



Voltammetric Investigation of Thioridazine using a Carbon Nanocomposite Electrode in Human Biological Samples

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Abstract

An electrochemical method employing a carbon nanocomposite electrode was developed to measure thioridazine. The electrode has been designed by incorporation of montmorillonite nanoclay into the carbon ionic liquid electrode. Surface morphology of the electrodes was done using scanning electron microscopy. Adsorptive stripping voltammetry was applied as a very sensitive analytical method for the determination of sub-micromolar amounts of thioridazine. The optimized linear working range and detection limit (S/N=3) are 0.1-50 μ M and 21nM, respectively. The nanocomposite electrode demonstrates several advantages such as simple preparation method, excellent activity in physiological conditions, low detection limit, long-term stability, and remarkable voltammetric reproducibility. As a result, it can find promising applications in blood serum and urine samples.

Keywords: Carbon ionic liquid electrode, Electrochemistry, Nanoclay, Thioridazine, Voltammetry.

Introduction

Thioridazine hydrochloride (TR-HCl) the hydrochloride of 10-[2-(1-methyl-2-piperidyl) ethyl]-2-methylthiophenothiazine is a phenothiazine neuroleptic drug used in the treatment of schizophrenia, and psychiatric disorders, as well as short-term treatment of

adults with major depression. Also, it may be used in the management of anxiety states, a behavior problem in children [1]. A serious side effect of TR is the potentially fatal narcoleptics malignant syndrome [1]. Various analytical methods have been reported for measuring thioridazine, such as high

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performance liquid chromatography (HPLC) [2], spectrophotometry [3], and flow-injection on-line oxidizing fluorimetry [4]. However, these methods suffer from noticeable disadvantages; for instance, HPLC is expensive and time consuming, spectrophotometry has the limitation of low selectivity and it suffers from interferences derived from others components of sample, and flow-injection on-line oxidizing fluorimetry is also expensive

and it suffers from serious interferences. On the other hand, electrochemical methods have attracted considerable attention due to their simplicity, short analysis time and require less expensive equipment than other methods. Some of methods for the determination of TR, as well as the electrochemical methods using for determination of TR, are summarized in Table 1.

Table 1. Different methods for the determination of TR.

Detection method	Linear range (μM)	Detection Limit (nM)	References
HPLC	0.1-7.0	20	[2]
Spectrophotometric	9.8-40, 6.0-54	-	[3]
Flow-injection on-line oxidizing fluorimetry	0.04-4.9	13.5	[4]
Voltammetry	100-1000	-	[5]
Voltammetry	0.1-100	50	[1]
Voltammetry	0.1-36	65	[6]
Voltammetry	12-850	1300	[7]
Voltammetry	3.2-750	205	[8]
Voltammetry	0.01-0.1	7	[9]
Voltammetry	0.1-50	21	This work

Recently, the application of nanomaterials has attracted considerable attention in electrochemical researches because of the unique properties of these materials [10]. Chemically modified electrodes (CMEs) have recently attracted considerable attention due to their significant advantages, such as increasing peak current and decreasing over potential for redox systems. Clays are stable aluminosilicates with high cation exchange

capacity, and exfoliated clay particles have a platelet shape with nanoscopic size [11]. Moreover, due to ionic exchange capacity, good catalytic support, large surface area, mechanical stability and low cost properties of clays, their application in the modification of the surface of electrodes has been extended [12]. In addition because of "green chemistry", environmentally friendly analytical protocols and devices, negligible

waste, and nontoxic materials have made clay minerals of considerable interest to the analytical community [13].

Clays are also mixed with carbon paste to enhance the adsorptive and ion-exchange properties of the electrode [14]. Montmorillonite nanoclay with its inherent layered inorganic nanostructure is a member in the smectite group of clays [15-17] and has a high affinity for several substances (e.g., heavy metals and organic molecules) [12, 17]. Carbon ionic liquid electrodes (CILE) exhibit properties for electrochemical applications, such as low cost, ease of preparation, antifouling effect and renewable surface [18]. CILE can be prepared by substituting the nonconductive organic binder with high conductive ionic liquids, which exhibit superior electrochemical performances over the traditional working electrodes [14]. The interaction of ionic liquids (ILs) and clays were studied and they were applied as a suitable matrix for immobilization of different biomolecules on the surface of electrodes [19-21]. In this work, montmorillonite nanoclay modified carbon ionic liquid electrode was applied for electrochemical detection of TR. The obtained results show that the electrode exhibits an excellent route to the sensitive determination of TR by using montmorillonite nanoclay. The proposed electrode is simple to prepare, reproducible, easily renewable and cost effective.

