ELECTROCHEMICAL DETERMINATION OF N,N-DIMETHYLTRYPTAMINE IN WATER BASED ON TETRARUTHENATED PORPHYRINS AND IONIC LIQUID MODIFIED ELECTRODES

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ABSTRACT

N,N-dimethyltryptamine (DMT) is a short-acting psychotropic agent when administered parenterally and is not active orally due to its rapid degradation by monoamine oxidase enzyme type A. There is growing interest in the therapeutic potential of DMT due to recent clinical data that has shown that produces antidepressant effects in humans. It has also been found that psychedelics that possess the central structure of DMT may have value as medications and cannot simply be labeled as drugs with the potential for abuse.

The incorporation of ionic liquid and tetraruthenated zinc porphyrin to glassy carbon electrode enhanced the voltammetric determination of DMT compared to the bare glassy carbon electrode. These modified electrodes showed good performance for DMT oxidation. These results translate into a low detection limit (1.75 µM), short response time, satisfactory linear concentration range, very good stability and reproducibility. Thus, his methodology can be a feasible and inexpensive way to detect DMT in water and presents potential to be applied in the detection of DMT in urine.

Keywords: Tetraruthenated porphyrins, Modified electrodes, DMT, Ionic Liquids.

1. INTRODUCTION

Tryptamine, N, N-dimethyltryptamine (DMT) and β-carboline derivatives are simple indole alkaloids that are commonly present in biota [1]. DMT is a short-acting psychotropic agent when administered parenterally and is not active orally due to its rapid degradation by monoamine oxidase enzyme type A (MAO-A). On the contrary, the DMT present in ayahuasca is active orally only because the alkaloids contained in that mixture reversibly inhibit the activity of MAO-A [1].

There is growing interest in the therapeutic potential of DMT due to recent clinical data that has shown that ayahuasca produces antidepressant effects in humans that resembles the effects of fast-acting ketamine [2]. It has also been found that psychedelics that possess the central structure of DMT may have value as medications and cannot simply be labeled as drugs with the potential for abuse. In addition to the classic serotonergic psychedelics, a variety of other bioactive compounds contains the structural motif of DMT [2]. These include the following triptans: sumatriptan, zolmitriptan and rizatriptan. These compounds are widely prescribed to treat migraines and cluster headaches. The latter compounds are still in the experimental phase, but have proven very promising in pre-clinical models and in clinical trials in relation to the improvement of cognitive deficits associated with Alzheimer's disease and depression [2].

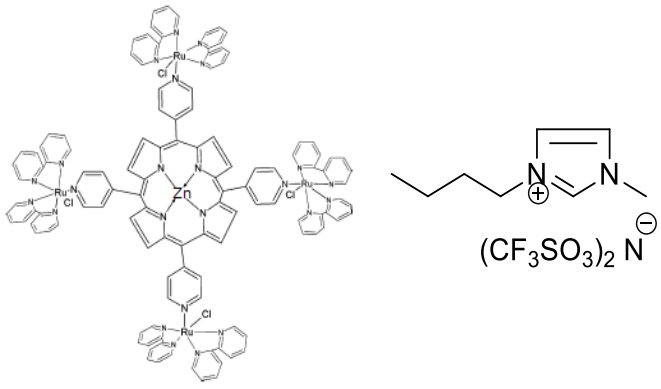
Despite its potential medical benefits, DMT in Chile is subject to Law No. 20,000 and therefore must be treated as an illicit drug. As a consequence, on May 24, 2019, the OS-7 of Antofagasta raided the first laboratory dedicated to the manufacture of this alkaloid [3,4]. The operation seized 270,945 doses, which was valued at more than $2,700 million Chilean pesos [3,4].

Therefore, in this context, it is important to have a DMT detection and quantification method. Over the last decade, several instrumental methods to detect and quantify have been reported, including high performance liquid chromatography (HPLC) [5,6], gas or liquid chromatography coupled with mass detectors [7-11] and other devices [12,13]. There is no doubt that instrumental methods are extremely sensitive and useful for laboratory measurements. However, these methods require pretreating the sample, processes that are very time consuming and require qualified and experienced staff in order to be performed properly. This makes these methods highly inconvenient for detection in the field [5,13].

