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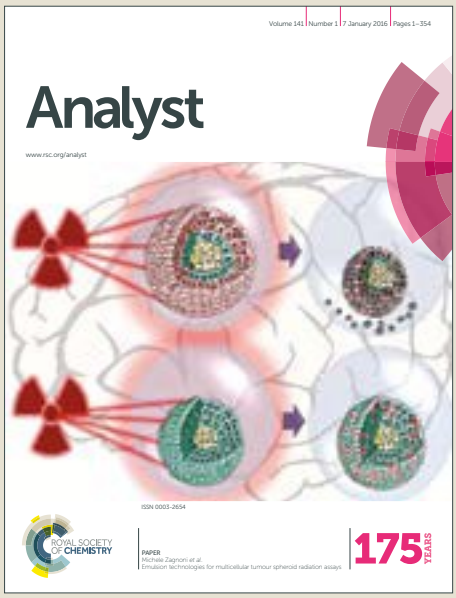

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Titanium nanoparticles (TiO_2) / Graphene oxide nanosheets (GO): an electrochemical sensing platform for the sensitive and simultaneous determination of benzocaine in the presence of antipyrine

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Abstract

An effective electrochemical sensing platform for the simultaneous determination of benzocaine (BEN) and antipyrine (ANT) based upon titanium dioxide nanoparticles (TiO_2) / graphene oxide nanosheets (GO) bulk modified carbon paste electrodes (TiO_2-GO/CPE) is reported. The TiO_2-GO/CPE electrochemical sensing platform is found to exhibit linear ranges from 1.0×10^{-6} to 1.0×10^{-4} M and 1.2×10^{-8} to 8.0×10^{-5} M for BEN and ANT respectively.

The TiO_2-GO/CPE sensor is explored towards the analysis of BEN and ANT in oral fluid (saliva) and pharmaceutical products. The synergy of the graphene oxide nanosheets and titanium dioxide nanoparticles result in a dramatic enhancement in the sensitivity of the sensor through a combination of increased surface area and improved electron transfer kinetics compared to other electrode alternatives. The fabricated TiO_2-GO/CPE is demonstrated to exhibit a high sensitivity, good stability towards the sensing of BEN and ANT and has potential to be used as a clinical assay and QA in pharmaceutical products.

Keywords: Electrochemical sensor; chemically modified carbon paste electrode; Graphene oxide; Titanium nanoparticles; Simultaneous determination of benzocaine and antipyrine; real samples

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1. Introduction

The Food and Drug Administration (FDA) have issued a Public Health Advisory warning about the risk of methemoglobinemia (MHb) related to topical benzocaine used during medical procedures [1] and the FDA have received over 70 cases of serious adverse events, with the number of deaths associated with MHb after the use of topical benzocaine, bringing the total reported cases to 319 [2]. Benzocaine (4-aminobenzoic ethyl ester), is a local anaesthetic and as such is commonly found in medicines for mild pain relief such as sore throat tablets and for reducing the pain in mucous membranes and teguments.

Various pharmaceutical products contain benzocaine, such as: sucking tablets, syrups, solutions etc. used for the treatment of oral or tongue ulcerations, gastric ulcers, as well as ointments and suppositories used for treating hemorrhoidal disorders [3]. Numerous experiments have shown that excessive doses of anaesthetic sprays containing BEN can result in cyanosis and life-threatening complications[4]. Research findings have reported that the administration of BEN containing sprays in endoscopy, intubation, bronchoscopy, and similar invasive procedures may cause MHb, with potentially serious consequences [4]. In addition, other research has revealed that topical BEN 20% spray used in endoscopy can be absorbed and lead to MHb [5]. There is a therefore a need to detect BEN in formulations for QA purposes as well as in the analysis of raw materials drugs and pharmaceutical preparations in addition to developing bioassays/biomonitoring. Several quantitative analytical studies for quantifying BEN have been reported, involving high-performance liquid chromatography (HPLC) [6-8], liquid chromatography–mass spectrometry (LC–MS) [9, 10], amperometric detection on a modified carbon-paste electrode [8, 11] and chemiluminescence [12, 13].

