Highly sensitive electrochemical azaperone sensor based on magnetic silica –NH₂-CS₂ in the ostrich meat and rat plasma and its comparison with HPLC–MS/MS

Faezeh Gandomi¹ · Mohammad Javad Taghizadeh² · Elnaz Marzi Khosrowshahi³ · Maryam Saleh Mohammadnia⁴ · Neda Irannejad⁵ · Esmail Sohouli² · Hermann Ehrlich⁶,⁷ · Yvonne Joseph⁶ · Mehdi Rahimi-Nasrabadi⁶,⁸,⁹

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Abstract
Azaperone is a very important phenylbutanone-based neurotransmitter used in the treatment of some animal (veterinary) clinics. This compound has various nerve and tendon stabilizing agents on livestock and animals. Muscular injection of azaperone is used to reduce stress in livestock and reduce their acting. In the present work, Fe₃O₄@SiO₂-NH₂-CS₂ nanocomposite was synthesized and thoroughly characterized using FE-SEM, FT-IR, and XRD technique. The glassy carbon electrode was then modified with nanocomposite to fabricate a sensor named GCE/Fe₃O₄@SiO₂-NH₂-CS₂. The application of modified electrode was tested for analysis of azaperone in ostrich meat and rat plasma. The electrochemical behavior of azaperone was tested using differential pulse and cyclic voltammetry. In Briton–Robinson buffer solution (pH = 6), azaperone had an oxidation peak at 0.82 V. Cyclic voltammetry studies indicated that the azaprone oxidation process on the modified electrode is irreversible. Experimental variables, including pH and accumulation time were optimized by monitoring the cyclic voltammetry responses toward azaperone peak current. Measurement of azaperone by differential pulse voltammetry technique showed linearity of anodic peak current vs. azaperone concentration in a range of 0.01–100.0 μM with detection and quantification limits of 3 nM and 10 nM, respectively. Also, the effect of disturbance of some species as possible interferers on the electrochemical response of azaperone was checked out. Finally, the capability of the fabricated sensor for azaperone measurement was successfully tested in ostrich meat and rat plasma as real samples.
Introduction

The history of the use of nanostructures in electrode surface modification dates back to the early 1990s. These structures were initially used to improve the electrochemical signal owing to their large surface area. In most cases, thanks to the increase in the effective surface area of the electrode due to modification with nanostructures, detection limit and the method sensitivity increases. Among different nanostructures, metal oxide nanoparticles are used more as modifiers [1–3]. The synergistic role of nanoparticles properties along with their catalytic properties has led to the introduction of a new generation of electrochemical sensors and biosensors. Many electrochemical reactions are facilitated by reducing the overvoltage in the presence of catalytic nanoparticles. This property of nanoparticles can also affect the reversibility of some oxidation–reduction reactions, which are typically irreversible on the unmodified electrode [4, 5].

In the present study, Fe$_3$O$_4$ and its nanocomposites based on silicate were used as a modifying agent of GCE (glassy carbon electrode) to measure low amounts of azaperone. Depending on the application of iron oxide nanoparticles, two categories of non-enzymatic sensors have been designed [6–13]. One group is non-enzymatic sensors in which pure or functional nanoparticles act as sensitive phases, and the other group acts as intermediates [14–16]. Silica has a special place in electrochemical applications with its unique properties such as high adsorption capacity, very high specific surface area and dramatic thermal stability. The most important drawback of silica is its non-conductivity [17, 18]. Therefore, pure silica cannot be used to make electrodes. To overcome these limitations, other nanoparticles such as iron oxide can be used as nanocomposites to improve the properties of Si-based sensors [19]. One of the most important applications of silicate is the improvement of chemical stability and the dispersion of Fe$_3$O$_4$ by creating a protective layer [20]. The role of SiO$_2$ film as ideal and suitable coatings for Fe$_3$O$_4$ nanoparticles is attributed to its chemical stability, compatibility, and reactivity with several binders. The outer silica shell stabilizes Fe$_3$O$_4$ nanoparticles in solution.
as well as provides reactive sites on its surface for functionalization [21, 22]. Amine groups are one of the active functional groups that can lead to the functionalization of the aforementioned SiO$_2$ surface or silicate shell through the reaction of aminomethyl triethoxysilane with the silica surface [23, 24]. In the following, other additives (like S-contents compounds) and compression reactions can be performed on this amino nucleophile group to reach the desired level [25]. Sulfur atoms are a very good choice in sensor modification. Sulfur atoms with strong adsorption on the electrode surface and creating a thin layer cause interaction with target analytes, which in turn leads to better adsorption and faster catalysis of oxidation and reduction processes [26].

