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A genosensor based on CPE for study the interaction between ketamine as an anesthesia drug with DNA

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ABSTRACT

The electrochemical oxidation of ketamine as an analgesia and anesthesia drug and its interaction with DNA was studied at carbon paste electrode (CPE) using voltammetric techniques. Ketamine showed one irreversible oxidation peak nearly around +1.14 V vs. Ag|AgCl|KCl (3 M) only in Britton buffer (pH 7.00). The effect of scan rate on the cyclic voltammetric behavior of ketamine was investigated at the CPE and binding constant of ketamine and DNA was also calculated. The binding mode of DNA and ketamine was elucidated by differential pulse voltammetry and UV–vis spectroscopy techniques. Based on these results, interaction between ketamine and single-stranded DNA was of electrostatic mode, while between double-stranded DNA and ketamine was of groove binding. Ketamine showed a special affinity toward guanine bases of DNA. Also, ketamine was employed as an electrochemical indicator for detection of DNA hybridization. The difference between the oxidation peak current of the DNA probe modified CPE in the presence and absence of ketamine (ΔI) was enhanced with increasing ketamine concentration and a detection limit of 1.98 nM was evaluated. To further investigate the selectivity of this biosensor, some noncomplementary sequences were used. Finally, the proposed method was successfully used for voltammetric determination of ketamine in real samples.

1. Introduction

Ketamine (2-(2-chlorophenyl)-2-(methylamino)cyclohexanone) (Scheme 1) is a drug and a phencyclidine derivative. Side effects of ketamine are extended in humans, including anesthesia, hallucinations, elevated blood pressure, bronchodilation and it provides sedation/analgesia for chronic pains through blockade of N-methyl-D-aspartate (NMDA)-type glutamate receptors [1,2]. Commercial ketamine is a racemic mixture of two enantiomers; R-ketamine and S-ketamine. In humans, the more active enantiomer is S-ketamine or ketanest S that is twice as anesthetic as racemic ketamine [3,4]. The application of ketamine is not limited to medical usage and pharmaceutical preparations. It is increasingly being used in the parties as a "club drug" due to its anesthetic properties [5,6]. Ketamine abuse results a state of "dreamy intoxication", blurred vision and out of body experiences [7–12]. Recently, interaction of DNA and drugs has been a growing attractive topic in the electrochemical investigations [13–16]. For small molecules such as drugs, mechanism of interaction as well as evaluation of binding constant can be investigated by electrochemical studies.

Genosensors (or DNA biosensors) are consisting of a single-stranded (ssDNA), as a gene probe fixed on transducer surface. They are mostly applied for recognition of specific target DNA sequence upon hybrid formation of probe with complementary target sequence [17,18]. DNA hybridization biosensors can be employed for early and precise recognition of infectious agents in various environments [19,20]. In fabrication of genosensors, carbon paste electrodes (CPEs) became popular due to the regeneration of surfaces without memory effects, their ease of preparation and cost effectiveness [21–23]. Herein, CPE, as a transducing system, was used to achieve information about the interaction between DNA and ketamine. To the best of our knowledge, this is a first time that ketamine was used as an electrochemical indicator for fabrication of a genosensor and evaluation of its interaction with DNA by electrochemical and spectroscopic methods in biological pH. Thereafter, this biosensor was employed for determination of ketamine in serum samples. The DNA sequence used as a probe in this work belonged to a short sequence of p53 gene which has been considered to be one of the most important tumor suppressor genes that are frequently mutated in human cancers. This is due in part to the fact that over 50% of human cancers carry loss of function mutations in p53 gene [24,25]. To investigate whether ketamine

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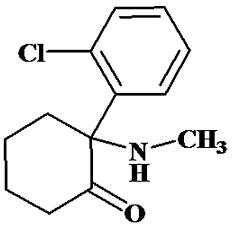


Scheme 1. Structure of ketamine.

has a specific affinity toward bases of DNA, cyclic voltammogram of ketamine was recorded in the absence and presence of the DNA sequence composed of pure bases. The hybridization was detected by differential pulse voltammetry method. Moreover, selectivity of the biosensor was investigated in binary mixture solution of complementary and noncomplementary sequences of target DNA. In the end, limit of detection was calculated for determination of ketamine at the surface of the genosensor.

2. Experimental

2.1. Reagents and materials

Ketamine was obtained from Sigma–Aldrich (AR grade). The stock solution of ketamine (10^{-2} M) was prepared using double distilled water and then diluted with 0.04 M Britton buffer solution (pH 7.00) and NaCl 0.1 M as a supporting electrolyte. Ketamine solution was stored in the refrigerator at 4 °C prior to use. The synthetic oligonucleotides were purchased from MWG-Biotech Company, with the following base sequences:

Probe DNA:	5′-TGGGGATGGAGAACT-3′
Complementary DNA:	5′-AGTTCTCCATCCCCA-3′
Noncomplementary DNAs:	(hpv5it): 5′-GTTACTGTTGTAGATACTAC-3′ (hil2): 5′-GGAGGAAGTGCTAAATTTAG-3′

The preparation of oligonucleotides stock solutions (10^{-2} M) was carried out using TE buffer solution (10 mM Tris–HCl, 1.0 mM EDTA, pH 8.00). The solutions of oligonucleotides were prepared by diluting their stock solution with 0.5 M acetate buffer solution (pH 4.80) containing 20 mM NaCl. All the experiments were carried out at room temperature in an electrochemical cell.