Antipyrine (ANT) and BEN are co-formulated in ear drops for relief of pain and reduction of inflammation in the ear in otitis externa and acute otitis media [14]. Antipyrine (1, 5-Dimethyl-2-phenyl-4-pyrazolin-3-one) is a non-steroidal anti-inflammatory drugs (NSAID) that can relieve mild to moderate pain. ANT is an official drug as reported in the United States Pharmacopeia (USP) [15] and British

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Pharmacopoeia (BP)[16]. Numerous analytical methods have been reported for the determination of ANT either alone or co-formulated with other drugs including spectrophotometric [17-20], chromatographic [21, 22], thin-layer chromatography (TLC) [23, 24], gas chromatography (GC) [25, 26], capillary zone electrophoresis [27] and non-aqueous titration [28] methods. Due to the side effects and harmfulness of BEN for humans, there is a need for the selective and fast high efficient analytical protocols for the determination of trace BEN and its co-formulated drug Antipyrine (ANT) in human fluids and pharmaceutical preparations.

Graphene oxide (GO) are being widely combined into an ever diversifying range of applications across several fields in the search for greatly improved device performance[29]. One area which receives significant interest is the field of electrochemistry where GO has been reported to be beneficial in various applications ranging from sensing through to energy storage, generation and carbon-based molecular electronics as well as water splitting to H_2[30-33]. Additionally, GO can work as a building block for the building of three dimensional (3D) graphene with various structures that can combine with other functional materials to yield new 3D-based hierarchical materials[30]. Graphene oxide (GO) has a high adsorption capacity, large surface area and good biocompatibility. In particular, GO contains a large number of hydrophilic functional groups, such as -OH, -COOH and epoxides on the basal plane and the sheet edge[34-36], resulting in good hydrophilicity that makes it easily dispersed in solvents with long-term stability. Due to these advantages, GO-based electrochemical sensors have been developed for the sensitive determination of various biological molecules[35]. The existence of oxygen functionalities at the surface of GO is very interesting as they provide reactive sites for chemical modification using well-known carbon surface chemistry [37]. One possible way to utilise the outstanding properties of GO in applications would be to integrate GO sheets in a composite material which is then applied as the basis of an electrochemical sensor.

In this paper, we utilise the reported benefits of GO and design and develop a GO bulk modified carbon paste electrode. To provide further enhancements, we incorporate nanomaterials, in this case, titanium dioxide (TiO_2) nanoparticles due to their reported physicochemical properties such as good biocompatibility, strong

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adsorptive ability, high surface area, thermal stability, non-toxicity and electrical / electrochemical properties [38]. To the best of our knowledge, and at the time of writing this, there are no studies reported to date that utilise TiO_2-GO for the electrochemical determination of BEN and ANT. We consequently report the first ever example of using TiO_2-GO/CPE electrode for the bio-monitoring of BEN and ANT in oral fluid (saliva) and pharmaceutical products. The TiO_2-GO/CPE based electrochemical sensing platform provides a simple, selective and reliable sensor for simultaneous determination of BEN and ANT.

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2. Experimental

2.1. Instrumentation

A Bio-logic SP 150 electrochemical workstation was used for all electrochemical measurements. A one compartment cell with a three electrode set-up was connected to the electrochemical workstation through a C3-stand from BAS (USA). A platinum wire from BAS (USA) was employed as the auxiliary electrode. All the cell potentials were measured with respect to an Ag/AgCl (3.0 M NaCl) reference electrode from BAS (USA). A Cyberscan 500 digital (EUTECH Instruments, USA) pH-meter with a glass combination electrode served to carry out pH measurements. All the electrochemical experiments were performed at an ambient temperature of 25^{o} C. Impedance spectra were recorded over a frequency range from 100 mHz to 100 kHz. Scanning electron microscopy (SEM) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company). FT-IR spectra were recorded on an IR-Affinity-1 Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan).The crystalline phases were detected and identified using an X-ray diffractometer (XRD) on an X'Pert PRO MRD with a copper source at a scan rate (2θ) of 1^{o} s^{-1}. Sigma Plot 10.0 was used for all statistical data.