Azaperone as pyridinyl piperazine and butyrophenone neuroleptic drug is mainly used as a sedative in the field of veterinary medicine. This drug facilitate venipuncture and induce anaesthesia in ostriches. In lieu of providing a whole day sedative for birds transferring and/or getting them used to the new location, Azapron provides a little muscle relaxation. It is also used to prevent the breeding and grafting of pig breeds in the time of transportation to the abattoir. Pigs are very sensitive to stress syndrome, which leads to high mortality and low-quality meat called pale soft exudative. Azaperone injection in pigs reduces blood pressure and CO$_2$ pressure at all dose levels. A maximum residue limit for azaperone, when administered to pigs, has been established by the European Medicines Agency [27]. Azaperone was approved for use in pigs in the USA in 1983, under NADA 115–732 [28]. Azaperone is often used in combination with other drugs to manage animal excitement and/or to capture or treat animals. Different doses of a mixture of botophanol-azaperone-medometidine are used to immobilize and capture deer, elephants, sheep, and others. Therefore, to ensure that the meat of these animals meets the required standards or not, it is important to determine the residues of azaperone in the tissues and biological fluids of animals, especially animals in the human food chain. Various articles have been published for the determination of azaperone in different samples, including ELISA [29], chromatography and fluorescence [30, 31].

Although they have low detection limits and sensitivity, many factors limit their use, like their high cost, complexity, and time. These days, electrochemical sensors have revolutionized sensing systems due to their excellent selectivity and sensitivity, simple operation, time-saving, and low cost [32, 33]. To the best of our knowledge, there is only two paper on the electrochemical measurement of azaperone in the literature [34, 35]. Therefore, in this research work, to increase the sensitivity of the GCE in the detection of azaperone, its surface was modified with a magnetic core–shell based on modified SiO$_2$.

### Experimental

#### Chemicals and solutions

Azaperone, iron (III) chloride, phosphoric acid, iron (II) chloride, tetraethoxysilicate, potassium chloride, acetic acid, potassium hexacyanoferrate(II), sodium hydroxide, (3-aminopropyl)triethoxysilane, potassium hexacyanoferrate (III), boric acid and carbon disulfide were purchased from Merck Company (analytical grade, Germany). The Briton–Robinson buffer solution (0.04 M) was used as the electrolyte. Mix potassium hexacyanoferrate (III) and potassium hexacyanoferrate (II) (1.0 mM) containing potassium chloride was used as the redox probe.

#### Apparatus

The nanocomposite and other nanoparticles morphology was studied by XRD, FE-SEM, FT-IR, TEM and impedance electrochemical spectroscopy (EIS). The µ-Autolab with nova software and FRA using a three-electrode configuration with the application of the Pt and Ag/AgCl was used for all electrochemical tests. The bare and modified GCE were used as the working electrodes.

LC–MS/MS analysis was performed using an HPLC (2695 Waters, Milford, MA) separation system using a C$_{18}$ column (USA, Agilent). The MS detector probe was run under the following conditions: cone spray 50 L/h, desolvation flow 600 L/h, positive ES mode, extractor voltage + 3 V, spray voltage + 3 kV, collision voltage + 20 V, con voltage + 30 V, capillary voltage + 2 kV, desolvation temperature 100 °C, and source temperature 300 °C. The pressure of Ar collision gas was set to 0.2 Pa and the MS/MS transition tuning was performed with the analytes infusion (1 ppm, 30 μL/min). A direct infusion technique was utilized for the optimization of the MS and MRMs conditions for quantitative analysis of the compounds. The 328.4→165.3 and 328.4→121.4 transitions were monitored as quantifier and qualifier transitions ions, respectively [36].