2.2. Instrumentation

AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, Netherlands) were used for electrochemical measurements. Three-electrode system was made up of a CPE as the working electrode, an Ag|AgCl|KCl (3 M) as the reference electrode and a platinum wire as the auxiliary electrode. A computing double beam UV-Vis spectrophotometer (CECIL 5000, elegant technology) with quartz cell was used to measure the absorbance.

2.3. Procedures

The whole procedures of DNA biosensor fabrication were demonstrated in Scheme 2.

2.3.1. Preparation of the CPE

The carbon paste electrode used as working electrode was fabricated as usual way with graphite powder and paraffin oil in a ratio of 70:30 (w/w) by hand mixing in a mortar. A portion of the resulting paste was filled firmly into a glass tube. A copper wire was used for the creation of electrical connection.

2.3.2. Electrochemical activation of CPE

The surface of the CPE was polished on a weighing paper and was washed with distilled water, then oxidized at an optimized potential of 1.5 V vs. Ag|AgCl|KCl (3 M) for 3 min in a 0.5 M acetate buffer solution (pH 4.80) containing 20 mM of NaCl without stirring.

2.3.3. Immobilization of DNA probe on CPE

Immobilization of the DNA probe at the electrochemical pretreated of CPE was achieved applying a potential of +0.50 V vs. Ag|AgCl|KCl (3 M) for 5 min in 1.0 μM probe in 0.50 M acetate buffer solution (pH 4.80) and 20 mM of NaCl with stirring [26–35]. Then, the electrode was washed with distilled water. Thereafter, CPE modified with ssDNA (ssDNA/CPE) was prepared.

2.3.4. DNA hybridization

The hybridization assay or double-stranded (dsDNA) helix formation was performed by immersing the ssDNA/CPE in hybridization solution (0.50 M of acetate buffer; pH 4.80) containing 1.0 μM of the target ssDNA sequence and 20 mM of NaCl with stirring through applying +0.50 V potential vs. Ag|AgCl|KCl (3 M) during 5 min. The electrode surface was washed with distilled water to eliminate the non-hybridized target ssDNA. This step was followed for hybridization of probe with noncomplementary sequences.

2.3.5. Accumulation of ketamine on CPE

Ketamine was accumulated on bare CPE, ssDNA/CPE before and after hybridization with complementary and noncomplementary sequences by immersing the working electrode into stirred ketamine solution (10^{-3} M) containing 0.1 M of NaCl in open circuit potential for 5 min. The electrode was washed with distilled water after accumulation procedure.

2.3.6. Electrochemical investigation

Cyclic voltammetric studies were performed by scanning the working electrode potential between −0.3 and 1.4 V vs. Ag|AgCl|KCl (3 M) in ketamine solution (10^{-3} M) at scan rate of 100 mV s^{-1}. Differential pulse voltammetry measurements were carried out in 0.04 M of Britton buffer solution (pH 7.00) containing 20 mM of NaCl and scanning the working electrode potential between 0.6 and 1.35 V vs. Ag|AgCl|KCl (3 M) at pulse amplitude of 50 mV.

The raw data were treated using the Savitzky and Golay filter (level 2) of GPES software, followed by the GPES software moving average baseline correction using a "peak width" of 0.01. Repetitive measurements were carried out following renewing the electrode surface by cutting and polishing.

3. Results and discussion

3.1. Electrochemical oxidation of ketamine

The cyclic voltammogram of ketamine (10^{-3} M) in Britton buffer solution (pH 7.00) containing 0.1 M NaCl at the CPE is shown in Fig. 1. Ketamine represented an oxidation peak at 1.14 V vs. Ag|AgCl|KCl (3 M) in the potential range of −0.3 to 1.4 at potential scan rate of 100 mV s^{-1}. No reduction peak was obtained in the reverse scan of potential. As can be seen, the oxidation of ketamine at CPE is irreversible. In this work, the electrochemical behavior of ketamine was studied by cyclic voltammetry in the various buffer solutions such as phosphate (pH 7.00), acetate (pH 4.80) and Britton (pH 7.00 and 8.00). The results revealed the best cyclic voltammogram for ketamine was obtained only in Britton buffer and no signal is detected in other buffers. Our assumption is that the phenomena can be related to the materials containing Britton buffer which are H_3BO_3, H_3PO_4 and CH_3COOH. The only difference with two other kinds of used buffer is Boric acid that is

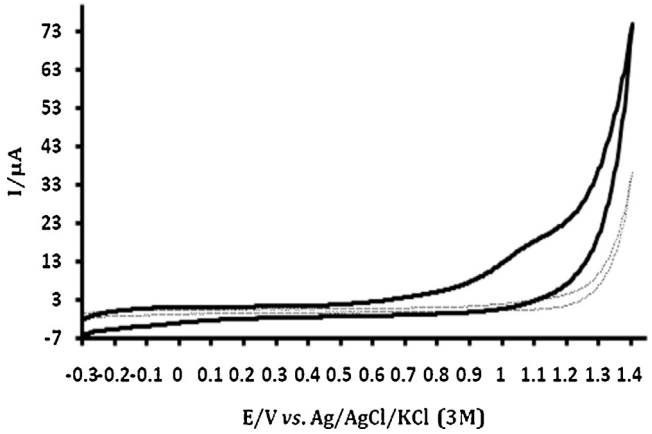


Fig. 1. Cyclic voltammograms of 10^{-3} M ketamine (solid line) in 0.04 M Britton buffer solution (pH 7.00) containing 0.1 M NaCl and blank solution (dotted line).

