ORIGINAL

Electrochemical detection of L-serine and L-phenylalanine at bamboo charcoal-carbon nanosphere electrode

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Abstract A carbon nanosphere electrode (CN) has been fabricated for the electrochemical detection of L-serine and L-phenylalanine. Electrochemical behavior of amino acids was investigated using cyclic voltammetry, differential pulse voltammetry, square wave voltammetry and linear sweep voltammetry. In optimal conditions, the peak current of both amino acids on the CN electrode was found to enhance greatly. A sensitive oxidation peak at 0.085 V was observed in the determination of L-serine and 0.06 V for L-phenylalanine. A linearity between the oxidation peak current and the concentration of both amino acids was obtained in the range of 1–100 μ M ($R^2 = 0.99783$ for L-serine, 0.99618 for L-phenylalanine). The practical application of the carbon nanosphere electrode in the determination of the amino acids possesses high selectivity, sensitivity and stability.

Keywords Carbon nanospheres · L-Serine · L-Phenylalanine

Introduction

L-Serine is an amino acid which contributes to several bodily processes, most notably in the functioning of RNA and DNA, formation of muscles, metabolism of fat/fatty acids, and the maintenance of a healthy immune system. L-Serine is also a component of cell membranes and is

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vital for the neurotransmission process that takes part between the brain and the various nerve endings in the body [1]. A deficiency in serine manifests itself through such symptoms as delayed cognitive and physical skills and seizures. Under development of the brain may also be traced to L-serine deficiency. In recent years, L-serine and the products of its metabolism have been recognized not only to be essential for cell proliferation, but also to be necessary for specific functions in the central nervous system [2].

L-Phenylalanine is an essential amino acid and used for the biochemical formation of proteins, coded for DNA. Phenylalanine is a precursor for tyrosine, the monoamine signaling molecules dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), and the skin pigment melanin. L-Phenylalanine is an antagonist at α2δ Ca²⁺ calcium channels with a Ki of 980 nM [3]. At higher doses, this may play a role in its analgesic and antidepressant properties. Hyper-phenylalaninemia (HPA) serves as the most common inherited disorder of amino acid metabolism

Amino acids are usually analyzed by liquid chromatographic methods [5], FTIR studies [6], differential capacitance, radioactive indicators, solid-phase extraction, flow injection [7, 8] electrochemiluminescence [9]. These methods are quite accurate, but they are dependent on multi-step sample clean-up procedures and are therefore relatively expensive and time consuming. For this reason, there is an interest in developing faster, simpler and lowcost procedures for amino acid analysis. In recent years, electrochemical detection has gained prominence as a sensitive and selective detection technique for the electroactive compounds. Various electroanalytical methods and sensors for the detection of amino acids have been reported [10-14].



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In continuation of our earlier studies [15–19], we have now reported the fabrication of a new L-serine and L-phenylalanine biosensor based on carbon nanospheres (CN), which was obtained by pyrolysis of bamboo. Electrochemical studies have been carried out using cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and linear sweep voltammetry (LSV) for the detection of L-serine and L-phenylalanine in phosphate buffer solution (PBS) at pH 6.8 and 7, respectively. The studies revealed that the nanospheremodified electrode showed excellent sensitivity, selectivity, high stability, low potential along with a fast response towards the detection of these amino acids.

Experimental section

Reagents and apparatus

L-Serine, L-phenylalanine, sodium dihydrogen phosphate (NaH₂PO₄) and disodium hydrogen phosphate (Na₂HPO₄) and phosphoric acid (H₃PO₄) were purchased from Sigma Aldrich. Pharmaceutical samples (Medett Products, India) of L-serine and L-phenylalanine were used for real sample analysis. All solutions were prepared with deionized water. Bamboo was carbonized in muffle furnace (Tanco, PL Tandon & Company). The structure and morphology of CN were characterized by scanning electron microscope (SEM) (Hitachi X650, Japan), transmission electron microscope (TEM) (JEOL JEM 1400) and atomic force microscopy (AFM) (afm + Anasys). X-ray diffraction (XRD) patterns were recorded with JSO ISO DEBYE-FLEX 2002 model X-ray powder diffractometer. Electrochemical studies were performed using a mini potentiostat (Dropsens ustat 100). Pocket-sized pH meter (HANNA instrument) was used to maintain the pH of the sample solution.

