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Citrate stabilized Fe₃O₄/DMG modified carbon paste electrode for determination of octamethylcyclotetrasiloxane in blood plasma and urine samples of cement factory workers

Rashid Heidarimoghadam¹ and Abbas Farmany^{2*}

Abstract

In this paper, a novel mercury-free electrochemical probe was constructed for the trace determination of octamethylcyclotetrasiloxane (D₄) in some biological fluids by adsorptive stripping voltammetry. The platform is based on the adsorptive accumulation of Ni(II) onto a carbon paste electrode modified with citrate stabilized Fe₃O₄ (Cit-Fe₃O₄) and dimethylglyoxime (DMG). It was shown that trace levels of D₄ enhance the electrochemical adsorptive stripping signal of Ni(II) on the electrode platform. It was shown that electrochemical signals are proportional to concentrations of D₄. The supporting electrolyte, pH and instrumental parameters associated with the electrode response, including scan rate, accumulation potential and deposition time were optimized. The electrode platform demonstrated well resolved, reproducible peaks, with relative standard deviation (RSD) of 3.8% and detection limit (3S_b/m) of 27.0 ng/mL. The sensor exhibited good D₄ detection and quantification in human blood plasma and urine samples.

Keywords: Octamethylcyclotetrasiloxane, Voltammetry, Human fluids

Introduction

As low molecular weight (LMW) compounds, siloxane derivatives play a vital role in human life. Today, the ever-increasing development of silicon technology has resulted in more than 150,000 products in pharmaceuticals, medicine, cosmetics, and food industry [1–3]. It is estimated that global siloxane market reached over 19 billion dollars [3, 4]. Many devices and tools used by infants, adults as well as the elderly are made of siloxane derivatives. Today, 50% of new skin care products contain one of silicon derivatives [3, 5]. Recent studies showed that

some of siloxane derivatives may interfere with the function of the endocrine glands and may adversely affect the fertility [5–8]. It was shown that siloxane derivatives such as octamethylcyclotetra-siloxane (D₄), decamethylcyclopentasiloxane (D₅) and dodecamethylcyclohexa-siloxane (D₆) can cause cancer or inflammation in the glands [5–7]. The molecular structure of D₄ is shown in Scheme 1. Most common siloxane polymer used in medical products is PDMS, which has been widely used in breast implants. It has been reported that silicon migrates from the implant to specific tissues such as plasma and blood [6, 7]. So the monitoring of siloxane derivatives, especially in human body fluids, is important.

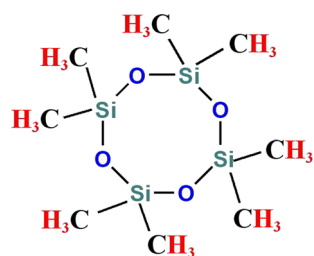
Siloxane derivatives may be detected using inductively coupled plasma spectroscopy (ICP), gas chromatography (GC), and high performance liquid chromatography

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Scheme 1 Molecular structure of D₄

(HPLC) [6–16]. However, some methods can detect the silicon element alone, and detection of organosilicon compounds remain an issue.

Recently, electrochemical methods with excellent selectivity and sensitivity have been developed for trace analysis of micro/macro molecules in biological samples. At this time, carbon based electrodes are modified using different nanomaterials to achieve the best sensitivity. Superparamagnetic iron oxide nanomaterial (Fe₃O₄) is a typical solid of layered metal oxides of Fe₂O₃ and FeO. In the structure of this metal oxide nanocrystal, one of two tetrahedral interstitial sites is filled by Fe³⁺ cations and other site is occupied by Fe²⁺ and another half of Fe³⁺. In these typical metal ions, valence mixing decreases the resistivity and possesses an excellent conductivity [17]. Also, surface to volume ratio and specific surface area of this nanomaterial accelerates the electron flow and increases its effective contact with electrolyte, which leads to excellent electroensing ability [18–21].

To the best of our knowledge, at this time no electrochemical method was reported for the quantification of siloxane derivatives in real samples. The aim of this work was to obtain a mercury-free electrode for quantification of octamethylcyclotetrasiloxane (D₄) in some biological fluid samples using a modified carbon paste electrode (CPE). To enhance the sensitivity, carbon paste electrode was modified with Cit-Fe₃O₄ and dimethylglyoxime (DMG) as selective chelating agent. The developed Cit-Fe₃O₄-DMG CPE sensor exhibited excellent electrochemical performance and obtained high sensitivity toward D₄. Meanwhile, the interference of some coexisting ion/molecules was investigated. The reproducibility and stability of the fabricated electrode were also investigated. Moreover, the potential of the sensor was verified by analyzing D₄ in blood plasma and urine samples.