a weak, monobasic Lewis acid of boron. Therefore, the connection between boron atoms and ketamine is the only clue. It seems that the oxygen atoms in ketamine have high affinity to the unoccupied orbital of boron atoms. However, the amount of oxygen atoms accessible from water molecules are enough for such intermolecular interaction with boron atom and it can decline the chance of this interaction for very few oxygen atoms of ketamine molecules. Nevertheless, the electronegativity of oxygen atoms of ketamine is much bigger than of water molecules, because they are connected to a cycle that is attached to a nitrogen group and a benzene ring. This is the winning chance of ketamine that conquers the oxygen atoms of water.

The proposed mechanism for the oxidation of ketamine is illustrated in Scheme 3. In this reaction, the oxidation is a one-electron and one-proton process. In other words, one mole of ketamine loses an electron to yield a radical cation which further deprotonates to form a radical. Such types of radical cations are observed in the reported literature for oxidation of gemcitabine hydrochloride (GMB) [36–39]. However, GMB is different from ketamine in that GMB is a pyrimidine-base compound. Nevertheless, it has a NH_2 functional group similar to ketamine that has a main role in this proposed mechanism. In the next step, two molecules of radicals combined together rapidly to form a dimer product. Therefore, the final number of electron transfer in the whole procedure to produce one mole dimer is two electrons which is in agreement with the earlier study by Zhou et al. [40].

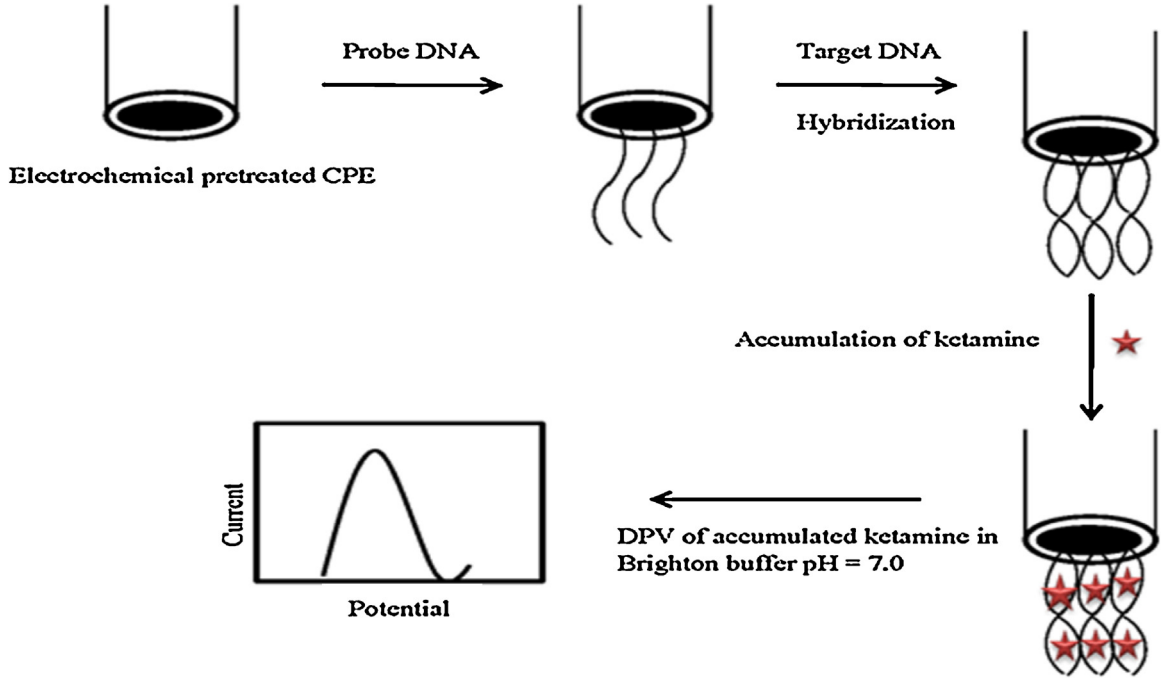
3.2. The effect of potential scan rate on the electrochemical behavior of ketamine

To investigate the effect of potential scan rate (ν) on the electrochemical behavior of ketamine, cyclic voltammogram of 10^{-3} M ketamine solution was recorded at CPE in scan rate of 10–180 mV s^{-1} in Britton buffer (pH 7.00). As shown in Fig. S1, the oxidation peak potential shifts to more positive potentials with increasing the scan rates of potential. Simultaneously, increasing of its oxidation peak current was also observed. These results were referred to electrochemical activity of ketamine. Inset of Fig. S1 shows the plot of i_p vs. $\nu^{1/2}$ provided a straight line, which is as expected for diffusion-controlled electrode process [41]. The electron transfer coefficient α for the irreversible-diffusion controlled reactions can be calculated from the difference between peak potential (E_p) and half wave potential ($E_{p/2}$). For this purpose, we used Eq. (1) [41]:

