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Adsorptive stripping voltammetry for simultaneous determination of hydrochlorothiazide and triamterene in hemodialysis samples using a multi-walled carbon nanotube-modified glassy carbon electrode

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ABSTRACT

Hemodialysis is the most commonly used method for the treatment of chronic kidney disease. In this procedure, some patients use diuretics to control weight gain and blood pressure. In this work, a voltammetric sensor based on a glassy carbon electrode modified with carbon nanotubes (GCE/MWCNT) is described for the simultaneous determination of the diuretics hydrochlorothiazide (HCT) and triamterene (TRT). The oxidation of the diuretics on the GCE/MWCNT surface was observed at 1.01 and 1.17 V for HCT and TRT, respectively, allowing simultaneous determination, which was not possible with the unmodified glassy carbon electrode. The GCE/MWCNT electrode provided 6-fold and 10-fold gains in anode peak intensity for HCT and TRT, respectively, compared to the unmodified electrode. After optimization of the conditions (pH, accumulation time, and accumulation potential), analytical curves were constructed for the analytes in the range from 1.0×10^{-7} to 2.0×10^{-5} mol L^{-1}. The detection limits for HCT and TRT were 2.8×10^{-8} and 2.9×10^{-8} mol L^{-1}, respectively. A high performance liquid chromatography method with diode array detection was also developed for the determination of HCT and TRT in hemodialysis samples, for comparison with the electroanalytical method.

1. Introduction

Hemodialysis is a treatment for chronic kidney disease (CKD), which affects 10% of the world's population [1,2]. Ensuring the quality of dialysis water can be a challenge, since each patient is exposed to between 18,000 and 36,000 l of water per year [3], and high water quality is extremely important in order to avoid exposure of these individuals to chemical and microbial contaminants [4]. The literature reports several analytical methods for the detection of metal contaminants, with the aim of minimizing the possibility of patient exposure [5–8]. However, to the best of our knowledge, there have been no reported studies concerning the detection of pharmaceutical compounds such as diuretics in the water used for dialysis. The control of diuretics is important, since these drugs may be used as part of the hemodialysis treatment, in order to control weight gain and blood pressure [9]. Therefore, it is necessary to develop methods able to detect and quantify diuretics such as hydrochlorothiazide (HCT) and triamterene (TRT) in samples of hemodialysis water.

Methods have been reported for the determination of HCT and TRT based on chromatography [10,11], spectrophotometry [12], capillary electrophoresis [13], amperometry [14], and voltammetry [15,16]. In addition to the need for methods for the determination of these diuretics, in recent years increasing attention has been given to the development of green analytical methods, with the goal of reducing environmental pollution [17].

Electrochemical analytical techniques are attractive because in addition to avoiding the generation of large amounts of residues, they offer high sensitivity, good reproducibility, low cost, and the possibility of miniaturization [18–22]. Further improvements in these techniques in terms of sensitivity, selectivity, enhanced surface area, and catalytic effects can be achieved by modifying the surfaces of the electrodes with specific materials [23–26]. Multi-walled carbon nanotubes (MWCNT), first discovered in 1991 [27], are especially attractive as electrode modifiers, due to their excellent electronic, thermal, mechanical, and catalytic properties [28,29]. From the electrochemical perspective, their use increases the active area and improves electron transfer reactions, resulting in higher detection capability and better peak separation [30,31].

This work describes the development of an electrochemical sensor based on a glassy carbon electrode modified with carbon nanotubes for the simultaneous determination of hydrochlorothiazide and triamterene at very low concentration in hemodialysis water, synthetic urine,

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and other aqueous samples of environmental interest. In addition, a high performance liquid chromatography method with diode array detection (HPLC-DAD) was developed for determination of the compounds, and the figures of merit were compared to those for the proposed electrochemical sensor.