Experimental

All experimental protocols were approved by the Ethics and Animal Handling Committee of the Hamadan University of Medical Science (IR.UMSHA.REC.1395.99). All methods were carried out in accordance with relevant guidelines and regulations. Oral consent was obtained from all participants.

Apparatus

Electrochemical experiments were made using an Autolab potentiostat/galvanostat (Ecochemie, Netherland). A personal computer was used for data storage. A carbon paste electrode was used as working electrode. A platinum wire and a silver/silver chloride electrode were utilized as counter and reference electrode, respectively.

Materials

Ferric chloride hexahydrate (FeCl₃·6H₂O) and nickel chloride hexahydrate (NiCl₂·6H₂O) were purchased from Merck (Germany). Octamethylcyclotetrasiloxane (D₄) was purchased from Sigma (USA). Dimethylglyoxime (DMG) was purchased from Sigma-Aldrich (USA). Stock solution of Ni²⁺ was prepared by dissolving an appropriate amount nickel chloride in double distilled water. Acetate-acetic acid (HAc-NaAc) buffer was used to adjust the pH of solutions. D₄ solution was prepared by dissolving 25 mg of D₄ in 25 mg of ethyl acetate and H₂O (50:50 w/w). Different concentrations of D₄ were prepared by diluting the stock solution with double distilled water.

Synthesis of Cit-Fe₃O₄

Fe₃O₄ nanocrystal was synthesized using an improved solvothermal method [21]. Briefly, 0.32 g FeCl₃·6H₂O and 0.1 g trisodium citrate were dissolved in ethylene glycol. 0.6 g sodium acetate was added to the solution and it was homogenized ultrasonically for 1 h at room temperature. The solution was transferred into a chemical autoclave and aged for 10 h at 200 °C. After cooling at room temperature, the nanocrystals were decanted and washed with acetone and pure ethanol. Finally, the synthesized Fe₃O₄ was ethanol evaporated using a reduced pressure chamber.

Sensor preparation

The unmodified carbon paste electrode was prepared by mixing 0.1 g graphite powder and 0.2 mL paraffin oil. A portion of the resulting paste was then firmly inserted into the electrode cavity (2.6 mm diameter). Electrical contact was made through a copper wire. The Cit-Fe₃O₄-DMG CPE electrode was prepared by mixing appropriate amounts of graphite powder, Cit-Fe₃O₄ nanoparticles,

DMG powder and paraffin oil. The surface of modified/unmodified electrode was thoroughly washed before each measurement by double distilled water.

