ELECTROCHEMICAL OXIDATION OF
10*H*-PHENOTHIAZINE-1-CARBOXYLIC ACID

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ABSTRACT. The electrochemical behavior of 10*H*-phenothiazine-1-carboxylic acid was investigated by square wave anodic stripping voltammetry (SWASV) at a glassy carbon electrode, which evidenced the first monoelectronic oxidation step at low potentials (around 0.25 V), slightly modulated by the nature of the solvent. The effect of three different solvents (acetonitrile, dimethyl sulfoxide and chloroform) on the strength of intramolecular hydrogen bonding associations was evidenced by DFT calculations. The computed electron density at the heterocyclic nitrogen atom appeared well correlated to the recorded oxidation potential. The possibility to use of 10*H*-phenothiazine-1-carboxylic acid as potential mediator for electrochemical detection of Malachite Green was explored, but the phenothiazine derivative appeared not suitable for the detection of the dye.

Keywords: 10H-phenothiazine-1-carboxylic acid, SWASV, intramolecular hydrogen bonds

INTRODUCTION

A significant characteristic of phenothiazine derivatives appeared to be their ability to readily generate various oxidation products under chemical, photochemical, enzymatic, or electrochemical conditions. The redox properties of several phenothiazine derivatives with important medicinal applications were largely exploited in analytical procedures tailored for the advanced detection of phenothiazine based neuroleptic drugs [1,2]. The electrochemical oxidation of phenothiazine and its derivatives proceeds on conventional

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electrodes (Pt, glassy carbon electrode and Au) either in organic or in aqueous acidic medium. The oxidation of the phenothiazine core takes place by the intermediate stage of radical cation with a stability influenced by the nature and position of the substituents on the heterocyclic core, the acidity and the presence of various salts in the reaction medium. Further oxidation steps imply the formation of the phenothiazinium cation which usually generates a colorless phenothiazine sulfoxide, colored hydroxy-substituted or polymeric derivatives [3]. Based on the reversibility of its first monoelectronic redox process, phenothiazine was described to be an efficient mediator in various electrochemical oxidation processes. For instance, it displayed excellent electrochemical catalytic activities for oxidase enzymes (glucose, lactate and cholesterol oxidase) even when immobilised on the surface of the electrode [4,5] and enzyme glucose dehydrogenase [6]. Several phenothiazine derivatives, were studied as electron transfer mediators in different electrochemical systems [7,8]. On the other hand, a series of 1-carboxyphenothiazine derivatives was synthesized and characterized [9], but there are no reported evidences for their utility as mediators for electrochemical systems.

Considering our previous interest in the electrochemical detection of Malachite Green (MG) [10], the aim of this work was to explore the electrochemical reactivity of 10*H*-phenothiazine-1-carboxylic acid and its potential as mediator for the electrochemical detection of the dye. Based on the electrostatic attraction between a carboxy functionality attached to the phenothiazine core and the positively charged MG cationic dye, it was expected that the diffusion/adsorption of the dye to a carboxyphenothiazine modified electrode surface would be facilitated.

RESULTS AND DISCUSSION

10*H*-Phenothiazine-1-carboxylic acid **1** was synthesized by optimizing a previously reported synthetic protocol [9] based on the thiation of N-phenyl-antranilic acid (scheme 1).

Scheme 1

The structure of **1** was confirmed by spectroscopic methods. ^{1}H-NMR spectrum of **1** displayed a deshielded signal characteristic to the acidic proton situated at 10.15 ppm, accompanied by the signals of aromatic protons situated in the region 6.7-7.6 ppm.

The position of the carboxyl group in the neighborhood of the heterocyclic NH group favors the formation of intramolecular hydrogen bonds. Density functional theory (DFT) calculations were applied to emphasize the possible intramolecular hydrogen bond formation. Thus, the structure of **1** was initially optimized using Spartan 06 with DFT B3LYP 6-31 G* method in gas phase. Free rotation of the carboxyl substituent generated two conformers of **1** with computed minimal energy which are presented in Figure 1. Conformer **1a** is characterized by hydrogen bond interaction between heterocyclic NH and carboxyl substituent, while conformer **1b** does not contain a hydrogen bond.
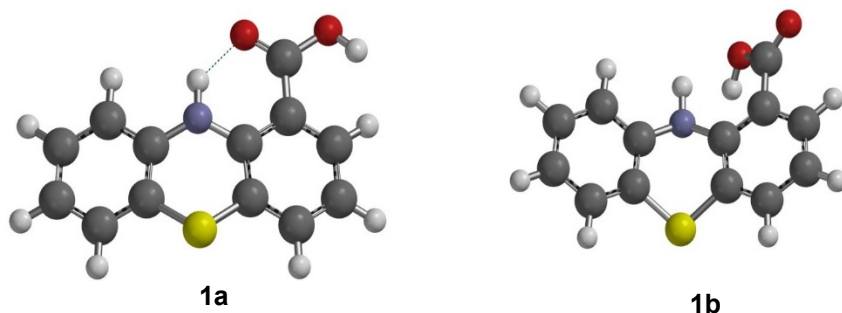

