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Sensitive detection of brucine an anti-metastatic drug for hepatocellular carcinoma at carbon nanotubes – nafion composite based biosensor

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ABSTRACT

A neoteric approach for the electrochemical analysis of brucine in biological environment has been developed. The glassy carbon electrode modified with single walled carbon nanotubes and nafion composite film is delineated for the first time to determine brucine employing square wave voltammetry. The quantification of brucine at physiological pH 7.2 manifests remarkable performance at the developed biosensor. The effect of several operational parameters has been studied in the present investigation. Under optimized conditions, a dynamic linear range from 1 nM to 8 μM with a high sensitivity of 340.8 μA μM^{-1} and limit of detection corresponding to 0.11 nM was achieved. The interfering effect of some coexisting metabolites on the current response of brucine has been reported. The method was successfully applied for the detection of brucine in traditional pharmaceutical formulations. The biological relevance of the present method has been demonstrated by the analysis of the alkaloid in human serum and urine samples. The analytical utility was further assessed by the selective determination of brucine in *Strychnos nux-vomica* seeds exhibiting considerable potential as a diagnostic tool.

1. Introduction

Alkaloids are the cornerstone of the pharmaceutical industry which has emerged in the forms of antiarrhythmic, antihypertensive, muscle relaxant, analgesic, anticancer drugs and other variants. Brucine is one such natural alkaloid which has shown to possess variety of biological activities. Research investigations have shown cytotoxic (Agrawal et al., 2011a), anti-proliferative (Rao et al., 2009), antitumor (Agrawal et al., 2011a) and antiangiogenic (Agrawal et al., 2011b) effects of brucine on human cell lines such as multiple myeloma and LoVo cell line from human colon (Zheng et al., 2013). Hepatocellular carcinoma (HCC) is one of the most pernicious and seventh most common forms of cancer related mortality worldwide (Yang and Roberts, 2010). Prognosis of HCC is largely influenced by metastasis and remarkably brucine was found to inhibit HCC migration *in vitro* and metastasis *in vivo* (Shu et al., 2013). Brucine is also reported along with thermosensitive liposomes in combination with local hyperthermia for thermo-chemotherapy of tumor (Chen et al., 2014). Though brucine demonstrates impressive preclinical results for the pharmacological activities, the major hurdle in its clinical application still exists due to severe toxicity in central nervous system. There is a thin line of safety between a therapeutic and a toxic dose thereby indicating that an improved method for the determination of trace levels of brucine in biological fluids is required for toxicological research, clinical study and forensic analysis.

In view of the prominence of brucine in clinical applications, there are several methods reported for its determination. Various analytical techniques have been described extensively in the scientific literature for the quantitative determination of brucine; especially through the use of capillary electrophoresis (Feng and Li, 2002), thin layer chromatography (Rathi et al., 2008), high performance liquid chromatography (Song et al., 2013; Van Eenoo et al., 2006), liquid chromatography-mass spectroscopy (Chen et al., 2012; Lin et al., 2016), ultra-performance liquid chromatography–tandem mass spectrometry (Liu et al., 2011b) and ionic liquid-based electromembrane extraction (Sun et al., 2014). However, these techniques had some disadvantages which involve complex procedures, expensive instrumentation and high running costs. Unlike the above mentioned techniques, electrochemical techniques offer hassle-free procedure and are relatively inexpensive which have been incorporated for the sensitive detection of a wide range of analytes (Bui et al., 2016; Chatterjee et al., 2013). The electrochemical detection provides an advantage to couple with portable instrumentation. Literature survey unveils that electrochemical techniques have been explored earlier for the detection of brucine involving diverse chemically modified sensors (Liu et al., 2013; Mumin et al., 2016; Wang and Xu, 2005; Wang et al., 2007; Zhang et al., 2004,

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2006). Sensors developed using carbon-paste electrode had narrow linear range (Behpour et al., 2012) while a molecularly imprinted composite film crafted sensor had good selectivity but lower sensitivity (Liu et al., 2012). Another research group investigated brucine by molecularly imprinted polymers on multi-walled carbon nanotubes, which was not compatible with physiological pH of human body (Zhao et al., 2014). Hence, a need for the development of detection technology for brucine has aroused which possess enhanced sensitivity and improved selectivity in the desired environment.