2.2. Chemicals and reagents

BEN was kindly supplied from Sabaa International Company (Egypt), purity: 99.98 %. ANT was kindly supplied from Pharma SWEDE Egypt, purity: 99.88 %. Graphite powder was obtained from Aldrich. The dosage form, Otosept ear drops (labelled to contain 300 mg of antipyrine and 100 mg of benzocaine) are manufactured by Amriya for pharmaceutical industries, Egypt. Zora-C lozenges (labelled to contain Benzalkonium chloride 0.001 mL, Benzocaine 1.0 mg and Vitamin C 50.0 mg) is manufactured by CID Company. Titanium dioxide nanoparticles were obtained from Nano-Lab (Waltham, MA, USA). Paraffin oil from Merck was used as the pasting liquid for the preparation of the paste electrodes. Britton-Robinson buffer (BRB) was prepared by mixing different volumes of 0.04 M in H_3PO_4, 0.04 M acetic acid and 0.04 M boric acid with the appropriate amount of 0.2 M NaOH to obtain the desired pH 2.0 - 10.0. All solutions were prepared from analytical grade chemicals and sterilized Milli-Q deionized water was used.

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2.3. Preparation of the GO-TiO_2-CPE

Carbon paste unmodified electrode (CPE) were prepared by mixing graphite powder (0.5 g) with paraffin oil (0.3 mL) in a pestle and mortar. The carbon paste was packed into the hole of an electrode body and smoothed on a filter paper until it has its shiny appearance. Graphene oxide (GO) was prepared by the Hummer's method [39]. TiO_2 were dispersed with GO in water by adding 100.0 mg TiO_2 and 100.0 mg GO in 25 mL volumetric flask (1:1, w:w%) and sonicated for 45 min to produce a homogeneous dispersion. The TiO_2-GO nanocomposite was then leaved to dry at 80 ^oC in the oven for 4 hours. Next, 30.0 mg of TiO_2-GO nanocomposite (1:1) was mixed with 970.0 mg graphite. The paste was extensively mixed with an appropriate amount of paraffin oil (0.35 mL) for 40 mins until a uniform and homogeneous wetted paste was obtained. This paste was packed into the hole of the electrode body and smoothed on a filter paper until shiny appearance. A new surface was obtained by pushing excess of the paste out of the syringe and polished with weighing paper. Other modified CPE were prepared for the comparative studies. Accordingly, TiO_2/CPE and GO/CPE were made using 15.0 mg of TiO_2 and GO separately with 985.0 mg of graphite. The same amount of paraffin oil was used for preparation of the partially bulk modified CPEs.

Prior to any voltammetric measurements, the modified electrode TiO_2-GO/CPE was cycled between 0.0 to -1400 mV at a scan rate of 100 mV s^{-1} in a pH 2 BRB several times until a reproducible response was achieved. Following this, the modified TiO_2-GO/CPE electrode was transferred into another cell containing pH 2 BRB.

2.4. Analysis of real samples

Otosept ear drops: A volume of ear drops equivalent to 1.0 x 10^{-3} M BEN and ANT was transferred into a volumetric flask (10 mL) and made up to the mark with double distilled water. An appropriate dilution was made using double distilled water to obtain solutions of final concentrations of BEN and ANT.

Five tablets were weighed and transferred to a clean, dry mortar. An accurately weighed amount equivalent to two tablets were dissolved in 10 mL of distilled water

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to give a solution containing 1.2 x 10^{-3} M of BEN; this was then diluted to give 1.0 x 10^{-3} M BEN, which was then filtered through a Nylon membrane filter.

Oral fluid (saliva): Collection of saliva samples was achieved using a salivette from a healthy volunteer who received repeated doses of Zora-C lozenges (once daily) within a pharmacokinetic study [40]. The saliva samples were centrifuged for 5 mins at 4000 rpm, then diluted to 5.0 mL with pH 2 BRB and were analysed using the standard addition method. The solution was transferred into the voltammetric cell to be analysed without any further pretreatment. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional ethics committees have approved these experiments.

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2. Results and discussion

Electrochemical characterisation of the TiO_2-GO/CPE

The GO-TiO_2 used in the fabrication of the TiO_2-GO/CPE electrochemical sensors is fully characterised and presented in the ESI (Figures S1 and S2) confirming the presence of nanometre sized TiO_2 supported upon graphene oxide (GO) nanosheets where the GO acts to electrically wire and support the TiO_2 electroactive centres. To demonstrate the benefits of using the TiO_2-GO/CPE as the basis of a sensor, the electrochemical response was explored using not only the TiO_2-GO/CPE, but also a bare CPE, TiO_2/CPE and GO/CPE. The cyclic voltammetric responses are shown in Figure 1A where in all cases, two voltammetric peaks are observed corresponding to the electrochemical oxidation of BEN and ANT. It is evident that the largest voltammetric response is observed with slight improvements in the voltammetric peak potentials using the TiO_2-GO/CPE, which is likely due to a combination of increased surface area and favourable electrochemical activity largely from the TiO_2 and GO. The electroactive area of each of the electrodes were calculated (see ESI Figure S3). The calculated areas were found to correspond to 0.055, 0.074, 0.095 and 0.144 cm^2 for CPE, TiO_2/CPE, and GO/CPE, and TiO_2-GO/CPE, respectively. This confirms that the TiO_2-GO/CPE exhibits the largest electrode surface area.