Experimental

Materials and apparatus

Thioridazine hydrochloride, pyridine, diethyl ether, paraffin oil (Merck), montmorillonite nanoclay, graphite powder (particle size <100 nm) (Aldrich), ammonium hexafluorophosphate and iodooctane (Fluka) were used as received. The IL, octylpyridinium iodide, was synthesized as described in Ref. [22]. For preparing all solutions, deionized distilled water was used and Briton-Robinson buffer solution was utilized in order to study the effect of pH on the analytical signal. In addition, we made use of phosphate buffer solution (PBS 0.07 M, pH 7.0) as supporting electrolyte. For providing the experimental samples, we got daily-based fresh frozen female blood sample, from Central Blood Transfusion Organization (Shiraz, Iran) and urine sample obtained from healthy individuals were stored frozen until assay.

Electrochemical measurements were done with a galvanostatic potentiostat Behpajoo Co. model BHP2063+. The electrochemical cell was assembled with a conventional three-electrode system; a saturated calomel electrode as a reference electrode (SCE), a platinum disk as a counter electrode, and CILE (Nanoclay modified carbon ionic liquid electrode (NC-CILE)) as the working electrode. The cell was a one-compartment cell with an internal volume of 10 mL. All experiments were typically conducted at room temperature.

Scanning electron microscopy (SEM) images were observed using SEM (Philips XL 30 and S-4160) gold coated, and by using energy dispersive X-ray spectroscopy (EDX).

Preparation electrode

In the first step, NC-CILE was prepared by hand-mixing of the graphite powder, ionic liquid and nanoclay in the proportion (40%:50%:10%, wt %). Therefore, a portion of the resulting paste was packed firmly into the cavity (1.8 mm i.d.) of a Teflon holder. The electrode was then heated (e.g. by using a hair drier) to a temperature above the melting point of IL (m.p. ~ 65 °C) prior to use [14]. The electric contact was established with a copper

wire. A new surface of CILE or NC-CILE was obtained by smoothing the electrode onto a weighing paper.

Results and discussion

SEM micrographs of nanoclays (as received) and NC-CILE are shown in figure 1 (A) and figure 1 (B), respectively. Figure 1 (B) depicts nanoclays are in the form of large and small aggregates. In fact, after the incorporation of nanoclays into the CILE, nanoclay particles are dispersed in the form of homogeneous nanostructures with a dimension about 30 nm. Certainly, the narrowly dispersed nanoparticles enhance the contact surface area of the clays with the solution.

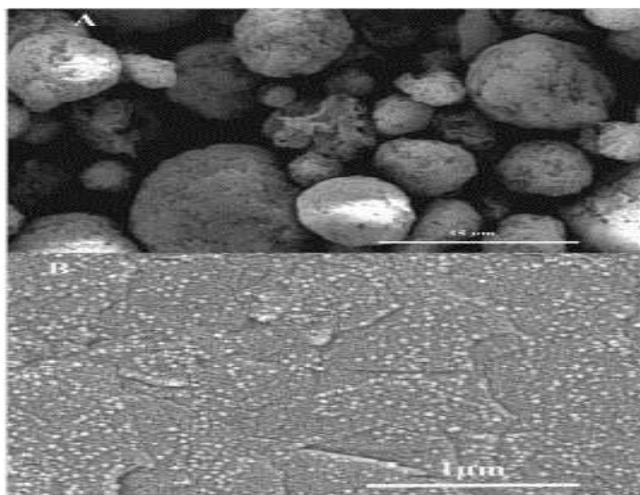


Figure 1. (A) SEM micrographs of nanoclays as received, (B) nanoclay modified carbon ionic liquid electrode (NC-CILE).

The cyclic voltammograms of 0.1 mM TR in PBS (0.07 M, pH 7.0) obtained at a potential sweep rate of 50 mVs^{-1} for CILE and, NC-CILE (10%, wt %), are given in Figure 2 for both CILE (curve b) and NC-CILE (curve

c). As it can be seen from Figure 2, three oxidation peaks were obtained around 0.54, 0.75, 0.87 V. Under the same conditions and in the absence of TR, no peak was observed on bare NC-CILE (curve a) and CILE (not

shown) in the working potential range. The first oxidation peak potential of TR on both CILE and NC-CILE are slightly lower than those reported for a carbon paste electrode modified with ZnS nanoparticles (~ 0.65 V)

[6], a GC electrode modified with Nitrogen-doped carbon nanotubes/gold composites (~ 0.58 V) [7], a GC electrode (~ 0.59 V) [8], a carbon paste electrode modified with β -cyclodextrin (0.56 V) [9].

