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Electrochemical Behavior and Direct Quantitative Determination of Tanshinone ⅡA in Micro-emulsion

Jiao-jiao Wu[1,2], *Wen-ting Wang*[1,2], *Miao Wang*[1,2], *Hong Liu*[1,2,*] & *Hong-chun Pan*[1,2,*]

[1] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China.
[2] Chongqing Engineering Research Center for Pharmaceutical Process and Quality Control, Chongqing 400715, China.
[*]E-mail: lhphch@126.com, panhongchun216@126.com

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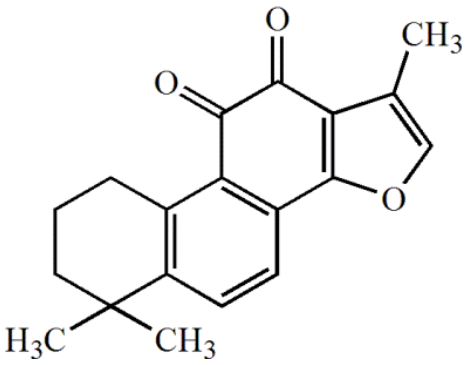
Tanshinone ⅡA (TAN), an insoluble drug prepared to form micro-emulsion, was electrochemically investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at glassy carbon electrode. Its electrochemical behavior was proved to be quasi-reversible in acetate buffer (pH4.0), besides, on the basis of CV, the electrochemical redox mechanism of TAN has proved two protons and two electrons involved in the electrode surface reaction. DPV method was developed for its quantitative determination in real sample, and the linear relationship between the peak current and the TAN concentration was obtained in the concentration range of 0.2 to 1.2 μg/mL with a detection limit of 50.25 ng/mL. The reproducibility and repeatability of the method were determined and validated feasibly. The accepted RSD (1.82%) and recovery studies (96.01-101.23%) were obtained, and the result was agreed well with that by HPLC. The present study provides guidance for TAN electrochemical behavior investigation and direct determination of micro-emulsion.

Keywords: Tanshinone ⅡA, Micro-emulsion, Quantitative determination, Voltammetry, Electrochemical behavior.

1. INTRODUCTION

Tanshinone ⅡA (TAN, Phenanthro [1,2-b]furan-10,11-dione,6,7,8,9-tetrahydro-1,6,6-trimethyl, chemical structure as given in scheme. 1), one of the major lipophilic bioactive ingredients isolated from the roots of Labiatae Chinese herb *Salvia miltiorrhiza Bunge* (Danshen), exhibits a variety of biological activities, such as expansion coronary artery, increase myocardial blood flow, anti-platelet, anti-oxidation, anti-bacterial, etc.[1-5]. However, TAN has low solubility in water (2.8 ng/mL) [6], poorly oral bioavailability [7], easily decomposed in light [8-9], sensible to temperature [9-10], etc.,

which all affect its clinical application. Many colloidal carriers including polymeric micelles [11], micro-emulsions [12], emulsions [9], liposomes [13] and nanoparticles [14] have been used to improve the stability and solubility of TAN.



Scheme 1. The chemical structure of TAN.

A lot of new drug candidates display a low solubility in water, hence, solubilization delivery systems have been given more and more attention in the past few decades [15-16]. Micro-emulsions were defined as "a dispersion system of water, oil and surfactant, which spontaneously formed to the isotropic, transparent and thermodynamically stable liquid solution" by Lindman and Danielsson in 1981 [17]. Micro-emulsions, spontaneous formed by the self-microemulsifying of amphiphilic copolymers and oil, have been used to deliver hydrophobic drugs and improve drug solubility. The premise of this study is to dissolve TAN and prepare to micro-emulsion, which was composed of polysorbate 80 and Kolliphor® HS 15 (HS 15) as surfactant and caprylic/capric triglyceride (GTCC) as the oil phase.

Surfactants has been widely used in chemical, especially influence several electrochemical reactions [18-20]. The electrochemical active compounds solubilized in micellar aggregation or surfactant adsorption in the electrode surface can greatly change the properties of electrode surface, even heavily affect the electrochemical reaction process of electroactive materials [21]. Surfactants are usually used to improve the selectivity and sensitivity of electrochemical active materials [22], but there are no available electrochemical data concerning the voltammetric behavior of TAN wrapped micro-emulsion.

In this paper, the primary purpose was to investigate the cyclic voltammetry (CV) behavior of the redox reaction of tanshinone ⅡA in micro-emulsion (TAN-ME) at surface of glassy carbon electrode (GCE). Test conditions, including supporting electrolyte and its concentration, pH, scan rate, etc., were optimized. A quasi-reversible electron transfer reaction of TAN-ME at GCE has been found, including two electrons and two protons transfer, which was different from the reported literature [23] because the degree of electron transfer was different under different conditions. Furthermore, Micro-emulsion structure hampered the electronic transfer of electroactive species, namely TAN in this study. The second purpose was to direct quantitative determine TAN by differential pulse voltammetry (DPV) under optimal conditions. A lot of literature has been reported for the determination of TAN in pharmaceuticals or plasma samples by HPLC [24], LC-MS/MS [25], HPLC-MS [26], UFLC-MS/MS

[27], but a few reports are available for the determination of TAN by electrochemical method [28]. As above said, the surfactant will affect peak measurement, therefore, drug delivery systems of hydrophobic drugs were destroyed and then determined in organic solvent. However, the present work established a direct quantitative determination method for hydrophobic ingredients in micro-emulsion form, based on a fixed component and prescription. Although this method can only be used to determinate this prescription, the present work remains provide a reference for the electrochemical behaviors studies of quinones and the determination of hydrophobic drugs.

