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Analysis of Nicotine Degradation Using a Chitosan and Carbon Nanotube-Modified Carbon-Glass Electrode: Impact of Oxygen and Nitrogen Gases at Bio-pH Conditions via Cyclic Voltammetry.

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Abstract

In this study, we explore the pathways of nicotine degradation (NICs) using a glassy carbon (GC) electrode modified through cyclic voltammetry (CV). The surface of the GC electrode was enhanced with electrospinning and hybrid nanofibers techniques. These hybrid nanofibers were composed of carboxylated carbon nanotubes (MWCNT-COOH) dispersed within a polymer matrix and chitosan (CS), resulting in a unique morphology and a large surface area. The electrochemical behavior of NIC was examined with the GC-CS/MWCNT-COOH electrode. When utilizing the CS/MWCNT-COOH electrode, the NIC process, which is governed by 2 protons and 2 electrons, demonstrated an irreversible reduction in the presence of oxygen and nitrogen gases. The oxidation signal at a lower potential and higher current was more pronounced with the modified electrode compared to the unmodified GC electrode. This effect is amplified in the presence of oxygen gas, indicating that the carbon nanotubes facilitate electron transfer, thereby supporting NIC's potential for electrocatalytic applications. Under optimal conditions, CV exhibited NIC oxidation in the presence of 0.74 V oxygen and 0.81 V nitrogen in a phosphate buffer solution with a pH of 7.4. A linear calibration curve was obtained, ranging from 0.1 to 200 µM for oxygen and 0.05 to 200 µM for nitrogen, with $R^2 = 0.99$ for both, and a detection limit of 7.1 nM for oxygen and 9.2 nM for nitrogen. For 100 parallel detections of 10 µM NIC over 10 cycles, the electrode achieved 98% replication with a standard deviation of 4.08% RSD, maintaining stability over the first cycle, which indicates excellent repeatability and stability of the CS/MWCNT-COOH electrode.

Keywords: ***Nicotine, Electrochemical properties, Cyclic voltammetry***

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1. Introduction

Start

Nicotine (NIC) is a highly toxic alkaloid substance and is commonly found in tobacco products such as cigarettes and cigarettes [1] [2]. NIC is the only drug that can be stimulating or sedative. Receptors are widespread almost everywhere in the body, and NIC receptors interact with the central nervous system and facilitate the release of neurotransmitters such as serotonin, norepinephrine, acetylcholine, dopamine, and glutamate [3]. Due to the rapid transfer of smoke to the lungs as well as the rapid uptake of NIC into them, it can be detected in the brain tissue within 10-20 seconds after a cigarette puff, which is the fastest route of measurement from the vein [4]. Therefore, early detection and monitoring of NIC levels in the body is important from a toxicological and pharmacological point of view. Particularly important is the tobacco industry, which must determine the maximum NIC content in its products [5].

To date, various analytical methods including chromatography [6], spectrophotometry [7] and electrochemical [8] have been used to determine NIC in different samples. Although chromatographic methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) are very common, these techniques require expensive solvents, a variety of equipment, highly skilled personnel and long analysis times [9]. Spectrophotometric methods also include toxic reagents such as cyanogen bromide [10] and require initial extraction and purification which results in the loss of analyte. Electrochemical techniques are simpler, cheaper and more reliable than other methods [11].

In recent years, various researchers have been conducting electrochemical experiments on biosensors and have attempted to improve their electrical responses. In 2010, Huawei Zhiyong et al. Prepared electrochemical methods using multi-walled carbon nanotubes immobilized on glass carbon electrode and were able to achieve a precision of 0.62 mmol 10 times [12]. In another study in 2015, copper nanoparticles were used to improve the GC electrode. They used multi-walled carbon nanotubes on the glass carbon electrode surface. The modified electrode was scanned by electron microscopy and tested under CV. They showed different sensitivities between the two linear regions. This sensitivity was recorded at 1.121 ($R^2 = 0.982$) in the range of 1×10^{-6} to 9×10^{-5} mM and reported accuracy of 1 µmol. For 6 trials the 1 mmol standard deviation was 5.68%, which indicates that this modified layer gave good accuracy. Other parameters including pH, correction focus, and scan rate showed that the optimum values were 0.7, 2 mg / ml and 80 mV / s, respectively [13]. In 2013, colleagues developed a NIC-sensitive and neuricotin-sensitive electrode that could detect the analytes in fungi by amperometry method. At an operating voltage of 0.95 V, they were able to maintain the linear area of 0.2-0.1 g/ml for 8 minutes [14].