1a   **1b**

Figure 1. Conformers of 10*H*-phenothiazine-1-carboxylic acid **1**
with optimized geometry

Due to the fact that solvents may influence the hydrogen bonding [11], conformer **1a** has been optimized by B3LYP/6-31++G(d,p) Gaussian method using three data sets for the following solvents: acetonitrile, chloroform and DMSO. The results are illustrated in Table 1.

As it may be seen from table 1, **1a** appears to be more stable than **1b** (molecular energy difference 5.25 kcal/mole in gas phase) and the interactions with the aprotic dipolar solvents suggested further stabilization effects upon **1a.**

Square wave anodic stripping voltammetry experiments were performed in order to explore the electrochemical reactivity of 10*H*-phenothiazine-1-carboxylic acid dissolved in three different solvents and deposited on the surface of a glassy carbon electrode. The voltammograms presented in Figure 2 are showing well defined oxidation peaks corresponding to phenothiazine radical cationic species.

Table 1. Gaussian B3LYP/6-31++G(d,p) computational results for 10*H*-phenothiazine-1-carboxylic acid: optimized geometry, molecular energy (E) and electrostatic charge on heterocyclic nitrogen atom in gas phase and different solvents

Conformer	Environment	E (kcal/mol)	Electrostatic charge on N atom
1b	Vacuum	-692898.748	-0.709
1a	Vacuum	-692904.000	-0.630
	Acetonitrile	-693049.921	0.126
	Chloroform	-693047.637	0.132
	DMSO	-693050.015	0.125

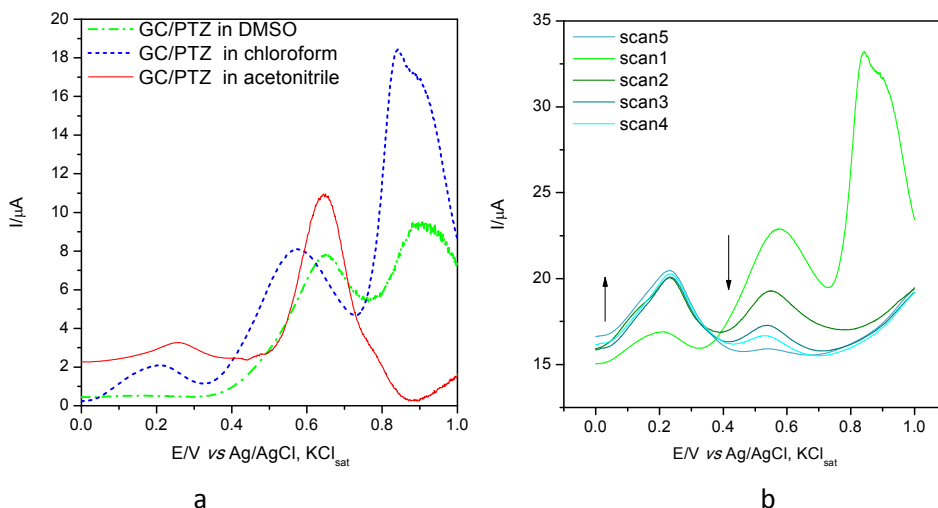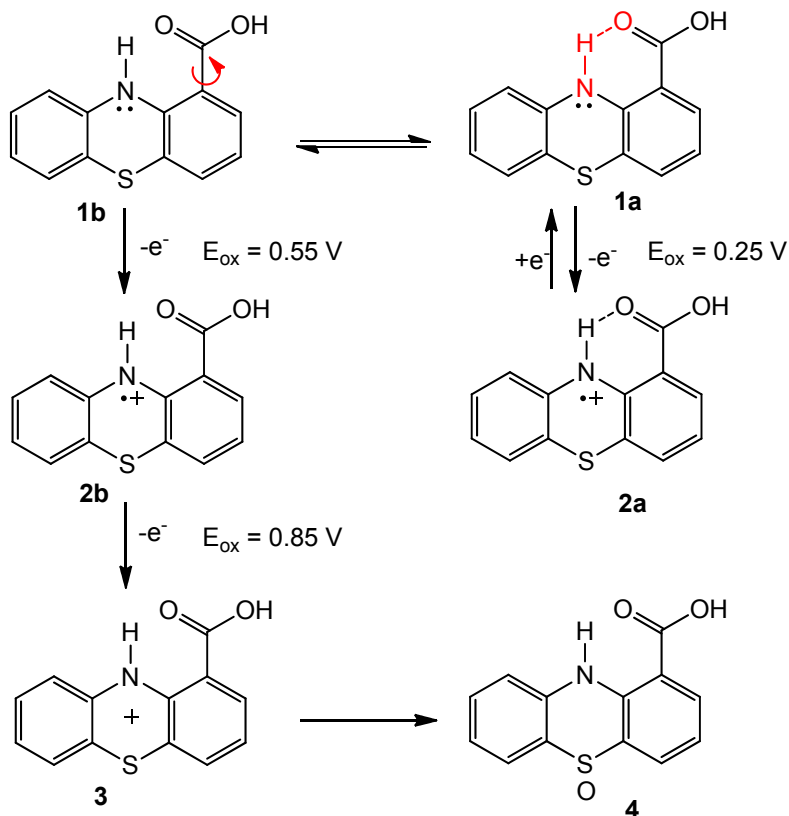a   b