2. EXPERIMENTAL

2.1. Reagents and materials

Tanshinone ⅡA was obtained from Nanjing King Bamboo Biotechnology Co. Ltd (Nanjing, China). Kolliphor® HS 15 was provided by BASF (Shanghai, China). Polysorbate 80 was manufactured by Wei Er Chemical Co., Ltd (Nanjing, China). Caprylic/capric triglyceride was purchased from Tieling North Medicinal Oil Co. Ltd (Liaoning, China). Acetonitrile was purchased from Spectrum Chemical Mfg. Corp (the port of Long Beach, USA). Other reagents and materials, such as hydrochloric acid, potassium dihydrogen phosphate, sodium hydroxide, dipotassium phosphate, acetic acid, potassium chloride, were purchased from Chengdu Kelong Chemical Reagent Company (Sichuan, China) and were analytical reagent. Samples were prepared in ultrapure water.

2.2. Instrumentation

All the electrochemical measurements including CV and DPV were performed with a CHI610E Chenhua electrochemical workstation (Shanghai, China). A traditional three-electrode system consisting of a glassy carbon electrode (3mm in diameter) as a working electrode, a saturated calomel electrode as the reference electrode and a platinum electrode as the counter electrode was used. To improve the reproducible and sensitivity of peak current, the surface of the working electrode should be polished with 1μm, 0.3μm, 0.05μm successively alumina power on a nylon polishing cloth, and then washed in an ultrasonic bath of ethanol and water for five minutes, respectively. It is worth noting that the electrode should be washed five time in ethanol by CV method before each measurement due to strong adsorption at GCE surface.

pH measurements were carried with a FE20 FiveEasy™ Mettler Toledo pH meter (Shanghai, China). The lyophilized samples were prepared by a PiloFD8-4.3V lyophilizer, which was manufactured by Gold Sim Instrument Co., Ltd (Beijing, China). Water was prepared and purified with Millipore Milli-Q (Bedford, USA). All electrochemical experiments were performed at 25 ± 0.1°C.

2.3. Procedure

2.3.1. Sample preparation

TAN-ME was prepared by Shah method according to our previous research. Briefly, HS 15, polysorbate 80 and GTCC were mixed to obtain a clear solution after stirring 15 min in a 50 °C thermostatted water bath. Then, TAN was added and completely dissolved in the mixed solution for 1 h. Finally, ultrapure water was introduced for diluting the micro-emulsion to certain concentration.

The inter-day and intra-day assay samples preparation method was described above. The former was placed at room temperature for 0, 2, 4, 6, 8, 10, 12, 24 h and the latter was treated at room temperature for 0, 0.5, 1, 2, 3 days. Thus treated samples were diluted to suitable concentration and analyzed by DPV for the quantitative determination of TAN.

According to the above drug-loaded micro-emulsion prescription, samples before freeze-drying were prepared as diluting with 2 mL ultrapure water added excipients. Next, the real samples were prepared by lyophilization process and went through pre-freezing, sublimation and twice sublimation. The real test solutions were prepared by redissolved about 10 mg of analyte in 100 mL ultrapure and then diluted to a suitable concentration.

The test solutions for electrochemical experiments were consisted of 80 vol.% supporting electrolyte and 20 vol.% micro-emulsion and they was stored in the dark and cool environment. The supporting electrolyte contained 0.1 mol/L NaOH, 0.1mol/L KCl, 0.1 mol/L HCl, 0.2 mol/L PBS (prepared according to the Chinese pharmacopoeia [29]), 0.1-5 mol/L acetate buffer (prepared according to the reported method [30]), and the final sample concentration was in the concentration range of 0.2-1.2 μg/mL. Besides, test solutions were deoxygenated before measurements by purging with nitrogen for 5 min.

2.3.2. Voltammetry parameters

The parameters for CV were initial potential E and high potential E: 0.4 V; low initial potential E and final potential E: -0.6 V; sample interval: 0.001 V; scan rate: 0.05 V/s; quite time: 2 s; sensitivity: 1.0×10^{-5} A/V.

The parameters for DPV were initial potential E: 0.2 V; final potential E: -0.4 V; increment potential E: 0.004 V; pulse width: 0.05 s; sample width: 0.0167 s; amplitude: 0.05 V; pulse period: 0.5 s; quite time: 2 s; sensitivity: 1.0×10^{-5} A/V.