Modified polymer electrodes are a class of modified electrodes in which films or polymer nanofibers are used to improve electrocatalytic properties, sensitivity, stability and repeatability of electrodes [15] [16]. Among the various methods available for placing nanofibers on the electrode surface, electrospinning is the most appropriate method for simplicity, control of nanofluid thickness, affordability and flexibility. However, a review of past research has shown that there are no studies on the modification of carbon-glass electrodes using polymeric nanofibers electronically to determine NIC. In the present work, we have described the electrochemical properties of NIC and followed the nicotine reactive pathways in living organisms by the CV and GC-CS / MWCNT-COOH electrodes.

Nicotine Degradaion Pathways

Nicotine found in tobacco leaves and in plant-derived products is mostly in isomeric (S) -nicotine form [17]. However, tobacco smoke contains a maximum of 10% (R) - nicotine. This isomer is thought to be the cause of combustion in some tobacco races [18]. Nicotine is an addictive compound in tobacco and an active psychoactive drug. Through smoking, tobacco is absorbed by the body through the mouth, trachea and chewing tobacco [19] [20] . It can also be

imported as a pure drug. Nicotine as a major component of cigarette smoke has been the subject of much research [21].

The nicotine pathway in the body of living things

Nicotine has complex physiological effects [22]. In cigarette smoke, nicotine is transported to the body on the cigarette smoke particles. It is rapidly absorbed into the small airways and alveoli of the lung [23]. In this environment, the pH is neutral, non-ionized, soluble and can be transferred to the blood via the cell membrane. In the pulmonary venous circulation, it is transmitted to the left ventricle of the heart and to the systemic arterial circulation [24]. Within a few seconds, it reaches the brain, which binds to nicotinic cholinergic receptors and activates the dopaminergic reward system [25]. Other forms of nicotine (such as nicotine gum, inhalation and sublingual tablets) are usually activated at alkaline pH buffer and pass through cell membranes rapidly, but sometimes reach the blood and brain slowly. Due to the delay in the effect of nicotine and the activity of the brain dopamine system, they cause addiction. In humans, the main nicotine metabolism is the liver. Despite the presence of nicotine-metabolizing enzymatic isoforms in other tissues, it is also limited and its metabolites are excreted in the urine [26]. In smokers, it is estimated that 70% of the dose of nicotine is metabolized to quinine [28]. It reaches high levels in the blood and has a long half-life [28]. The trans-3'-hydroxyquinine metabolite is the most abundant metabolite in the urine [28] and is the major part of the biosynthetic intermediate in the liver by microsomal enzymes [30]. For example, cytochrome P450 is one of the dominant enzymes in the liver and plays an important role against extracellular substances such as nicotine [31]. The known reactions are as follows:

Nicotine to Cotinine: The conversion of nicotine to quinine involves the oxidation of the pyrrolidine ring. Quinine is the major metabolite of nicotine in most mammals. Nicotine is rapidly metabolized and cotinine is slowly metabolized [26] Nicotine and cotinine are reduced by the same enzyme in chain metabolism [28]. The presence of a nicotine-Δ1 '(5') -iminium ion-mediated reaction is involved in this reaction [32]. The enzymatic reaction in the liver has been shown to require oxygen for this reaction by the involvement of an oxidase concomitant with NADPH as the preferred cofactor. The reaction is inhibited by carbon monoxide. The synthesis and structure of the crystalline iminium salt is shown to be in equilibrium with 5'-hydroxynicotine [33]. Iminium ion is also an alkylating agent and may play a role in nicotine pharmacology [34]. The formation position of iminium ions has also been described as nicotine hydroxylation in the face of unstable 5'-hydroxynicotine [35]. The reaction is catalyzed by cytochrome P450 2A6, but the 2B6 and 2D6 isoforms may also help. Therefore 5'-hydroxy nicotine may be the aldehyde oxidase substrate for cotinine formation. Another natural metabolite of the nicotine pathway is the production of cotinine. This bio-oxidation involves related changes in intramolecular deformation and chemical reaction on the carbon atom of nicotine [36].