Recently, carbon nanomaterials have been extensively used for research and development in the burgeoning field of electrochemical sensors (Chen and Chatterjee, 2013). The attraction of such materials in the form of single walled carbon nanotubes (SWNTs) has myriad striking characteristics. The high efficiency of electron transfer is one of the significant features of the SWNTs (Goyal et al., 2010). Large electroactive surface area of the SWNTs also enhances the adeptness of the sensor (Liu et al., 2011a). The modification of carbon nanomaterials on the electrode as a substrate boosts the sensitivity when utilized for the determination of various analytes (Chatterjee and Chen, 2012). With the advent of polymeric films for dispersing SWNTs, the biocompatibility of the sensors has been stimulated. The unique ion-exchange and discriminative properties, of polymers like nafion have been used extensively for the modification of electrode surfaces (Wang et al., 2003). Nafion has exhibited magnified activity of the sensor employing various electrochemical techniques.

In the present investigation, SWNTs along with Nafion has been used to modify the glassy carbon electrode (GCE), which provides a useful avenue for preparing SWNTs based biosensors. Literature survey unveils that no attempt has been made to investigate brucine using SWNTs and nafion composite on GCE exploiting square wave voltammetric technique till date. Nafion, which is a kind of perfluorosulfonic acid and cation exchange polymer has the micropore structure, the fixed charge sites, and the movable counterions. It can form membranes that are highly permeable to cations but almost impermeable to anions. Hence, nafion has been utilized as a matrix in the present work for the construction of SWNTs composite biosensor. A homogenous dispersion of SWNTs in nafion solution engenders hydrophobic side chains and polar head groups which permits a variety of manipulations, including modification of electrode surfaces and preparation of biosensors, where association of nafion does not impair the electrocatalytic properties of SWNTs. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used in a comparative investigation for the electrochemical oxidation of brucine and the tentative reaction mechanism was suggested. The developed biosensor displayed an electrocatalytic effect in anodic oxidation of brucine at physiological pH with a sensitivity augmentation. Moreover, in the present work real samples such as human serum and urine have been analyzed for the determination of brucine in order to elucidate the performance of the developed biosensor in human physiology. The investigation of brucine concentration has been satisfactorily carried out in *S. nux vomica* seeds which are the natural source of brucine. To the best of our knowledge, the developed biosensor has reported the lowest detection limit till date for the quantification of brucine. The experimental results revealed that this method is simplistic, selective and expeditious; thus providing an influential tool for the analysis of brucine. Enhancements in the quantification of brucine will instigate the employment of brucine in the pharmaceutical formulations for the metastasis of hepatocellular carcinoma.

2. Materials and methods

2.1. Chemicals and reagents

Brucine was purchased from Sigma Aldrich and the stock solution was prepared using double distilled water. The SWNTs of > 99.5% purity and nafion were obtained from Sigma Aldrich as well. All other reagents were of analytical grade and used as supplied. The experiments were performed in a 1 M phosphate buffer solution (PBS) with different pH values maintained with Na_2HPO_4 and NaH_2PO_4 which were obtained from Merck. Human serum samples were collected from Dr. Sangita Shetty's pathology laboratory, Mumbai, India. Blood that contained EDTA as an anticoagulant was ultra-centrifuged and the supernatant obtained was extracted for the analysis of brucine. Human urine samples were acquired from healthy volunteers. *S. nux-vomica* seeds were bought from an ayurvedic store located in Ahmedabad, India. Gujinwan and Yaotongning capsules were procured from a pharmaceutical store in China.

2.2. Instrumentation

All the voltammetric experiments were performed using electrochemical analyzer Autolab PGSTAT 302 of Metrohm. CV and SWV was accomplished using a three-electrode single compartment cell, which was equipped with GCE that had a diameter of 3.0 mm as the working electrode, a platinum coil as the counter electrode and an Ag/AgCl (3.0 M KCl) as the reference electrode. The platinum coil was flame-annealed prior to each experiment. All of the potentials quoted are versus an Ag/AgCl electrode at an ambient temperature of 25 °C. The pH measurements were made with Eutech pH meter, Singapore. A Tescan MIRA 3 model was employed for field-emission gun scanning electron microscopy (FEG-SEM) experiments. Transmission electron microscopy (TEM) analysis was done utilizing CM-200 Philips instrument with operating voltages of 20–200 kV.

2.3. Procedure

The stock solution of brucine was prepared by dissolving required amount of the analyte in double distilled water and stored at 4 °C. The individual working standards were prepared daily by diluting the aliquots of the stock solution. High purity nitrogen was purged for 10–12 min to deoxygenate the solutions before recording the cyclic voltammograms to prevent the oxygen interference. The optimized operating conditions to record SWV were: initial E: 500 mV, final E: 1100 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV, square wave frequency (*f*): 15 Hz.