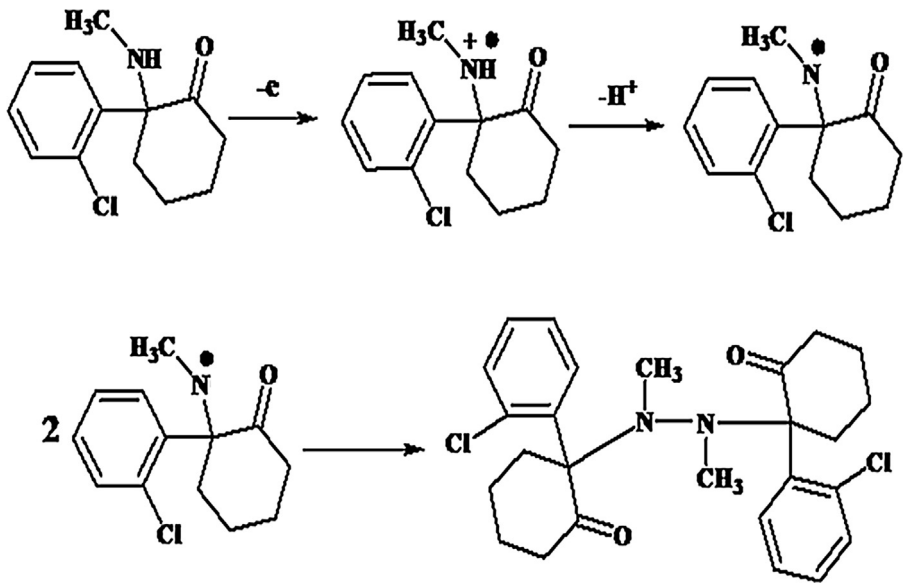
$$\Delta E_{pa} = E_p - E_{p/2} = \left(\frac{47.7}{\alpha}\right) \text{ mV} \quad \text{(diffusion-controlled, at 298 K)} \tag{1}$$

The value of α was calculated to be 0.54. Other parameters such as the number of electron transferred (n) and standard rate constant (k^0) for an irreversible anodic reaction were calculated from Laviron equation (Eq. (2)) [42]:

$$E_{pa} = E^{0'} - \left(\frac{RT}{(1-\alpha)nF}\right) \ln\left(\frac{RTk^0}{(1-\alpha)nF}\right) + \left(\frac{RT}{(1-\alpha)nF}\right) \ln \nu \tag{2}$$



Scheme 2. Procedure of DNA biosensor fabrication.



Scheme 3. Oxidation mechanism of ketamine.

where $E^{0'}$ is the formal potential of the anodic peak. The value of $E^{0'}$ was achieved from the plot of the E_{pa} vs. ν by extrapolation to the vertical axis at $\nu = 0$. The plot of E_{pa} vs. ln ν yielded the values of n and k^0 which were found to be 1 and 4.1 s^{-1}, respectively.

3.3. Electrochemical investigation of interaction between DNA and ketamine

There are three modes of interaction between DNA and small molecules such as drugs. These interactions involve intercalative binding, groove binding and electrostatic binding [43,44]. Among them, the electrostatic interaction occurs out of the groove of the DNA. Accordingly, the interaction capability would decrease in the presence of double strand DNA. In contrast, intercalative and groove interactions occur on DNA double helix. If a negative shift or positive shift occurs in peak potential, the interaction between DNA and molecules are considered to be electrostatic and intercalative, respectively [45]. The differential pulse voltammetry (DPV) technique was utilized to further study the interaction of DNA with ketamine, because it provides high sensitivity, high peak resolution and can reduce the background effects in studying the electrochemical behavior of biological systems. Fig. 2A shows the DPV signal of ketamine 10^{-3} M in absence (curve a) and presence of increasing concentration of ssDNA (curve b–e) in Britton buffer solution (pH 7.00). As it can be seen, after addition of ssDNA to ketamine solution, the oxidation peak current of ketamine increased and shifted to more negative potentials (from 1.14 V to 1.04 V). On the other hand, in Fig. 2B, ssDNA concentration is constant (10^{-6} M) and ketamine concentration is variable. The oxidation peak current of ssDNA decreased with increasing in added ketamine. As shown in Fig. 2A, the negative shift in oxidation peak potential of ketamine in the presence of ssDNA indicating the electrostatic interaction between ketamine and ssDNA [45]. As mentioned above, DNA shows an oxidation peak around 1.00 V that related to oxidation of guanine. In Fig. 2A, initially there is a ketamine solution in beaker and DNA is added step by step to the solution under stirring. Then, the DPV increases sequentially after addition of DNA. Our assumption is that with increasing the amount of DNA in the solution, its amount is increased in diffusion layer of the working electrode leading to an increase in the signal. In Fig. 2B, firstly we have DNA in beaker and is adding ketamine to the beaker step by step. Then the DPV decreases sequentially. In this case, we have enough DNA in the solution and there is a chance to form ketamine-DNA complex that is electrochemically inactive [36].