2. Experimental

2.1. Reagents and equipment

All chemicals used in this work were analytical grade and the solutions were prepared using ultra-pure water (Milli-Q® system, Millipore). Triamterene (purity ≥ 99.9%), hydrochlorothiazide, and dimethylsulfoxide were obtained from Sigma-Aldrich. Acetic acid, phosphoric acid, boric acid, and potassium chloride were from Merck. Sodium hydroxide and N,N-dimethylformamide were from Synth. Multi-walled carbon nanotubes (MWCNT) were obtained from DropSens. Standard solutions of 0.01 mol L^{-1} triamterene and hydrochlorothiazide were prepared by dissolution of the compounds in dimethylsulfoxide and acetonitrile, respectively, and were subsequently diluted in 0.10 mol L^{-1} Britton–Robinson (B − R) buffer, used as supporting electrolyte. The B − R buffer was prepared by mixing appropriate amounts of 0.10 mol L^{-1} sodium hydroxide with orthophosphoric acid, acetic acid, and boric acid (all at 0.10 mol L^{-1}). Measurements of pH were performed with a Tecnopon mPA 210 pH meter. The electrochemical experiments (using voltammetric techniques and electrochemical impedance spectroscopy) employed an Autolab PGSTAT 302 N potentiostat equipped with a FRA32 AC module and controlled using NOVA software. The chromatographic analyses were performed using a Shimadzu LC10ATVp high performance liquid chromatograph equipped with a diode array detector (HPLC-DAD) and controlled using CLASS-VP software.

2.2. Preparation of the modified electrode

A 1 mg portion of MWCNT was macerated in a Petri dish for 15 min and was then placed in 1 mL of N,N-dimethylformamide, with ultrasonication for 30 min. A 2.5 µL aliquot of the resulting MWCNT composite was placed on the surface of a previously cleaned glassy carbon electrode, followed by drying of the film at 60 °C for 20 min in an oven.

2.3. Sample preparation and analysis

2.3.1. Tap water sample

An aliquot of tap water fortified with HCT and TRT was transferred to a 10 mL electrochemical cell, giving a final concentration of 3 µmol L^{-1} of the diuretics. Voltammograms were recorded in 0.10 mol L^{-1} B–R buffer (pH 4.0), under magnetic stirring, using linear sweep adsorptive stripping voltammetry with application of an accumulation potential of 0.80 V for 40 s. The concentrations of HCT and TRT were obtained using the standard additions method.

2.3.2. Water treatment plant sample

A 10 mL sample of water collected from a water treatment plant in the city of Araraquara (São Paulo State) was fortified with 15 µmol L^{-1} of HCT and TRT. A 2 mL aliquot of the sample was transferred to an electrochemical cell containing 8 mL of B-R buffer solution, resulting in final concentrations of 3 µmol L^{-1} of the diuretics, and analysis was performed as described in Section 2.3.1.

2.3.3. Artificial urine sample

The artificial urine sample was prepared by mixing the following reagents in 250 mL of water: 0.73 g NaCl, 0.40 g KCl, 0.28 g $CaCl_2 \cdot 2H_2O$, 0.56 g Na_2SO_4, 0.35 g KH_2PO_4, 0.25 g NH_4Cl, and 6.25 g urea [32,33]. A 10 mL aliquot was then fortified with 8.40×10^{-7} and 9.87×10^{-7} mol L^{-1} of HCT and TRT, respectively (equivalent to 250 ng mL^{-1}). For the electrochemical analysis, 4 mL of the urine sample, without any previous treatment, was placed in an electrochemical cell containing 6 mL of 0.10 mol L^{-1} B–R buffer (pH 4.0), resulting in concentrations of 3.36×10^{-7} and 3.95×10^{-7} mol L^{-1} of HCT and TRT, respectively. Analysis was performed as described in Section 2.3.1.

2.3.4. Hemodialysis sample

The hemodialysis sample was collected during the hemodialysis of a patient at the Araraquara Regional Hemodialysis Reference Center (São Paulo State). A 10 mL aliquot was fortified with 15 µmol L^{-1} of HCT and TRT.