2.4. Fabrication of glassy carbon electrode

The surface of a bare GCE was carefully polished with 0.3 μm alumina powder using micro-cloth pads prior to modification and then rinsed thoroughly with double distilled water until a mirror like surface was obtained. A 0.5 mg mL^{-1} suspension was prepared by dispersing 0.5 mg SWNTs in 1.0 mL N, N-dimethylformamide via ultrasonic agitation. A 0.1% solution of nafion in ethanol was added to the SWNTs dispersion. The GCE surface was then coated with a known volume (12 μL) of this suspension and permitted to dry at room temperature. The working electrode surface with a well-coated layer of SWNTs and nafion composite was then ready for use, denoted as SWNT/Naf/GCE. The surface morphology of the bare and modified electrode was studied by recording FEG-SEM images and is presented in Fig. 1, which clearly shows the deposited SWNTs on the surface of the bare glassy carbon electrode. A TEM image of the bifunctional nanocomposite has also been showcased in Fig. 1C.

3. Results and discussion

3.1. Electrochemical characterization of the electrodes

The electrochemical properties of the four electrodes, namely bare GCE, GCE modified with Nafion, GCE coated with SWNTs and SWNT/Naf/GCE was assessed using cyclic voltammetry. A comparison of the voltammetric response obtained at the four different electrodes in