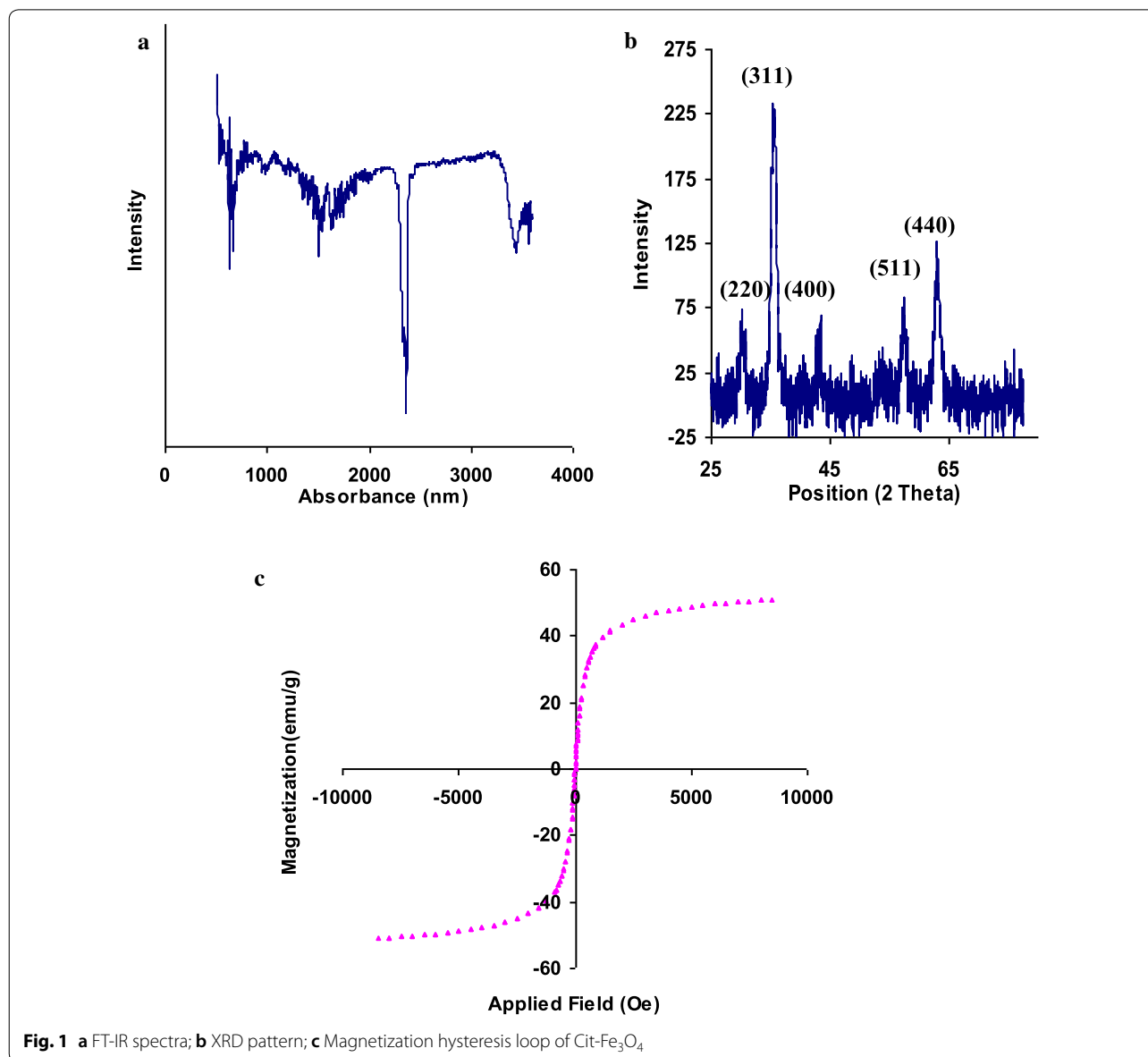
Procedure

Ni^{2+} and 0.05 mol L^{-1} HAc-NaAc buffer (pH 4.65) were transferred into a three electrode electrochemical cell setup. Cit- Fe_3O_4 -DMG-CPE was immersed into the cell as working electrode. The electrochemical cell was degassed with nitrogen gas for 2 min. An accumulation potential of -0.2 V was applied to working electrode. After 100 s stirring, the potential was scanned at rate of 120 mV s^{-1} in differential pulse waveform mode.

Results and discussion

Synthesis and characterization of Cit- Fe_3O_4

The Cit- Fe_3O_4 nanocrystals were solvothermally synthesized and characterized using Fourier-transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), vibrating-sample magnetometer (VSM), scanning electron microscope (SEM) and transmission electron microscope (TEM). FTIR spectra of Cit- Fe_3O_4 is shown in Fig. 1a. The characteristic peak at 578 cm^{-1} is related to the vibration of Fe-O bond which confirms the magnetic phase of nanoparticles. Absorption peaks at 1371 - 1540 cm^{-1} confirms the stretching vibration (symmetric and asymmetric) of carbonyl groups [21].



The microstructure of Cit-Fe₃O₄ nanocrystal was characterized by the x-ray diffraction (XRD) technique. Figure 1b shows the XRD pattern of magnetic nanocrystals. Indexing 2θ diffraction peaks at 30.0, 35.6, 43.8, 54.2, 57.2 and 62.5 to (220) (311) (400) (511) and (440) shows the cubic spinel structure of nanocrystals. The result of this study is in accordance with the standard PDF card (JSPDS 86-2343) of magnetite. The magnetic characteristic of Cit-Fe₃O₄ nanocrystals was evaluated by a vibrating-sample magnetometer (VSM). The nanocrystals exhibit superparamagnetic behavior and have lower saturation magnetization value than the bulk magnetization (~92 emu/g) [22]. Magnetization hysteresis loop of

Cit-Fe₃O₄ is shown in Fig. 1c. VSM data shows a superparamagnetic behavior of synthesized nanocrystals with a saturation magnetization of 50.7 emu/g. The saturation magnetization and susceptibility of Cit-Fe₃O₄ seems to be smaller than Fe₃O₄. This is due to the existence of citrate diamagnetic shell surrounding the Fe₃O₄ which quench the magnetic moment [23]. However, Cit-Fe₃O₄ showed superparamagnetic behaviors, which indicate that magnetite nanocrystal is incorporated in the composite particles, which exhibited no residual magnetism effect at applied magnetic field from the hysteresis loops. Surface micromorphology and topography of as-prepared Cit-Fe₃O₄ nanocrystals was characterized by