For the electroanalytical measurements, a 2 mL aliquot of the sample (without previous treatment) was placed in an electrochemical cell containing 8 mL of 0.10 mol L^{-1} B–R buffer (pH 4.0) and was analyzed as described in Section 2.3.1. For measurements using the HPLC-DAD method, the sample was pretreated by filtering first through a 0.45 µm filter and then through a 0.22 µm filter. The analysis was performed using an injection volume of 20 µL of the sample, a Phenomenex Kinetex PFP column (150 × 4.6 mm; 5 µm) heated at 35 °C, and a mobile phase consisting of methanol (eluent A) and 0.01 mol L^{-1} acetate buffer (pH 5.6) (eluent B), at a flow rate of 1.2 mL min^{-1}. The eluent gradient was as follows: 0–2 min 5% A, 2–12 min 5–100% A, 12–14 min 100% A, 14–20 min 100–5% A.

3. Results and discussion

3.1. Characteristics of the GCE/MWCNT modified electrode

Electrochemical impedance spectroscopy (EIS) measurements were recorded using 5 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ in 0.1 mol L^{-1} KCl. The charge transfer resistance (R_{ct}) of the unmodified GCE was 288 Ω (Fig. 1). After modification of the electrode with MWCNT, R_{ct} decreased to 36.2 Ω, which could be explained by the excellent intrinsic conductivity of MWCNT. The Nyquist plots for all electrodes were fitted using the Randles circuit (Fig. 1 insert).

3.2. Electrochemical behavior of HCT and TRT at the GCE/MWCNT electrode

The electrochemical behaviors of 100 µmol L^{-1} HCT and TRT were first investigated individually by cyclic voltammetry in 0.10 mol L^{-1} B–R buffer (pH 4.0), as shown in Fig. 2. For the glassy carbon electrode (GCE), HCT (Fig. 2.I.a) showed an anodic peak (E_{ap}) at 1.06 V, corresponding to oxidation of the –NH– group [34], while no peak was observed in the cathodic scan, indicative of irreversible behavior [35]. For

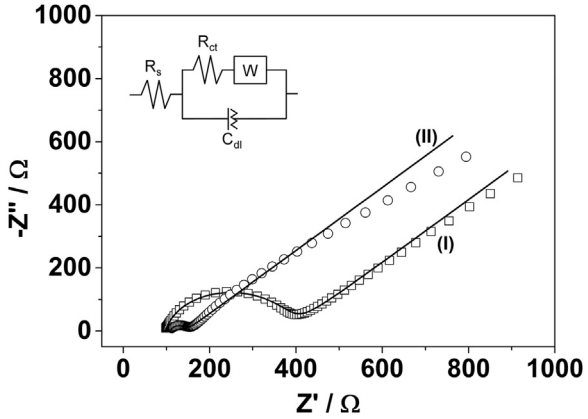

Fig. 1. Nyquist plots using 5.0×10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ redox probes in 1.0 mol L^{-1} KCl solution for GCE (a) and GCE/MWCNT (b). Conditions: 10 kHz–0.03 Hz; 5 mV rms sinusoidal modulation at OCP.