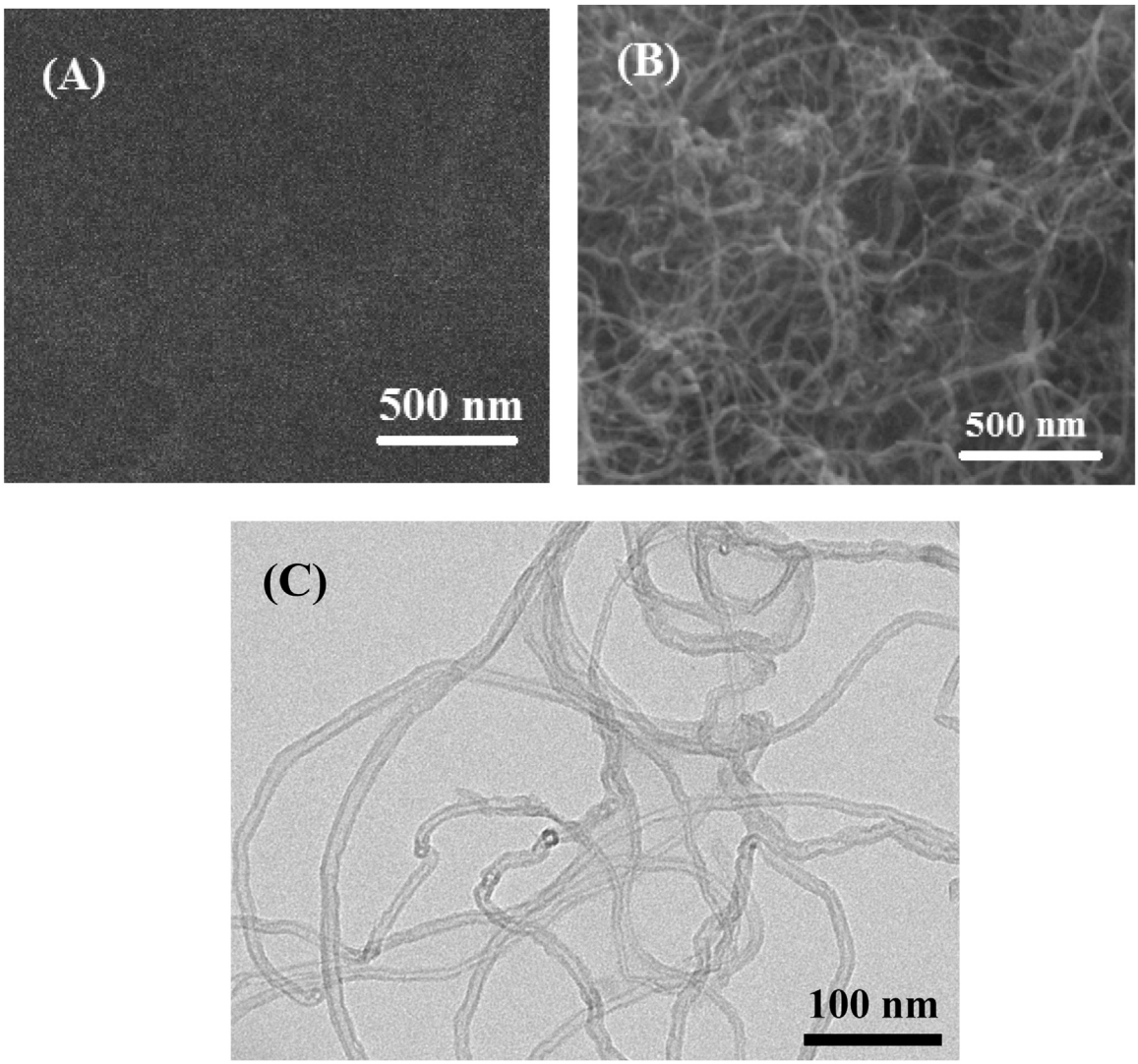

Fig. 1. FEG-SEM image of **(A)** bare GCE and **(B)** SWNT/Naf/GCE. **(C)** TEM image of the single walled carbon nanotube and nafion composite.

0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe at the scan rate of 25 mV s^{-1} is illustrated in Fig. S-1. It is observed that bare GCE and nafion modified GCE (curves a and b) exhibited broad redox couple with minimal current response. The amplification of the current intensity for SWNT modified GCE (curve c) followed by further increase in signal response at SWNT/Naf/GCE (curve d) was explicitly observed. The developed biosensor had ΔE_p of ~ 55 mV and that at SWNT modified GCE ΔE_p is ~ 110 mV which demonstrate that electron transfer is faster in the former electrode due to the synergic effect of nafion and SWNTs composite.

3.2. Surface area determination of the modified electrode

The effective surface area of the bare GCE and SWNT/Naf/GCE was determined in order to ascertain the efficacy of surface modification procedure. Chronocoulometry technique was applied in 1 mM $K_3[Fe(CN)_6]$ solution using 0.1 M KCl as the supporting electrolyte. The integrated Cottrell expression denotes the charge as a function of time which is represented as below:

$$Q = 2nFAcD^{1/2}t^{1/2}\pi^{-1/2} + Q_{dl} + nFA\Gamma_0$$

where n stands for the number of transferred electrons (n = 1 in this case), F is the Faraday constant, A is the effective surface area of electrode, c is the concentration of $K_3[Fe(CN)_6]$, D is the diffusion coefficient (7.6×10^{-6} cm^2 s^{-1} for 1 mM $K_3[Fe(CN)_6]$). Besides, Q_{dl} is the capacitive charge, and $nFA\Gamma_0$ is the charge from the reduction of adsorbed species. The resultant chronocoulograms displayed linear relationship between Q and $t^{1/2}$, with slope of 30 μC $s^{-1/2}$ and 252 μC $s^{-1/2}$ corresponding to bare and SWNT/Naf/GCE respectively. From the above equation, the effective surface area of bare and modified GCE is calculated to be 0.020 and 0.166 cm^2 respectively. The electroactive surface area of the SWNT/Naf/GCE is approximately 8.3 fold larger than that of the bare GCE which evidently demonstrates that the large specific surface area increases the electrochemical active sites of bare GCE.

3.3. Electrochemical oxidation of brucine

3.3.1. Cyclic voltammetry

In order to investigate the electrochemical performance of the biosensor, CVs of brucine at SWNT/Naf/GCE as well as bare GCE were recorded in 1 M PBS (pH 7.2). As shown in Fig. 2, the developed sensor revealed an oxidative peak at ~ 846 mV while the broad peak observed at bare GCE was at ~ 910 mV when analyzed in the employed CV scanning window at scan rate of 25 mV s^{-1}. The peak current response at the modified electrode was ~ 10 times higher in comparison to the bare electrode. Here, the shift in the peak potential between modified electrode and bare GCE can be explained by the employment of SWNTs and Nafion which has given rise to the recognition sites complementary

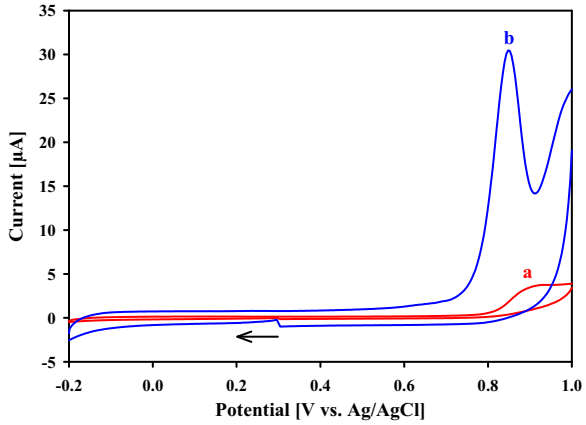

Fig. 2. Cyclic voltammograms obtained for 2 μM brucine in PBS of pH 7.2 at (a) bare GCE and (b) SWNT/Naf/GCE at 25 mV s^{-1}.