#### Fe$_3$O$_4$ nanoparticles preparation

The co-precipitation method was used to prepare magnetite nanoparticles, using a mixed iron (II) chloride and iron (III) chloride. FeCl$_2$·4H$_2$O (3.0 g, 0.015 mol) was completely dissolved in 20 mL water to prepare solution S1. 6.0 g of FeCl$_3$·6H$_2$O (0.22 mol) was solved in 30 mL of water to prepare solution S2. Solution S2 was added to solution S1 and the mix was stirred for 45 min at 70 °C. The pH of the solution in the same hot condition was reached 10 by adding ammonia solution (25%) and a black precipitate was
obtained. The solution was stirred at 60 °C for more than 2.5 h. The resulting precipitate was thoroughly washed with water and ethanol and finally dried in an oven for 8 h after separation with a magnet [3].

**Magnetic silica preparation**

Stober method was employed for the preparation of magnetic silica (Fe$_3$O$_4$@SiO$_2$). A solvent mixture of ethanol and distilled water is added to 2.2 mol (0.5 g) of synthesized magnetite nanoparticles. The magnetite mixture was sonicated for 15 min. 0.2 mL of tetraethoxy orthosilicate and then 5 mL of sodium hydroxide (10% W/V) were added to the mentioned solution, then stirred for 1 h. The resulting precipitate is washed three times with water: ethanol solution and placed in an oven for 10 h at 80 °C [37].

**Magnetic silica-NH$_2$ preparation**

0.2 g of magnetic silica was added to a solution of 0.2 mL (3-aminopropyl) triethoxysilane, in 4.8 mL of dry toluene. Fe$_3$O$_4$@SiO$_2$ was then dispersed in a perfect uniform solution for 15 min. The resulting mixture was refluxed at 110 °C for 24 h. The resulting product was washed three times with water: toluene and finally, three times with ethanol. The resulting precipitate was collected by magnets and oven-dried for 12 h at 80 °C [37].

**Magnetic silica-NH$_2$/CS$_2$ preparation**

3 mL of 3-amino propyl trimethoxysilane and carbon disulfide (0.4 mmol, 0.74 ml) were added and allowed to be stirred for 15 min. Then, 0.5 g of magnetic silica and desired amount of DMF were added to the resulting mixture and refluxed for 24 h [38].

**GCEs surface modification**

To achieve the mirror surface of the electrode, GCE was polished with Al$_2$O$_3$ (≤ 0.05 µm) powder following by rinsing with distilled water and ethanol as well as water and acetone for several min and then washed with water. For further cleaning by electrochemical method, the GCE was placed in sulfuric acid (0.5 M) solution as an electrolyte and was scanned (with ten scans) in the potential range from −1.5 to 1 V and a scan rate of 100 mV s$^{-1}$. Finally, the electrode was washed with distilled water to prepare for modification.

To modify the GCE, 3 μL of homogeneous suspension of Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ nanocomposite (3.0 mg/mL) in water was dropped on the GCE surface. The electrode was dried in an oven at 50 °C. Therefore, GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ was prepared. Other modified electrodes with Fe$_3$O$_4$, Fe$_3$O$_4$@SiO$_2$, and Fe$_3$O$_4$@SiO$_2$-NH$_2$ were prepared in the same way.

### Real samples analysis

2.0 g of fresh ostrich meat samples were grounded and mixed in a Briton–Robinson buffer solution in a ratio of 1:10 (v/v). After stirring (20 min) and centrifugation (15,000 rpm for 120 min), the supernatant was separated and stored for analysis [39]. Blood serum samples were mixed thoroughly with 2% methanol solution (v/v) following by mixing and centrifugation for 10 min (> 5000 rpm). The protein agglomerations were separated from the solution. The supernatant solution was diluted ten times with Briton–Robinson buffer and used for analysis.