2.3.3. Area of electrode

The GCE area was performed by CV using 1 mM $K_3Fe(CN)_6$ as a probe at different scan rates. The following Randles-Sevcik formula can be used for a reversible process [31]:

$$I_p = 0.4463(F^3/RT)^{1/2}An^{3/2}D_R^{1/2}C_0v^{1/2}$$

where *F* is Faraday's constant (96485 C/mol), *R* is the gas constant (8.314 J/mol K), *A* refers to the electrode surface area (cm^2), i_p is the peak current (Ampere), for 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl

electrolyte, $D_R = 7.6 \times 10^{-6}$ cm^2 s^{-1}, $n = 1$, T is the absolute temperature (298 K), v is scan rate (V/s) and C_0 is the concentration of $K_3Fe(CN)_6$ in mol/L. The surface area was calculated from the slopes of the *ip* versus $v^{1/2}$ plots and found as 0.067 cm^2 for the GCE.

3. RESULTS AND DISCUSSION

3.1. Effect of supporting electrolyte and its concentration

Sample peak are strongly dependent upon the electrolyte. Thus, the effect of the supporting electrolyte on the TAN voltammetric signal was performed. Cyclic voltammograms were recorded at GCE using 0.1mol/L NaOH, 0.1mol/L KCl, 0.1 mol/L HCl, pH7.0 PBS or 0.1 mol/L acetate buffer, respectively, as supporting electrolyte. Other electrolyte did not present apparent voltammetric peak, whereas the best voltammetric peak was obtained using acetate buffer as supporting electrolyte. The test solutions contained 20 vol.% TAN-ME and 80 vol.% supporting electrolyte, and such composition of the solutions is very necessary because acetic acid has low dielectric constant (ε=6.15 at 25 °C [32]), resulting in a slight ionization of electrolytes, and thus lead to the ohmic potential drop significantly.

Preliminary tests showed that the supporting electrolyte concentration didn't change the peak shape of CV curves for TAN, but the higher concentration of acetate buffer was in favor of electron transfer. Thus, we choose the acetate buffer concentration of 5.0 mol/L for the following test.

3.2. The cyclic voltammetric studies of TAN-ME

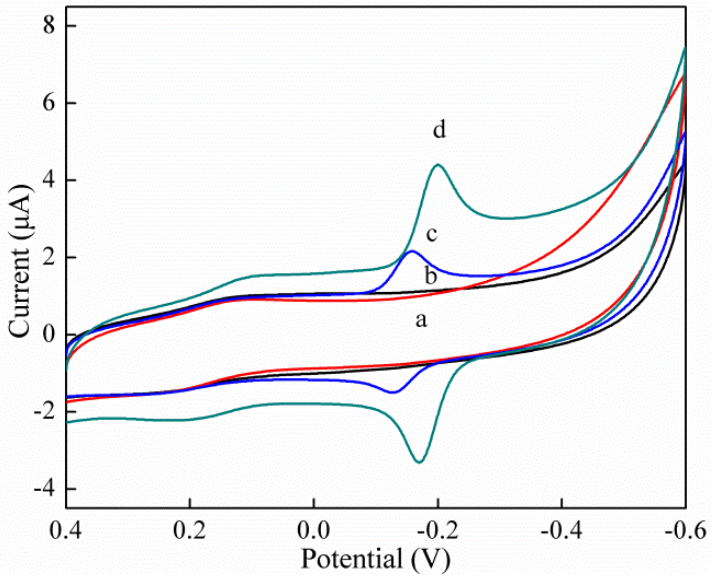


Figure 1. Comparison of cyclic voltammograms of (a) pH 4.0 acetate buffer, (b) blank micro-emulsion, (c) 20 μg/mL TAN-ME, (d) 20 μg/mL TAN dissolved in acetonitrile at GCE; scan rate 0.05 V/s; pH 4.0 acetate buffer as supporting electrolyte.

The cyclic voltammograms recorded with the electrochemical behavior at GCE in 5.0 mol/L acetate buffer (pH 4.0) in the absence (a, b) and in the presence (c, d) of TAN are shown in Fig. 1. It's observed that the electrode reaction cannot take place in the potential range of 0.4 V to -0.6 V in the absence of TAN. Adding TAN to the solution, the obvious redox reaction emerged in the potential range between -0.1 V and -0.2 V.

The peak current of TAN directly dissolved in acetonitrile (d) was 2-3 times than wrapped in micro-emulsion (c) for the same concentration of analyte. Besides, the cathodic and anodic peak current ratio (I_{pc}/I_{pa}) was 1.13 for the former, closing to the theoretical value 1 of reversible reaction, but the latter was 1.67. When the TAN was wrapped by surfactant and oil phase forming micro-emulsion (c), the cathodic peak potential (E_{pc}), and anodic peak potential (E_{pa}), shifted toward positive values, but the separation between anodic and cathodic peak potentials (ΔE_p) was basically unchanged at 30 mV at 50 mV/s scan rate compared with TAN directly dissolved in acetonitrile (d). The result is attributed to the formation of micro-emulsion (consisted of nonionic surfactants) hampered electron transfer of the electroactive species, namely TAN in this reaction, at the electrode surface [20].