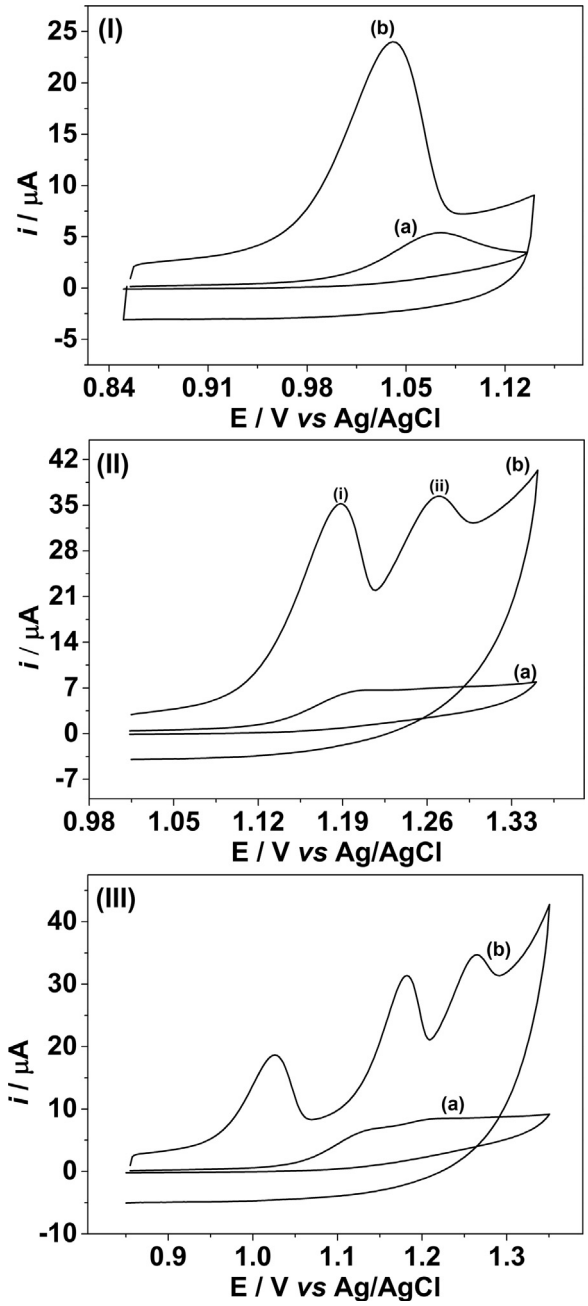

Fig. 2. Cyclic voltammograms for 100 µmol L^{-1} of HCT (I), TRT (II), and HCT + TRT (III) using GCE (a) and GCE/MWCNT (b) in 0.10 mol L^{-1} B–R buffer (pH 4.0). Conditions: υ = 75 mV s^{-1}; modifier volume 2.5 µL (1 mg mL^{-1}).

oxidation of HCT at the GCE/MWCNT electrode (Fig. 2.I.b), there was a shift of E_{ap} to 1.01 V and a 6-fold increase in the anodic peak intensity (i_{ap}). In the case of TRT, use of the GCE (Fig. 2.II.a) resulted in a single oxidation peak at 1.21 V, due to oxidation of the R–NH_2 group [15], with irreversible characteristics [35]. At the GCE/MWCNT electrode (Fig. 2.II.b), the E_{ap} of the R–NH_2 group appeared at 1.17 V (peak i), with a 10-fold increase in i_{pa}. In addition, another anodic peak was observed at 1.25 V (peak ii), which was not found in the scan using the GCE and was probably due to subsequent oxidation of the amine group [36].

For the simultaneous detection of the two compounds at the GCE (Fig. 2.III.a), the oxidation peaks for HCT and TRT were present, but peak overlapping made it impossible to quantify the analytes. However, use of the GCE/MWCNT (Fig. 2.III.b) resulted in separation of the HCT and TRT peaks, with ΔE = 160 mV, enabling simultaneous determination of the diuretics.

In order to identify the charge transport control in the system, cyclic voltammograms were recorded for oxidation of 100 µmol L^{-1} HCT and TRT in 0.10 mol L^{-1} B–R buffer (pH 4.0), with scanning from 2 to 100 mV s^{-1} at different rates. As shown in Fig. S1, for HCT (I) and TRT (II), when the scan rate (υ) was increased, there were small displacements of the anodic peak potentials (E_{ap}) of the two compounds towards more positive regions, which is behavior typical of an irreversible process. The linear relationships obtained could be described by the following equations: i_{ap} / µA = 2.41 × 10^{-4} υ / V s^{-1} + 1.03 × 10^{-6} (R^2 = 0.996) and i_{ap} / µA = 1.34 × 10^{-4} υ / V s^{-1} + 9.76 × 10^{-7} (R^2 = 0.990) for HCT (insert, Fig. S1.I) and TRT (insert, Fig. S1.II), respectively. From this, it could be concluded that the charge transfer was controlled by the adsorption process [35]. Nevertheless, both compounds could be monitored at all the scan rates tested. A scan rate of 100 mV s^{-1} was employed in the subsequent experiments.