250 ml 0.1 M PBS (pH 7) was prepared by mixing 0.22 g of Na_2HPO_4 and 0.3 g of NaH_2PO_4 in 250 ml deionized water and adjusted the pH by the addition of H_3PO_4 . The stock solution of 100 ml 10^{-3} M L-serine was prepared by dissolving 0.010 g L-serine in 100 ml of PBS (pH 6.8) and 100 ml 10^{-3} M L-phenylalanine was prepared by dissolving 0.016 g of L-phenylalanine in 100 ml of PBS (pH 7). Standard solutions were prepared by diluting the stock solution in PBS by maintaining the pH. Deionized water was used throughout this study.

Preparation, functionalization and characterization of carbon nanospheres from bamboo (CN)

CN was prepared by our own reported method [16] by carbonizing the pieces of bamboo at 600-650 °C under

insufficient flow of air in a muffle furnace. For purification, carbon soot was collected in a thimble and then placed in a soxhlet extractor for sequential purification with petroleum ether, acetone, ethyl alcohol and finally with water. Unburnt bamboo and other unwanted form of carbon can be removed by this method. For functionalization, 250 ml 2 M HNO₃ was mixed with the carbon soot and stirred for several minutes and then kept undisturbed overnight. To remove excess nitrate, it was then repeatedly dissolved in water and then evaporated to dryness. Complete removal of nitrate was confirmed by Griess's test [20]. For further purification, it was re-precipitated with alcohol and water. It was then characterized by SEM, TEM, AFM, XRD.

Fabrication of CN electrode

As reported earlier [19], the CN electrode was designed like the commercially available standard electrode DS110CNT. It was fabricated on Teflon material containing three silver wires, where both working and counter electrodes were made of CN and third silver wire was acted as reference electrode. For the fabrication of the electrode, polystyrene solution was first prepared in chloroform (9:1 ratio). Then, CN was mixed with polystyrene solution using mechanical stirrer followed by sonication. A drop of the slurry was then deposited as a very fine thin film on the two silver wires, serving as working and counter electrodes. The electrode has a dimension $3.5 \times 1.0 \times 0.5$ cm (length × width × height) and it was ideal for working with 50 ul volume like the standard electrode.

L-Serine and L-phenylalanine detection with fabricated CN electrode

For the detection of L-serine, electrochemical studies such as CV, DPV, and LSV studies were performed with the sample solutions in phosphate buffer solution at CN electrode and CV, SWV and LSV studies were performed for L-phenylalanine detection. pH 6.8 was maintained for L-serine detection and pH 7 was maintained for L-phenylalanine detection. To optimize the conditions of electrochemical detection of both the amino acids at CN electrode, effect of pH, scan rate, time interval were studied in detail. Then, the linearity, detection limit, sensitivity, reproducibility and stability of the sensor were also investigated. To determine the selectivity of the sensor, the effect of the presence of some small biomolecules on the current responses of L-serine and L-phenylalanine at CN electrode was evaluated under optimized experimental conditions. The utility of the CN electrode was tested by determining the amino acids in some pharmaceutical samples.





Fig. 1 a SEM image of carbon nanospheres (CN), b TEM image of carbon nanospheres (CN)

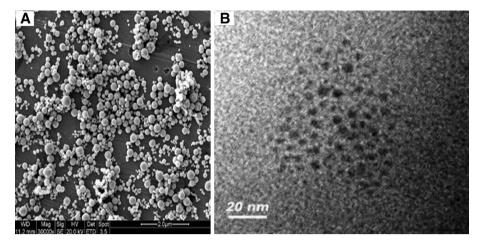
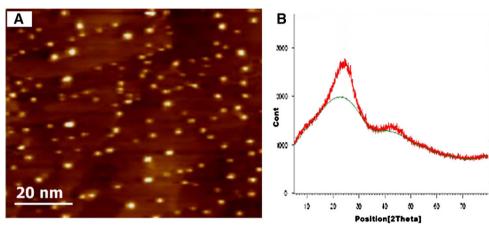