### Result and discussion

#### Characterization

Figure 1a shows the FT-IR analysis of nanoparticles and nanocomposite. The peak appear at 3425 cm$^{-1}$ features the O–H stretch bond adsorbed at the Fe$_3$O$_4$ nanoparticles surface. Furthermore, Fe–O vibrational bond of Fe$_3$O$_4$ occurred at 567 cm$^{-1}$ and 1384 cm$^{-1}$. The absorption bands at 488 cm$^{-1}$, 587 cm$^{-1}$, and 804 cm$^{-1}$ are linked to the vibration of the Fe–O–Fe group. The peak at 1085 cm$^{-1}$ is ascribed to symmetric Si–O–Si stretching vibrations implying successful silica coating on the Fe$_3$O$_4$. The presence of a peak at 3404 cm$^{-1}$ is attributed to the O–H vibration of the water absorbed on the surface. Also, Fig. 1 shows the main absorption bands of hydroxyl (OH) at 3470 cm$^{-1}$ and Fe–O–Fe groups at 590 cm$^{-1}$.

The broad absorption bands at 2850–2920 cm$^{-1}$ and 1626 cm$^{-1}$ were related to the N–H stretching vibration and NH$_2$ bending of APTES as coupling agents, respectively. Comparison between the Fe$_3$O$_4$@SiO$_2$–NH$_2$ and Fe$_3$O$_4$@SiO$_2$–NH$_2$/CS$_2$ FT-IR spectra reveals the presence of dithiocarbamate groups in Fe$_3$O$_4$@SiO$_2$–NH$_2$/CS$_2$ nanocomposite corresponding to the disappearance of the 3436 cm$^{-1}$ stretching band and apparition of the 2100–2170 cm$^{-1}$ and 2900–3600 cm$^{-1}$ broad bands [37, 38, 40].

The XRD patterns of the Fe$_3$O$_4$@SiO$_2$–NH$_2$/CS$_2$, Fe$_3$O$_4$@SiO$_2$, Fe$_3$O$_4$@SiO$_2$–NH$_2$, and Fe$_3$O$_4$ are shown in Fig. 1b. The typical peaks of Fe$_3$O$_4$ were detected at 2θ = 62.94°, 57.25°, 43.26°, 35.62°, and 30.38°. The diffraction peaks in the range of 2θ = 20–30 are due to the amorphous state of the SiO$_2$ shells, which proved the silica coating on the iron oxide nanoparticles [38, 40, 41]. The diffraction patterns of Fe$_3$O$_4$@SiO$_2$, Fe$_3$O$_4$@SiO$_2$–NH$_2$, and Fe$_3$O$_4$@SiO$_2$–NH$_2$/CS$_2$ were in agreement with the XRD pattern of Fe$_3$O$_4$@SiO$_2$.

This displayed that the Fe$_3$O$_4$ nanoparticles remained the
The morphology of Fe₃O₄ nanoparticles after functionalizing with SiO₂, NH₂, and CS₂ was studied by FE-SEM (Fig. 2). As shown in the images, the formation of nanoparticles is approximately uniform and in a spherical shape, with homogeneous dispersion. In other words, morphological features of the nanoparticles cannot be markedly changed during the functionalization of Fe₃O₄@SiO₂-NH₂ with the NH₂ group. The FE-SEM image of the Fe₃O₄@SiO₂-NH₂/CS₂ nanocomposite is different from the Fe₃O₄@SiO₂ and Fe₃O₄@SiO₂-NH₂ images. White spots of sulfide on the surface of the composite are shown in Fig. 2. Such a difference can be attributed to the presence of CS₂ on the surface Fe₃O₄@SiO₂-NH₂. In the SEM image of the nanocomposite (Fig. S1), completely spherical nanoparticles with a medium size of ~20–40 nm are observable. The chemical composition of the Fe₃O₄@SiO₂-NH₂/CS₂ nanocomposite was determined by EDS as shown in Fig. 2. The results revealed 35.20% O, 37.25% Si, 11.54% Fe, 9.55% S, 4.93% C, and 1.54% N according to the expected values for the approval of the chemical structure of Fe₃O₄@SiO₂-NH₂/CS₂ [38].