3.3. Effect of pH

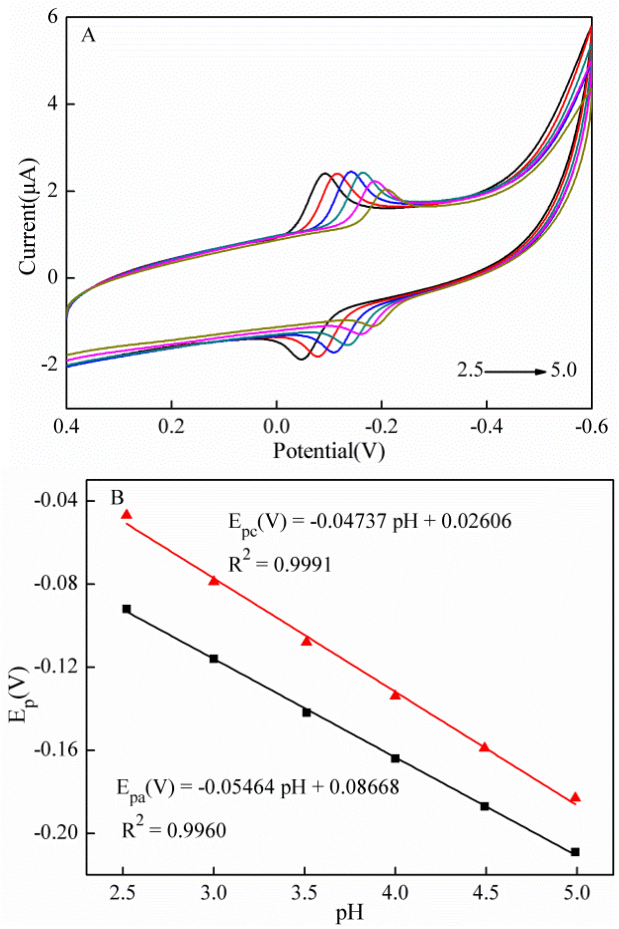


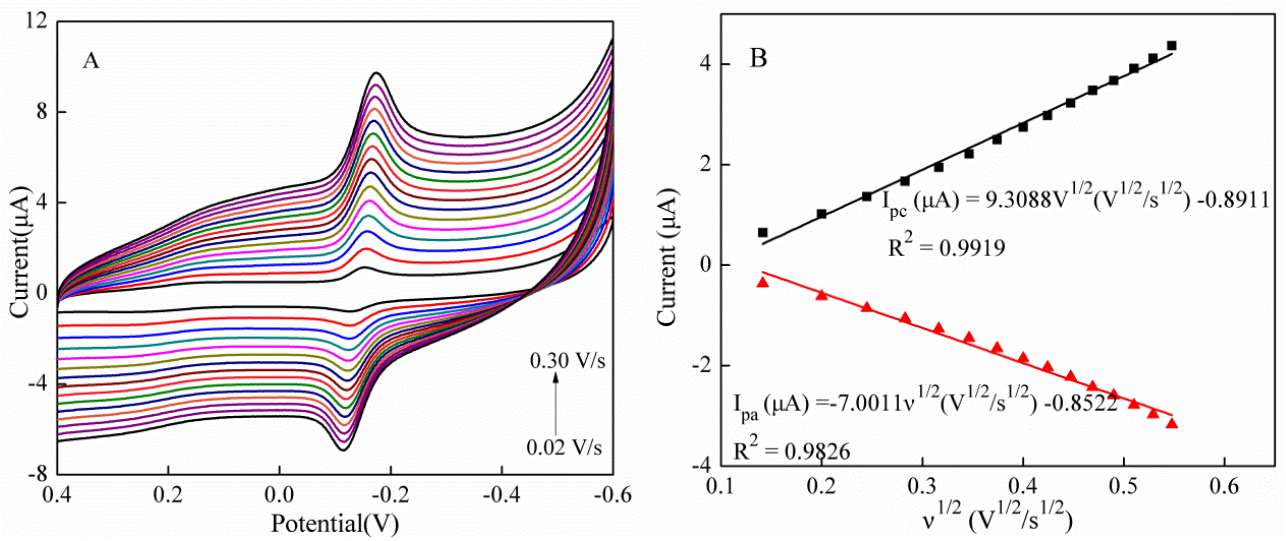
Figure 2A. Influence of pH on the redox peaks, the pH from left to right: 2.5, 3.0, 3.5, 4.0, 4.5, 5.0; **Fig. 2B.** Dependence of pH on the potential E_{pa} (V) and E_{pc} (V) of TAN-ME. Scan rate: 50 mV/s. The concentration of TAN is 20 μg/mL

The cyclic voltammograms of TAN exhibited the well-defined redox peak which shifted to positive potentials as the pH values of supporting electrolyte decreasing as can be found from Fig. 2A. The redox peak of TAN was studied over the pH range of 2.5-5.0. When the pH values of supporting electrolyte was more than 5.0, the solution would be turbidity once mixing the micro-emulsion with supporting electrolyte. The phenomenon indicated that the increasing pH damaged the formation of micro-emulsion. Besides, the peak current has little effect on pH values range 2.5-4.0, but it showed a decreasing trend when the pH values more than 4.0. Therefore, we chose pH 4.0 for the further test.

The linearity correlation of peak potential of TAN vs. pH values of supporting electrolyte corresponded to the following equation: E_{pc} (V) = -0.04737 pH + 0.02606 (R^2 = 0.9991), E_{pa} (V) = -0.05464 pH + 0.08668 (R^2 = 0.9960). The slope of this equation is found to be 47.37 mV/pH (E_{pc} vs pH) and 54.64 mV/pH (E_{pa} vs pH), which is very close to the expected value of 59 mV/pH [33] suggesting that the transfer number of electrons and protons in the reaction were equal.