Fig. 2 a AFM image of carbon nanospheres (CN), **b** XRD pattern of carbon nanospheres (CN)



Results and discussion

Characterizations of CN

As reported earlier [16], surface morphological studies were done by SEM, TEM studies. Figure 1a shows the SEM image of carbon nanospheres in the range of 300–600 nm. Figure 1b shows the TEM image of uniformly distributed carbon nanospheres and it clearly indicates the formation of carbon nano particles of spherical like structures. AFM image in Fig. 2a further confirms the formation of carbon nanospheres. XRD pattern of CN (Fig. 2b) shows two intensed peak at 25° and 44° which were assigned for (002) and (001) reflections, respectively, and confirms CN formation.

Effect of pH on the peak current of L-serine and L-phenylalanine at CN electrode

Effect of solution pH on the oxidation of 10^{-5} M L-serine and L-phenylalanine at CN-modified electrode was investigated by LSV in the pH range from 6.5 to 8.5 by mixing Na₂HPO₄, NaH₂PO₄ and H₃PO₄. Figure 3a, b show that there is a gradual decrease of the oxidation peak current with

the increasing pH for both amino acids, beyond the value of 6.8 for L-serine and 7 for L-phenylalanine. *I/µA* vs. pH graph shows that for L-serine, maximum peak current is observed at pH 6.8 and for L-phenylalanine at pH 7. Therefore, the pH 6.8 and 7 were chosen for the subsequent analytical experiments for L-serine and L-phenylalanine, respectively.

CV studies to determine the effect of scan rate on the peak current and peak potential of L-serine and L-phenylalanine at CN electrode

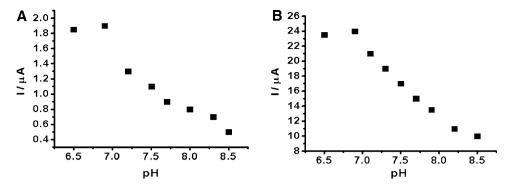
In both cases, CV studies were performed with 10^{-5} M L-serine (pH 6.8) and L-phenylalanine (pH 7) solutions in PBS at 100 mV s^{-1} on bare silver electrode (Blank) (Fig. 4). No peak current was observed at the potential range 0.05-0.09 V, which indicates that on bare silver electrode no reactions took place.

The effect of the scan rate on the electrochemical detection of L-serine and L-phenylalanine at the CN electrode was investigated by voltammetric techniques. Figure 5a, b displays the overlaps of cyclic voltagrams of 10^{-5} M L-serine (pH 6.8) and L-phenylalanine (pH 7.0), respectively, at various scan rates in PBS, while the inset shows the 3D representation of CVs. The current (I/μ A) vs.



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Fig. 3 a Effect of solution pH on the oxidation peak current of 10^{-5} M L-serine in PBS (pH 6.8) at 100 mV s⁻¹. b Effect of solution pH on the oxidation peak current of 10^{-5} M L-phenylalanine in PBS (pH 7) at 100 mV s⁻¹



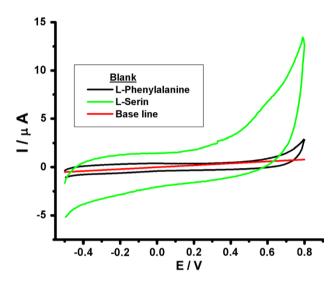


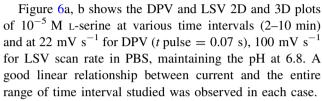
Fig. 4 CVs of 10^{-5} M L-serine and L-phenylalanine at silver electrode in PBS at 100 mV s⁻¹ scan rate (*blank*)

scan rate ($v/mV s^{-1}$) plot, shown in the inset, exhibits a linear relationship with $R^2 = 0.99589$ for L-serine and $R^2 = 0.99719$ for L-phenylalanine.