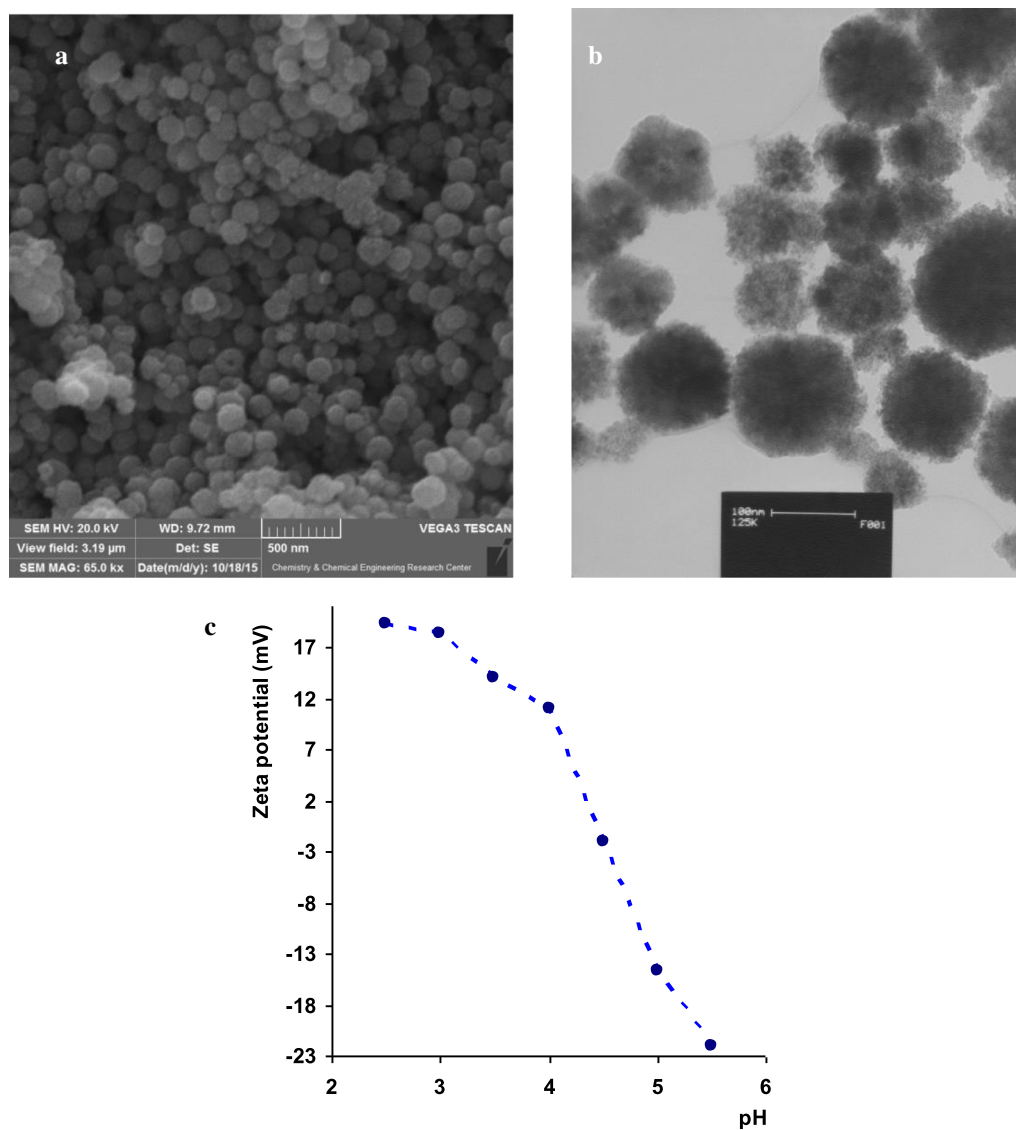
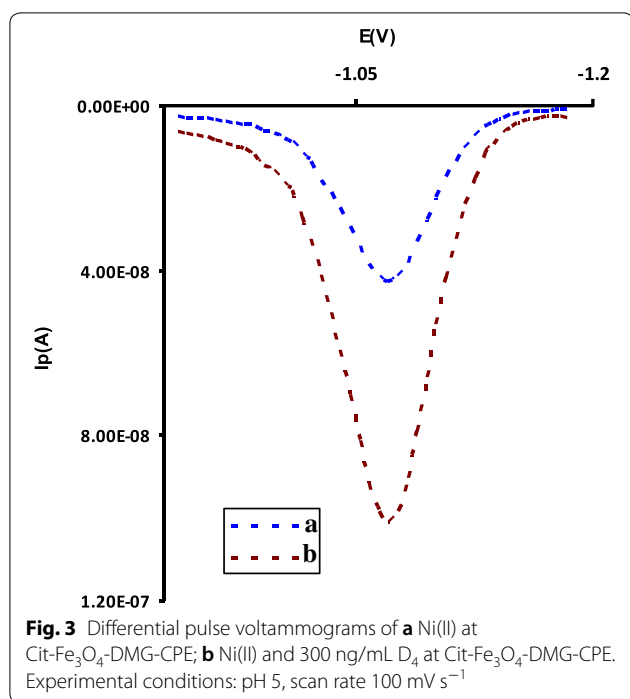