Electrochemical techniques provide reasonable results under laboratory conditions. In electroanalysis, modified electrode fields present a potential method that is capable of fulfilling the requirements of a fast and precise sensor [15, 17]. The methods of preparing these electrodes include adsorbed monolayers, layer-by-layer adsorption and electropolymerization, among others [18 – 20]. For an efficient modification of the electrode surface, it is necessary to use an electroactive substance like porphyrins, which are capable of forming stable, complex structures with a large variety of metals [18-20]. For example, tetraruthenated porphyrin (TRP) consists of a tetrapyridylporphyrin (TPyP) along with four Ru (II) complexes in the periphery of the macrocycle. These kinds of macrocycles are particularly attractive because they display unusual electrocatalytic [21,22] and photoelectrochemical [23,24] properties. In particular, these porphyrins have been used in the electroanalytical detection of S (IV) oxoanions [25-27] and the electrocatalytic reduction processes of O_2 [28] and CO_2 [29, 30]. In all cases, a multielectron transfer is essential to the enhancement of the catalytic activity [25-28, 31].

Ionic liquids (**IL**) are ionic salts formed from large and very asymmetric ions, whereby their attractive cation-anion forces are weaker than those of inorganic salts and this makes them take a liquid form over a wide range of temperatures [32,33]. Hydrophobicity, high viscosity, ionic structure, ionic conductivity, very low volatility and biocompatibility are among the properties of ionic liquids that make them attractive for electrode modification [34]. In most cases, electrodes modified with IL droplets or film are prepared by the direct deposition of IL on the electrode surface or of its diluted solution combining IL with a volatile solvent.

The aim of this paper is to study a glassy carbon modified electrode with Zn(II) tetraruthenated porphyrin and 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide as an electrocatalyst (See Figure 1) with the goal of the electrochemical detection of DMT in neutral media. Differential Pulse Voltammetry (DPV) was used as the determination technique. The analytical application of the proposed technique was successfully used to determine the detection and quantification limit of the electroanalytical data.



A **B**

Figure 1. Structure of A) Zn (II) µ-{meso-5,10,15,20 tetrapyridyl)porphyrin} tetrakis {bis(bipyridine) (chloride) ruthenium(II)}$(PF_6)_4$ (ZnTRP) and B) 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($BMIMNTF_2$).

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2. MATERIALS AND METHODS

2.1. Extraction and isolation of N,N-dimethyltryptamine from *Mimosa Tenuiflora*.

The extraction and purification of this substance was carried out according to the protocols previously used by Gaudac *et al.* [35]. It should be noted that the initial quantity of plant material powder used was 50 g.

2.1.1 Purification and characterization of N,N-dimethyltryptamine

The traditional liquid–liquid procedure for the extraction of indole alkaloids from plant matrices was employed [35,36]. The alkaloids formed salts in acidic aqueous media and showed both greater solubility and enhanced stability at low pH values [35,36]. The acid extract was basified with sodium hydroxide and then extracted with hexane. This resulted in approximately 500 mg of crude alkaloids [35,36]. The final purification was conducted via recrystallization from hexane.

2.1.2 Characterization of N,N-dimethyltryptamine

Gas chromatography coupled with mass spectrometry (GC/MS) was carried out using a Shimadzu GCMS-QP2010. The GC/MS was equipped with a fused silica RTX-5 capillary column (30 m × 0.25 mm id, 0.25 mm film, Restek, Bellefonte, PA, USA). The oven was programmed from 50 °C for 5 min and then at 8 °C min^{-1} to 270 °C. The GC was operated in split mode, with an injector temperature of 250 °C. Helium was used as the carrier gas. Electron impact mass spectra were acquired at 70 eV.

2.2. Preparation of modified glassy carbon electrode

The supramolecular complexes of Zn (II) μ-{*meso*-5,10,15,20-tetra(pyridyl)porphyrin}tetrakis{bis(bipyridine)(chloride) ruthenium(II)} $(PF_6)_4$ were prepared by the methods described in the cited literature [37, 38]. The purity of these compounds was checked through optical absorption spectroscopy, elemental analysis and ^{1}H-NMR. The ionic liquid 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide was purchased from Merck.

After each experiment, the GC electrode was cleaned by polishing it with 0.3 μm and 0.05 μm alumina slurries. The electrode was rinsed with double distilled, deionized water and then, rinsed in an ultrasonic bath for 30s to remove any remaining alumina. Next, it was rinsed again with abundant deionized water.

The procedure for the preparation of the modified electrodes (ME) is briefly described below.

GC/BMIMNTF$_2$/ZnTRP-modified electrode. A 1: 9 mixture of IL and 1mM ZnTRP is prepared and is then sonicated for 5 minutes. Eight microliters of this mixture was placed on the surface of the GC electrode and it was allowed to dry at room temperature (drop coating) [3, 40].