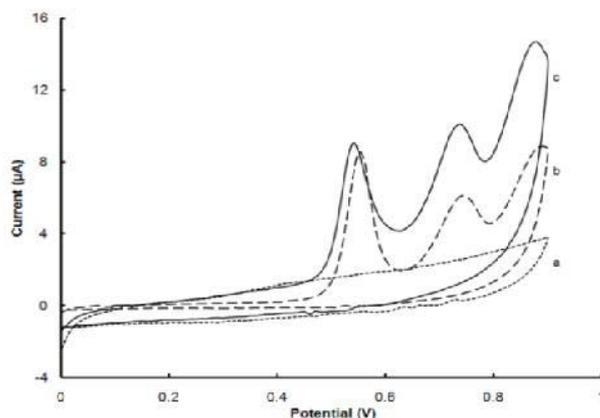


Figure 2. CVs of NC-CILE (a, c) and CILE (b) in 0.07M PBS (pH 7.0) in the presence (b, c) and in the absence (a) of 0.1mM TR at a scan rate of 50 mVs⁻¹.

The use of accumulation period (240 s) results in peak current enhancement for both CILE and NC-CILE that which is larger for NC-CILE compared to the ones of CILE. This is because montmorillonite possesses a physical structure consisting of sheets of aluminosilicates [23], which is neutralized by the intercalation of compensating, exchangeable Na⁺. The Na⁺ ion can be exchanged with a wide variety of hydrated inorganic or organic cations [24]. Several other researchers also applied the cation exchange property of montmorillonite for the adsorption of some cationic electroactive compounds on the surface of electrode [25, 26]. Since TR is protonated and has positive charge in the working solution (0.07 M PBS, pH7.0), its interactions with

montmorillonite nanoclays is similar to the other organic cationic species in this matrix. Actually, this feature causes an increase in faradic currents and, consequently, sensitivity enhancement due to the synergetic effect of both nanoclays and IL which can effectually increase the rate of drug preconcentration from aqueous solution to the electrode surface by ion exchange and adsorption. Therefore, NC-CILE was selected for further studies.

Effect of pH

The influence of the pH on the oxidation peak current of 0.1 mM TR was investigated in the pH range of 4–7 applying the Britton–Robinson (B. R.) buffer, as can be seen from figure 3. At pH values higher than 8, the

solubility of the drug is somewhat lowered due to the hydrophobic character of deprotonated molecule of TR. Therefore, the range of pH selected was from 4 to 7. As seen, with solution pH raising the peak current increased and reached to the maximum value at pH 7.0. Since the pH 7 PBS gave the same response in terms of the peak current and the peak shape for TR, PBS with pH 7.0 was used as the supporting electrolyte in all voltammetric determinations.

Over the pH range of 4–7, E_p of TR was a linear function of pH, as shown in the inset, figure 3. From the plot of E_p versus pH, slope of -0.028 V was obtained, corresponding to the following equation: E_p (V) = $0.744 - 0.028$ pH. This result revealed that a mechanism comprising two electrons and two protons in the electrochemical reaction. This is consistent with the previously proposed mechanism for the TR oxidation [6, 27].

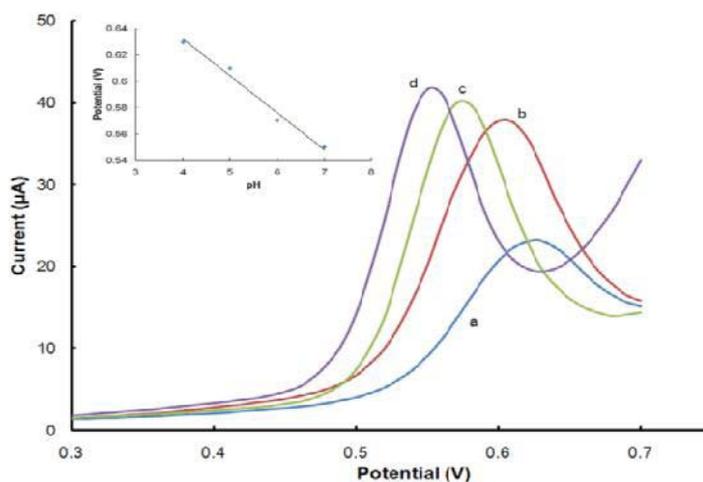


Figure 3. CVs of 0.1mM TR at the NC–CILE. pH: (a) 4.0; (b) 5.0; (c) 6.0 ; (d) 7.0; at 50mVs^{-1} . Inset: plot of oxidation peak potential vs. pH.