Figure 2. SWASV at glassy carbon (GC) electrodes modified with 10*H*-phenothiazine-1-carboxylic acid (PTZ) a) in different solvents; b) several scans in acetonitrile. Experimental conditions: pH=3, accumulation time 300 s; frequency 25 Hz; potential oxidation processes interval 0-1 V.

In scheme 2 are described the oxidation steps proposed in order to assign the SWASV peaks observed in figure 2. The first monoelectronic oxidation step generates the radical cation **2** and the values of the typical potentials may be correlated to the computational results related to electron density at the heterocyclic nitrogen atom presented in table 1. The lowest oxidation potential (0.25 V) may be assigned to **1a** characterized by higher electron density as compared to **1b,** which in turn may be responsible for the oxidation peak situated at 0.55 V. Further step requires a higher oxidation potential and generates the cation **3** which may end up as a colourless sulfoxide **4**. As it may be seen in figure

2b, after several scans the intensity of the first oxidation peak increases (maybe due to the reversibility of the oxidation processes of **1a**)**,** while the oxidation of **1b** proceeding towards the chemical reaction may explain the consumption of the radical cationic species **2b** and consequently the decrease in intensity of the second oxidation peak.

Scheme 2

A characteristic signal of the sulfoxide was recorded by ^{1}H-NMR (in deuterated acetonitrile solution) at chemical shift 11.65 ppm.

The SWASV experiments performed in the presence of MG indicated a decrease in intensity of the first oxidation peak (figure 3a), which may be correlated to steric interactions between **1** and the bulky cationic dye in control for weakening the intramolecular hydrogen bond associations. Calibration curves presented in figure 3b indicate a feeble correlation with the MG concentration and do not sustain the possibility of elaborating an analytical procedure for MG detection.
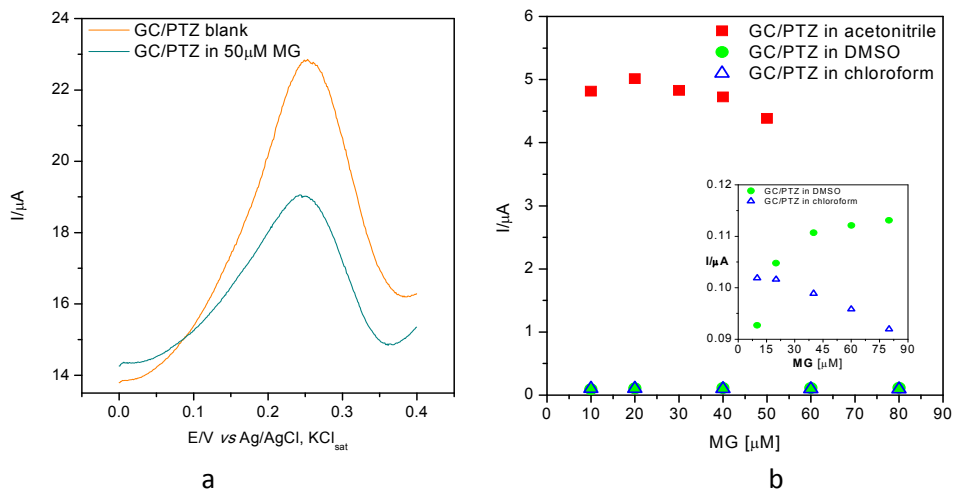
a   b