3.4. Effect of scan rate

Scan rate has a great influence on the redox process of electrode surface. Therefore, cyclic voltammograms of 20 μg/mL TAN-ME at GCE were recorded in the scan rate range from 0.02 to 0.30 V/s (Fig. 3A). The linear relationship of the square root of scan rate and scan rate on the peak current were shown in Fig. 3B and Fig. 3C. A dependence of i_p on $v^{1/2}$ for cathodic and anodic peak corresponded to the following equations: I_{pc} (μA) = 9.3088 $v^{1/2}$ ($V^{1/2}/s^{1/2}$) – 0.8911 (R^2 = 0.9919), I_{pa} (μA) = -7.0011 $v^{1/2}$ ($V^{1/2}/s^{1/2}$) – 0.8522 (R^2 = 0.9826). This is a typical diffusion controlled reaction. However, i_p and v also have a good linear correlation, which relationship can be presented by the following equations: I_{pc} (μA) = 12.9500 v (V/s) + 0.5887 (R^2 = 0.9940), I_{pa} (μA) = -9.8038 v (V/s) – 0.2505 (R^2 = 0.9987). Therefore, the redox reaction also controlled by adsorption. With regards to this, the other approach was used to study whether the electrode reaction is diffusion or adsorption controlled.



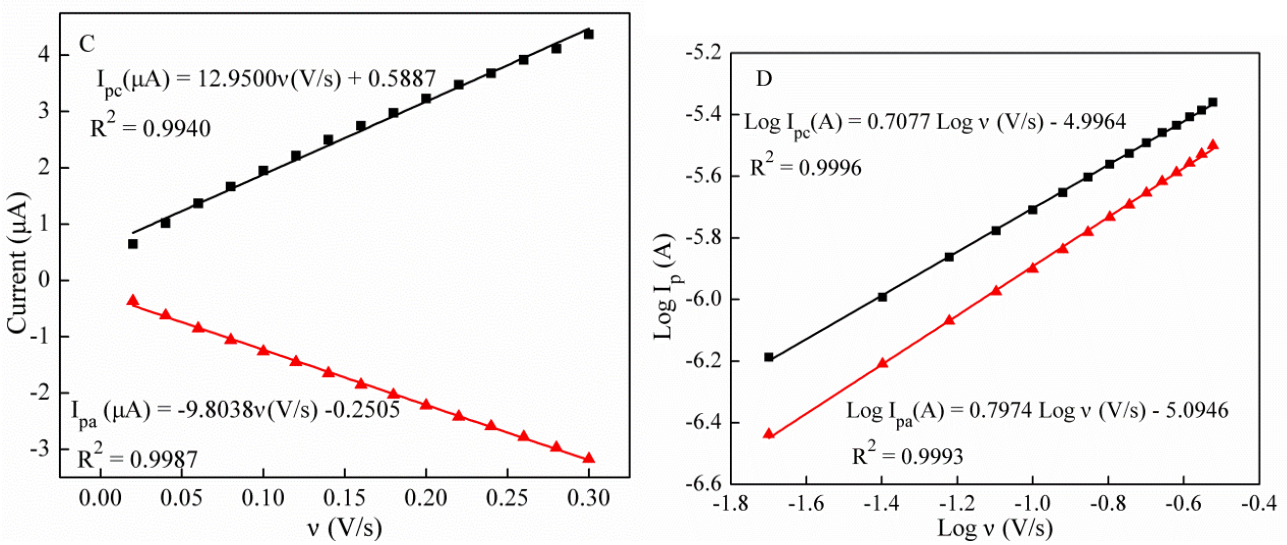
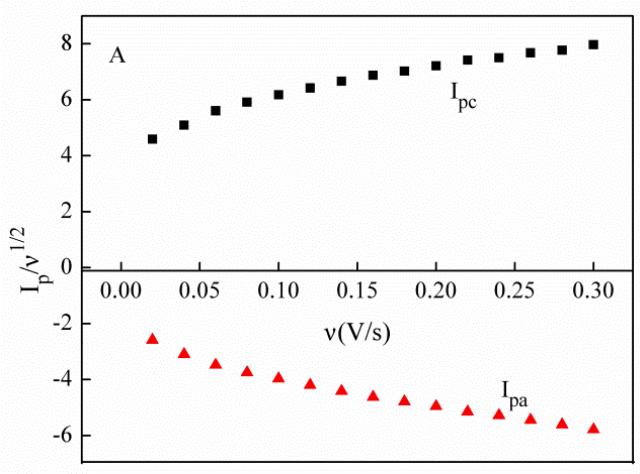


Figure 3A. Cyclic voltammograms for the reduction and oxidation of 20 μg/mL TAN-ME at GCE at different scan rates: 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28, 0.30 V/s. **Fig. 3B**. and **Fig. 3C.** Dependence of redox peak current on the square root of scan rate or scan rate. **Fig. 3D.** Linear relationship between logarithm of redox peak current and logarithm of scan rate

Fig. 3D shows the relationship between logarithm of redox peak current and logarithm of scan rate, which conformed the following equations: Log I_{pc} (A) = 0.7077 Log v (V/s) – 4.9964 (R^2 = 0.9996), Log I_{pa} (A) = 0.7974 Log v (V/s) – 5.0946 (R^2 = 0.9993). As we all know, the slope close to 0.5 is explained for diffusion-controlled process, whereas close to 1.0 is for adsorption-controlled process [34-36]. Nevertheless, The slope for the two dependence in this paper is 0.7077 and 0.7974, respectively, between 0.5 and 1.0, suggesting diffusion and adsorption joint control.

