A new modification method of a Cetyl Trimethyl Ammonium Bromide/Nano-ZnO and Multi-walled Carbon Nanotubes Electrode for Determination of Anti Doping in Urine

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Abstract. The preparation and application of Cetyl Trimethyl Ammonium Bromide/Nano-ZnO and Multi-walled Carbon Nanotubes (CTAB/ ZnO-MWNTs) Modified Electrodes was studied, establishing a new electrochemical method for determination of carteolol hydrochloride in urine. After its pre-enrichment by adsorption and extraction on modified electrodes, electrochemical behaviors of carteolol hydrochloride on the modified electrodes were studied by CV and DPV. The response is linear at the range of $1\times10^{-3} \sim 2\times10^{-1}$ g/L, with a detection limit of 2×10^{-4} g/L. Under appropriate conditions, the content of carteolol hydrochloride in urine can be determined directly by the method, which had strong anti-interference ability and the recovery is 96.5% - 110.5%. In addition, extraction and adsorption behaviors of the modified electrodes for carteolol hydrochloride were studied by chronocoulumetry, and the results showed that extraction during the enrichment process played a major role at low concentrations, and contribution of surface adsorption became greater with the increase of concentrations.

1. Introduction
Carteolol hydrochloride (CH) is a beta blocker which has intrinsic sympathomimetic activity. It is used extensively in clinical practice for treatments with hypertension, arrhythmia, myocardial infarction and cardiac neurosis[1]. CH has been listed as forbidden drugs for athletes by WADA in some sport events. At present, there are several analytical techniques for determination of CH, such as liquid chromatograph-electrospray ionization mass spectrometry analysis[2,3], HPLC[4], NMR spectroscopy[5] and fluorescence method[6]. However, these methods are usually complex, time-consuming, solvent intensive, or require expensive devices. Because of these reasons, development of a simple, highly sensitive, selective, easily miniaturized, low cost, and rapid analytical method for the determination of CH is of great significance. To our best knowledge, electrochemical techniques can offer all the advantages.

Carbon nanotubes has received considerable attention by its particular mechanical, electrical, chemical properties and special tubal molecular structures in recent years[7,8]. Nano-ZnO has been used extensively in electroanalytical chemistry research because of its special properties, such as electrochemical activity, catalytic activity and biocompatibility. Chemical sensors and biosensors based on this material with high sensitivity/selectivity have obtained extensive applications[9,10], and

Cationic surfactant Cetyl Trimethyl Ammonium Bromide (CTAB) has a function of hydrophobic adsorption[11]. In this study, a kind of CTAB/ZnO-MWNTs modified electrodes was prepared. The ability of the modified electrodes for direct detection of pH in urine samples was evaluated. The studies showed that the modified electrodes had better catalytic oxidation performance for CH comparing with bare glassy carbon electrode.

2. Materials and methods

2.1 Reagents

The CATB was purchased from Shanghai Aibi Chemical Co, Ltd. The MWNTs (diameter 8-15 nm, length 50 μm, purity > 95%) was purchased from Chengdu Organic Chemicals Co, CAS. Potassium ferricyanide was purchased from Beijing Chemical Plant. Carteolol Hydrochloride(CH), Terbutaline Sulfate(TES), Clenbuterol(CLE) and salbutamol(SAL) were purchased from Beijing Shanglifang Association Chemical Industry.Co, CAS. Phosphate buffer solution (PBS) was home-made. All the chemicals in this study were of analytical grade. All the solutions were prepared with twice distilled deionized water.

2.2 Apparatuses

Electrochemical measurements were carried out on a CHI 600C Electrochemical Analyzer (CHI Co., Shanghai, China). A standard three-electrode cell was employed. The working electrodes were either bare glassy carbon electrode (GCE) or modified electrode. A platinum wire and Ag/AgCl were used as counter and reference electrodes, respectively. All of the electrodes were from CHI Co. And all the potentials referred to the Ag/AgCl reference electrode. The pH values were measured with a PHB-1 precision acidity meter(Instrument Co., Ltd Shanghai Yu Long). All experiments were carried out at room temperature.

2.3 Preparation of modified electrodes

A 3-mm (in diameter) GCE was sanded with metallographic abrasive papers, then sequentially polished by 0.1, 0.3 and 0.05-μm alumina pastes on suede pad, and finally fully cleaned in an ultrasonic cleaner with double distilled water, diluted nitric acid (volume ratio 1:1), alcohol, and double distilled water, respectively. The freshly cleaned GCE was electrochemically activated in 0.5 M H_2SO_4 at a scanning rate of 50 mV s^{-1} and in the potential range from -0.5 to +1.4 V for 20 cycles[12].