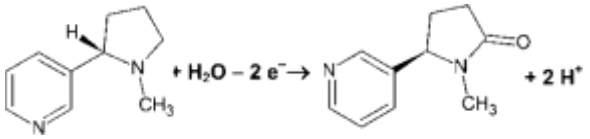

Figure 1: Nicotine bio-oxidation pathways [37]

Reactions to this compound	Needs	Pathways
(*S*)-nicotine + a reduced [NADPH-hemoprotein reductase] + oxygen → nicotine-$\Delta^{1'5'}$-iminium ion + an oxidized [NADPH-hemoprotein reductase] + 2 H_2O	P450 2A6	nicotine degradation IV, nicotine degradation V
nicotine-Δ1'5'-iminium ion+ Aldehyde Oxidase→Cotinine	Aldehyde Oxidase	

As a result, this pathway is a two-step reaction requiring enzyme and aqueous substrate. Of course, with the production of water and if there is residual nicotine in the solution, over time they will become other pathways. In total, these reactions produce 2 electrons and 2 hydrogen with a positive charge and reach the electrode surface.

Nicotine to Nornicotine: Nicotine can also be oxidized by the loss of the methyl group [26]. Nuricotin is found in the urine of smokers. Although nornicotin is a minor alkaloid in tobacco, most of the narcotic in the smoker is excreted in the urine [28]. In rabbit liver microsomes, an N'-methylen-iminium

ion intermediate is formed by the formation of N-(cyanomethyl) N-adducts. The authors of the paper stated that this intermediate is a product of the microsomal mixed oxidase function system. They proposed mechanisms related to the oxidation of nicotine to N- (hydroxymethyl) noricotin, ionization to nicotine-N-methyleniminium ion, and the cyanide reaction to form a cyanide compound [38]. In human [26] and animal [39] tests, there has been qualitative evidence for this reaction. The conversion of neurotinicin to 4-oxo-4- (3-pyridyl) -butanamide has been shown in rat liver microsomes [40] .3-Pyridyl acetate has been discovered in human urine after oral cotinine administration [41]. The hydroxylation of the nicotine pathway in the nitrogen atom was investigated by Safredini et al. They investigated the dimethylation hypothesis by hydroxylating the nitrogen atom in the pyrrolidine ring as shown in the drawing below:

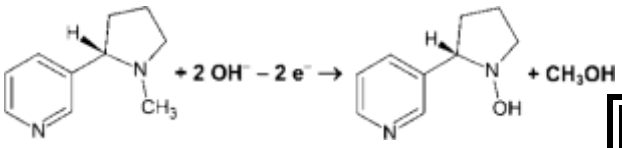

Figure 2: Hydroxylation of nitrogen atoms in the pyrrolidine ring [37]

Reactions to this compound	Needs	Pathways
(*S*)-nicotine + 2 a reduced [NADPH-hemoprotein reductase] + 2 oxygen → nornicotine + 2 an oxidized [NADPH-hemoprotein reductase] + formate + 2 H_2O + H^+	O_2	nicotine biosynthesis, superpathway of nicotine biosynthesis
(*S*)-nicotine + an oxidized electron acceptor + H_2O → nornicotine + formaldehyde + a reduced electron acceptor	H_2O	nicotine degradation IV

Nicotine to nicotine 1'-N-oxide: Oxidation of the pyrrolidine ring can also produce nicotine 1'-N-oxide [26]. Nicotine 1'-N-oxide is found in the urine of smokers [42]. In animals, both CIS and its trans form. In humans, the enzyme dimethylaniline monooxygenase (containing flavin-containing monoxygenase 3) (FMO 3) (formerly FMO II) selectively forms the trans isomer. In the microsomal preparation of human liver, nicotine 1'-N-oxide formation is NADPH dependent [43]. Nicotine 1'-N-oxide is only metabolized in one step, although evidence has been provided to reduce this compound to nicotine in the intestine [44].

Reactions to this compound	Needs	Pathways
(*S*)-nicotine + a reduced [NADPH-hemoprotein reductase] + oxygen → nicotine-$\Delta^{1'5'}$-iminium ion + an oxidized [NADPH-hemoprotein reductase] + 2 H_2O	FMO3 & O_2	nicotine degradation IV, nicotine degradation V

Nicotine to nicotine ion isomethonium: Formation of nicotine isomethonium ion involves non-oxidizing pyridine nitrogen methylation [26]. This pathway was first found in dogs [45]. Animal models and homogenates of human liver showed that S-adenosyl-L-methionine is the source of the methyl group. In the human liver, nicotine (R) -isomers are methylated more rapidly than (S) -isomers, but both enantiomers are methylated [46]. Small amounts of nicotine isomethionine ions have been observed in the urine of smokers [47].