Then, it's necessary to research whether the irreversibility of electrode reaction is owing to a sluggish heterogeneous electron transfer or to a coupled homogeneous chemical reaction. Hence, Fig. 4A showed the dependence of $i_p/v^{1/2}$ and v for 20 μg/mL TAN-ME. The current function ($i_p v^{-1/2}$) is independent on v for irreversible and reversible processes [36].



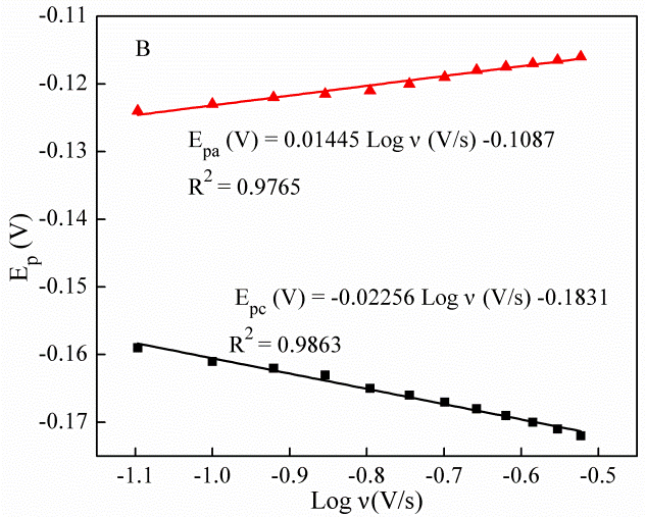


Figure 4A. Dependence of current function ($i_p v^{-1/2}$) on the scan rate. **B.** Linear relation between redox peak potential and logarithm of scan rate.

However, if the irreversibility is because of a coupled chemical reaction, the current function changes with v and the value can be observed depending on the properties of the chemical reaction. Therefore, the increasing current function with v hinted a chemical reaction coupling to the electrode process [34, 36]. Therefore, the electrode reaction of 20 μg/mL TAN-ME at GCE in pH 4.0 acetate buffer is a quasi-reversible reaction.

A dependence of peak potential on logarithm of scan rate was also recorded for TAN-ME in Fig. 4B. It can be found from Fig. 4B that, with an decreasing scan rate, the cathodic peak potential shifts to more positive potential whereas the anodic peak potential shifts negatively. At a low scan rate, the difference between cathodic potential and anodic potential hints the quasi-reversible process. If the scan rate continues to increase, the reaction would be more irreversible. The relationship between peak potential and logarithm of scan rate corresponded to the following equations: E_{pc} (V) = -0.02256 Log v (V/s) – 0.1831 (R^2 = 0.9863), E_{pa} (V) = 0.01445 Log v (V/s) – 0.1087 (R^2 = 0.9765).

The overall electron transfer coefficient (αn) for TAN-ME redox process can be obtained from the equation as follows [34, 37, 38]:

$E_p = (b/2) \log v + \text{constant}$

The slope of E_p vs. log v is shown in Fig. 4B, based on the above equation the Tafel slope b = -0.02256 for reduction and 0.01445 for oxidation peak. According to Laviron [39], a graph of $E_p = f(\log v)$ yields two straight lines with a slope equal to -2.3 RT/αnF for the cathodic peak, and 2.3 RT/(1 – α)nF for the anodic peak. The value of the cathodic transfer coefficient (α) for the electrode reaction can be obtained according to the slopes of E_p vs. log v using the following equation [34, 40]:

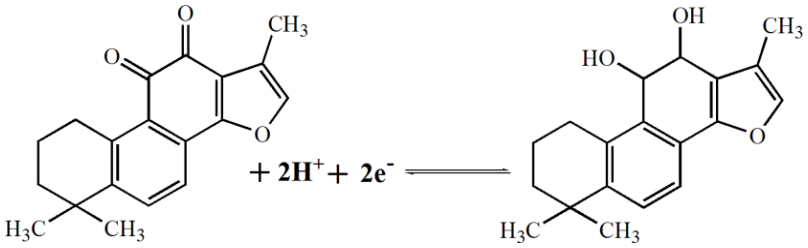
$\log \kappa_a/\kappa_c = \log \alpha/(1-\alpha)$ or $\kappa_a/\kappa_c = \alpha/(1-\alpha)$

where κ_a and κ_c is the slope of straight lines for E_{pa} vs. log v and E_{pc} vs. log v, respectively. The cathodic transfer coefficient (α) was calculated to be 0.39. The width of mid-height ($W_{1/2}$ = 74 mV) becomes independent of α and trends towards 62.5/αn [39], so n was found to be 2, implying that two electrons were caught up in the electrochemical redox reaction of TAN-ME. The apparent

heterogeneous electron transfer rate constant (κ_s), κ_s = 1.52 s^{-1} can be calculated according the following equation [39]: $\kappa_s = \alpha n F v_c / RT$