37 mg of $Zn(CH_3COO)_2$ $2H_2O$ and 100 mg of polyethylene glycol were added into 15 mL twice distilled deionized water. 8 mL of 0.04 mmol L^{-1} $Li(OH)_2$ H_2O solution was slowly added in the above mixture at 50 ℃, then treated by ultrasound at a constant temperature for 10 minutes. After centrifugal separation, the precipitation was washed for several times with twice distilled deionized water and dried at 70 ℃ under vacuum. The final product nano-ZnO was placed in porcelain crucible for 2 hours at 300 ℃. The product was cooled down at room temperature before use.

MWNTs were ultrasonicated in mixed acids (98% H_2SO_4/HNO_3 3:1v/v) for 12 h, and then washed with distilled water and 0.01 M NaOH solution until pH reached 7.0[13]. The washed MWNTs were centrifuged and dried under vacuum at room temperature.1 mg of nano-ZnO and 2 mg of prepared MWNTs were added in 10 mL DMF and prepared by ultrasonic dispersion for 30 minutes. The stable dispersion liquid was obtained.
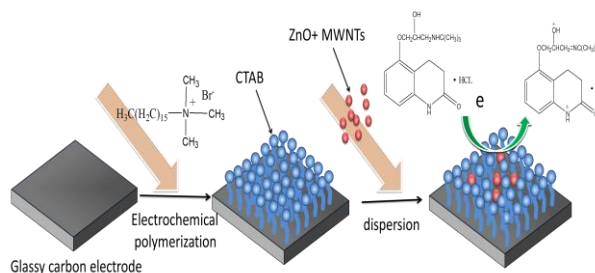
Figure 1. Preparation of CTAB/ ZnO-MWNTs modified electrodes.

As was shown in the Fig.1, GCE was chosen as the basal electrode, and the pretreated electrode was put in PBS (pH = 5.6) containing 5 mmol/L CTAB. The electrodes were treated by cyclic voltammetry in the potential range from -1.6 ∽ +1.8 V for 15 cycles until stable voltammetric responses were obtained. After the electropolymerization, the modified electrodes were rinsed fully with distilled water and dried at room temperature. Then nano-ZnO and MWNTs dispersion liquid was dropped on CTAB/GCE by micropipette. After dried under infrared lamp, the CTAB/ZnO-MWNTs/GCE was obtained. The electrodes have good electrical catalytic ability for CH.

2.4 Experimental methods
The electrodes were placed in buffer solution before testing, in the potential range from -0.5 ∽ 1.2V for several cycles until stable voltammetric responses were obtained. Then the electrodes were dipped into sample solution to enrich for a while, using differential pulse and cyclic voltammetry to study the electrochemical behaviors of CH on the electrodes. Potential sweep range was 1.0 ∽ 1.5 V, with scanning speed 100 mV/s, pulse height 50 mV, and pulse width 50ms. The potential step range of chronocoulometry was -1.0 ∽ 1.0V, enriched for 20 min with pulse width 20 s.