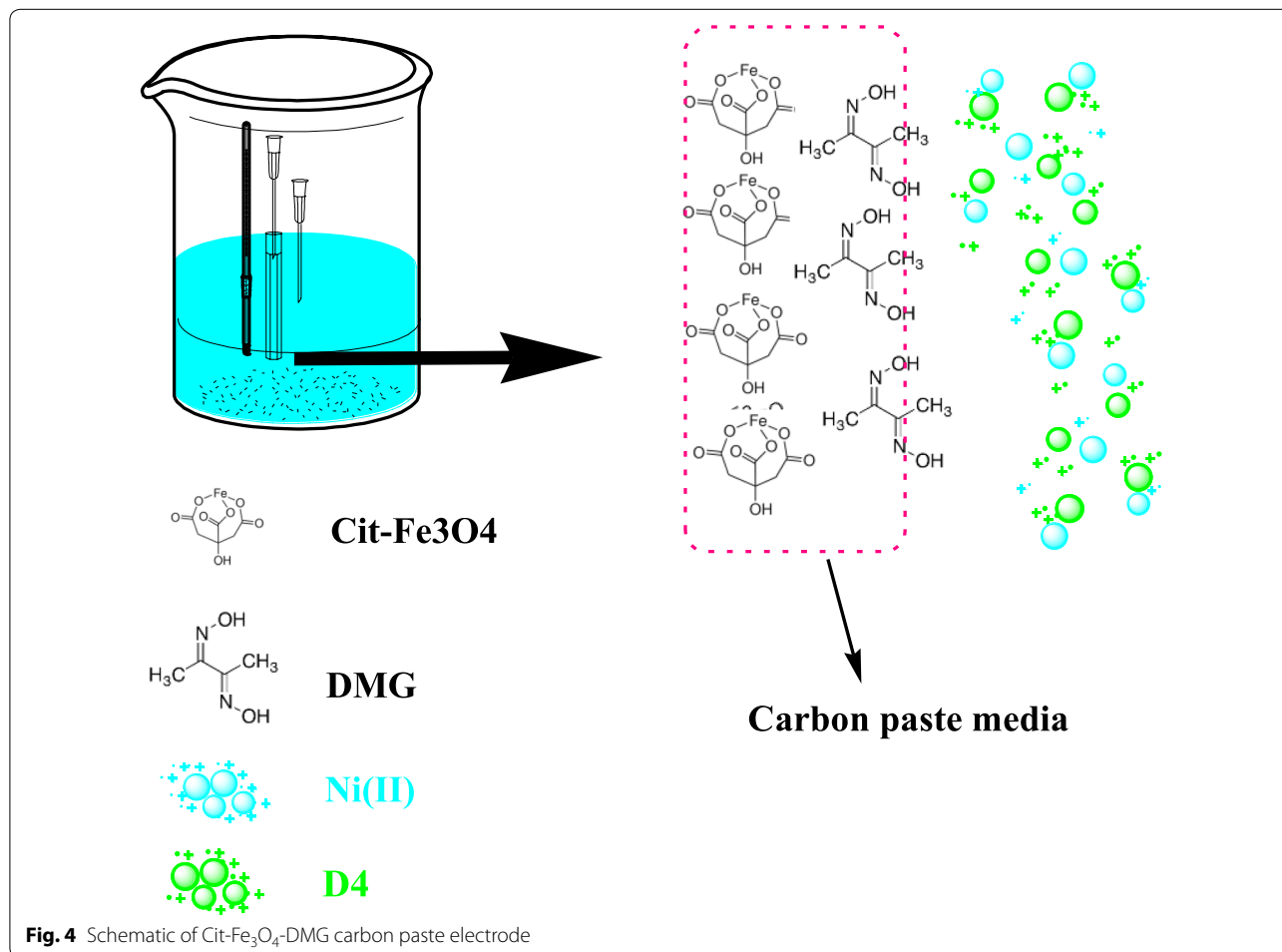
Fig. 2 a SEM; b TEM micrograph; c Zeta potential of Cit-Fe₃O₄



scanning electron microscopy (SEM). As indicated in Fig. 2a, the irregular shape of nanocrystals with excellent dispersity is observed. TEM image of nanocrystal is shown in Fig. 2b. The size of Cit-Fe₃O₄ nanocrystals is estimated as 150 nm. Surface zeta potential of Cit-Fe₃O₄ at different pHs was evaluated and the surface charge of nanocrystals was obtained. As shown in Fig. 2c, the zeta potential of Cit-Fe₃O₄ at optimum pH of this method (pH 4.65) is - 9.0 mV.