Figure 3. SWASV at GC/10H-phenothiazine-1-carboxylic acid (PTZ) electrodes:
a) in the presence of malachite green (MG). b) Calibration curves obtained for increasing
amounts of MG. Experimental conditions: accumulation time 300 s; frequency 25 Hz;
potential interval 0-0.4 V; electrolyte phosphate buffer (pH 3).

CONCLUSIONS

The oxidation potential of 10*H*-phenothiazine-carboxilic acid appeared to
be lowered by intramolecular hydrogen bond associations. The first monoelectronic
oxidation step generating the radical cation occurred around 0.25 V, this
value being slightly modulated by the nature of the solvent.

No favourable interactions between MG and 10*H*-phenothiazine-carboxilic
acid occurred and, consequently the tested electrochemical system based on
GC/10*H*-phenothiazin-carboxilic acid is not suitable for the detection of MG.

EXPERIMENTAL SECTION

Chemicals

All the chemicals used for the experiments in this study were of analytical
grade quality and were used as received, without further purification or alteration.

Malachite Green oxalate salt was purchased from Penta, Czech Republic
and all solutions were made in pH 3 phosphate buffer (PB). Phosphate buffer
solution was made starting from $NaH_2PO_4*H_2O$ and Na_2HPO_4 salts and pH
adjusted with *o*-H_3PO_4 acid, all from Merck, Germany.

10H-Phenothiazine-1-carboxylic acid 1 preparation

N-phenylanthranilic acid 6.4 g (0.03 mole), sulfur powder 1.92 g (0.06 mole) and iodine 0.8 g (0.006 mole) were dissolved in 60 ml 1,2-dichlorobenzene. The reaction mixture was heated at 175 °C for 6 h under constant stirring. After cooling at room temperature, 10 ml solution of sodium thiosulphate 40% was added to the reaction mixture. The organic layer was separated and the organic solvent was removed by vacuum distillation. The product was purified by column chromatography on silica gel using eluent toluene to give orange coloured 10*H*-phenothiazine-1-carboxylic acid (1.4 g, yield 19%). M.p. 248 °C (lit. [9])

^{1}H NMR(DMSO-d_6, 400 MHz, ppm): 6,88-7,04 (m, 5H, Ar-H), 7,18 (d, 1H, Ar-H), 7,65 (d, 1H, Ar-H), 9,63(s, 1H, -N-H), 10,15 (s, 1H, -COOH)

Electrode preparation

Before conducting any measurements, the glassy carbon (GC) working electrode was thoroughly cleaned on a piece of felt with γ-alumina slurry until mirror-like shine aspect was obtained. In order to remove any traces of impurities, further cleaning was applied by sonication in acetone and distilled water respectively.

For modifying the electrode, saturated 10*H*-phenothiazine-1-carboxylic acid solutions were prepared, in three different solvents: chloroform, acetonitrile and dimethyl-sulfoxide. Each solution was applied by drop-casting on the electrode's active surface and allowed to dry freely. In the case of DMSO solutions, a warm air current was applied to accelerate the evaporation process. Constant 5μL volumes of solutions were employed for all electrode modification purposes. No other protective coatings were applied on the modified electrode as the evaporated phenothiazine carboxylic acid layer had a good stability during measurements in aqueous solutions.

Electrochemical measurements

All electrochemical experiments were performed on a Metrohm Autolab PGSTAT 302N, electrochemical workstation (Eco Chemie, Netherlands). A three-electrode system composed of a Ag/AgCl, KCl_{sat} reference electrode, a platinum counter-electrode and a bare or modified glassy carbon (GC) working electrode was used. The electrolyte solution contained 0.1 M phosphate buffer adjusted with o-H_3PO_4 at pH 3. All experiments were performed at a room temperature of 25 °C.

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