The influence of scan rate was investigated within the range of $50\text{--}140~\text{mV s}^{-1}$ for L-serine and $50\text{--}150~\text{mV s}^{-1}$ for L-phenylalanine. The linearity was observed over the entire range of scan rates studied and it indicates that the electrochemical kinetics reaction is adsorption controlled [21]. Potential required for the detection of L-serine on CN electrode was found to be 0.085~V and for L-phenylalanine is 0.06~V.

DPV, LSV and SWV studies to determine the effect of time interval on the peak current and peak potential of L-serine and L-phenylalanine at CN electrode

Beside CV studies, other electrochemical studies were also performed to check the nature of peak current and peak potential and also to check the maximum accumulation time for both the amino acids. Interestingly, it was observed that sharp intense peaks were obtained without changing the peak potential.



$$I/\mu A = 4.22 + 0.23 \text{ min}, R^2 = 0.99438,$$

 $SD = 0.089, N = 5 \text{ (DPV)},$
 $I/\mu A = 1.28 + 0.055 \text{ min}, R^2 = 0.99589,$
 $SD = 0.018, N = 5 \text{ (LSV)}.$

Figure 7a, b shows the SWV and LSV 2D and 3D plots of 10^{-5} M L-phenylalanine in PBS (pH 7) with various time intervals. For SWV studies, the frequency was adjusted to 12 Hz and $E_{\rm ampl} = 0.010$ V and LSV was performed at a scan rate of 100 mV s⁻¹. For both SWV and LSV, the studies were conducted up to 20 min and here also, a good linear relationship between current and the time interval was observed in each case.

$$I/\mu A = 20.656 + 0.536 \,\text{min}, R^2 = 0.99719,$$

 $SD = 0.278, N = 8 \,(\text{SWV})$
 $I/\mu A = 20.426 + 0.183 \,\text{min}, R^2 = 0.99822,$
 $SD = 0.070, N = 10 \,(\text{LSV}).$

In each and every case, the peak current increases linearly with time up to a certain period of time, but when these studies were conducted over a more extended time interval range then there was a breakdown in the linearity relationship which may be due to the stabilization of current with time. Therefore, for 10^{-5} M L-serine and L-phenylalanine, the maximum accumulation times, 10 and 20 min, respectively, were employed.

Linearity, detection limit, stability and sensitivity of the L-serine and L-phenylalanine sensor

Figure 8a shows the CV of different concentrations of L-serine at pH 6.8 and Fig. 8b shows the LSV of different concentrations of L-phenylalanine in PBS at pH 7. As LSV studies gave more intensed peak than CV in case of





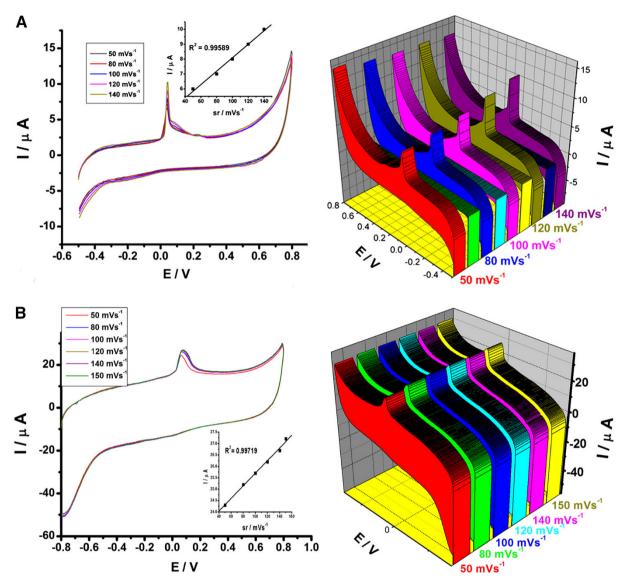


Fig. 5 a 2D and 3D plots of CVs of 10^{-5} M L-Serine in PBS (pH 6.8) at various scan rates (50, 80, 100, 120 and 140 mV s⁻¹) on CN-modified electrode. *Inset* the plot of peak current (I/μ A) versus scan rate (v/mV s⁻¹). **b** 2D and 3D plots of CVs of 10^{-5} M