The Fe₃O₄@SiO₂-NH₂/CS₂ nanocomposite morphology and size were also established by TEM. The TEM images of Fe₃O₄@SiO₂-NH₂/CS₂ nanoparticles at different magnifications are shown in Fig. 2. As stated in this figure, the nanoparticles had a spherical morphology and aggregated. In these images, the core of the nanoparticles (Fe₃O₄) is seen as a dark area and the lighter regions are related to the shell structure of (SiO₂-NH₂/CS₂) nanoparticles [38].

**Electrochemical behavior of modified electrode**

The electrochemical behavior of the modified electrode was evaluated using the EIS technique. Therefore, the EIS properties of GCE, GCE/Fe₃O₄, Fe₃O₄@SiO₂-NH₂, and GCE/Fe₃O₄@SiO₂-NH₂/CS₂ in a solution of 1.0 mM redox pairs in the 0.1 Hz to 10 kHz range, were recorded and shown as the Nyquist plot − Z″ vs Z′ plot in Fig. 3a. In the Nyquist plot of unmodified electrode, a large semicircular section is observed at high frequencies. The results show high charge transfer resistance (Rct) according to the reduced charge transfer rate and mass at the surface of the modified electrode. By modifying the electrode with Fe₃O₄@SiO₂-NH₂/CS₂ nanocomposites, the semicircle was smaller (0.12 kΩ) at high frequency, than the GCE (2.67 kΩ), GCE/Fe₃O₄ (1.45 kΩ) and Fe₃O₄@SiO₂-NH₂ (0.67 kΩ). In fact, with the presence of the above modifiers, the kinetics of electron transfer on the electrode has increased and this reduces the load transfer resistance. In the case of the modified Fe₃O₄@SiO₂-NH₂/CS₂ electrode, there is a very sharp increase in charge transfer, which can be related to the high conductivity of the modifier [42–46].

Cyclic voltammograms (CVs) of the modified electrodes in 1 mM [Fe(CN)₆]³⁻/⁴⁻ in 0.1 M KCl were evaluated to investigate the surface characteristics of these electrodes (Fig. 3b). As shown in Fig. 3b, a pair of reversible peaks of [Fe(CN)₆]³⁻/⁴⁻ are appeared with peak separation of 160 mV and 220 mV at the GCE/Fe₃O₄, and GCE surfaces, respectively. At the surface of the Fe₃O₄@SiO₂-NH₂ modified electrode, ΔEp is equal to 120 mV and at the modified electrode with Fe₃O₄@SiO₂-NH₂/CS₂ in addition to decreasing ΔEp to 90 mV, peak current also increased, due to the better conductivity and electron transfer at the presence of Fe₃O₄@SiO₂-NH₂/CS₂. Therefore, these voltammograms confirm the placement of Fe₃O₄@SiO₂-NH₂/CS₂ on the surface.

**Modified electrodes surface area calculation**

Equation 1 was used to calculate the effective surface area of the electrode (A) which unit is cm². For this purpose,
CVs of redox probes with a penetration coefficient of $7.5 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ were used [42].

$$I_p = 2.69 \times 10^5 n^{3/2} AC (D_o)^{1/2}$$

(1)

The GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ (0.211 cm$^2$) surface area is larger than that of GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$ (0.170 cm$^2$), GCE/Fe$_3$O$_4$@SiO$_2$ (0.123 cm$^2$), GCE/Fe$_3$O$_4$ (0.093 cm$^2$), and GCE (0.069 cm$^2$) which can be related to the high specific surface area of the compound.