3. Results and discussions

3.1 The polymerization and characterization of modified membrane
The cyclic voltammetry curve of GCE electrochemical polymerization in CTAB is shown in Fig.2, indicating that oxidation peak b current decreased and disappeared sharply around potential 1.5 V, due to the oxidation of Br- to Br_2. Based on the reducing reactivity of the active groups in the reduction processes, peak c indicates the reduction peak of Br_2, and the reduction product was Br-. Because the concentration of Br_2 was low in solution, the reduction peak of Br_2 was low. The concentration of cetyl trimethyl ammonium bromide kept almost unchanged, so peak a and peak d were slightly lower. With the number of scanning cycle increased, peak current decreased constantly, indicating polymer film formed gradually on the GCE surface.
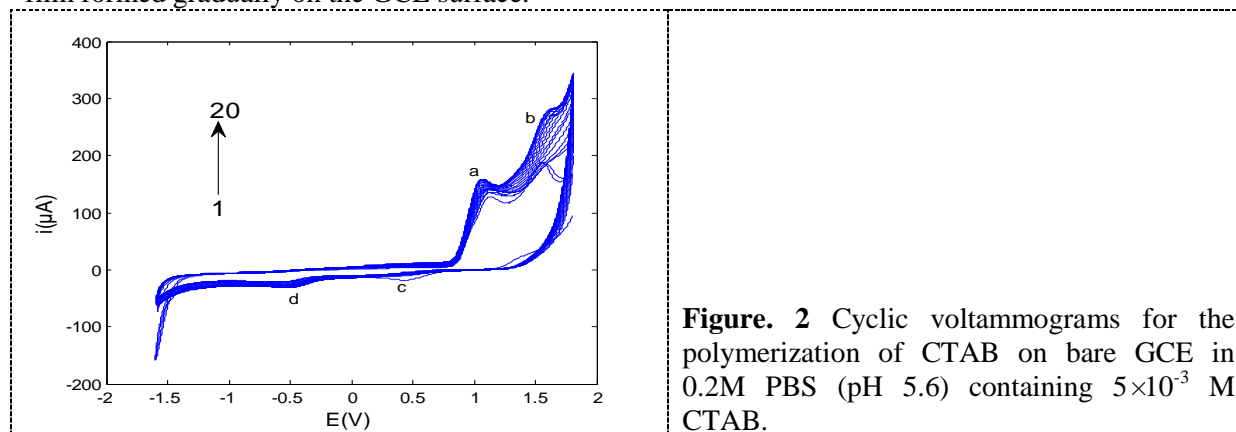

Figure. 2 Cyclic voltammograms for the polymerization of CTAB on bare GCE in 0.2M PBS (pH 5.6) containing 5×10^{-3} M CTAB.

In the potential range from -0.2 ～ +0.7 V at a scanning rate of 100 mV s^{-1} , GCE, CTAB/GCE, and CTAB/ ZnO-MWNTs/GCE were scanned with cyclic voltammetry in $K_3Fe(CN)_6$ solution, respectively. In Fig. 3, the peak current of curve c is lower than that of curve b. It was caused by CTAB polymer film. The peak current of curve a obviously was the highest, indicating CTAB/ ZnO-MWNTs/GCE electrodes can transfer electrons effectively.
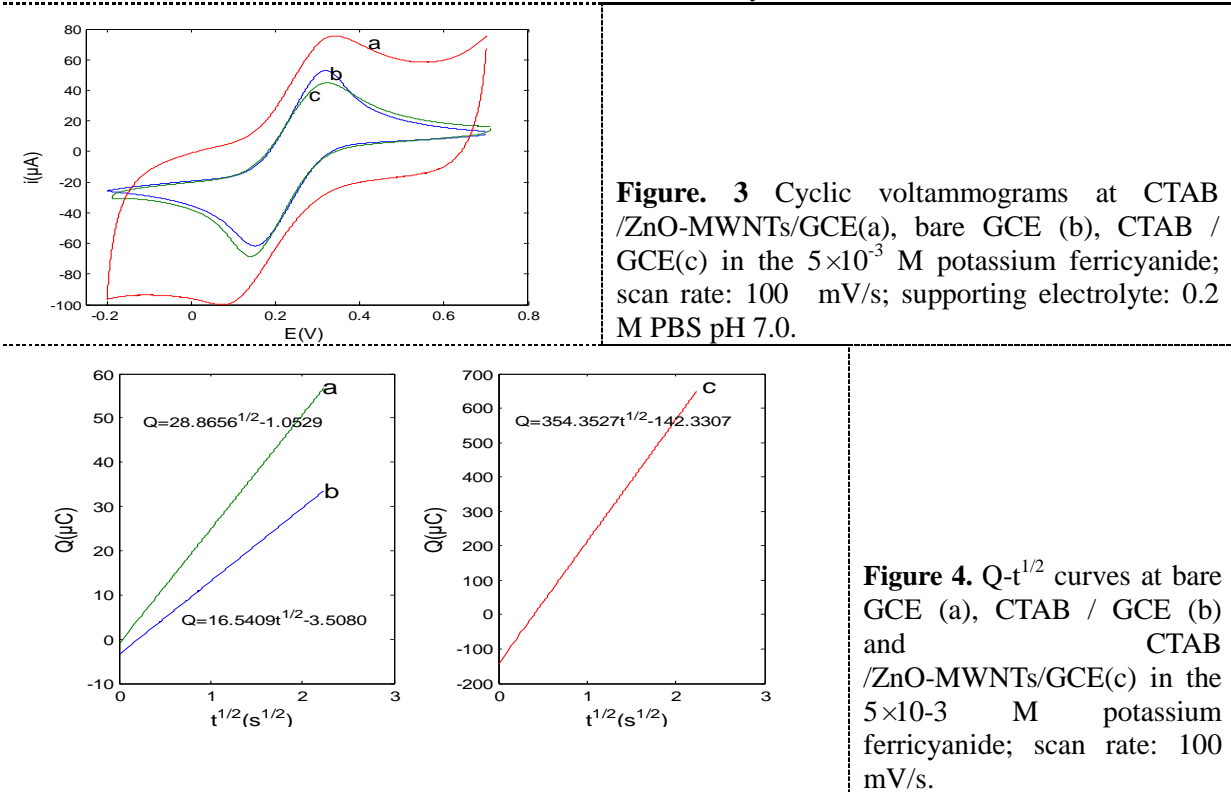

