon paste electrodes modified with nanobentonite and nano TiO₂ in a composition ratio of 3:4:1:2 (carbon:nanobentonite: nano TiO₂:paraffin) demonstrated the highest reduction current response in the electrochemical analysis of tartrazine using cyclic voltammetry. This composition was found to provide the most favorable interaction between the analyte and the electrode surface, likely due to the increased surface area and enhanced conductivity introduced by the nanomaterials. Under these conditions, the optimal electrochemical behavior was observed at pH 7, where the redox activity of tartrazine reached its peak, indicating that this pH facilitated the most efficient electron transfer processes at the modified electrode

interface. These findings suggest that the developed electrode has strong potential for application in real food sample analysis and further development into electrochemical sensors for synthetic dyes.

Author's Contribution

Rahmalia Indah: Collect data and compile the article; Pirim Setiarso: Responsible person and article compiler

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