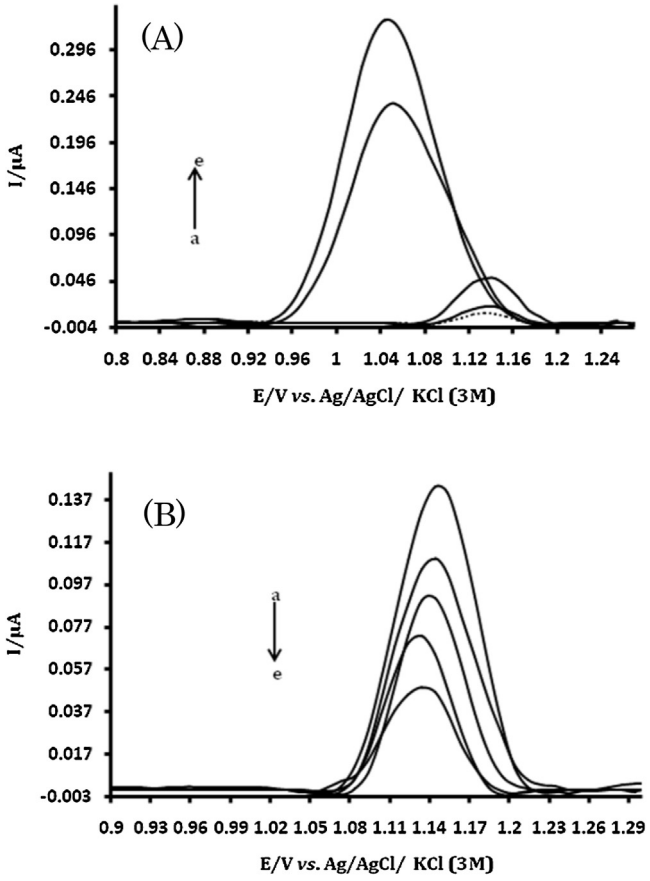


Fig. 2. Differential pulse voltammogram of (A) 10^{-3} M ketamine at CPE in the absence (a) and presence of 10^{-7} (b), 10^{-6} (c), 10^{-5} (d) and 10^{-4} M (e) ssDNA in Britton buffer (pH 7.00) and (B) 10^{-6} M ssDNA in the absence (a) and presence of 10^{-6} (b), 10^{-5} (c), 10^{-4} (d) and 10^{-3} M (e) of ketamine.

Various types of DNA base sequences were examined for further study. The ssDNA sequence used in this work belonged to a short sequence of p53 gene, which has seven guanines, four

adenines, three thymines and one cytosine. To understand whether ketamine has special affinity to different types of DNA bases, we have recorded cyclic voltammogram of ketamine in the presence of the DNA sequence composed of pure bases. Fig. S2 shows the cyclic voltammograms of CPE in the ketamine solution in presence of the DNA sequence involved only adenine (A), cytosine (B), thymine (C) and guanine (D). Dotted line in these figures is the cyclic voltammogram of each base sequence in the absence of ketamine. As it can be seen, guanine shows an oxidation peak at 1.07 V (Fig. S2D, dotted line); adding ketamine made this peak potential shift to 1.13 V and increase the peak current, which is probably due to specific affinity of ketamine toward the guanine base rather than other bases of DNA sequence. Therefore, the interaction between DNA and ketamine showed a dependency on the types of base.

3.4. Electrochemical detection of hybridization

To investigate the hybridization detection, the differential pulse voltammetry (DPV) experiments were employed with ketamine as an electroactive indicator on the surface of ssDNA/CPE in Britton buffer solution (pH 7.00) containing 20 mM of NaCl. Fig. 3 shows DPV signals of accumulated ketamine at the surface of (a) ssDNA/CPE, (b) after interaction of ssDNA with noncomplementary oligonucleotide (hpv5it), (c) after interaction of ssDNA with noncomplementary oligonucleotide (hil2) and (d) after hybridization of ssDNA with complementary target DNA sequence. The interaction among noncomplementary oligonucleotides and ssDNA/CPE lead to decrease in the oxidation peak current of ketamine, but as not well as complementary sequences, that may attribute to negligible hybridization. The results showed that the oxidation peak current of accumulated ketamine decreased in presence of DNA double helix, which is probably due to the prevention of the interaction between ketamine and guanine bases and lower accessibility of the guanine base for binding to ketamine in the double helix of DNA [46].

3.5. Selectivity study

To investigate the selectivity of the proposed biosensor based on ketamine electroactivity response via hybridization to the target sequence, the solution containing both complementary and noncomplementary sequences was used as a target sample. Fig. 3 curve e shows DPV of the accumulated ketamine at ssDNA/CPE after hybridization with complementary in a binary mixture solution of target DNA and hpv5it sequences. Comparing ketamine signal in the binary mixture, it can be observed that the signal was decreased after hybridization with a complementary sequence in the mixture solution of target and noncomplementary sequences. The interaction between these sequences in solution has a smaller effect on the hybridization event, suggesting that only the complementary sequence as the target DNA can interact with immobilized probe and subsequently cause an appreciable decrease in ketamine signal.