Figure. 3 Cyclic voltammograms at CTAB /ZnO-MWNTs/GCE(a), bare GCE (b), CTAB / GCE(c) in the 5×10^{-3} M potassium ferricyanide; scan rate: 100 mV/s; supporting electrolyte: 0.2 M PBS pH 7.0.

Figure 4. Q-t$^{1/2}$ curves at bare GCE (a), CTAB / GCE (b) and CTAB /ZnO-MWNTs/GCE(c) in the 5×10^{-3} M potassium ferricyanide; scan rate: 100 mV/s.
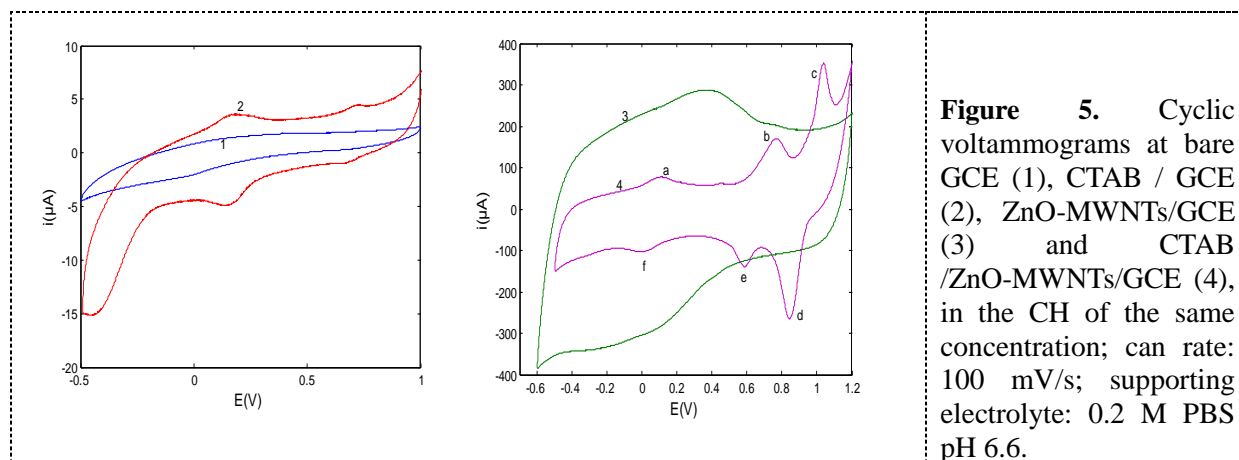
3.2 The comparison of different electrodes

Curves of GCE (a) , CTAB/GCE (b), or CTAB/ZnO-MWNTs/GCE (c) drawn in 5.0×10^{-3} mol/L of K3[Fe(CN)6] solution (Fig. 4), according to Anson formula[14]:

$$Q=2nFCA\pi^{-1/2}D^{1/2}t^{1/2}+Q_{ads}+Q_{dl} \qquad （1）$$

A is the effective surface area of working electrodes, C is concentration of electrolyte, n is electronic transmission number, D is diffusion coefficient of electrolyte. N and D of K3[Fe(CN)6] is 1 and 7.6×10^{-6} cm^2 s^{-1} [15], respectively. Based on the slope of the linear relationship of Q-t$^{1/2}$, the effective surface areas of GCE, CTAB/GCE, and CTAB/ZnO-MWNTs/GCE could be calculated, which were 0.0192 、0.0111 and 2.361 cm^2, respectively. These results demonstrated that ZnO-MWNTs had enlarged the surface areas of GCE.