3.3. Optimization of the method

In order to be able to simultaneously detect HCT and TRT at low levels, investigation was made of the effects of pH, accumulation potential, and accumulation time. Firstly, the behaviors of HCT and TRT were evaluated at pH 3–10 in 0.10 mol L^{-1} B–R buffer. For both diuretics, the highest i_{ap} values were observed at pH 3 and 4, with decreases at higher pH and the lowest values at pH 10 (Fig. S2.I). The best peak separation for detection of both analytes was achieved at pH 4, which was selected for use in the subsequent analyses. As the pH decreased, E_{ap} shifted to more positive values for both HCT and TRT (Fig. S2.II), according to the following equations: E_{ap} / V = −70.4 × 10^{-3} pH + 1.30 (R^2 = 0.995) for HCT and E_{ap} / V = −51.0 × 10^{-3} pH + 1.37 (R^2 = 0.994) for TRT. The results indicated the same e^- / H^+ ratios for oxidation of the diuretics [15,34].

Since both diuretics showed charge transfer controlled by the adsorption process, preconcentration studies were performed using 50 µmol L^{-1} of HCT and TRT. The effect of the accumulation potential (E_{ac}) on i_{ap} was first evaluated in the range from 0.80 to −0.40 V, after stirring for 15 s, as shown in Fig. S3.I. For both analytes, the highest i_{ap} was observed at E_{ac} of 0.80 V, which was therefore selected in the subsequent analyses. The effect of the accumulation time (t_{ac}) on i_{ap} was studied in the range from 15 to 70 s, using 50 µmol L^{-1} of HCT and TRT (Fig. S3.II). For both compounds, i_{ap} increased as t_{ac} was increased from 15 to 40 s. At longer t_{ac}, a plateau was observed. Therefore, prior to each measurement, the solution was submitted to a preconcentration during 40 s at E_{ac} of 0.80 V.

3.4. Analytical performance

Under optimized conditions of pH (4.0), E_{ac} (0.80 V), and t_{ac} (40 s), linear sweep adsorptive stripping voltammograms (LSAdSV) for HCT and TRT were recorded in 0.10 mol L^{-1} B–R buffer (pH 4.0), as shown in Fig. 3. For both HCT and TRT, two linear relationships were found in the concentration range from 1.0 × 10^{-7} to 2.0 × 10^{-5} mol L^{-1}. The first was between 1.0 × 10^{-7} and 5.0 × 10^{-6} mol L^{-1}, described by the equations: i_{ap} / µA = 0.565[HCT] / µmol L^{-1} + 2.06 × 10^{-8} (R^2 = 0.996) and i_{ap} / µA = 0.895[TRT] / µmol L^{-1} + 2.95 × 10^{-7} (R^2 = 0.997). The second linear relationship was between 5.0 × 10^{-6} and 2.0 × 10^{-5} mol L^{-1}, described by the equations: i_{ap} / µA = 0.412[HCT] / µmol L^{-1} + 6.62 × 10^{-7} (R^2 = 0.997) and i_{ap} / µA = 0.416[TRT] / µmol L^{-1} + 2.38 × 10^{-6} (R^2 = 0.999). The limits of detection (LD) and quantitation (LQ) were calculated using the expressions LD = 3*std*/*m* and LQ = 10*std*/*m*, where *std* is the standard deviation of 10 LSAdSVs obtained for the supporting electrolyte alone (0.10 mol L^{-1} B–R buffer solution at pH 4.0) and *m* is the slope of the curve. The LD values were 2.8 × 10^{-8} and 2.9 × 10^{-8} mol L^{-1} and the corresponding LQ values were 9.5 × 10^{-8} and 9.6 × 10^{-8} mol L^{-1} for HCT and TRT, respectively.