Reactions to this compound	Needs	Pathways
(*S*)-nicotine + an oxidized electron acceptor → *N*-methylmyosmine + a reduced electron acceptor	-	nicotine degradation II (pyrrolidine pathway)

Nicotine to nicotine glucuronide: Nicotine glucuronide formation involves non-oxidative glucuronidation [26]. This route, of course, requires the UDP.

Reactions to this compound	Needs to	Pathways
UDP-α-D-glucuronate + (*S*)-nicotine → nicotine-glucuronide + UDP	UDP-α-D-glucuronate	nicotine degradation IV

Nicotine can also produce 2'-hydroxy nicotine as an intermediate in the catalyzed conversion of cytochrome P450 2A6. This reaction is first

metabolized to 4- (methylamino) -1- (3-pyridyl) -1-butanone by action on nicotine. Then 2'-hydroxy nicotine spontaneously produces nicotine-Δ1 '(2') -iminium ions, which is in equilibrium with 4- (methylamino) -1- (3-pyridyl) -1-butanone. The end product is 3-pyridyl acetate [41].

Reactions to this compound	Needs to	Pathways
(*S*)-nicotine + an oxidized electron acceptor + H_2O → (*S*)-6-hydroxynicotine + a reduced electron acceptor	H_2O &CYP450 2A6	nicotine degradation I (pyridine pathway), nicotine degradation III (VPP pathway)

Consequently, if nicotine is soluble only in water (water buffer) and no enzyme is present in the medium, in the presence of air (dissolved oxygen in the buffer medium, nicotine turns into nicotine light) the multistep pathway II) and nicotine isomethonium ion (pathway IV). But by blowing nitrogen into the environment and evacuating the environment from nicotine oxygen, it will become more of an isomethonium nicotine ion.

2. Material and Methode

High molecular weight chitosan (CS), standard nicotine (NIC), phosphate buffered saline monopotassium phosphate (KH_2) and dipotassium phosphate (K_2 HPO4) were prepared by Sigma Aldrich. Acetic acid is manufactured by the German Merck Company and is a multiwalled carbon nanotube carbon nanotube (MWCNT-COOH) made by Nanolab USA.

Preparation of chitosan hybrid nanofibers / functionalized carbon nanotubes (CS / MWCNT-COOH)

To prepare CS / MWCNT-COOH nanofibers, 2% CS solution was prepared first. The mixture was heated on a hysteresis for 18 h at 65 ° C to obtain a homogeneous and uniform solution. Then different amounts of functionalized carbon nanotubes (1, 1.5, 2 and 2.5 wt.%) Were added to the solution. The nanotubes were placed in an ultrasonic bath for 20 minutes to fully disperse the carbon nanotubes. Each of the final mixtures was transferred into a plastic syringe for electrostatic operation. Electrification was performed under a 25 kV constant electric field using a nanomagnetic technology company electrode on an aluminum layer as a collector. The needle head distance was set to 10 cm and the feed rate of polymer was 0.7 ml / h.

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3. Result

The surface morphology of the nanofibers was investigated by FESEM. FESEM images of CS and CS / MWCNT-COOH nanofibers (2 wt%) are shown in Fig. 1. Research by other researchers has produced CS nanofibers of similar molecular weight and electrospun conditions used in this study [48]. If dispersed nanoparticles are made in the proper

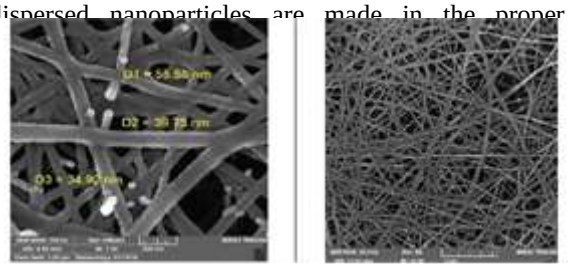

Figure 3:FESEM image of MWCNT-COOH CS / (2wt.%) Nanofibers suitable for electrode surface coating

The size distribution diagrams of different nanofibers are shown in Fig. 2. The mean diameter of CS and CS / MWCNT-COOH nanofibers with 2 wt.% Is 131/01 nm, which can be stated that increasing the amount of MWCNT-COOH resulted in increased crosslinking with the polymer solution. This confirms the images from the electron microscope. Similar results have been reported in other studies [37].