2.3. Cyclic and Differential Pulse Voltammetry measurements

Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) were carried out using a CH Instrument 920D. Working electrodes were glassy carbon discs purchased from CH Instrument (r = 1.5 mm), the auxiliary electrode was a Pt wire and the reference electrode was Ag/AgCl and both were purchased from CH Instruments. All potentials refer to this reference electrode.

The Cyclic and Differential Pulse Voltammetry experiments were performed using a 0.10 M $NaClO_4$ (pH 6) as a supporting electrolyte. All the experiments were carried out at room temperature. Differential Pulse Voltammetry was then performed, applying the potential range of 0 to 1 V, a step potential of 4 mV, a modulation amplitude of 50 mV, a modulation time of 0.05s and an interval time of 0.1 s.

3. RESULTS AND DISCUSSION

3.1 Characterization of N,N-dimethyltryptamine

DMT was characterized by using gas chromatography coupled with mass spectrometry (GC/MS). The spectrum obtained was compared with those that appear in the cited literature [41].

3.2 Morphological characterization of modified electrodes

In previous studies, modified electrodes were morphologically characterized by SEM [42]. When ZnTRP is incorporated onto the surface of the GC, it is covered by irregular nodules. However, the addition of $BMIMNTF_2$ drastically changes the morphology, which is evidence of a kind of ZnTRP encapsulation in the ionic liquid. This phenomenon can be interpreted as resulting from the interactions between the ionic liquid and the extended π ring of the macrocycle, giving rise to strong interactions that impede the proper precipitation of ZnTRP on the electrode surface [42].

The modified surface is too soft and fragile to be characterized using an atomic force microscope and/or Raman spectra.

3.3 Voltammetric studies of DMT oxidation

The electrocatalytic activity of GC and GC/BMIMNTF$_2$/ZnTRP was evaluated by comparing the voltammetric responses obtained in the absence and presence of 6.5μM DMT (see Figure 2).

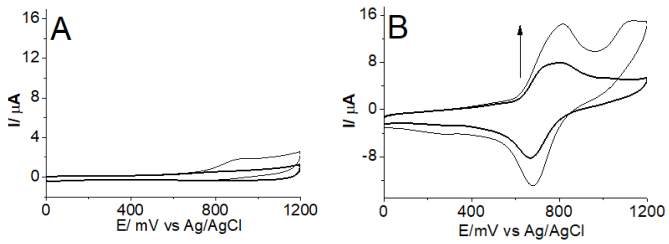

Figure 2. Cyclic Voltammetry of A) GC and B) GC/BMIMNTF$_2$/ZnTRP in absence (thin line) and presence (thick line) of 6.5μM of DMT in 0.1M $NaClO_4$.

Voltammograms containing ZnTRP (See Figure 2B) exhibit a characteristic redox process that is associated with porphyrin and is present near 0.8V, corresponding to the Ru(III)/Ru(II) redox couple. In the absence of DMT, neither electrode exhibits the relevant voltammetric signals (except those mentioned in the previous paragraph). However, when DMT is added, there is an increase in the current that is possibly related to its oxidation. It should be noted that this increase is considerable in the modified electrode (8 μA) in comparison to the less than 2 μA of the unmodified GC.

Additionally, the electrode is very stable when exposed to air for 30 days. The reproducibility of the modification protocol was calculated from ten independent runs and a RSD of 5% was obtained.

3.4 Differential Pulse Voltammetry study of DMT oxidation on GC/BMIMNTF$_2$/ZnTRP modified electrodes.

Since ionic liquids are highly viscous, mass transport is limited between the electrode and the solution. This leads to a high capacitive current that could affect the determination and quantification of the analyte. To solve this issue, Differential Pulse Voltammetry (DPV) was used because it is clear that DPV is much more sensitive to currents than Cyclic Voltammetry. Figure 3 shows the corresponding calibration curve obtained from sequential additions of DMT to the modified electrodes (inset).