L-phenylalanine in PBS (pH 7) at various scan rates (50, 80, 100, 120, 140 and 150 mV s⁻¹) on CN-modified electrode. *Inset* the plot of peak current (I/μ A) versus scan rate (v/mV s⁻¹)

L-phenylalanine, therefore LSV studies were performed for LOD, sensitivity determination. Both the figures showed that the current increases linearly with the increase of concentrations of L-serine and L-phenylalanine. Calibration plots (inset) showed a linear dependence of the anodic peak current of concentrations in range of 1–100 μ M in both the cases.

$$I/\mu A = 6.824 + 0.664 \,\mu M, \,R^2 = 0.99783,$$

 $SD = 0.144, \,N = 24 \,(L - serine)$
 $I/\mu A = 4.748 + 0.482 \,\mu M, \,R^2 = 0.99618,$
 $SD = 0.092, \,N = 17 \,(L - phenylalanine).$

The active surface area of CNA electrode was calculated with the help of CV studies of 5×10^{-4} M K₄[Fe(CN)₆] in

0.1 M H₂SO₄ as a redox probe at various scan rate and using Randles–Sevcik equation, $I_{\rm P}=(2.69\times 10^5)$ $n^{3/2}AD^{1/2}$ C $v^{1/2}$, where n is the number of electrons participating in the redox reaction, A is the electroactive surface area (cm²), D is the diffusion coefficient (cm² s⁻¹), C is the concentration of the redox probe molecule (mol cm⁻³) and v is the scan rate (mV s⁻¹). The calculated electroactive surface area of CN electrode was found to be 0.125 cm², where $C=5\times 10^{-4}$ M, $D=7.1\times 10^{-6}$ cm² s⁻¹, n=1 for [Fe(CN)₆]^{3-/4-}system.

The sensitivity was calculated using the slope of the current versus concentration calibration plot (Fig. 8a, b inset) divided by the active surface area of CN according to the following equation [22]:



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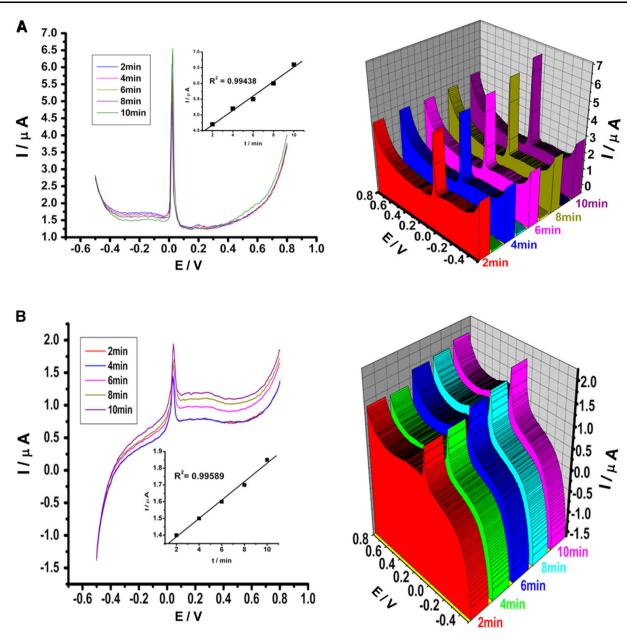


Fig. 6 a 2D and 3D plots of DPVs of 10^{-5} M L-serine at CN electrode in PBS (pH 6.8) in various time interval (2, 4, 6, 8 and 10 min) at 22 mV s⁻¹ scan rate (t pulse = 0.07 s). *Inset* the plot of peak current (I/μ A) versus time interval (t/min). **b** 2D and 3D plots of