Next, electrochemical impedance spectroscopy (EIS) was used to study the electrochemical response of the CPE, TiO_2/CPE, and GO/CPE, and TiO_2-GO/CPE; the results are depicted in Figure 1B. In order to obtain detailed data of the impedance spectroscopy, a simple equivalent circuit model was used to fit the results. In this circuit, R_s, C and R_{CT} represent solution resistance, a capacitance for the double-layer and electron transfer resistance, respectively. Through fitting the data, the values of R_{CT} were estimated to correspond to 4207.0 Ω at the bare CPE, which decreases to 2509.0 and 1877.0 Ω for the TiO_2/CPE and GO/CPE, respectively, which is then observed to further decrease to 160.0 Ω for TiO_2-GO/CPE, indicating the significantly lower electron-transfer resistance of TiO_2-GO/CPE compared with other electrodes. The results are consistent with the CV results presented above, demonstrating that the TiO_2-GO/CPE composite likely provides higher electron conduction pathways due to the synergistic effects of its constituents. Overall, the electrochemical results

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demonstrate that the TiO_2-GO/CPE provides enhancements over other possible electrode alternatives.

The influence of solution pH upon the electrochemical oxidation of BEN and ANT using the TiO_2-GO/CPE was next explored; pH is an important parameter, which can affect the electrochemical (analytical) sensitivity and voltammetric peak separation. Figure S4 depicts the response upon the electrochemical oxidation of BEN and ANT from changing the solution pH from 2 to 8 which shows that the electrochemical oxidation peak potential for BEN and ANT is pH dependant as indicated by the following equations: BEN: E_p(V) = 1.17-0.062 *pH* (R^2= 0.992); ANT: E_p(V) = 1.45-0.052 *pH* (R^2 = 0.991). Both responses are close to the anticipated Nernstian value of 59.2 mV for an electrochemical process with equal electron and protons. Additionally shown in ESI Figure S4 is the response of peak current as a function of pH where it is observed that pH 2 BRB gives rise to the largest peak currents / analytical signal for both BEN and ANT.

Furthermore, the effect of voltammetric scan rate upon the electrochemical signal of BEN and ANT was explored; these results are presented in the ESI Figure S5, which demonstrate that the electrochemical oxidation of both BEN and ANT are diffusion-controlled processes. Additionally, chronoamperometric measurements were performed providing information on the diffusion coefficients, which are calculated to be 4.95 x 10^{-6} cm^2 s^{-1} and 2.34 x 10^{-6} cm^2 s^{-1}, for BEN and ANT, respectively; further details are provided in the ESI.

Last, the influence of the amount of TiO_2 and GO used in the fabrication of the TiO_2-GO/CPE upon the magnitude of the analytical signal / peak current was explored. The anodic peak current for both drugs were investigated towards changing the quantity of TiO_2-GO over the range 5.0 mg to 30.0 mg. Results indicated that the anodic peak current was optimal when the CPE was modified with quantity equivalent to 30.0 mg of TiO_2-GO nanocomposite (15.0 mg of TiO_2 and 15.0 mg of GO). More quantities of TiO_2-GO increased the background current and poorer repeatability of the BEN and ANT response was observed.

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Individual and simultaneous voltammetric determination of BEN and ANT using square wave voltammetry (SWV)

