



## Short communication

Nano-TiO<sub>2</sub> modified carbon paste sensor for electrochemical nicotine detection using anionic surfactantM. Shehata <sup>a,\*</sup>, S.M. Azab <sup>b</sup>, A.M. Fekry <sup>a</sup>, M.A. Ameer <sup>a</sup><sup>a</sup> Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt<sup>b</sup> Pharmaceutical Chemistry Dept., National Organization for Drug Control and Research [NODCAR], 6 Abu Hazem Street, Pyramids Ave. 29, Giza, Egypt

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## ABSTRACT

A newly competitive electrochemical sensor for nicotine (NIC) detection was successfully achieved. Nano-TiO<sub>2</sub> with a carbon paste electrode (CPE) were used for the sensor construction, where Nano-TiO<sub>2</sub> was considered as one of the richest and highly variable class of materials. The sensor showed electrocatalytic activity in both aqueous and micellar media toward the oxidation of NIC at Britton–Robinson (B–R) buffer solution ( $4 \times 10^{-2}$  M) of pH range (2.0–8.0) containing (1.0 mM) sodium dodecylsulfate (SDS) using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. Scanning electron microscope (SEM) and Energy Dispersive X-Ray Analysis (EDX) techniques were also used. The linear range of detection for NIC using the new Nano-TiO<sub>2</sub> Modified Carbon Paste sensor (NTMCP) was detected using differential pulse voltammetry (DPV) technique and it was found between  $2 \times 10^{-6}$  M and  $5.4 \times 10^{-4}$  M with a detection limit of  $1.34 \times 10^{-8}$  M. The obtained results clarified the simplicity, high sensitivity and selectivity of the new NTMCP for nicotine determination in real cigarettes and urine samples.

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## 1. Introduction

Nicotine (NIC) represents one of the pyridine derivative alkaloid family that exists in tobacco leaves (Meyer and Quenzer, 2005). It is well known to be used in cigarettes (Watson et al., 2004), natural insecticide at agriculture and an anti-parasitic in veterinary practice in the past (Rodgman and Perfetti, 2013), dietary nicotine (Siegmund et al., 2001), fertilizer (Nota et al., 2000) rather than for their therapeutical and pharmaceuticals applications (Zuo et al., 2004). For all of these, nicotine determination was very important in medicine, toxicology, human body and tobacco industry. Many methods for NIC determination in the human body or in tobacco samples have been used like HPLC technique with various detectors such as electrochemical (HPLC–EC) (Mahoney and Al-Delaimy, 2001) and ultraviolet detectors (HPLC–UV) (Page-Sharp et al., 2003), and high-sensitivity gas chromatography mass spectrometry (GC–MS) (Shin et al., 2002). HPLC technique still normally employed in clinical laboratories for accurate sensing (Maurer, 1998; Nakajima et al., 2000; Papadoyannis et al., 2002) with disadvantage of time-consumption, very sophisticated, require preliminary extraction and purification of nicotine from the sample matrix and involves many steps.

Electrochemical sensing methods have a great interest among

researchers because of its fast response, relatively highly sensitive, simple, low cost, reliability and could be miniaturized (Yang et al., 2004; Suffredini et al., 2005; Lin et al. 2008; Sims et al., 2010; Švorc et al., 2014). In a previous work Fekry et al. constructed a sensor based on Ce-nanoparticles modified carbon paste electrode which used as a high sensitive nicotine detection tool (Fekry et al., 2015).

In the recent work a chemical mixing of Nano-TiO<sub>2</sub> with CPE was an effective strategy to enhance the detection of nicotine. A modification made by surfactant solution improved the sensitivity and selectivity of the voltammetric measurements (Levent et al., 2009). The simplicity, high sensitivity and selectivity of the new Nano-TiO<sub>2</sub> Modified Carbon Paste sensor (NTMCP) for nicotine determination using very low anionic surfactant concentration (1.0 mM SDS), very low amount of Nano-TiO<sub>2</sub> at pH=5.0, made the method very highly convenient and satisfactory results were obtained.