LSVs of 10^{-5} M L-serine at CN electrode in PBS (pH 6.8) in various time interval (2, 4, 6, 8 and 10 min) at 100 mV s⁻¹ scan rate. *Inset* the plot of peak current (I/μ A) versus time interval (t/min)

Sensitivity

= slope of the plot/active surface area of the electrode (1)

The sensitivity of the CN L-serine sensor was calculated to be $\sim\!5.21~\mu A~\mu M^{-1}~cm^{-2}$ (slope = 0.664 $\mu A~\mu M^{-1})$ and L-phenylalanine to be $\sim\!3.83~\mu A~\mu M^{-1}~cm^{-2}$ (slope = 0.482 $\mu A~\mu M^{-1}).$

The limit of detection (LOD) for both amino acids was calculated using the following equation: LOD = 3 s/m;

where s is the standard deviation of the peak currents of the blank (five runs), and m is the slope of the calibration curve [23], and the values were found to be 0.54 μ M for L-serine and 1 μ M for L-phenylalanine.

Series of five successive LSV measurements with 10^{-5} M L-serine (pH = 6.8) and L-phenylalanine in PBS (pH = 7), each recorded on a new modified CN electrode, yielded relative standard deviations of 2.8 and 2.5 %, respectively (Fig. 9). The results indicate that CN electrode provides good reproducibility towards oxidation of both





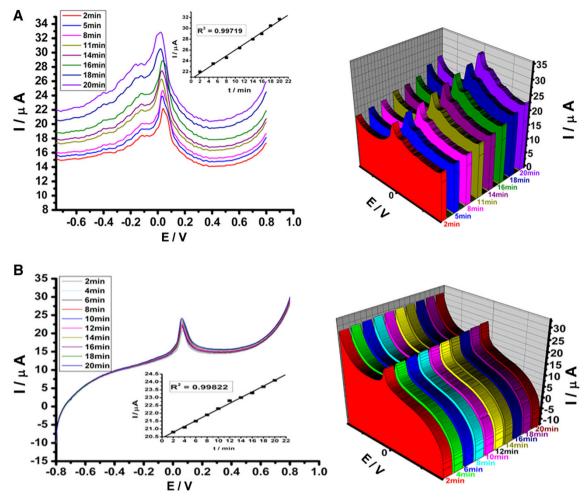
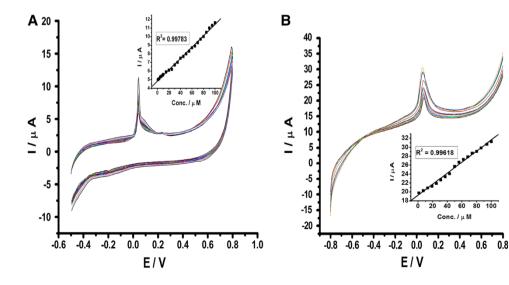


Fig. 7 a 2D and 3D plots of SWVs of 10^{-5} M L-phenylalanine at CN electrode in PBS (pH 7) in various time interval (2, 5, 8, 11, 14, 16, 18 and 20 min) at 100 mV s⁻¹ scan rate. *Inset* the plot of peak current (I/μ A) versus time interval (t/min). **b** 2D and 3D plots of LSVs of

 10^{-5} M L-phenylalanine at CN electrode in PBS (pH 7) in various time interval (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 min) at $100~\text{mV s}^{-1}$ scan rate. *Inset* the plot of peak current (I/μ A) versus time interval (t/min)

Fig. 8 a CVs at CN electrode in PBS (pH 6.8) with different concentrations of L-serine (1–100 $\mu M)$ at 100 mV s^{-1} scan rate. Inset calibration curve of response current (I/μA) versus L-serine concentration (Conc./µM). **b** LSVs at CN electrode in PBS (pH 7) with different concentrations of L-phenylalanine (1-100 μM) at 100 mV s⁻¹ scan rate. *Inset* calibration curve of response current (I/μA) versus L-phenylalanine concentration (Conc./µM)