3.6. Spectroscopic investigation

Absorption spectroscopy was widely employed to monitor the mode of interaction and intensity binding of drug to DNA [47]. Fig. 4A shows the UV–vis absorption of ketamine (curve a), ketamine-dsDNA (curve b) and ketamine-ssDNA (curve c). To synthesize dsDNA, the same amounts of two ssDNA sequences were mixed initially then the solution heated at 95 °C for 5–10 min and the solution cooled down at room temperature. As can be seen, the absorbance of ketamine decreased in presence of DNA that can be due to the interaction between ketamine and DNA. A decrease in the absorbance of ketamine in presence of ssDNA is more noticeable than dsDNA, which implied that ssDNA could stabilize ketamine more than dsDNA [48].

The intercalative binders have been characterized by a significant shift in wavelength ($\Delta\lambda \geq 15$ nm) due to the interaction of a DNA helix π stack and ligand π system while a small shift in wavelength usually correlates with the groove binding mode of molecules to DNA helix ($\Delta\lambda \leq 8$ nm) [49]. Fig. 4B and C shows the absorbance of ketamine in presence of different concentrations of dsDNA and ssDNA, respectively. Ketamine exhibits bands

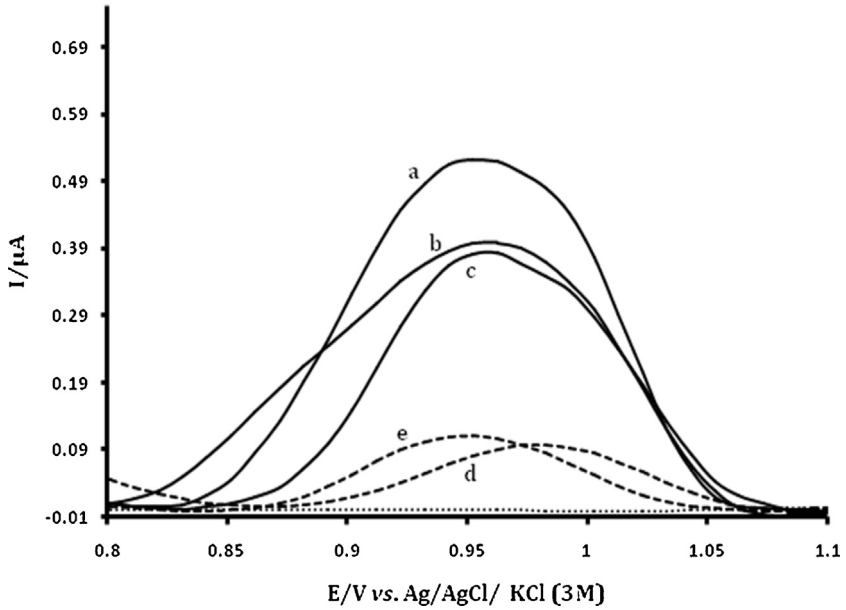


Fig. 3. Differential pulse voltammograms of the accumulated ketamine at the surface of (a) ssDNA/CPE, (b) after interaction of ssDNA with noncomplementary oligonucleotide (hpv5it), (c) after interaction of ssDNA with noncomplementary oligonucleotide (hil2) and (d) after hybridization of ssDNA with complementary target DNA sequence and (e) after hybridization of ssDNA with complementary oligonucleotide in binary mixture of target DNA and hpv5it and complementary target DNA in Britton buffer solution (pH 7.00) containing 20 mM of NaCl. A dotted line shows the blank signal of electrode.