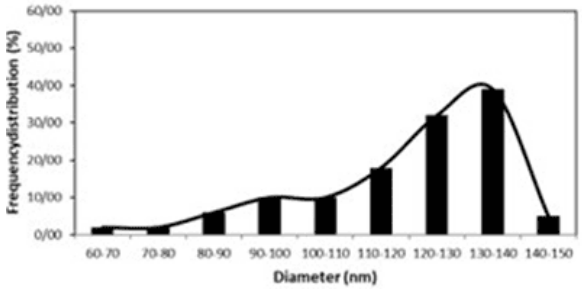

Figure 4: Distribution diagram of different nanofibers for optimum conditions of CS / (2wt.%) MWCNT-COOH production.

Electrochemical studies

Effect of oxygen and nitrogen gases

Nicotine was prepared in 10 µM phosphate buffer with pH = 7.4 one tenth molar. The test used deionized distilled water from the market. Then, for approximately two minutes, we placed nicotine with 15 cc of deionized distilled water inside an aluminum-coated human (for lack of light) to thoroughly mix nicotine with deionized distilled water. Human beings were completely covered with light and air. We then transferred 15 cc of nicotine-containing buffer into the autolab test vessel. This glass container was also completely screwed up to avoid exposure to light. The test was conducted at -1V to +1. But for the sake of simplicity, this review is magnified from 400 mV to 1 V:

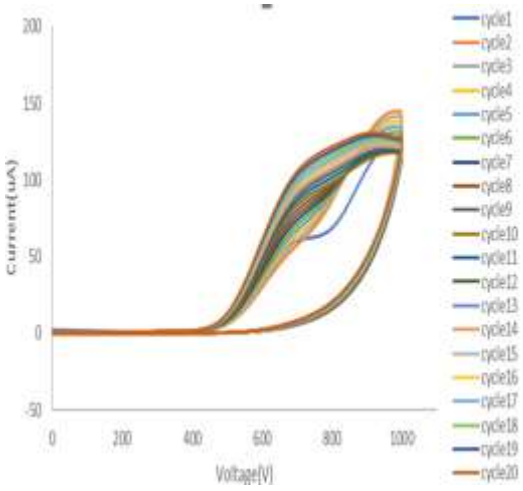

Figure5: Twenty cycles at a scan rate of 50 mV / 10-10 mM phosphate buffer pH 7.4 = tenth molar GC-CS / MWCNT-COOH electrode

4. Discussion

The glassy carbon electrode was modified in an easy manner by CS / MWCNT-COOH nanofibers and studied to study NIC catalytic oxidation. An irreversible cycle was obtained in which the peak was higher than that of the polycarbonate polycarbonate electrode compared to the modified electrode. Oxygen allowed the reaction to proceed from several different paths, while the presence of nitrogen allowed only one pathway for the nitrogen reduction reaction. This higher surface area was due to the presence of carbon nanotube particles in the electrode and its electrospinning. The amounts of transferred electrons and protons 2 were calculated. Under optimal conditions, the CV shows NIC oxidation in the presence of 0.74 V oxygen and 0.81 V nitrogen in phosphate buffer solution with pH = 7.4. Linear calibration curve ranging from 0.1 to 200 µM for oxygen and 0.05 to 200 µM in nitrogen state, with R ^ 2 = 0.99 for both with a detection limit of 7.1 for oxygen and 9.2 nM Indicates for nitrogen. For 100 parallel detection of 10 µM NIC for 10 times 98% replication with standard deviation of 4.08% RSD maintain its stability over the first cycle indicating that CS / MWCNT-COOH electrode has excellent repeatability and stability. Using this method, low

detection limits, appropriate fixation and repeatable measurements were obtained. As a result, nicotine in the presence of air (dissolved oxygen in the buffer medium) is converted to nicotine light and nicotine isomethonium ions. But by blowing nitrogen into the environment and evacuating the environment from nicotine oxygen, it will become more of an isomethonium nicotine ion. In fact, the result of cyclic voltammetry in the presence of oxygen is a combination of nitrogen and air.

Acknowledgments

Acknowledgments should be inserted at the end of the paper, before the references, not as a footnote to the title. Use an unnumbered section heading for the Acknowledgments, similar to the References heading.

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