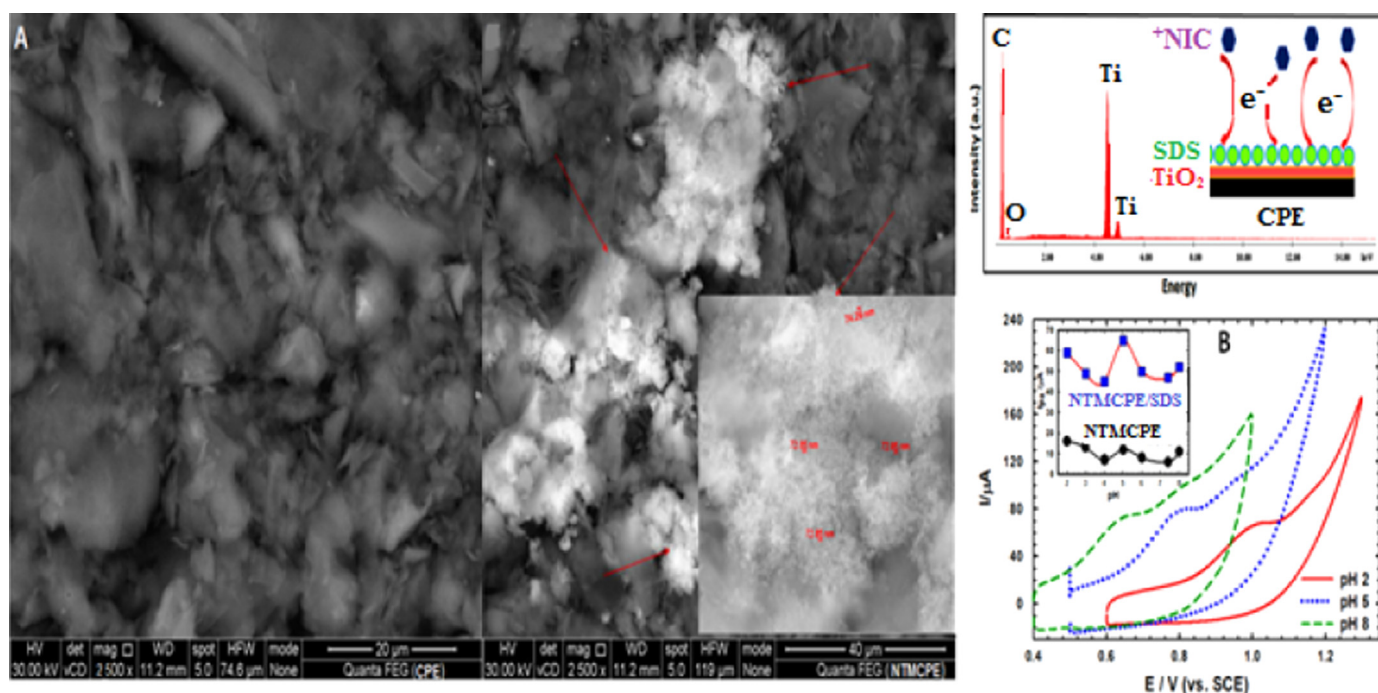
## 2. Experimental

## 2.1. Reagent and chemicals

NIC standard samples (99%) were provided by Egyptian Eastern Company for Smoking. NIC stock solutions of about 1.62 g/L were freshly prepared in water then stored in a dark container since the

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**Fig. 1.** (A) SEM images of CPE and Nano-TiO<sub>2</sub>-CPE, and EDX spectra of TiO<sub>2</sub> nanoparticles, including NIC/SDS interaction model. (B) CVs of NTMCPe/SDS in B-R buffer of different pH values containing  $1.0 \times 10^{-3}$  M NIC. Inset: variation of  $I_{pa}$  for Nano-TiO<sub>2</sub>-CPE and NTMCPe/SDS values with pH values.

compound is sensitive to light. Britton–Robinson (B–R) ( $4.0 \times 10^{-2}$  M) supporting electrolyte buffer solutions of pH range (2.0–8.0) ( $\text{CH}_3\text{COOH} + \text{H}_3\text{BO}_3 + \text{H}_3\text{PO}_4$ ) were used for preparing the standard solutions of nicotine. pH values were adjusted using 0.2 M NaOH. SDS and all solutions were prepared from analytical grade chemicals and triply distilled water. Cigarette samples were purchased from the local supermarkets. Two products of different international cigarette brands: L&M<sup>®</sup> and Marlboro<sup>®</sup> were chosen.

## 2.2. Construction of Nano-TiO<sub>2</sub> Modified Carbon Paste sensor (NTMCP)

CPE was prepared using few drops of paraffin oil mixed with graphite powder (0.5 g) and the mixture was blended homogeneously using the mortar (Chaki and Vijayamohan, 2002). The resulting CP was chemically mixed with Nano-TiO<sub>2</sub> powder (0.01 g) and then used to fill into a Teflon tube to prepare the Nano-TiO<sub>2</sub>-CPE. The electrode surface was pre-treated by applying a potential of +1.3 V for 30 s in the blank supporting electrolyte without stirring, in order to increase the hydrophilic properties of the electrode.