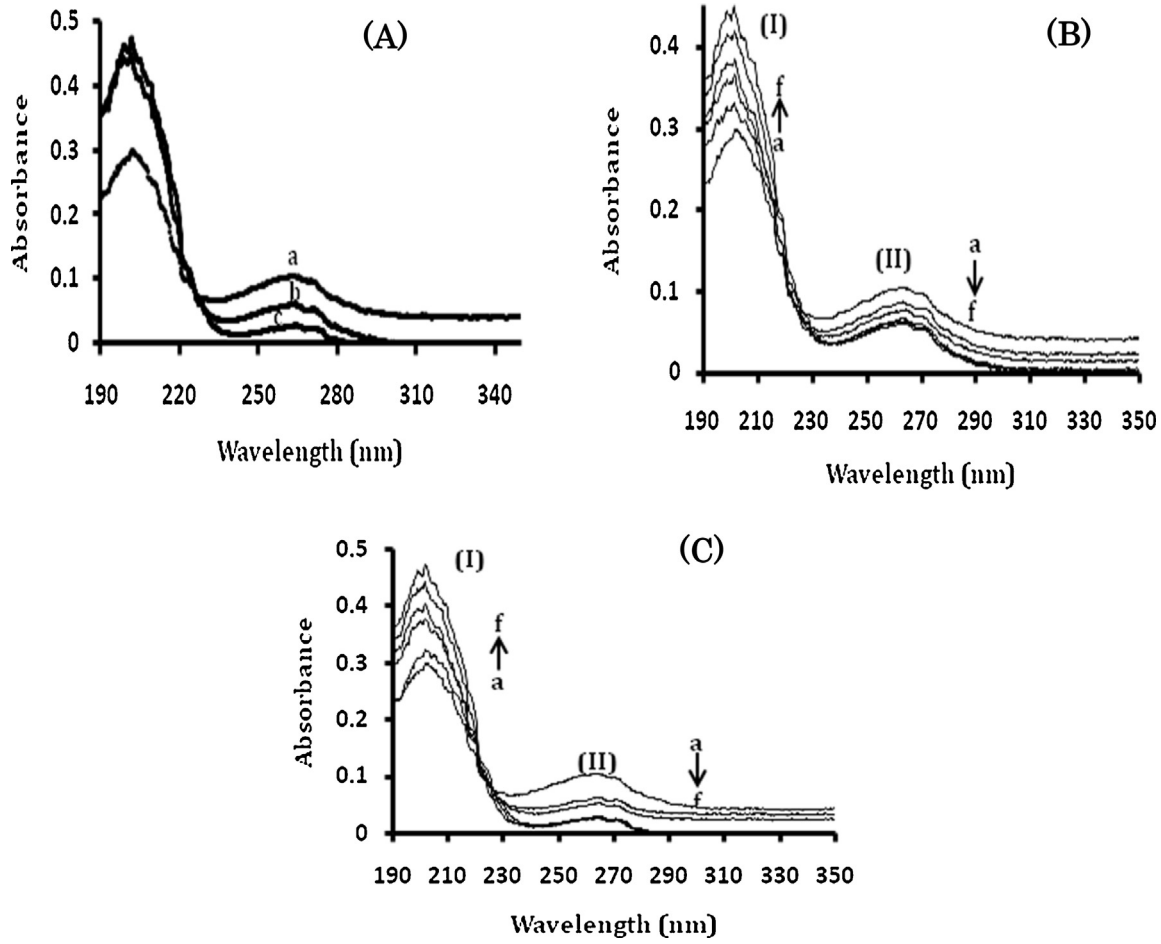


Fig. 4. (A) UV–vis absorption spectra of ketamine (a), ketamine-dsDNA (b), ketamine-ssDNA (c). (B, C) UV–vis of ketamine in absence (a) and presence of different concentrations of DNA 9.9×10^{-9} (b), 2.91×10^{-8} (c), 4.76×10^{-8} (d), 6.54×10^{-8} (e) and 8.26×10^{-8} M (f) for dsDNA and ssDNA, respectively.

at 203 (I) and 263 (II) nm in Fig. 4B and C. The absorbance of ketamine increased at 203 nm while decreased at 263 nm with the addition of ssDNA. With an injection of ssDNA in each step, the absorbance of ketamine at 263 nm (II) reduced due to the decrease in concentration of free ligands. On the other hand, the peak at 203 nm (I) increased because of the more interaction of ssDNA with ketamine. This result is in good agreement with electrochemical study results. The shift in ketamine absorption peak at 263 nm (II) in Fig. 4B is approximately 3 nm. Therefore, the interaction between ketamine/dsDNA is suggested to be out-binding mode or groove binding. The binding constant, k, can be calculated using Eq. (3) [50]:

$$\frac{A_0}{A - A_0} = \left(\frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G}\right) + \left(\frac{\varepsilon_G}{(\varepsilon_{H\text{-}G} - \varepsilon_G)k[DNA]}\right) \quad (3)$$

where A_0 and A are the absorbance of ketamine in absence and presence of DNA, ε_G and $\varepsilon_{H\text{-}G}$ are absorption coefficients of ketamine and its complex with DNA, respectively. The value of binding constant is achieved from the plot of $A_0/(A - A_0)$ vs. 1/[DNA] and found to be 1.0×10^7 and 3.0×10^7 for binding of ketamine to dsDNA and ssDNA, respectively. Consequently, ketamine can highly interact with ssDNA rather than dsDNA causing to a greater decrease in its adsorption.

3.7. Voltammetric determination of ketamine

The DPV technique was employed to measure the ketamine in serum sample. Fig. 5A shows DPV of different concentrations of accumulated ketamine at ssDNA/CPE. DNA concentration is constant (10^{-6} M) and ketamine concentration is increasing from 0.0 to 6.0×10^{-7} M. DNA shows an oxidation peak at 0.98 V on the surface of modified CPE and as previous results shown in Section 3.3, with an increase in ketamine concentration, the peak current decreased. The variation of voltammetric response vs. ketamine concentration is shown in Fig. 5B in which ΔI (the difference between the oxidation peak current of the DNA probe modified CPE in presence and absence of ketamine) is enhanced with increasing ketamine concentration. The limit of detection (LOD) (Eq. (4a)) and limit of quantification (LOQ) (Eq. (4b)) are calculated using the plot of ΔI vs. ketamine concentration ($n = 3$). The equations are follows [51,52]:

$$\text{LOD} = 3s/m = 1.98\,\text{nM} \quad (4a)$$

$$\text{LOQ} = 10s/m = 6.53\,\text{nM} \quad (4b)$$

s and m are the standard deviation of the blank and the slope of the calibration curve, respectively.

A summary and a comparison of our proposed biosensor with the other analytical techniques have been depicted in Table 1.

Table 1
Comparison of the proposed biosensor with the other techniques earlier reported for determination of ketamine.