to the molecular shape, size, and functionality of brucine molecules. The oxygen in the brucine molecule would interact with the hydrogen of the sulfonic acid group in nafion through hydrogen bond. The pores in nafion allow the movement of cations so nafion could ensure better accessibility and selective recognition for the template in complex matrix. CV was further employed to study the reaction mechanism of the electrochemical oxidation of brucine. At SWNT/Naf/GCE, an initiative potential of 0.3 V and the scanning potential between − 0.2 and 1.0 V was applied. The two successive scans clearly indicated that a sharp irreversible oxidation peak on first anodic scanning followed by a pair of redox couple at lower potential in the second scan was observed as shown in Fig. S-2. The proposed mechanism for the oxidation of brucine has been included in the inset of Fig. S-2 (Zhang et al., 2004). The amelioration in the peak current response of brucine at the modified electrode clearly indicated that synergistic effect of SWNTs and Nafion composite magnified the current response signal by boosting electron transfer and intensifying specific surface area on the electrode interface.

3.3.2. Square wave voltammetry

The electrocatalytic activity of the developed sensor is further demonstrated by the comparison of the square wave voltammograms of 1 μM brucine in 1 M PBS (pH 7.2) recorded at four different working electrodes as depicted in Fig. 3. The voltage applied during the square wave voltammetry on the electrode provides energy for the exchange of electrons. The oxidation of brucine takes place and the product formed gives the maximum current at the specific voltage. On scanning from potential 0.4–1.1 V, one well-defined peak is obtained at ~ 810 mV at SWNT/Naf/GCE as shown in Fig. 3 (curve b). Under similar conditions, a small peak is noticed at potential ~ 875 mV at bare GCE (curve

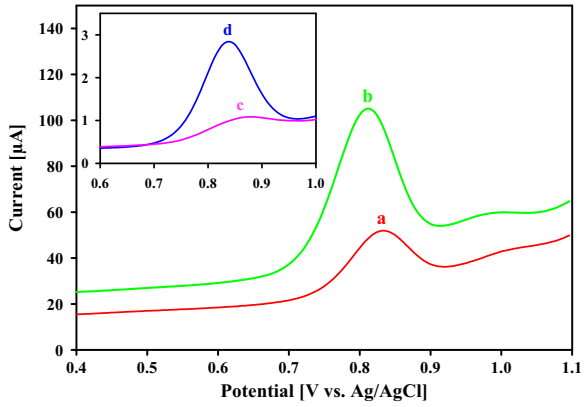

Fig. 3. Square wave voltammograms obtained for 1 μM brucine in PBS of pH 7.2 at (a) SWNT/GCE, (b) SWNT/Naf/GCE, (c) bare GCE and (d) Nafion modified GCE at 15 Hz.

c). The significant improvement of peak current together with the sharpness of the peak clearly demonstrates that SWNT/Nafion composite acts as an efficient promoter to enhance the kinetics of the electrochemical process. Further, a comparison of the voltammetric response of brucine was made at SWNTs modified GCE and nafion modified GCE. At SWNTs modified GCE, the oxidation peak was obtained at ~ 830 mV which is close to the peak potential obtained at SWNT/Naf/GCE but the current signal was ~ 2.6 times lower than that at the SWNT/Naf/GCE (curve a). The nafion modified GCE on the other hand exhibited the oxidation peak at ~ 840 mV with considerably very low current response in comparison to the developed sensor (curve d). Thus, the lowered peak potential and the increase in current response demonstrate the superiority of SWNT/Naf/GCE towards the oxidation of brucine as compared to the other working electrodes. Hence, further studies were carried out at SWNT/Naf/GCE employing square wave voltammetry.