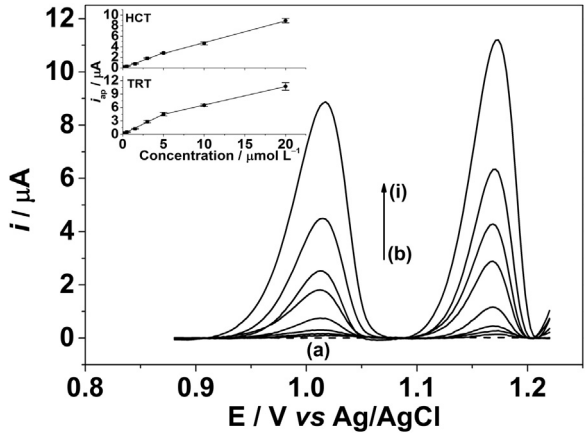

Fig. 3. LSAdSV using the GCE/MWCNT in 0.10 mol L^{-1} B–R buffer (pH 4.0) (a) and with HCT and TRT concentrations of 0.100 (b), 0.200 (c), 0.500 (d), 1.00 (e), 5.00 (f), 10.0 (g), and 20.0 µmol L^{-1} (h). Figure insert: linear relationships for i_{ap} vs. [HCT] and i_{ap} vs. [TRT]. Conditions: υ = 100 mV s^{-1}; E_{ac} = 0.80 V; t_{ac} = 40 s.

Table 1 provides a comparison of the figures of merit of the proposed method and other techniques reported in the literature. The proposed sensor presented a lower limit of detection, compared to the values found elsewhere [15,17,34,37]. Although a lower limit of detection and higher linear range was obtained for the method described by Nezhadali [38], the novelty of the proposed sensor is that it enabled simultaneous determination of the analytes.

The repeatability of the method was evaluated using 10 analyses at concentrations of 0.500 and 15.0 µmol L^{-1} of HCT and TRT. The relative standard deviations obtained were 4.25% (HCT) and 5.02% (TRT) at 0.500 µmol L^{-1}, and 5.98% (HCT) and 3.92% (TRT) at 15.0 µmol L^{-1}, indicating that the proposed electrode was not poisoned during consecutive analyses, since the solution was stirred between the measurements.

3.5. Interference study

In order to evaluate possible interferences in the i_{ap} of the analytes, evaluation was made of the effects of the possible interferents glucose (GLI), urea (UR), furosemide (FUR), ascorbic acid (AA), uric acid (UA), creatinine (CR), and bumetanide (BMT), using concentrations ranging from 0.1-fold to 10-fold the concentrations of HCT and TRT (in this case between 0.3 and 30 µmol L^{-1}). Table 2 shows the concentration range studied for each possible interferent and the percentage effects on the i_{ap} of HCT and TRT. In the potential range studied, no significant effects on i_{ap} were observed for GLI, UR, AA, UA, and CR, at any of the concentrations tested, indicating that these substances did not interfere in the responses of the analytes. FUR and BMT showed oxidation peaks in the potential window employed, at 0.965 and 1.13 V for FUR and at 0.876 and 0.948 V for BMT. In the case of FUR, at concentrations in the range from 0.30 to 6.0 µmol L^{-1}, there were no significant alterations in the i_{ap} values for HCT and TRT. At higher concentrations, the oxidation peak of FUR began to interfere in the oxidation of the analytes. BMT showed no interference in the i_{ap} of HCT, despite the existence of an oxidation peak in the range employed. However, BMT showed interference in the analysis of TRT when the interferent was present at a concentration 5-fold higher than the TRT concentration (when the BMT concentration exceeded 15 µmol L^{-1}).

Table 2
Results of interference assays employing glucose, urea, furosemide, ascorbic acid, uric acid, creatinine, and bumetanide.

Interfering	Analyte	Concentration	% signal
GLI	HCT	0.30–30	92.1–109
	TRT	0.30–30	90.8–99.1
UR	HCT	0.30–30	98.8–108
	TRT	0.30–30	90.4–102
FUR	HCT	0.30–6.0	91.6–106
	TRT	0.30–6.0	90.4–106
AA	HCT	0.30–30	92.4–109
	TRT	0.30–30	96.4–109
UA	HCT	0.30–30	90.7–103
	TRT	0.30–30	93.9–109
CR	HCT	0.30–30	90.5–100
	TRT	0.30–30	90.7–111
BMT	HCT	0.30–30	94.2–108
	TRT	0.30–15	92.8–111

3.6. Application of the GCE/MWCNT sensor

The GCE/MWCNT sensor was applied in the analysis of three types of sample: artificial urine, since HCT and TRT are regulated and monitored by the World Anti-Doping Agency (WADA) [39], and in tap water and water treatment plant water, due to concerns about the release of these drugs and their presence in wastewater [40]. In all cases, the concentrations obtained were close to the fortified concentrations (Table 3). Application of the student's *t*-test [41] showed that there were no significant differences (at a 95% confidence level) between the fortified and recovered concentrations. The results demonstrated the satisfactory applicability of the method for the simultaneous determination of HCT and TRT.