Fig. 5 shows the CV curves when GCE 、CTAB/GCE 、ZnO-MWNTs/GCE and CTAB/ZnO-MWNTs/GCE measured the CH standard solution of the same concentration. The peak current of CTAB/GCE (curve 2) was higher than that of GCE (curve 1), indicating that CTAB had better electrochemical response to CH. Although the peak current of ZnO-MWNTs/GCE (3) increased, the electrochemical response to CTAB/ZnO-MWNTs/GCE (curve 4) was worse, indicating that CTAB and ZnO-MWNTs had sensitizing effect on CH. Fig. 5 also shows that there were three steps that in the oxidation and reduction reactions of CH on the CTAB/ZnO-MWNTs/GCE, which were quasi-reversible processes.
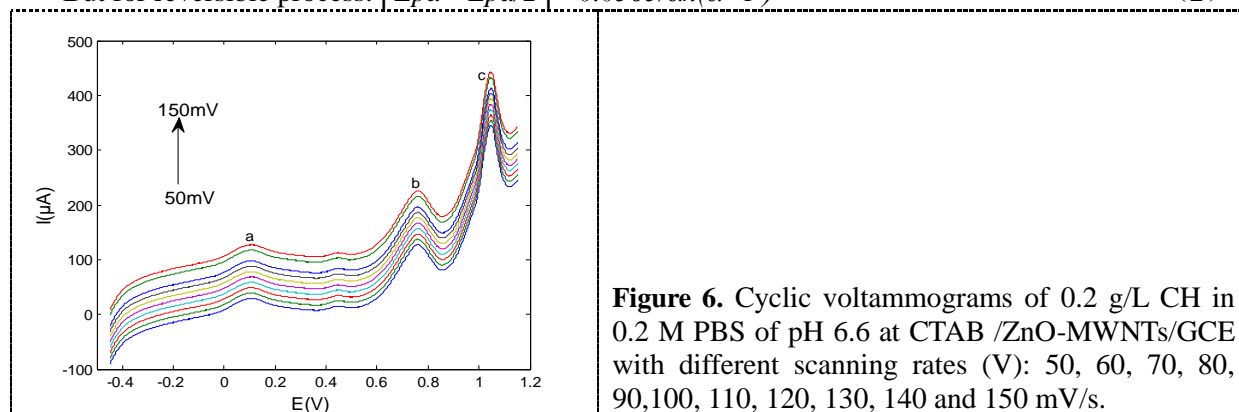
Figure 5. Cyclic voltammograms at bare GCE (1), CTAB / GCE (2), ZnO-MWNTs/GCE (3) and CTAB /ZnO-MWNTs/GCE (4), in the CH of the same concentration; can rate: 100 mV/s; supporting electrolyte: 0.2 M PBS pH 6.6.

3.3 Electrochemical behaviors of CH on CTAB /ZnO-MWNTs/GCE

At a scanning rate of 50 $\sim$ 150 mV/s, the electrochemical mechanism of 0.2 g/L CH was studied on modified electrode surface using cyclic voltammetry (Fig. 6). The oxidation and reduction reactions of CH were under similar conditions. So the reactions of CH on modified electrodes were quasi-reversible processes. Tab. 1 shows the relationship and electrode processes between current peaks Ip of a, b, c and scanning speed.

The electron transfer number of CH electrochemical reaction on CTAB/ZnO-MWNTs/GCE can be measured by cyclic voltammetry.

For peak a, $E_{pa}(a)=0.1107V$, $E_{pa/2}(a)=0.053V$, for b, $E_{pa}(b)=0.766V$, $E_{pa/2}(b)=0.815V$, for c, $E_{pa}(c)=1.051V$, $E_{pa/2}(c)=1.081V$.

But for reversible process: $\left| E_{pa} - E_{pa/2} \right| = 0.0565/\alpha n (\alpha=1)$ （2）

Figure 6. Cyclic voltammograms of 0.2 g/L CH in 0.2 M PBS of pH 6.6 at CTAB /ZnO-MWNTs/GCE with different scanning rates (V): 50, 60, 70, 80, 90,100, 110, 120, 130, 140 and 150 mV/s.

E_{pa} is peak potential，$E_{pa/2}$ is half peak potential，α is electron transfer coefficient, and n is number of electron transfer. The number of electron transfer of peak a, b and c were 1, 1 and 2, respectively.