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**Investigation of the electrochemical behavior of azaperone**

Oxidation of azaperone was studied on the surface of GCE, GCE/Fe$_3$O$_4$, GCE/Fe$_3$O$_4$@SiO$_2$, GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$, and GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ to investigate the catalytic role of the modifiers. The GCE voltammogram in Briton–Robinson buffer solution (0.04 M, pH = 7.0) was plotted as the underlying electrolyte in the presence of 50.0 µM azaperone. The observed oxidation peak at 0.82 V (Fig. 3c), is indicating the electroactive behavior of the
azaperone at the surface of GCE. Then, the voltammograms of 50.0 μM azaperone were recorded on the GCE, GCE/Fe₃O₄, GCE/Fe₃O₄@SiO₂, GCE/Fe₃O₄@SiO₂-NH₂, and GCE/Fe₃O₄@SiO₂-NH₂-CS₂ surfaces in Britton–Robinson buffer solution (pH = 6.0). In the presence of the modified electrodes, compared to the bare GCE, a significant increase in peak current was obtained. Also, the onset of oxidation potential has changed from ~0.68 V relative to the GCE electrode to ~0.56 V in modified GCE with Fe₃O₄@SiO₂-NH₂-CS₂. The decrease in azaperone overvoltage, as well as a large increase in the oxidative current, are related to the excellent catalytic activity of the modified Fe₃O₄@SiO₂-NH₂-CS₂ electrode.

**Effect of pH**

The influence of the azaperone electrochemical behavior in different pH was examined by cyclic voltammetry in the pH
range between 2 and 9 using Britton–Robinson buffer. The highest sensitivity was achieved at pH = 6.0 (Fig. 3d). As the pH of the buffer solution increases, displacement in the anodic peak potential is observed (Fig. 3f). The curve of the anodic peak potential vs. pH indicates a linear relationship with a slope of about 53 mV. Accordingly, electrons number and protons participating in the azaperone oxidation process is equal (mechanism in scheme 1) [42].

**Effect of potential scan rate on azaperone electro-oxidation**

Investigation of the potential scan rate on the oxidation of azaperone in the range 10–400 mV s⁻¹ shows a linear relationship between the anodic peak current logarithm and the scan rate logarithm with a slope of 0.9 (Fig. 4b), which indicates the adsorption is dominant in the oxidation process of azaperone. The adsorbed species is oxidized on the surface of the electrode. The Tafel curve was also plotted as potential vs. logarithm of current at a scan rate of 10 mV (Fig. 4c). The electron transfer coefficient for an oxidation species is obtained by Eq. 2 [42].

\[
\text{Slope}_{\alpha} = \frac{2.3RT}{(1 - \alpha)n_F}. 
\]  

(2)

In this relation, the general constant of gases \(R\) is equal to 8.314 kJ / mol K, \(T\) is the ambient temperature in K, \(\alpha\) is the electron transfer coefficient and \(F\) is the Faraday constant.

**Accumulation time effect on the voltammetric response of azaperone**

The significant increase in azaperone electro-oxidation peak current at the surface of the modified electrode is more attributed to the ability of azaperone to be adsorbed by GCE/Fe₃O₄@SiO₂-NH₂/CS₂ surface. Therefore, optimization of accumulation conditions can affect the sensitivity of the measurement. Consequently, the adsorption time of azaperone on the surface of the modified electrode was investigated by cyclic voltammetry. As the pre-concentration time increases, the anodic peak current increases rapidly (until the 5 min). While with increasing pre-concentration time, very little change is observed in the anodic peak current. Figure 4e shows a peak current of 50.0 μM azaperone in Britton—Robinson 0.04 M (pH = 7.0) at different times on the surface of GCE/Fe₃O₄@SiO₂-NH₂/CS₂. According to the results, 5 min was applied as optimal pre-concentration time for quantitative measurements under open-circuit conditions to achieve greater sensitivity.
Chronoamperometry

The diffusion coefficient of azaperone at the surface of the GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ in Britton–Robinson buffer solution (pH = 6.0) is calculated by the Cottrell equation and using chronoamperometry in a stationary solution. Figure 5 shows the chronoamprograms at 0.95 V potential for 0.1, 5.0, 15.0, 30.0, 60.0, and 75.0 µM of azaperone. As can be seen, the current drop at constant times is almost the same for all concentrations. The diffusion coefficient $D = 4.52 \times 10^{-6}$ cm$^2$s$^{-1}$ was obtained from the diagram’s slope of the current versus squared second. In Cottrell relation, $A$, $n$, $C$, $F$ are the electrode surface area, the number of electrons, concentration, and Faraday constant, respectively [42].