## 2.3. Apparatus and electrochemical measurements

All electrochemical measurements were investigated using electrochemical IM6e Zahner-elektrok, GmbH, (Kronach, Germany) workstation. A typical three-electrode cell with a large platinum sheet as a counter electrode (CE), saturated calomel electrode (SCE) as a reference electrode (RE) and Nano-TiO<sub>2</sub>-CPE as the working electrode (WE) were used. A HANNA 213 pH meter with glass combination electrode was used for pH measurements. SEM measurements were carried out using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit, with accelerating voltage 30 KV, magnification  $14 \times$  up to 1,000,000 and resolution for Gun.1n (FEI company, Netherlands). EIS measurements were recorded between 0.1 Hz and 100 kHz at 10 mV amplitude. Analyses of the experimental spectra were made using Thales software provided with the workstation. All the electrochemical

experiments were carried out and repeated for 3 times and reproducible results were obtained.

## 2.4. Analysis of urine

Urine samples were diluted 400 times using 100 ml of B–R buffer pH 5 to make a stock solution. Standard additions of NIC with different concentrations were carried out from a solution containing NIC in B–R buffer of pH 5 and 1.0 mM SDS to obtain the calibration graph (Fekry et al., 2015).

## 2.5. Analysis of cigarettes samples

Cigarettes were taken out of their rolling paper and dried for 30 min at 40 °C oven. 0.1 g of tobacco collected from mixture of 10 cigarettes taken from two packs of the same brand was added to 10 mL water, and then the contents of the vial were sonicated for 3 h in an ultrasonic water bath and filtered. Appropriate volume (100  $\mu\text{L}$ ) of the filtrate was mixed with the B–R buffer (pH 5.0) containing 1.0 mM SDS in the voltammetric cell and analyzed under the same conditions used to obtain calibration graph (Levent et al., 2009).

# 3. Results and discussion

## 3.1. Morphologies of the different electrodes

Fig. 1A showed SEM images of CPE and Nano-TiO<sub>2</sub>-CPE. CPE exhibited a microstructure with a heterogeneous grain growth (Fekry et al., 2015), while Nano-TiO<sub>2</sub>-CPE surface showed TiO<sub>2</sub> nanoparticles located at different places over the CPE surface with a cloudy aggregates shape and uniform distribution, exhibiting a large surface area. The EDX data of the TiO<sub>2</sub> nanoparticles, indicated the presence of Ti and O peaks, and confirmed that TiO<sub>2</sub> nanoparticles were indeed coated on the CPE surface. A model for the interaction process that occurs between NIC and SDS was also presented.

### 3.2. Optimization of measurement variables

The effect of modifying Nano-TiO<sub>2</sub>-CPE by adding SDS solution was noticed through comparing the voltammetric response of the electrode before and after modification. The sensitivity of the modified electrode (NTMCPE/1.0 mM SDS) was about 5.42 times higher than the unmodified electrode because of the positive charged nicotine that accumulate in the negatively charged crown of anionic SDS micelles, which may increase the electron transfer rate (Wen et al., 1999). The SDS concentration of 1.0 mM was enough to complete saturation of electrode surface with SDS charged species [See Supplementary material (Fig. 1)].

Cyclic voltammetry (CV) technique was processed to study the effect of solution pH on the electrocatalytic oxidation of NIC at the NTMCPE/SDS by using B–R buffers within the pH range of 2.0–8.0 (Fig. 1B). It was observed the anodic peak potentials ( $I_{pa}$ ) shifted negatively as the pH increases, indicating that the electrocatalytic oxidation of NIC at the NTMCPE/SDS was a pH-dependent reaction.

The highest oxidation peak current was obtained at pH 5.0 (inset). NIC is a weak diacidic base having  $pK_{a1}=8.02$  and  $pK_{a2}=3.12$  which correspond to the protonation of pyrrolidine nitrogen (monoprotonated form) and pyridine nitrogen (diprotonated form) present in nicotine molecule, respectively (Yang and Smetena, 1995). These results strongly proved that the oxidation step of nicotine was located on the pyrrolidine ring and attributed to the oxidation of tertiary nitrogen.