The pH of buffer solution has important effect on electrochemical response of CH. Therefore, voltammetry behaviors of CH in PBS buffer solutions with different pH values. The peak profile is the best at pH 6.6. With pH changed. The peak potentials of a, b and c changed accordingly, with the changes of –0.0524 V pH-1, –0.0524 V pH-1, and -0.0563 V pH-1, respectively. The changes were close to the theoretical value –0.059 V pH-1, indicating that the number of electron transfer and proton transfer was 1:1.

3.4 The adsorption and extraction behaviors of CH on CTAB/ZnO-MWNTs/GCE

The electric quantity-time curve of CTAB/ZnO-MWNTs/GCE was determined by chronocoulometry after pre-enrichment. Using Anson formula, total electric quantity Q could be calculated, which was

generated after potential step.   When the time of enrichment is certain, Anson formula[16] can be simplified as :

$$Q = Q_{ext} + Q_{ads} + Q_{dl}$$

（3）

Q_{ext} is electric quantity of extraction, and its value is proportional to extraction quantities. Q_{ads} is electric quantity generated by surface adsorption. Q_{dl} is the electric quantity of electric double-layer charges. After 20 min of enrichment, electric quantities were determined in several different concentrations of CH standard solutions. The Q_{ext} and the $Q_{ads} + Q_{dl}$ of CTAB/ZnO-MWNTs/GCE were calculated according to Q-t1/2 curves of different CH concentrations. Tab. 1 shows that at low concentrations, the enrichment of electrode to CH mainly came from extraction reaction. With the increase of CH concentrations, the relative contribution of surface absorption to all enrichment process gradually increased.

Table 1. Extraction and adsorption capacity of the different concentrations of CH

Concentration of CH（g/L）	Q_{ext} (C)	$Q_{ads} + Q_{dl}$ (C)	$Q_{ext} / (Q_{ads} + Q_{dl})$ (C)
0.2	11419.71	242.39	47.11
0.1	11676.35	240.43	48.56
0.05	12965.11	114.36	113.38
0.02	13644.49	81.97	166.46
0.01	13797.71	77.26	178.60
0.005	19066.93	47.98	397.42
0.001	20668.95	14.52	1423.07

3.5 Selectivity experiment
0.2 g/L CH, 0.1 g/L TES, 0.1 g/L CLE, 0.1 g/L SAL and 0.1 g/L mixed solution (CH+TES+CLE+SAL) were prepared using PBS buffer solution at pH 6.6. After pre-enrichment, the solutions were scanned with cyclic voltammetry. In Fig. 7, for peak c, CTAB/ZnO-MWNTs/GCE almost had no response to SAL (curve 5), and very small to CLE (curve 3). The current peaks of the mixed solution (curve 1) and CH (curve 2) differed little, indicating that the electrodes had good selectivity to CH.