3.5. Mechanism

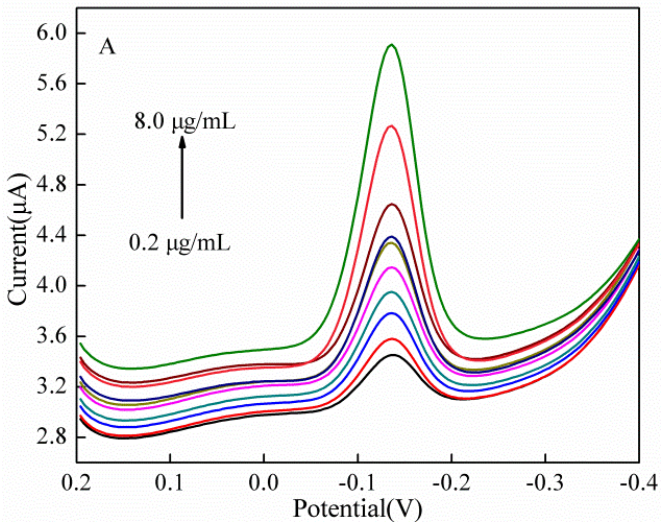
In the previous studies, the electrochemical reaction involved in two electrons and two protons transfer process was verified and the mechanism was proposed in scheme. 2. TAN is the derivative of 9,10-phenanthrenequinone, and ortho carbonyl is its main functional group. Furthermore, some researches indicated that the ortho carbonyl existed in 9,10-phenanthrenequinone molecules proceeded addition reaction to generate hydrogen phenanthrene quinone with two electron and two proton transferring. The redox reaction mechanism of phenanthrenequinone has been reported in some literature [30, 41-43], and in the present study, the TAN molecule is highly aromatic in nature. So there was π–π stacking force and was more conducive to the process of releasing or capturing protons and electrons.



Scheme 2. Electrode reaction mechanism of TAN.

3.6. Electrochemical determination of TAN-ME

DPV was used for quantitative determination of TAN-ME in the present study, because it has higher sensitivity and lower limit detection than cyclic voltammetry.



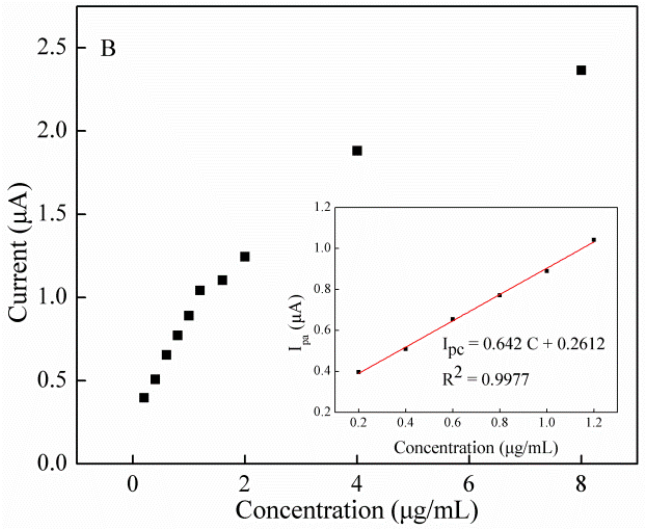


Figure 5A. Differential pulse voltammograms for the variation of cathodic peak current with increasing concentration of TAN-ME at GCE: 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2, 4, 8 μg/mL. **Fig. 5B.** The dependence of current against the concentration of TAN-ME. Inset: The linear relation between TAN-ME cathodic peak current and concentration from 0.2 to 1.2 μg/mL .

Table 1. Characteristics of TAN-ME calibration plot using DPV at GCE.

Parameter	
Regression equation	I_{pc} (μA) = 0.6420 C (μg/mL) + 0.2612
Linearity range (μg/mL)	0.2-1.2
Slope	0.6420
Intercept	0.2616
Correlation coefficient (R^2)	0.9977
RSD of intercept (%)	1.072
RSD of slope (%)	1.376
LOD (μg/mL)	0.05025
LOQ (μg/mL)	0.1675
Number of data points	6
Reproducibility of peak current (RSD %)	1.27
Reproducibility of peak potential (RSD %)	1.20
Repeatability of peak current (RSD %)	0.57
Repeatability of peak potential (RSD %)	0.89

Fig. 5A shows the DPV plot of cathodic peak current with different concentration from 0.2 to 8.0 μg/mL at GCE. As observed, there is a good linear relationship between cathodic peak current (I_{pc}) and TAN-ME concentration (C) from 0.2 to 1.2 μg/mL (inset of Fig. 5B), and its linear equation showed as follow: I_{pc} (μA) = 0.6420 C (μg/mL) + 0.2612 (R^2 = 0.9977). However, the cathodic peak current deviates from the calibration curve when the concentration of TAN-ME is more than 1.2 μg/mL, which may be due to the saturation for electroactive material.