Electrochemical experiments

Cit-Fe₃O₄-DMG-CPE electrode was prepared and its indirect electrochemical response to D₄ molecule was studied. Accumulation potential applied onto the electrochemical cell pre-concentrates the nickel ion on the working electrode surface in an open circuit conditions. The possible mechanism for enhanced stripping signal of D₄ could be related to high electron density on the D₄ molecule surface which follows the reversible redox process of Ni²⁺/Ni⁰ pair. Nickel redox peak is registered at -0.91 V at Cit-Fe₃O₄-DMG-CPE electrode surface. In the presence of nickel ion, introducing trace levels of D₄ onto



the electrochemical cell enhance the electrochemical signal (Figs. 3 and 4). Increasing the current value can be attributed to the D_4 concentration.

Effect of supporting electrolyte, pH, depositing time & potential and scan rate

To achieve the high sensitivity, the effect of supporting electrolyte, pH, accumulation potential and scan rate on the electrode response were studied. To investigate the effect of electrolyte media and pH on the electrode response, various electrolytes such as sodium borate-boric acid, Britton-Robinson (B-R) and sodium acetate-acetic acid (HAc-NaAc) buffers were tested. The highest peak signal of D_4 existed in the HAc-NaAc solution (0.05 M). While in sodium borate-boric acid, B-R solutions, weak peak signal were obtained. Consequently, in further experiments, HAc-NaAc (0.05 M) was chosen as the electrolyte. The pH of buffer solution has significant effect on the electrochemical signal of sensor. The influence of pH on the electrochemical response of Cit- Fe_3O_4 -DMG-CPE electrode to D_4 was

studied. The results are presented in Fig. 5a. The results of this study show that by increasing the pH value up to 4.65, the peak signal of D_4 increases. Then, the D_4 electrochemical signal decreased with pH value up to 6.0. This result could be related to the deprotonation of HAc-NaAc buffer solution which promote the generation of H^+ , the concentration of D_4 adsorbed on the Cit- Fe_3O_4 -DMG-CPE surface would be less. Thus, the current signal of D_4 would be increased with increase of pH till 4.65. With increase of pH from 4.65 to 6.0, the corresponding stripping signal was decreased due to the prevailing effect of D_4 hydrolysis and formation of $Ni(OH)_2$, $Ni(OH)^+$, and $Ni(OH)_3^-$. Thus, pH 4.65 of HAc-NaAc buffer solution (0.05 M) was chosen in further experiments. In the electrochemical experiment, the accumulation potential is particularly vital for achieving good signals. In voltammetry, the deposition time has a significant impact on the electrochemical signals and sensitivity. The results of this study showed that the current signal D_4 increased by increasing the accumulation time from 25 to 250 s (Fig. 5b).