3.4. Optimization of operational parameters

The pH of supporting electrolyte is one of the most influential parameters for the oxidation peak potential of brucine, investigated in the pH range of 2.9–11.0 at a square wave frequency of 15 Hz. The peak potential (E_p) decreased linearly with increasing pH as shown in Fig. S-3A. The linear relationship could be expressed as follows:

$$E_p(\text{pH } 2.9\text{–}11.0) = [\, -0.0306\text{pH} + 1.0197] \text{ V versus Ag/AgCl}$$

with a correlation coefficient of 0.9975 and a slope of 0.0306 V pH^{-1}. According to the Nernstian equation, E_p= 59.16 (m/n) pH (m: proton number; n: electron number), which infers that the number of electrons transferred is twice as that of protons transferred in the electrochemical reaction of brucine. Additionally, the effect of pH on the peak current response of brucine was investigated and the maximum peak current was observed at pH 7.2 (Fig. S-3B) which was then selected as the supporting electrolyte.

The peak current (i_p) for the oxidation of brucine was found to increase linearly with the increase in square wave frequency (f) in the range 10–100 Hz at pH 7.2 at the SWNT/Naf/GCE as seen in Fig. S-3C. The relation between i_p and f may be expressed by the following equation:

$$i_p(\mu A) = 4.4284f + 6.066$$

having a correlation coefficient of 0.9907. The effect of the square wave frequency on the peak potential was also examined in the range 10–100 Hz using the SWNT/Naf/GCE sensor. The plot of E_p versus log f was linear and found to shift towards more positive potentials with an increase in the square wave frequency as depicted in Fig. S-3D. The variation of E_p with log f can be expressed by the equation:

$$E_p(V) = 0.0457 \log f + 0.7544$$

with a correlation coefficient of 0.9903. These observations are in good agreement with the properties of an irreversible electrochemical process which is adsorption controlled (Massaroppi et al., 2003; Radi et al., 1999).

3.5. Quantitation of brucine

The quantitative evaluation of brucine was investigated by recording the square wave voltammetric response of varying concentration of brucine at SWNT/Naf/GCE. Under the optimum conditions, the oxidation peak current increased linearly with elevation in concentration of brucine. The voltammograms thus obtained depicts the systematic increase in the peak current values with increase in the concentration of brucine in the range 1–80 nM and 0.1–8 μM as shown in Fig. 4. The linear calibration plot for the aforementioned ranges is depicted in the inset of the respective figures. The current values were

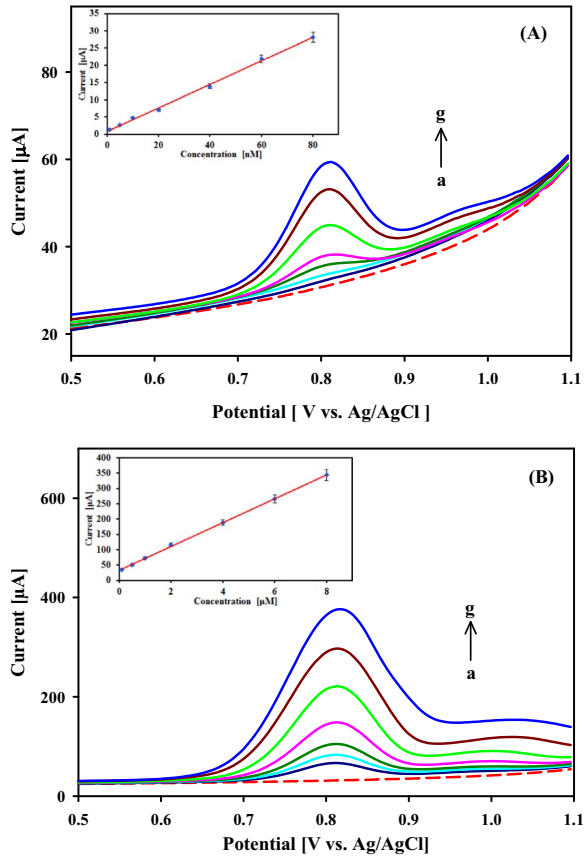

Fig. 4. (A) Square wave voltammograms recorded for (i) PBS (background) at SWNT/Naf/GCE (- - -) and (ii) increasing concentration of brucine at SWNT/Naf/GCE (—) [Curves were recorded at (a) 1, (b) 5, (c) 10, (d) 20, (e) 40, (f) 60 and (g) 80 nM concentration in PBS of pH 7.2]. Linear calibration plot of brucine in the range 1–80 nM observed at SWNT/Naf/GCE is presented in the inset. **(B)** Square wave voltammograms recorded for (i) PBS (background) at SWNT/Naf/GCE (- - -) and (ii) increasing concentration of brucine at SWNT/Naf/GCE (—) [Curves were recorded at (a) 0.1, (b) 0.5, (c) 1, (d) 2, (e) 4, (f) 6 and (g) 8 μM concentration in PBS of pH 7.2]. Linear calibration plot of brucine in the range 0.1–8 μM observed at SWNT/Naf/GCE is presented in the inset.