### 3.3. Electrochemical impedance spectroscopy (EIS) studies

Fig. 2A and B showed the Nyquist plots for Nano-TiO<sub>2</sub>-CPE and NTMCPE/SDS electrodes, respectively using  $1 \times 10^{-3}$  M NIC solution. The experimental results were fitted with two time constant model (Fig. 2A inset) with an average error of 1%.  $CPE_1$ ,  $CPE_2$  are the inner and outer layer capacitances, respectively, while  $R_{CT}$  is the outer layer charge transfer resistance related to the small semicircle in Nyquist plot (Fekry, 2010; Ameer and Fekry, 2011). A Warburg component ( $Z_w$ ) is due to diffusion process (Heakal and Fekry, 2008; Heakal et al., 2009). CPE used instead of ideal capacitance and an empirical exponent ( $\alpha=0$  to 1) used to account for the deviation from the ideal capacitive behavior and both are used due to surface heterogeneity (Retter et al., 2003).

NTMCPE/SDS showed lower impedance values (18.61 k $\Omega$  cm<sup>2</sup> at pH 2, 13.72 k $\Omega$  cm<sup>2</sup> at pH 5 and 20.33 k $\Omega$  cm<sup>2</sup> at pH 8) compared to Nano-TiO<sub>2</sub>-CPE (26.51 k $\Omega$  cm<sup>2</sup> at pH 2, 15.34 k $\Omega$  cm<sup>2</sup> at pH 5 and 29.33 k $\Omega$  cm<sup>2</sup> at pH 8) indicating a more conducting behavior due to electrode surface SDS ionic adsorption.  $Z_w$  values had higher

values for NTMCPE/SDS (26.28 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 2, 50.05 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 5 and 10.79 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 8) than for Nano-TiO<sub>2</sub>-CPE (9.32 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 2, 19.69 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 5 and 7.56 k $\Omega$  cm<sup>2</sup> s<sup>-1/2</sup> at pH 8). The lowest  $R_{CT}$  or highest CPE values obtained at pH=5 for both tested electrodes indicated higher conductivity confirming highest oxidation peak current obtained from CV's results.

### 3.4. Calibration curve

Fig. 3A represented the calibration curve obtained over  $2.0 \times 10^{-6}$  to  $5.4 \times 10^{-4}$  M concentration range. Calibration experiments processed on NTMCPE for the determination of successive additions of NIC in B–R buffer pH 5.0 containing 1 mM SDS solution (optimized conditions) and scan rate of 10 mV s<sup>-1</sup> were represented by DPV technique at Fig. 3A inset.

The concentration of NIC was calculated from the linear regression equation of the standard calibration curve:  $i_{pa}(\mu A) = 0.0224C(\mu M) + 1.5216$ . The limit of detection (LOD) and limit of quantification (LOQ) values were found to be  $1.34 \times 10^{-8}$  M and  $4.48 \times 10^{-8}$  M respectively. Comparisons of the data obtained for NIC determination by various methods and electrochemical techniques against NTMCPE were given [See Supplementary material (Table 1)].

### 3.5. Application of NTMCPE sensor in urine

The calibration plot for the usage of NTMCPE sensor at real samples as urine was shown in Fig. 3B, which gave a straight line on the concentration range of  $8.0 \times 10^{-6}$  M– $4.8 \times 10^{-4}$  M. From calibration curve equation,  $I_{pa}(\mu A) = 0.0259C(\mu M) + 2.3786$ , the NIC concentration in urine samples was calculated. LOD =  $1.17 \times 10^{-8}$  M and LOQ =  $3.91 \times 10^{-8}$  M were also calculated. The precision and accuracy of the proposed method using four different concentrations on the calibration curve that are repeated for five times was evaluated and the recovery was found to be in the range of 99.92–100.38.

### 3.6. Analysis of real cigarette brands samples

In order to ensure the validation of the proposed method at real samples for practical application, two products of different cigarette brands were analyzed (L&M and Marlboro). The method used in this study was a slight modification of the method described by Suffredini et al. (2005). Recovery studies were performed by adding the appropriate volume of standard nicotine