$$I = nFAD^{1/2}/\pi^{1/2}t^{1/2}.$$  

(3)

Another parameter calculated in this section was the electrocatalytic reaction rate ($K_{cat}$) of azaperone on the GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ surface. To this purpose, the ratio of catalytic current in the presence ($I_{cat}$) and absence ($I_0$) of azaperone was obtained by Eq. 4 [42] employing the slope of the current–time diagram. The obtained value was $4.9 \times 10^5$ cm$^3$mol$^{-1}$s$^{-1}$, which demonstrates the electrocatalytic reaction.

**Fig. 4** a CVs of 50.0 µM Azaperone solution on the GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ surface in the presence of Britton–Robinson buffer electrolyte solution 0.04 M at pH = 6.0, scan rates from up to down are 10 to 400 mV s$^{-1}$, b Log I versus Log $\nu$, c Potential changes with the logarithm of current a scan rate of 10 mV s$^{-1}$, d CVs of azaperone at different times and e anodic peak current curve in terms of pre-concentration time in 50.0 µM solution of azaperone.
Fig. 5  a Azaperone chronoamperometry in different concentration on the GCE/Fe₃O₄@SiO₂-NH₂/CS₂ surface, b current plot versus time square, c slopes of figure b versus of different concentrations of azaperone, and d plot of \( I_{cat}/I_{l} \) versus time square, e differential pulse voltammograms for different concentrations of azaperone on the GCE/Fe₃O₄@SiO₂-NH₂/CS₂ surface (from top to bottom: 0.01–100.0 μM), f The linear calibration curve of peak current versus azaperone on the surface of modified electrode

Table 1  Determination of azaperone in real samples

| Sample         | Spiked (μM) | Developed method | HPLC–MS/MS | \( t \) statistical |
|----------------|-------------|------------------|------------|-------------------|
|                | Obtained (μM) | Recovery (%) | RSD % (n = 4) | Obtained (μM) | Recovery (%) | RSD % (n = 4) | p (t value) |
| Ostrich meat   |             |              |             |                 |              |             |             |
|                | <LOQ        | –               | –           | <LOQ           | –               | –           | –           |
| 0.50           | 0.42        | 82.00          | 3.93        | 0.45           | 92.00          | 4.1         | –2.4        | 0.18        |
| 5.00           | 4.30        | 86.00          | 4.12        | 4.43           | 88.67          | 2.9         | –0.76       | 0.71        |
| 10.00          | 9.11        | 91.00          | 3.64        | 9.28           | 92.80          | 3.4         | –0.74       | 0.36        |
| Rat plasma     | 0           | 0.09            | 4.81        | 0.095          | –               | 2.5         | –           | –           |
|                | 0.50        | 0.53            | 90.00       | 4.62           | 0.52           | 84.00       | 4.4         | 1.31        | 0.47        |
|                | 5.00        | 5.01            | 96.40       | 4.84           | 4.72           | 92.40       | 3.1         | 1.50        | 0.25        |
|                | 10.00       | 9.80            | 97.10       | 4.41           | 10.12          | 100.03      | 2.8         | –1.26       | 0.08        |
of azaperone is associated with an excellent electron transfer rate at the modified electrode surface.

$$\frac{I_{\text{cat}}}{I_{l}} = \pi^{1/2} \left( K_{\text{cat}} C_0 l \right)^{1/2}.$$  \hfill (4)