3.7. Comparison with the HPLC-DAD method and application using a hemodialysis sample

After optimization and application of the GCE/MWCNT sensor, an HPLC-DAD method was developed for the determination of HCT and TRT (based on modified literature method [42]), for comparison with the proposed electroanalytical method.

The best chromatographic conditions for separation of the diuretics were found using a mobile phase consisting of methanol and

Table 1
Comparison between the figures of merit of the proposed GCE/MWCNT method and other techniques reported in the literature for the determination of HCT and TRT.

Analyte	Electrode	Technique	Concentration range (mol L^{-1})	LD (mol L^{-1})	Reference
TRT	[a]GECE/MPs-To	SWV	5.00 × 10^{-7} to 9.98 × 10^{-5}	1.4 × 10^{-7}	[15]
	[b]MIPs/MWCNTs/PGE	DPV	8.00 × 10^{-8} to 2.65 × 10^{-4}	3.3 × 10^{-9}	[38]
	GCE/MWCNT	LSAdSV	1 × 10^{-7} a 2 × 10^{-5}	2.8 × 10^{-8}	This work
HCT	[c]BDDE	DPV	5.10 × 10^{-7} to 1.87 × 10^{-5}	3.7 × 10^{-7}	[17]
	[d]NMN	LSV	1.39 × 10^{-5} to 1.67 × 10^{-4}	7.9 × 10^{-6}	[37]
	BDDE	SWV	4.0 × 10^{-6} to 8.3 × 10^{-5}	1.0 × 10^{-6}	[34]
	GCE/MWCNT	LSAdSV	1 × 10^{-7} to 2 × 10^{-5}	2.9 × 10^{-8}	This work

[a] GECE/MPs-To: Graphite epoxy composite electrode modified by tosyl-functionalized magnetic particles.
[b] MIPs/MWCNTs/PGE: Molecularly imprinted polymer/multi-walled carbon nanotubes immobilized pencil graphite electrode.
[c] BDDE: Boron-doped diamond electrode.
[d] NMN: nickel hydroxide modified nickel electrode.

Table 3
Results of application of the GCE/MWCNT technique for the analysis of tap water, water treatment plant water, and artificial urine.

Tap water sample		
Analyte	HCT	TRT
Added (μmol L^{-1})	3.00	3.00
Found (μmol L^{-1})	3.08 ± 0.0805	3.12 ± 0.167
t calc.	0.827	1.21
Water treatment plant sample		
Analyte	HCT	TRT
Added (μmol L^{-1})	15.0	15.0
Found μmol L^{-1})	15.7 ± 1.32	13.4 ± 1.21
t calc.	0.997	2.22
Artificial urine sample		
Analyte	HCT	TRT
Added (μmol L^{-1})	8.40	9.87
Found μmol L^{-1})	7.84 ± 0.254	9.82 ± 0.690
t calc.	3.78	0.124

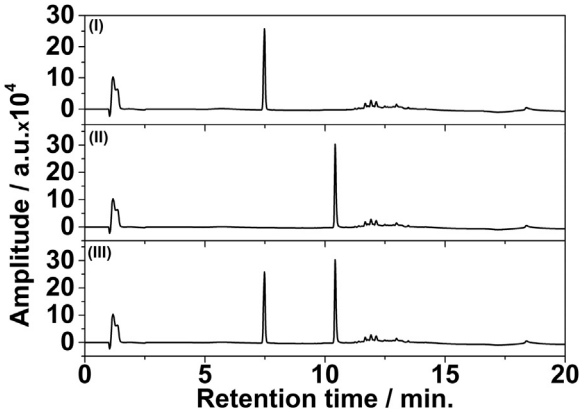

Fig. 4. Chromatograms for HCT (I), TRT (II), and HCT + TRT (III). Conditions: [HCT] = 5.04×10^{-5} mol L^{-1}; [TRT] = 5.92×10^{-5} mol L^{-1}.