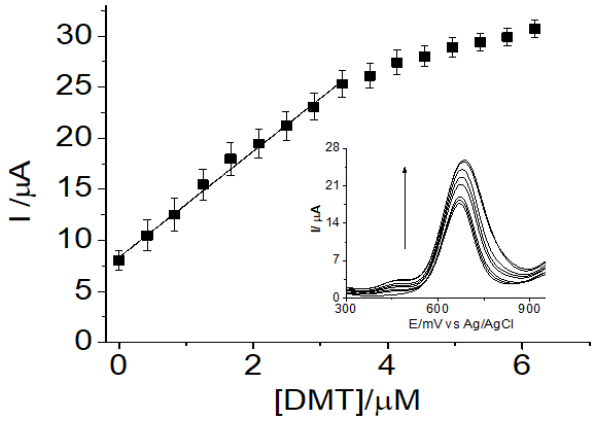

Figure 3. Calibration curve for differential pulse voltammetric responses of GC/BMIMNTF$_2$/ZnTRP inset: DVP for increasing DMT concentrations.

There is a linear relation between the current response and DMT concentration in the range 0 to 3.5µM for GC/BMIMNTF$_2$/ZnTRP (see Figure 3). Limit of detection (LOD) was calculated as three times the standard deviation of the blank over the sensitivity, in this case the value was 1.75 µM. Moreover, limit of quantification (LOQ) was calculated as ten times the standard deviation of the blank over the sensitivity, in this case the value was 2.1 µM. The regression equations obtained were y (µA) = $8.32*10^{-6}$ + 5.2x (µM) for the electrodes modified with ZnTRP. The graph presents a linear coefficient above 0.99. The results suggest that the modified electrode can detect and quantify DMT efficiently.

Several analytical methods have been develop for the determination of DMT, these correspond mainly to chromatography's such as gaseous (GC), liquid (LC) and high performance liquid (HPLC), always coupled to a mass detector (MS) [43-47]. These methods have very low detection limits, however, they are very expensive and difficult to carry and harder to implement when compared to electrochemical methods. Although the LODs presented in this work are higher (see Table 1), herein the preparation of the electrodes are simpler and faster, resulting in a considerable gain of time. It has been found that the concentration of DMT in urine samples varies in the range 5.3 and 42 µM, values well above our LOD.

The quick and easy preparation of the modified electrode, the low cost production in addition to its very good stability and catalytic effect, show that this modified electrode can potentially be used for the determination of DMT in urine.

Table 1. Comparison of the prepared modified electrode in this study to other analytical methods.

Material or Method	Linear Range/µM	LOD/µM	Reference
GC/MS	8.29 - 1590	4.14	[43]
LC/MS-MS	0.03 – 26.6	0.016	[44]
HPLC/ESI-MS-MS	*	0.00053	[45]
LC/MS	*	0.53	[46]
HPLC/Electrospray/MS	0.26 – 5.31	0.053	[47]
GC/BMIMNTF$_2$/ZnTRP	0 – 3.5	1.75	this study

* does not appear in this article.

On the other hand, monoamine oxidases (MAOs) are enzymes that catalyze the oxidation of monoamines and the degradation of -amine neurotransmitters (serotonin, norepinephrine, and dopamine). The role of MAO in the metabolic decomposition of DMT has been emphasized in the literature and is based on the presence of indole-3-acetic acid (IAA), which is formed by oxidative deamination as a product of DMT degradation [48]. However, oxidative deamination by MAO may not be the only metabolic pathway present in humans. *In vitro* and animal studies have described N-oxidation, N-demethylation and cyclization as alternative metabolic pathways, as shown in Figure 4 [48].

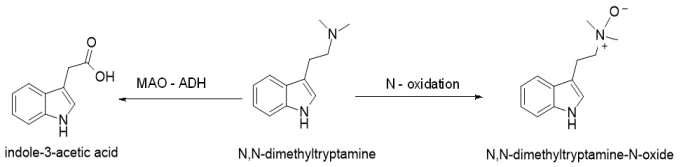

Figure 4. Possible oxidation products of N,N-dimethyltryptamine (DMT). MAO= monoamine oxidase; ADH= aldehyde dehydrogenase.

Due to the absence of MAO in this study, it was assumed that the oxidation product of DMT corresponded to N,N-dimethyltryptamine-N-oxide.

4. CONCLUSION

A simple procedure was used to modify glassy carbon electrodes using a Zn (II) tetraruthenated porphyrin and the ionic liquid BMIMNTF$_2$. The electrocatalytic activity of these modified electrodes were compared to the oxidation of DMT in neutral media (0.1M $NaClO_4$).

This modified electrodes showed good performance for DMT oxidation. These results translate into a low detection and quantification limit, high sensitivity, short response time, satisfactory linear concentration range, very good stability and reproducibility. Thus, its potential use for quantifying the amount of DMT in urine is promising.

CONFLICT OF INTEREST

No conflict of interest was reported by the authors.

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