Analytical method	Limit of detection	Reference
This method	1.98 nM	
Potentiometric sensor	7.3×10^{-6} M	[53]
Drug-selective electrode	5×10^{-6} M	[54]

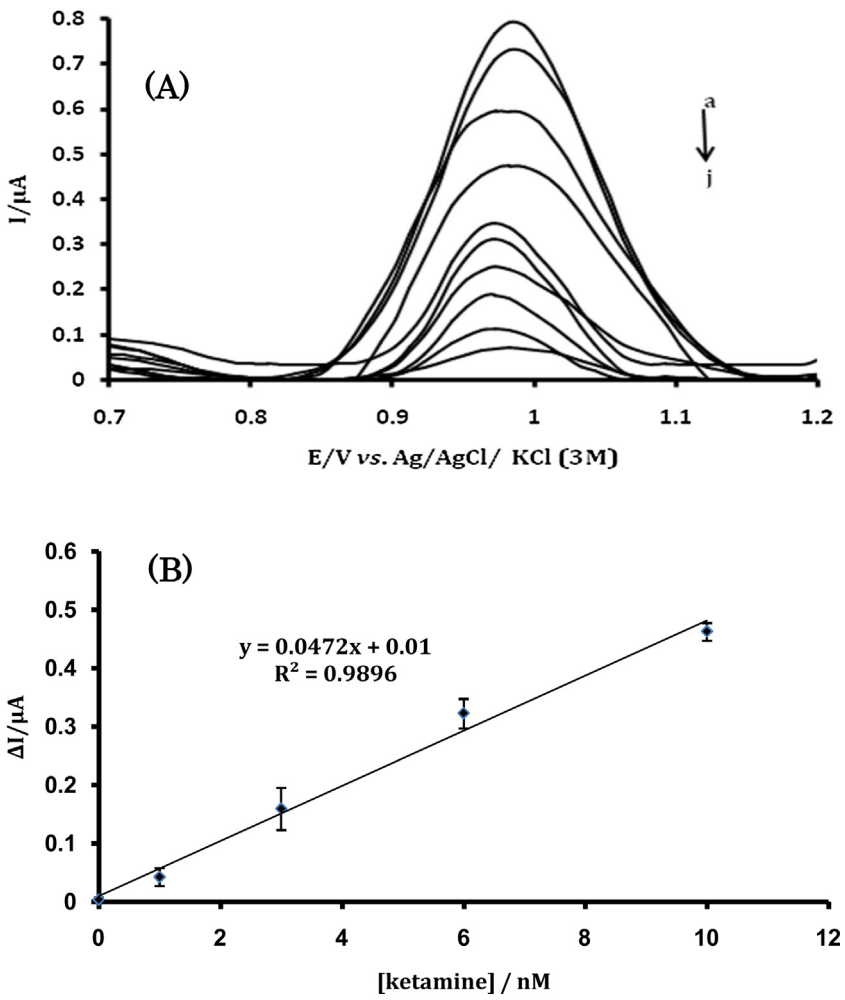


Fig. 5. (A) Differential pulse voltammogram of increasing levels of accumulated ketamine at ssDNA/CPE (a) 0.0, (b) 1.0×10^{-9}, (c) 3.0×10^{-9}, (d) 6.0×10^{-9}, (e) 1.0×10^{-8}, (f) 3.0×10^{-8}, (g) 6.0×10^{-8}, (h) 1.0×10^{-7}, (i) 3.0×10^{-7} and (j) 6.0×10^{-7} M in Britton buffer solution (pH 7.00) after immobilization of 10^{-6} M probe at the surface of CPE. (B) Variation of the difference between the oxidation peak current of the DNA probe modified CPE in the presence and absence of ketamine (ΔI) vs. ketamine concentration.

Table 2
Determination of ketamine in real sample.

Added[a]	Founded[b]	RSD%
1×10^{-8} M	1.3×10^{-8} M	3.44
5×10^{-8} M	4.6×10^{-8} M	3.31
1×10^{-9} M	1.5×10^{-9} M	4.12

[a] Added means the values that we add into the diluted serum sample.
[b] Founded means the amount of ketamine obtained according to regression equation from Fig. 5B.

Also, DPV method was employed in order to examine the ability of the proposed biosensor for the determination of ketamine in real samples. The diluted serum sample solution was containing a constant concentration of DNA (10^{-6} M) and different concentrations of ketamine (10^{-9}, 10^{-8} M); the peak current of the DPV signal was inserted in obtaining regression equation from Fig. 5B. Achieved results are given in Table 2. The low values of (RSD) can demonstrate good precision of this biosensor for determination of ketamine in real samples.

4. Conclusions

In the present study, the electrochemical properties of ketamine were investigated at CPE and ssDNA/CPE in Britton buffer solution (pH 7.00). The interaction between DNA and ketamine was studied both by voltammetric and spectroscopy methods. Types of DNA bases influence the interaction between DNA and ketamine. It was concluded that ketamine has a specific affinity toward guanine bases rather than other DNA bases. Also, the decrease in ketamine signal on the DNA double helix declared electrostatic interaction between DNA and ketamine. Spectroscopic investigation revealed that not only electrostatic interaction existed between DNA and ketamine, but also groove binding interaction mode was distinguished between ketamine/dsDNA. Various parameters such as values of electron transfer coefficient, standard rate constant and binding constant were also calculated. The analytical application of this biosensor was successfully investigated by DPV technique for determination of ketamine in serum sample.

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