Meanwhile, the results were obtained by repeating five measurements on the same day and in the same condition (repeatability) and over 5 days from the different solutions for other people

(reproducibility). The results of TAN-ME calibration plot were presented in Table. 1. The limit of detection (LOD) and limit of quantifications (LOQ) were calculated to be 50.25 ng/mL, 167.5 ng/mL, respectively, using the IUPAC definition:

$LOD = 3\ s/m$; $LOQ = 10\ s/m$.

where s is the standard deviation of the peak current of the blank (five experiments) and m is the plot of the calibration curve [44]. The LOD and LOQ compared with literature [24, 27-28] were found to be higher, which was because surfactant as the masking agent hindered electron transfer.

3.7. Recovery studies

To develop a reliable voltammetric determination method, recovery test was performed. TAN-MEs with three different concentration (80%, 100%, 120%, the prescription concentration as 100%) were prepared as described above. The recovery studies of the method were expressed as a recovery trial with nine determinations, which were calculated by the ratio of the measured mean and the concentrations labeled. The results recorded in Table. 2. implied that the present method showed good recoveries (96.01-101.23%) and acceptable precision (RSD = 1.82%). Therefore, the results illustrate the method is feasible.

Table 2. Recovery results of the TAN.

TAN marked [a] (mg)	TAN found (mg)	Recovery (%)
8.01	7.71	96.25
8.00	7.91	98.89
8.01	7.68	96.01
10.02	10.12	101.23
9.98	9.78	97.79
10.00	9.94	99.36
12.01	11.81	98.44
12.02	12.08	100.67
12.01	11.95	99.67
RSD (%)	1.82	

[a] Determined by HPLC

3.8. Precision and Accuracy

The precision and accuracy of the method were evaluated by intra-day and inter-day stability of TAN-ME, respectively. The intra-day accuracy was gotten by measuring the samples eight times in one day and was expressed as a recovery trial with five determinations per sample. Besides, the inter-day precision was obtained by measuring samples within three days and was calculated by a relative standard deviation with five determinations per sample. The results listed in Table. 3. showed that the intra-day and inter-day RSDs for TAN-ME were 1.08-1.42% and 1.17-1.55%, respectively. Hence, the reproducibility of the method was available.

Table. 3. The accuracy and precision results of TAN-ME at room temperature by DPV.

Sample	Marked [a] (mg)	Intra-day			Inter-day		
		Found [b] (mg)	Recovery (%)	RSD(%)	Found [b] (mg)	Recovery (%)	RSD (%)
A	10.01	9.85 ± 0.01	98.54	1.08	9.87 ± 0.03	98.65	1.17
B	10.02	9.88 ± 0.08	98.82	1.23	9.95 ± 0.05	99.49	1.15
C	10.01	9.91 ± 0.05	99.14	1.22	9.91 ± 0.07	99.12	1.08
D	9.98	9.95 ± 0.02	99.49	1.35	9.89 ± 0.05	98.87	1.43
E	10.03	9.92 ± 0.03	99.23	1.42	9.92 ± 0.11	99.23	1.55

[a] Determined by HPLC, [b] Average of five determinations

3.9. Effect of excipients

It is necessary to study the effect of excipients in real sample determination for an available method. In present work, the effect of excipient on the voltammetric signal was carried by measuring sample containing a fixed amount of TAN-ME (1μg/mL) and lyophilization excipient (200 μg/mL). The peak potential remains basically unchanged, however, the peak observed at a less decrease current (1.32%). Besides, the surfactant and oil did not change the curve shape, but 120% surfactant and oil, with respect to the original prescription, can reduce the peak height by six-percent. Therefore, proposed method can only apply to fixed prescription micro-emulsion system.

3.10. Application

Since the method only applies to my prescription, we prepared TAN-ME lyophilized product as described above. The three batches of lyophilized products contains 10 mg TAN were redissolved with water and diluted to 5 μg/mL, 20 vol.% of this diluted TAN-ME and 80 vol.% pH4.0 acetate buffer was mixed and then placed in the electrolytic cell for measuring. The recovery (97.86%-101.91%) and RSD (0.87%-2.79%) were summarized in Table. 4. The results revealed that the method may be useful in the determination of TAN-ME in real samples.

Table. 4. Application of the determination of real sample by DPV.

Real sample	Marked [a] (mg)	Found [b] (mg)	Recovery (%)	RSD (%)
A	10.01	9.79	97.86	2.79
B	9.97	10.13	101.29	0.87
C	10.00	10.19	101.91	1.82

[a] Determined by HPLC, [b] Average of five determinations

4. CONCLUSIONS

The electrochemical behavior of TAN-ME at glassy carbon electrode in pH4.0 acetate buffer was studied by cyclic voltammetry. Meanwhile, the reaction was quasi-reversible process at low scan

rate and was controlled by diffusion and adsorption simultaneously. Besides, DPV method was developed for the quantitative determination of TAN-ME in real samples, with excellent intra-day accuracy, inter-day precision, accepted RSD and recovery studies. Currently, there has not been reported a direct electrochemical method for quantitative determination of hydrophobic drug in micro-emulsion system. Furthermore, the method was inexpensive, rapid and sensitive. In summary, the present study provides guidance for direct determination of hydrophobic and investigation of the electrochemical behaviors of quinones.

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