The electrochemical sensing of BEN and ANT was first explored at the TiO_2-GO/CPE using SWV in pH 2 BRB individually, as shown in Figure 2. From inspection of Figure 2 is it clear that the voltammetric response / analytical signal increases with increasing concentrations of BEN and ANT with the corresponding calibration plots shown as insets within Figure 2. In the case of the electrochemical sensing of BEN, a linear response over the range 1.0×10^{-6} to 1.0×10^{-4} M was found with the following linear regression: I_p (μA) = 0.193C (μM) + 0.845; R^2 = 0.9991. The calculated limit of detection (LOD) was found to correspond to 2.48×10^{-7} M with a limit of quantitation (LOQ) of 8.26×10^{-7} M. For the sensing of ANT, a linear response was observed over the range 1.20×10^{-8} to 8.0×10^{-5} M I_p (μA) = 0.353C (μM) + 1.930; R^2 = 0.9992. The calculated values of LOD and LOQ were found to correspond to 3.00×10^{-9} and 9.99×10^{-9} respectively, with a repeatability and intermediate precisions of 0.81 - 0.90% and 0.95 - 1.09%, for BEN and ANT, respectively. Additionally the stability of the TiO_2-GO/CPE was investigated, where the peak current was not found to change following storage in air for 30 days. The modified electrode retained 98.80 - 99.11% of its initial response.

Simultaneous determination of BEN and ANT

To test the specificity of the TiO_2-GO/CPE proposed sensor, its electroanalytical response was explored towards the sensing of BEN in the presence of the frequently co-formulated drug ANT. This was performed by simultaneously changing the concentrations of the drugs and recording the SWVs using the TiO_2-GO/CPE. Figure 3 depicts the voltammetric response of using the TiO_2-GO/CPE sensor towards the sensing of BEN in the presence of ANT. The results demonstrate well-defined anodic peaks at potentials of +1.01 and +1.22 V (*vs.* Ag/AgCl), corresponding to the oxidation of BEN and ANT, respectively, indicating that simultaneous determination of these compounds is feasible. The inset of Figure 3 shows the plot of the peak current (I_p) as a function of BEN and ANT concentrations. The calibration plots (inset) were linearly related to BEN and ANT over the ranges 4.0×10^{-6} to 1.0×10^{-4} M and 2.9×10^{-6} to 73.5×10^{-6} M, respectively. The regression equations were: I_p(μA) =

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0.192C (μM) + 0.507, R^2= 0.9978; and I_p(μA) = 0.349C (μM) + 0.684, R^2 = 0.998; for BEN and ANT, respectively. The analytical sensitivities of the TiO_2-GO/CPE sensor towards the sensing of BEN and ANT are found to be 0.193 and 0.353 μA/μM respectively. These values are very close to the value obtained in the absence of interfering drug (0.192 and 0.349 μA/μM, see Figure 2 above), indicating that the electrochemical oxidation processes of these compounds on the surface of TiO_2-GO/CPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interference.

Reproducibility and stability of TiO_2-GO/CPE

The reproducibility and stability of the TiO_2-GO/CPE was examined by measuring the simultaneous electrochemical signal of 0.1 mM BEN and ANT. The relative standard deviation (RSD) for the peak current of BEN and ANT in six successive measurements was 1.9 % and 2.2%, respectively, Figure S8A. In addition, the fabrication reproducibility was examined by using three modified sensors prepared independently by the same way, the RSD was 2.5% and 1.8% for BEN and ANT, respectively, as shown within Figure S8B. Also, the inter-day precision was deduced using different drugs concentrations over a period of three days. Intra-day and inter-day precision were expressed as relative standard deviation (RSD %), Table S1. For all concentration levels, the RSD didn't exceed 2.78% which revealing good precision of the TiO_2-GO/CPE.

Analysis of real and spiked plasma samples

The applicability of TiO_2-GO/CPE for the sensing of BEN in oral fluid (saliva as real sample), both BEN and ANT in ear drops (Otosept ear drops (300 mg ANT 100 mg BEN, spiked samples) and BEN in Zora-C lozenges; 1 mg/tablet was explored. Concentrations were measured via the standard addition method. The results are shown in Table 1. The recovery values show a good accuracy of the proposed TiO_2-GO/CPE sensor. It is very clear that this TiO_2-GO/CPE sensor has potential for the determination of trace amounts of these compounds in biological fluids and pharmaceutical products.

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4. Conclusions

We have reported the simultaneous determination of BEN and ANT using a TiO_2-GO/CPE based electrochemical sensing platform that provides a simple, selective and reliable sensor for simultaneous determination of TiO_2-GO/CPE. We believe this to be the first example using this sensor towards the electrochemical detection of both BEN and ANT. The sensor is found to exhibit useful analytical ranges in model matrix and is applied for the sensing of BEN and ANT in oral fluid (saliva) and pharmaceutical products. The TiO_2-GO/CPE provides a simple, selective and economical sensor for BEN and ANT and precludes expensive and time-consuming pre-treatments such as those used in chromatographic methods.

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