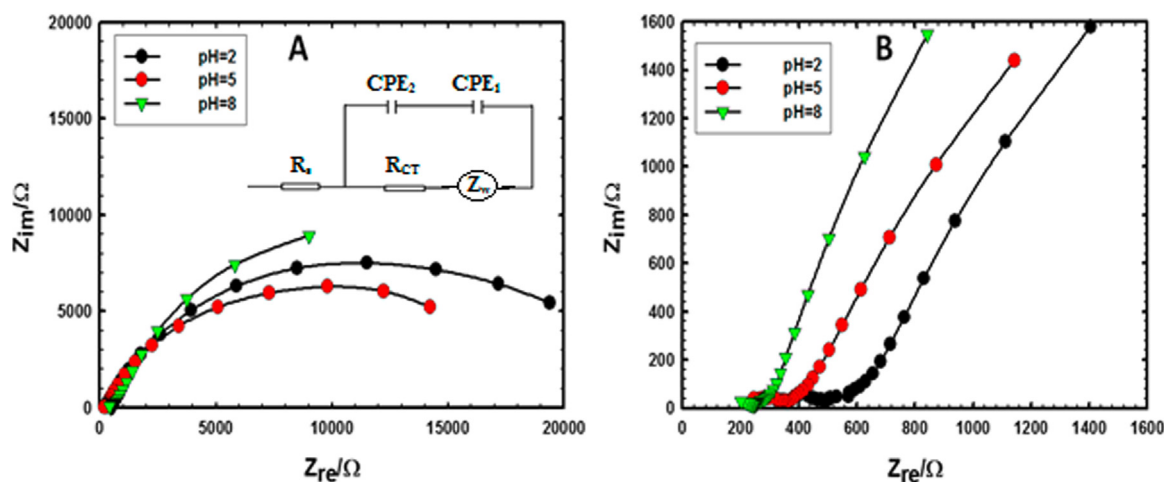
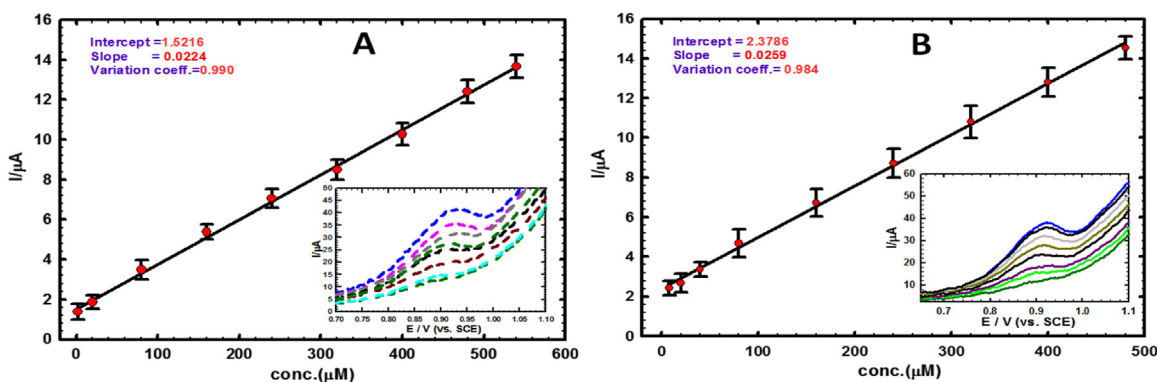


Fig. 2. Nyquist plots of NIC for: (A) Nano-TiO<sub>2</sub>-CPE including the equivalent circuit as inset. (B) NTMCPE/SDS at different pH values.



**Fig. 3.** (A) The calibration curve for NIC. Inset: the corresponding differential pulse plots of successive addition of NIC and 1.0 mM SDS in B–R buffer of pH 5.0 at scan rate = 10 mV/s using NTMCPE/SDS. (B) Calibration curve of NIC in urine. Inset: the corresponding differential pulse data obtained at NTMCPE/SDS for successive addition of NIC on 1.0 mM SDS in B–R buffer of pH 5.0 containing urine and a scan rate = 10 mV/s.

solution prepared in supporting electrolyte to the nicotine content previously determined at the tobacco sample. The recovery data ensure the accuracy of the voltammetric detection of nicotine in tobacco as it was found that nicotine amounts can be quantitatively recovered by the proposed method [See [Supplementary material \(Table 2\)](#)].

#### 4. Conclusions

NTMCPE was used as a sensor for electrochemical determination of NIC.  $D_{app}$  results proved that redox reaction of the NIC processed under diffusion control process. Under the optimum conditions, calibration plots for NIC were linear in the range of  $2.0 \times 10^{-6}$ – $5.4 \times 10^{-4}$  M with variation coefficient of 0.990 and detection limit of  $1.34 \times 10^{-8}$  M.

This new sensor exhibited a great progress toward NIC response in real samples with high stability, sensitivity and reproducible analytical performance as in urine and different brands of commercial cigarettes.

#### Study limitations

Alkaloids interference which may be present in tobacco was not performed in this study due to the ineffective low concentration level of other minor alkaloids (0.2–0.5% of total alkaloids).

#### Future work

A transfer from the success of nicotine detection to cotinine detection based on NTMCPE will broaden the analytical applications and have more impact towards future research. Cotinine is the metabolite of nicotine and structurally similar to it. Also other graphite derivatives like carbon nanotubes, graphene oxide or graphene for nicotine detection may be used in future studies.

#### Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [10.1016/j.bios.2015.12.090](https://doi.org/10.1016/j.bios.2015.12.090).

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