Effect of modifier

The effect of nanoclay as a modifier on the voltammetric response of the NC – CILE was optimized by varying its composition (2.5, 5, 10 and 15, % weight percent ratio). The results indicated that the peak currents increase with increasing nanoclay up to 10%, while further increase in the amount of nanoclay causes a decrease in the peak current. This is because the sites for desorption increase with the increase of nanoclay percentage in the modified electrode, while further nanoclay results in an

increase in the resistance of the electrode, and consequently, it enhances the electron-transfer resistance. As a result, a NC – CILE (10%, w/w) was used in further studies.

Study of the potential sweep rate effect

Cyclic voltammetry was carried out for 0.1 mM TR at different scan rates, as it can be seen from figure 4. As the inset, figure 4 depicts there is a linear relationship between the peak current (i_p) of TR and the scan rate (v) in the range of 10 to 100mVs^{-1} . Plot of log peak

current versus log scan rate with the equation of $\log i_p = 0.92 \log v + 1.84$ ($R^2 = 0.990$), and with the slope of 0.92. Therefore, the obtained value for the slope is close to the expected value of 1.00 in the condition of ideal reaction of surface species, which indicates that the

TR electrooxidation reaction is an adsorption controllable process. As figure 4 shows with scan rate increasing, the anodic peak grows and the oxidation peak potential shifts to more positive potentials confirming the kinetic limitation in the electrochemical reaction [6].

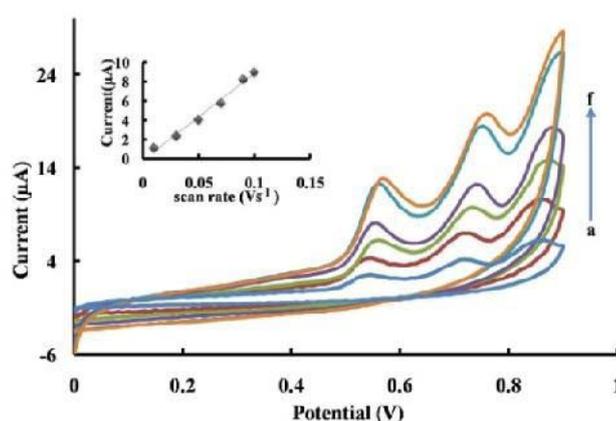


Figure 4. CVs of NC –CILE in 0.07M PBS (pH 7.0) containing 0.1 mM TR at various scan rates (a: 10 to f : 100 mVs^{-1}). Inset: plot of peak current vs scan rate.

Effect of accumulation potential and time

The accumulation potential, similar to the accumulation time, is an effective factor which affects the response sensitivity. The effect of accumulation potential on the peak current of TR was examined over the range of -0.5 to 1 V keeping the accumulation time 240 s. In this study, the oxidation peak current increased up to 0 V. Therefore, an optimal accumulation potential of 0 V was used for further studies. The accumulation time varied between 0 to 300 s employing optimized accumulation potential value. It was observed that the peak current increased with accumulation time; the more TR was adsorbed, the larger the peak currents became as it reached to a constant

value after a certain accumulation period. Therefore, an accumulation time of 240 s was chosen for further experiments.

Calibration curve

The proposed method was employed for the determination of TR. For this purpose, the relationship between the anodic peak current and the concentration of TR was studied using cyclic voltammetry under the optimum conditions, which is depicted in figure 5. The analytical curve shown in the inset, figure 5 has a linear calibration range of 0.1 – 50 μM , and it can be described with the equation of $I_p = 0.845 + 1.380C$ (μM) ($R^2 = 0.991$).

The limit of detection (LOD) of the proposed

modified electrode was found to be 21 nM. The responses of electrochemical methods for the determination of TR in terms of linear range and their detection limit are listed in Table 1 [1, 5, 6-9]. As it can be seen from Table 1, the detection limit of the proposed method

is considerably better than those of the other reported electrochemical methods, except for one case [9]. Also, the linear range of the calibration curve of our method is better than those of the most previously reported methods [6,9].