obtained by subtracting the background current and are reported as an average of at least three replicate determinations with a standard deviation of less than 3.9% for n = 3. The linear regression equation for both the calibration range is represented as:

$$i_p(\mu A) = 0.3408C\ (nM) + 0.8451 \quad (1–80\ nM)$$

$$i_p(\mu A) = 38.868C\ (\mu M) + 33.577 \quad (0.1–8\ \mu M)$$

where C is the concentration of brucine. The correlation coefficient for the expressions are 0.998 and 0.9996 respectively. The sensitivity was found to be 340.8 μA μM^{-1} for the proposed method and the limit of detection was estimated to be 0.11 nM (based on S/N = 3) with 0.37 nM as the limit of quantification.

3.6. Selectivity

The strong affinity of developed sensor towards brucine was confirmed with the voltammetric measurements performed in biological fluids, where the elemental substances act as potential interferents. An efficient study of interference effects initiated by dopamine, uric acid, xanthine and hypoxanthine of different concentrations was carried out in a standard 1 μM brucine solution. The tolerance limit was defined as the maximum concentration of extraneous species that would yield an inaccuracy, not exceeding ± 5%, in the detection of the analyte. According to the analysis, the metabolites up to 50 fold concentrations did not interfere with the peak current of brucine utilizing the SWNT/Naf/GCE. The deviation in peak current response of brucine with varying concentration of interferents is summarized in Table S-1. The evaluation of the obtained results endorsed that the major metabolites inherently present in biological fluids did not impede the brucine quantification.

3.7. Stability and reproducibility of the sensor

The stability and reproducibility are utmost important factors which signify the feasibility and application of the developed sensor. The stability was evaluated by storing the SWNT/Naf/GCE sensor in ambient atmosphere for 30 days and then assessed by the voltammetric measurements in 1 μM brucine solution. The relative standard deviation (RSD) was 3.46% which manifests the excellent long-term stability of the sensor. The reproducibility of the SWNT/Naf/GCE sensor was derived by recording repeated voltammetric response on five replicate sensors. They exhibited fair reproducibility with a RSD of 2.37% for 1 μM brucine solution. The voltammetric response of the developed sensor for seven consecutive days was measured in 1 μM brucine solution which showed the RSD of the peak current as 3.16%. Moreover, seven intraday measurements recorded in 1 μM brucine solution depicted RSD value of 2.51%. The stability and reproducibility plots have been presented in the supplementary information (Fig. S-4). Thus, the SWNT/Naf/GCE exhibits good stability and reproducibility for the electrochemical oxidation of brucine.

3.8. Recovery studies

The reliability of the sensor was demonstrated by the analysis of real samples, performed under optimized experimental conditions. The urine samples obtained from healthy human volunteers (samples 1–3) were analyzed which showed the absence of oxidation peak of brucine as depicted in Fig. S-5A (curve a). The samples were then spiked to affirm the consistency of the developed protocol with known concentrations of brucine, which was followed by recording their voltammograms as seen in Fig. S-5A (curves b, c and d). The recoveries varied in the range of 96.00–105.00% in normal human urine samples as shown in Table 1. The human serum samples were subsequently experimentally analyzed (samples 1 and 2), which showed the absence of brucine content as illustrated in Fig. S-5B (curve a). A standard spiking was

Table 1
Concentration of brucine in human urine and human serum sample at SWNT/Naf/GCE at pH 7.2.

Spiked	(μM)	Detected (μM)	Recovery (%)
Human urine			
Sample 1	0.00	–	–
	0.50	0.52	104.00
	1.00	1.05	105.00
	2.00	2.04	102.00
Sample 2	0.00	–	–
	0.50	0.51	102.00
	1.00	0.96	96.00
	2.00	2.04	102.00
Sample 3	0.00	–	–
	0.50	0.49	98.00
	1.00	1.03	103.00
	2.00	2.07	103.50
Human serum			
Sample 1	0.00	–	–
	1.00	1.01	101.00
	2.00	2.08	104.00
	4.00	4.01	100.25
Sample 2	0.00	–	–
	1.00	1.03	103.00
	2.00	1.94	97.00
	4.00	3.97	99.25

Table 2
Determination of brucine in pharmaceutical preparations and *Strychnos nux-vomica* seeds using SWNT/Naf/GCE.