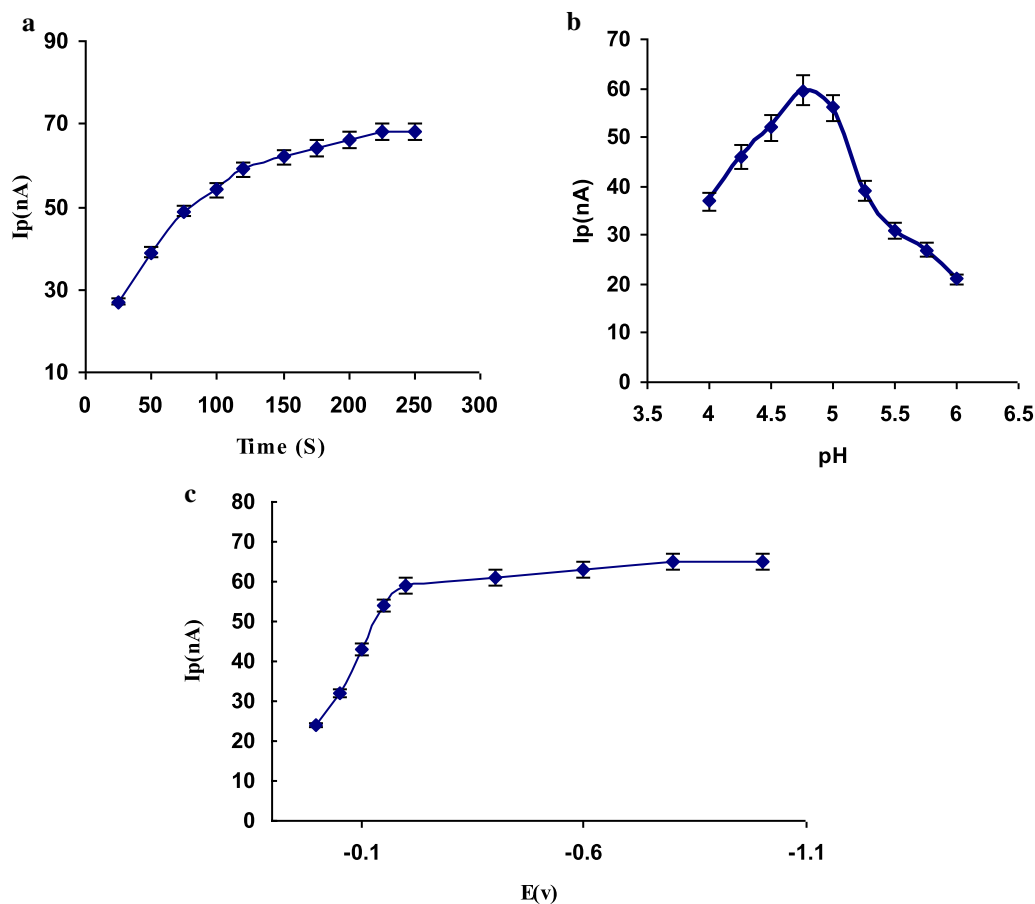


Fig. 5 Effect of **a** pH; **b** deposition time; **c** deposition potential on differential pulse voltammograms of 250 ng/mL D_4

After 120 s, the signal intensity of D_4 remained constant. Therefore the accumulation time of 120 s was selected for construction of the calibration curve and other experiments. The selection of 120 s as accumulation time was based on the development of a rapid method with a short real sample analysis time. In continue, the effect of accumulation potential on the electrode response was studied in the potential range of 0.0 to -1.0 V (Fig. 5c). The results of this study show that by increasing the accumulation potential from 0.0 to -1.0 V, the electrochemical signal is increasing till -0.2 V and remains constant at more potential. At more negative accumulation potentials, reduction of D_4 occurs, as indicated by decrease in the electrochemical signal of D_4 measured during the scanning. Thus, -0.2 V was adopted as the accumulate potential. Also, the effect of scan rate on the electrochemical signal of D_4 showed that till 100 mVs^{-1} , the response is increasing. It was shown that at scan rates from 0.1 to 100 mVs^{-1} , the dependence of redox response upon the scan rate was linear which demonstrate an adsorption controlled process [24, 26]. The results of this study show that the cathodic and anodic peak current was increased with increasing the scan rate which indicates the oxidation and reduction process of D_4 at Cit- Fe_3O_4 -DMG-CPE electrode. So, it can be concluded that D_4

was firstly absorbed on the electrode surface then the electrode reaction occurs.

Calibration equation

Differential pulse voltammograms of different concentration of D_4 at Cit- Fe_3O_4 -DMG-CPE electrode in 0.05 M HAc-NaAc buffer at pH 4.65 are recorded (Fig. 6). It was shown that peak current values are linear at 50.0 to 340.0 ng/mL of D_4 with regression equation of $y(A) = 3E^{-10}x(\text{ng/mL}) - 2E^{-08}$ and correlation coefficient of $R^2 = 0.9834$. The detection limit ($3S_b/m$) of the electrode was calculated as 27.0 ng/mL . The results of this study reveal that the electrochemical voltammetry platform is sensitive to D_4 determination.

Stability, selectivity and repeatability

The reproducibility of Cit- Fe_3O_4 -DMG-CPE electrode was excellent since the RSD ($n = 10$) was obtained as 3.8% which indicate the good repeatability of the electrode. The selectivity of electrode was evaluated by the determination of D_4 in the presence of some potential interfering ions/molecules at optimized instrumental and operational conditions. Selectivity coefficient was defined as a concentration of the other ion/molecule that causes $\pm 5\%$ relative error (RE) in the electrode response. The results of this study showed that most anions/cations and molecules Na^+ , Ca^{2+} , Cu^{2+} , Cu^+ , Fe^{3+} , Fe^{2+} , chlorate, iodate, bromate, chloride, bromide and thiosulfate don't interfere with the system while Zn^{2+} , EDTA, D_3 , D_5 and D_6 , showed interference in D_4 determination. However, the interference of EDTA and Zn^{2+} could be resolved by using them as potential masking agents for each others. As the results of interference study, major interference was found in the detection of low molecular weight (LMW) cyclic silicone families of D_4 as D_3 , D_5 , and D_6 . Due to easy-to-use, inexpensive, non-GC methods are becoming increasingly popular for the determination of organic/organometallic substances including D_4 . To examine the stability of platform, in the 0.05 M HAc-NaAc buffer 10 scan were made in the proposed potential range. The results show the voltammograms with clear background. Also, after 2 week storage of electrode in 0.05 M HAc-NaAc buffer, only 15% of electrochemical signals were decreased.