Figure 7. The selective electrode of CTAB/ZnO-MWNTs/GCE for CH+TES+CLE+SAL(1), CH(2), CLE(3), TES(4), SAL(5).
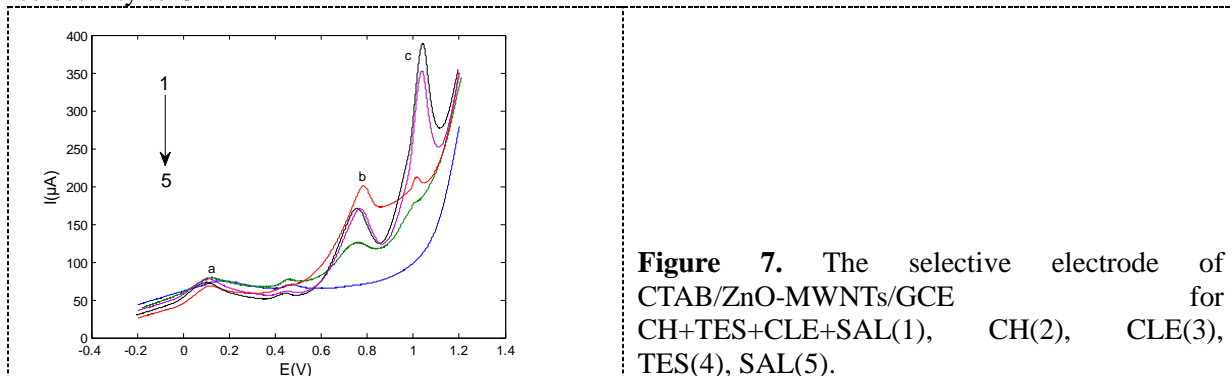
3.6 Working curve and stability of electrode
Under the best experimental conditions, CH standard solutions of different concentrations were tested using CV and DPV. Fig.8 and Fig. 9 show that peak c presented good linear relationship. Tab. 2 shows its linear range and linear regression equation. When the same electrode was used to determine the same 0.2 g/L CH standard solution ten times within a day, the relative standard deviation (RSD) of the current peak was 2.9%, indicating that the determination of electrode had good reproducibility. Stability tests were also carried out at room temperature. The current response decreased slightly after a period of 17 days of exposing the CTAB/ ZnO-MWNTs/GCE in air, which indicated that the CTAB/ ZnO-MWNTs/GCE had a good long-time stability.
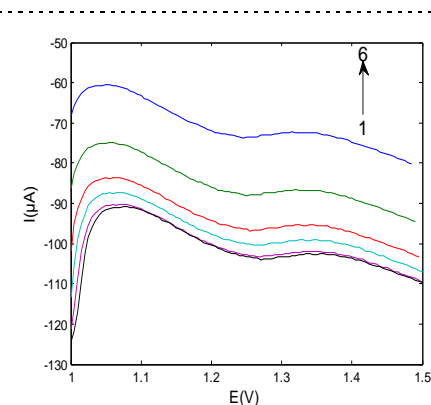
Figure 8. Differential pulse voltammetry in 0.2 M PBS of pH 6.6 on CTAB /ZnO-MWNTs/GCE with different concentrations of CH at 0.002, 0.01, 0.02, 0.05, 0.1, 0.2 g/L (1-6).
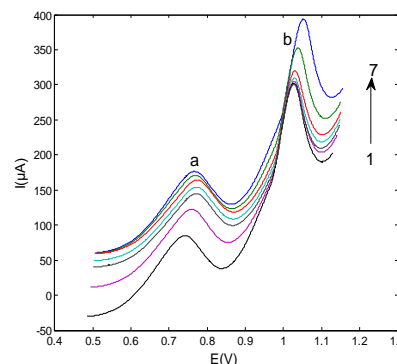

Figure 9. Cyclic voltammograms in 0.2 M PBS of pH 6.6 on CTAB /ZnO-MWNTs/GCE with different concentrations of CH at 0.002, 0.01, 0.02, 0.05, 0.1, 0.2 g/L (1-7).

Table 2. Linear ranges, equations and correlation coefficients for determination of CH

Method	Linear range(g/L)	Regression equation	Correlation coefficient
CV	$1\times10^{-3}\sim2\times10^{-1}$	$I_p=477.52C_{CH}(g/L)+299.93$	0.9937
DPV	$2\times10^{-3}\sim2\times10^{-1}$	$I_p=-154.18C_{CH}(g/L)-30.064$	0.9976

3.7 Sample analysis and recovery rate of standard addition experiment
The empty urine was picked form the volunteer who did not took carteolol hydrochloride .5 ml of urine was placed at room temperature for 5 min. 2 mL methanol was added into the urine and centrifuged for 10 min. The supernatant was added into PBS solution, and the pH was adjusted to 6.6. Then recovery rate of standard addition experiment was determined by CV methods to test the sample in parallel for five times. The results in Tab.3 show that recovery rate of standard addition for CTAB/ZnO-MWNTs/GCE was 98.4% ~ 108.1%, and the relative standard deviation was 1.97% ~ 4.29%, indicating that the electrodes have potential application value.

Table 3. Detection results of recovery rate in sample

Sample	Added (g/L)	Found(g/L)	Recovery(%)	RSD (%，n=5)
1	2×10^{-3}	0.00216	108.1	2.38
2	5×10^{-3}	0.00522	104.5	1.97
3	1×10^{-2}	0.00984	98.4	4.29

4. Conclusion
Using CTAB/ZnO-MWNTs to modify electrode, CTAB/ZnO-MWNTs/GCE were obtained. It can be used to determine carteolol hydrochloride in urine. The results of determination would not be influenced under the conditions when other doping reagents such as clenbuterol, terbutaline sulphate and salbutamol are present. The results demonstrated that the modified electrodes haav high sensitivity, good selectivity, rapid response, good stability as well as reproducibility. Furthermore, the proposed method provides advantages such as time-saving and simplicity of the reagents and apparetus. The modified electrode has hopeful application prospect.

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