**Analytical function of the modified electrodes for the azaperone determination**

To evaluate and determine the low values of azaperone concentrations on the GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ surface, differential pulse voltammetry was used (Fig. 5e). By increasing concentration, the oxidation peak current increases and the peak potential remains constant. In Fig. 5e, the differential pulse voltammograms are in the range of 0.01 to 100.0 μM, and the calibration diagram is shown in Fig. 5f. The linear relationship of this concentration and current is given in Eqs. 5 and 6:

$$I_{p,a}(\mu A) = 5.8392 \left( \mu M \right) + 4.2497 \left( R^2 = 0.9909 \right) \left( 0.01 - 1 \text{ mM} \right)$$  \hfill (5)

$$I_{p,a}(\mu A) = 0.5998 \left( \mu M \right) + 11.122 \left( R^2 = 0.9938 \right) \left( 1 - 100.0 \text{ mM} \right)$$  \hfill (6)

The detection limit of the developed method (LOD = 3S/m) was calculated as 3 nM. Table S1 compares the obtained results for measuring azaperone utilizing the proposed method with previously reported papers, which shows that the proposed method has lower or at least comparable detection limit to other analytical methods.

**Disturbance effect of various species on azaperone measurements**

The disturbance effect of disposed of species on the electrochemical response of 15.0 μM azaperone was checked out. The results revealed that in the presence of the concomitants such as citric acid, ascorbic acid (100.0 μM), iron sulfate, sodium hydroxide, dopamine (100.0 μM), l-cysteine, copper nitrate, uric acid (100.0 μM), and potassium cyanide, there is no interference effect on the simultaneous determination of azaperone. The results showed the deviation on the peak current in the presence of the investigated interferents is less than 5%, which confirms the selectivity of the proposed method in the azaperone measurement (Fig S2).

**Stability and repeatability of the modified electrode**

As an important parameter, the repeatability of the breeding process was investigated. The five electrodes were made separately and used to analyze 25.0 μM of azaperone. The relative standard deviation for the oxidation peaks was 2.52% (of five electrodes), which confirms the repeatability of the modification procedure. Stability over time for GCE/Fe$_3$O$_4$@SiO$_2$-NH$_2$/CS$_2$ was also investigated by placing the modified electrode in buffer medium under normal conditions for 30 days and analyzing the 25.0 μM of solution. The observed slight decrease in peak current (~ 3.45%), approves the long-term stability of the fabricated sensor.
Also, GCE/Fe3O4@SiO2-NH2/CS2 repeatability was measured by analysing 25.0 μM azaperone solution on electrode and re-modification after each analysis. Also good repeatability was observed for this electrode so that for six measurements the percentage of relative standard deviation (RSD) was calculated to be 2.26%.

**Measurement of azaperone in real sample and comparison with HPLC–MS/MS results**

The standard addition method was utilized in azaperone analysis of the ostrich meat. Table 1 represents relative recovery values obtained from the differential pulse voltammetry for added azaperone solution to the ostrich meat sample. The recovery values for concentrations of 0.5, 5.0, 10.0 μM were 82, 86 and 90%, respectively. The results show the applicability of the developed electrode in the measurements of azaperone in the ostrich meat sample. These experiments were performed for rat plasma samples and the results are presented in Table 1.

Finally, the obtained standard addition results in the real samples from the developed electrochemical technique were compared to the results of the HPLC–MS/MS method (Table 1). The results of t test at 95% confidence level show a good agreement between the two methods. The t values are smaller than the tabulated value (t_{0.95} critical = 2.353) in spiked samples. A typical chromatogram of rat plasma sample is shown in Fig. 6.

**Conclusion**

The design of new electrochemical sensors plays an important role in the diagnostic process and has a wide range of modifications for nanoscale materials that can be useful for portable clinical medical diagnostic devices. In this study, GCE modified with GCE/Fe3O4@SiO2-NH2/CS2 along with voltammetry method (cyclic and differential pulse) and chronoamperometry were utilized to measure azaperone in ostrich meat and rat plasma sample. The GCE/Fe3O4@SiO2-NH2/CS2 exhibited a vast linear quantification range (0.01–100.0 μM) and low detection limit (3 nM). The results of relative recovery tests showed that the developed method was useable for real samples with acceptable accuracy. In addition to the good stability of the sensor, the simplicity and ease of modification, cheapness, and availability of equipment for modifier synthesis and the short time required to analysis are the other benefits of this method.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human or animal subjects.

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