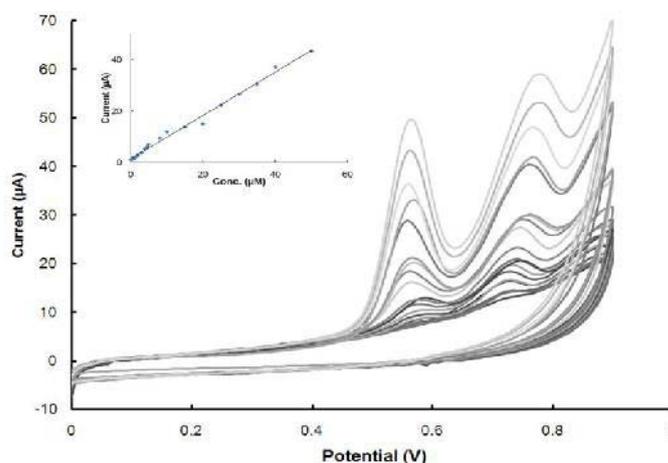


Figure 5. CVs of NC-CILE for different concentrations of TR in 0.07M PBS (pH 7.0), accumulation time 240 s and accumulation potential 0 V. Inset shows the calibration curve of peak current vs. TR concentration.

Stability, repeatability and reproducibility of the electrode

The stability of the proposed nanocomposite electrode was investigated by recording the response of the electrode in 2 μ M of TR after every few days. Finally, the NC-CILE showed high stability for TR detection and it retained 89% of its initial response to TR after 30 days of storage. The repeatability was estimated through the relative standard deviation of 7 replicate measurements of a 0.07 M PBS (pH7.0) containing 2 μ M of TR. The relative standards deviation (RSDs) of 2.24% revealed good repeatability. The responses of four similar electrodes were separately measured in 2 μ M TR, and consequently, RSDs of 2.7%

was obtained confirming high reproducibility of the fabrication method. The response of the proposed composite electrode was evaluated for some common species found in biological fluids such as glucose, ascorbic acid and uric acid. The tolerance limit was defined as the maximum concentration ratio of interfere/TR causing an error less than $\pm 5.0\%$ for the determination of TR. In the presence of 10 μ M TR the results showed that 120-fold excess of glucose; 100- fold excess of ascorbic acid and 50-fold excess of uric acid; did not interfere with the analysis of TR. The results demonstrated good selectivity for the proposed electrode.

Real sample analysis

In order to evaluate the analytical applicability of the proposed nanocomposite electrode, for the determination of TR, it was applied for the biological samples such as serum and urine. Standard addition method was used for measuring TR concentration in these samples. The blood serum sample was deproteinized by adding 2 ml of 10% (w/w) trichloroacetic acid solution to 10 ml sample, and then the solution was centrifuged. Therefore, then obtained sample was diluted 10 times with 0.07M PBS

with pH 7.0. In the next step, appropriate amount of prepared sample was transferred to the electrochemical cell for the determination of TR. The results are presented in Table 2. Urine sample was also diluted with 0.07 M PBS (pH 7.0) and then appropriate amount of this diluted sample was transferred to the electrochemical cell for the determination of TR, as reported in Table 2. The obtained results confirm that the NC-CILE retains its efficiency in the determination of TR in real samples.

Table 2. Determination of TR in body fluids by using the proposed method (n=3).

Sample	Added (μM)	Found (μM)	Recovery (%)
Blood serum	0	N.D.*	-
	0.5	0.49(± 0.03)	98.0
	1.0	1.02(± 0.04)	102.0
	1.5	1.49(± 0.06)	99.3
Urine	0	N.D.*	-
	0.5	0.517(± 0.02)	104.0
	1.0	0.96(± 0.03)	96.0
	1.5	1.52(± 0.06)	101.3

*Not detected

Conclusion

By using IL as a binder and montmorillonite nanoclay as a modifier, NC – CILE was fabricated, and it was used for sensitive determination of TR. Simultaneously, an effective accumulation of the drug molecules was discovered on the electrode due to synergistic effect of nanoclays and IL. In sum, high stability and reproducibility as well as

the ease of preparation, low cost and surface renewal have made this type of electrode ideal for the determination of TR in blood serum and urine sample.

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