0.01 mol L^{-1} acetate buffer (pH 5.6), in gradient mode at a flow rate of 1.2 mL min^{-1}, with the column heated at 35 °C. Both diuretics were detected at a wavelength of 227 nm. As shown in Fig. 4.I and .II, injections of the individual compounds under optimized conditions resulted in retention times (t_{ret}) of 7.46 min (HCT) and 10.4 min (TRT). The same retention times were obtained for simultaneous injection of the two diuretics (Fig. 4.III).

The analytical curve for HCT presented linearity between 8.40×10^{-6} and 1.68×10^{-4} mol L^{-1}, described by the equation: A / a.u. = 2.43×10^{10}[HCT] / mol L^{-1} + 28901.2 (R^2 = 0.999). For TRT, linearity was obtained between 5.92×10^{-6} and 3.95×10^{-4} mol L^{-1}, described by the equation: A / a.u. = 2.30×10^{10}[TRT] / mol L^{-1} + 16083.2 (R^2 = 0.999). The calculated LD values were 2.3×10^{-6} and 1.8×10^{-6} mol L^{-1} for HCT and TRT, respectively. The repeatability of the method was assessed using the relative standard deviations (RSDs) of inter-day and intra-day measurements. For HCT at concentrations of 8.40×10^{-6}, 16.8×10^{-6}, and 33.6×10^{-6} mol L^{-1}, the RSD values were 2.20%, 0.880%, and 0.890% (inter-day) and 4.03%, 2.15%, and 0.470% (intra-day), respectively. For TRT at concentrations of 5.92×10^{-6}, 19.7×10^{-6}, and 39.5×10^{-6} mol L^{-1}, the RSD values were 1.83%, 0.430%, and 0.400% (inter-day) and 5.01%, 1.93%, and 0.580% (intra-day), respectively.

After the development of the chromatographic method, the GCE/MWCNT and HPLC-DAD methods were compared for analysis of a hemodialysis sample. Similar HCT and TRT concentrations were obtained with the two methods (Table 4). Application of the Student's *t*-test (paired values) resulted in values of t of 2.18 (HCT) and 1.23 (TRT), which were lower than t_{crit} (4.30) [41]. This showed that there were no significant differences (at a 95% confidence level) between the concentrations obtained using the proposed voltammetric method and the HPLC-DAD method.

Table 4
Determination of HCT and TRT in a hemodialysis sample using the proposed GCE/MWCNT method and the HPLC-DAD technique.

Analyte	HCT		TRT	
Method	GCE/MWCNT	HPLC-DAD	GCE/MWCNT	HPLC-DAD
Found values (μmol L^{-1})	15.5 ± 0.108	15.2 ± 0.100	14.3 ± 0.0926	14.3 ± 0.129

n = 3.

The results demonstrated that the voltammetric method based on GCE/MWCNT could be used for the simultaneous determination of HCT and TRT in different types of sample, offering rapidity, low cost, low levels of detection, and no requirement for the organic solvents employed in chromatographic methods.

4. Conclusions

The findings of this work showed that a glassy carbon electrode modified with carbon nanotubes could provide excellent performance in the simultaneous determination of diuretics. The modification of the electrode resulted in a decrease in the charge transfer resistance and an increase in the analyte signal intensity, compared to the unmodified electrode. The technique enabled simultaneous determination of HCT and TRT, unlike most electroanalytical methods, where only individual determinations are possible. The method showed a wide linear range, low detection limits, and excellent performance in the simultaneous determination of HCT and TRT in tap water, water treatment plant water, and artificial urine samples. The detection limit of the electrochemical method was lower than that of the comparative HPLC-DAD method, and there was no significant difference (at a 95% level of confidence) between the methods when they were applied for analysis of a hemodialysis sample.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2017.11.071.

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