Sample	Spiked (μM)	Detected (μM)	Recovery (%)
Gujjinwan capsule	0.00	2.37	–
	0.50	2.84	98.95
	0.75	3.09	99.04
	1.00	3.40	100.01
Yaotongning capsule	0.00	3.27	–
	0.50	3.74	99.20
	0.75	4.05	100.75
	1.00	4.23	99.06
	Spiked (nM)	**Detected (nM)**	
Seeds sample	0.00	19.23	–
	50.00	47.69	95.38
	75.00	71.75	95.67
	100.00	98.45	98.45

thereafter carried out to the human serum samples to measure the recoveries at different concentration levels. The corresponding voltammograms are presented in Fig. S-5B (curves b, c and d). The recoveries of brucine in the human serum samples were calculated in the range of 97.00–104.00% which is showcased in Table 1. Satisfactory recoveries of these urine and serum samples indicated that this developed sensor is promising for applications in the practical detection of brucine in the biological matrix.

3.9. Pharmaceutical analysis

Pragmatic application provides definitive corroboration for the developed biosensor to be a potential diagnostic tool. Thus, the analysis of two commercial pharmaceutical formulations containing brucine viz. Gujinwan capsule and Yaotongning capsule was executed by the developed method. The capsules were crushed into fine powder and accurately weighed 300 mg individually. The resulting powder was then dissolved in 50 mL ethanol under ultrasonication for 1 h and thereafter stirred for 20 h. After filtration through a 5 μm nylon fabric filter, the resultant residue was dried and then dissolved in ethanol for the preparation of stock solution. These solutions were employed after suitable dilution for square wave voltammetry, recorded under optimized conditions. After deliberating over the dilution factor, the determined content of brucine in 300 mg of Gujinwan and Yaotongning capsule was found to be 2.80 mg/g and 3.87 mg/g, respectively. In addition, the accuracy of the proposed method was testified by performing a recovery test after spiking the samples. The value of recovery was between 98.95% and 100.75% as shown in Table 2. This suggests that the sensor has higher recognition selectivity for brucine which enhances its application for multi component analysis.

3.10. Assay of brucine in seeds of S. nux-vomica

In order to comprehend the capability of the developed biosensor, it was applied for brucine detection in *S. nux-vomica* seeds. The extraction of seeds, as discussed in the Supplementary material was performed and utilized for the voltammetric measurements under optimized conditions after appropriate dilution. The voltammetric response clearly indicated the oxidation peak of brucine as shown in Fig. S-6 (curve a). The quantity of brucine in processed seeds was estimated to be 5.05 mg/g. Thereafter, the recovery test was done for the determination of brucine in *S. nux-vomica* seeds. The voltammograms were recorded through SWV as depicted in Fig. S-6 (curves b, c and d) which gave recovery within ± 5% of known concentration of brucine as shown in Table 2. The results showed that the recoveries varied from 95.38% to 98.45%, which distinctly indicated that the fabricated sensor can be used as an effective and reliable sensing platform for brucine determination in plant samples.

4. Conclusions

The proposed method demonstrates the detection of brucine at SWNTs dispersed in nafion composite modified on GCE. The electrochemical response of SWNT/Naf/GCE was superior to the bare GCE, nafion modified GCE and even SWNTs modified GCE while performing voltammetric analysis of brucine. At optimum conditions in SWV, a good linear correlation between oxidation peak current and corresponding concentration of brucine was obtained in the calibration range of 1 nM – 8 μM. The observations revealed that the developed biosensor has reported the lowest limit of detection (0.11 nM) with highest sensitivity (340.8 $\mu A\ \mu M^{-1}$) so far in the electrochemical determination of brucine as shown in Table S-2. Reliability of this analytical method was displayed by its recognition ability for the targeted analyte even in the presence of 50 fold concentration of the interferents. The developed prototype was employed for the quantification of brucine in Gujinwan and Yaotongning medicinal capsules which clearly indicates that the method is feasible and pragmatic. The fabricated sensor provides explicit evidence as a powerful diagnostic tool for in vivo studies when applied for sensitive detection of brucine in human serum and urine samples. The applicability of this biosensor was satisfactorily evaluated by investigation of the *S. nux-vomica* seeds for the presence of brucine which illustrated promising analytical applications. The future perspective of this biosensor for the determination of brucine includes facilitation for understanding the activity of brucine in inhibition of HCC migration in vivo.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.07.011.

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