Determination of D_4 in urine and blood plasma

Blood and urine sample of workers of Ekbatan cement factory were collected. 1 mL of urine or blood plasma was transferred into a tube. After addition of 1 ml ethyl alcohol, each sample was vortexed. Then a certain amount of D_4 was added to the samples and vortexed for 2 min. After addition of 2 mL hexane, the samples were

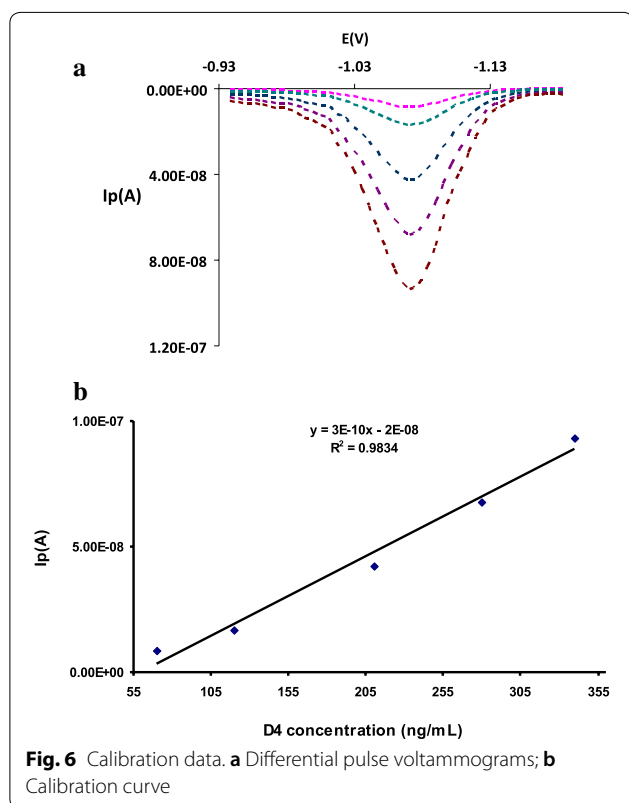


Fig. 6 Calibration data. **a** Differential pulse voltammograms; **b** Calibration curve

Table 1 Determination of D₄ in human blood plasma samples (n = 3)

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)	Standard method ^a (ng/mL)
Blood plasma	50.0	47.3	94.6	49.8
	100.0	92.0	92.0	96.3
	150.0	146.0	97.3	147.0
	200.0	193.2	96.6	197.4
	250.0	246.8	98.7	249.0

^a GC-mass**Table 2 Determination of D₄ in urine samples (n = 3)**

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)	Standard method ^a (ng/mL)
Urine	50.0	46.9	93.8	48.4
	100.0	101.0	101.0	99.4
	150.0	148.0	98.6	150.3
	200.0	197.1	98.5	201.0
	250.0	244.3	97.7	246.9

^a GC-mass

re-vortexed for 2 min. It was transferred to the electrochemical cell and related voltammogram was recorded [25]. The results are presented in Tables 1 and 2.

Conclusions

In conclusion, the Cit-Fe₃O₄-DMG modified carbon paste electrode show superior detection capabilities as a result of enhances the electron transfer kinetics and surface area to volume ratio following the incorporation of Cit-Fe₃O₄ nanoparticles. The electrode has been successfully applied to the quantification of D₄ in biological fluids.

Abbreviations

D₄: Octamethylcyclotetrasiloxane; Cit-Fe₃O₄: Citrate stabilized Fe₃O₄; RSD: Relative standard deviation; LMW: low molecular weight; D₅: decamethylcyclopentasiloxane; D₆: Dodecamethylcyclohexa-siloxane; ICP: Inductively coupled plasma spectroscopy; HPLC: High performance liquid chromatography; GC: Gas chromatography; HAc-NaAc: Acetate-acetic acid; FTIR: Fourier-transform infrared spectroscopy; XRD: X-ray powder diffraction; VSM: Vibrating-sample magnetometer; SEM: Scanning electron microscope; B-R: Britton-Robinson.

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Authors' contributions

AF performed the experiments. RH and AF provided the chemicals and conceived the idea. RH supervised the study and writes the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials could be available upon the request.

Competing interests

All authors declare that they have no competing or conflict of interests.

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