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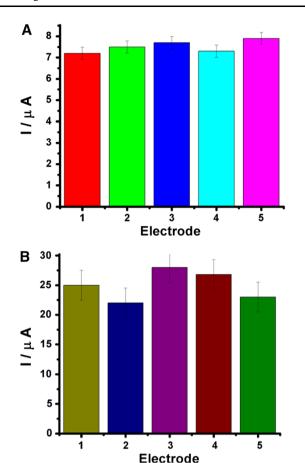
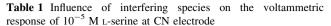


Fig. 9 Stability of the responses for 10^{-5} M L-serine (**a**) and 10^{-5} M L-phenylalanine (**b**), obtained at five different modified CN electrode. For L-serine N=5, RSD = 2.8 % and for L-phenylalanine N=5, RSD = 2.5 %

amino acids. The stability of the amino acid sensor was also explored when the modified electrode was stored in air for 15 days, where the current response of 10^{-5} M L-serine and L-phenylalanine was found to be stable maintaining ~ 90 % of its initial intensity.

Interference

During the electrochemical studies of a sensor, it is necessary to conduct the experiments at a particular potential, which can only drive the reaction of interest. Under optimized experimental conditions described above, the effects of some small biomolecules on the current responses of 10^{-5} M L-serine and L-phenylalanine have been evaluated. Uric acid, vitamin C, dopamine, L-alanine, glycine, tryptophan, and tyrosine have no influence on the current response at CN electrode in PBS at pH 6.8 and 7. The experimental results (Tables 1, 2) showed that ten times of each substance had almost no interference with the determination of L-serine and L-phenylalanine. So, it can be



L-Serine (10 ⁻⁵ M) + interfering species (10 ⁻⁴ M)	Observed potential (V)	Signal change (%)	Average signal change (%)
L-Serine	0.0852	0	
L-Serine + uric acid	0.0855	0.352	
L-Serine + vitamin-C	0.086	0.939	
L-Serine + dopamine	0.0859	0.939	0.7051
L-Serine + L-alanine	0.0847	0.587	
L-Serine + glycine	0.0852	0	
L-Serine + tryptophan	0.0845	0.828	
L-Serine + tyrosine	0.0863	1.291	

Table 2 Influence of interfering species on the voltammetric response of 10^{-5} M L-phenylalanine at CN electrode

L-Serine (10 ⁻⁵ M) + interfering species (10 ⁻⁴ M)	Observed potential (V)	Signal change (%)	Average signal change (%)
L-Phenylalanine	0.0613	0	
L-Phenylalanine + uric acid	0.0617	0.652	
L-Phenylalanine + Vitamin-C	0.0611	0.326	
L-Phenylalanine + dopamine	0.0621	1.305	0.675
L-Phenylalanine + L-alanine	0.0616	0.489	
L-Phenylalanine + glycine	0.0615	0.326	
L-Phenylalanine + tryptophan	0.0609	0.652	
L-Phenylalanine + tyrosine	0.0607	0.978	

concluded that the proposed method is able to assay both the amino acids in the presence of interfering substances and hence it can be considered specific. The average signal change was found to be 0.7051 % for L-serine and 0.675 % for L-phenylalanine.

Determination of amino acids in pharmaceutical samples

L-Serine and L-phenylalanine were determined in some pharmaceutical samples (Medett Products, India) using the proposed method. Preparation of sample solution was as same as mentioned in the "Reagents and apparatus" 10^{-5} M sample solution was prepared in PBS at desired pH and LSV studies were performed. Table 3 summarizes the results obtained from voltammetric determination of L-serine and L-phenylalanine along with the certified values of the analyzed pharmaceutical products.

The above results suggest that CN electrode is very reliable and sensitive enough for the determination of these amino acids ion real samples.





Table 3 Results obtained for the determination of L-serine and L-phenylalanine in pharmaceutical products using modified CN electrode

Sample	CN-modified sensor (g)	Certified value (g)	Recovery (%)	Bias (%)
L-Serine	1.47 ± 0.02^{a}	1.5	98	2
L-Phenylalanine	1.46 ± 0.01^a	1.5	97.3	2.6

a Average of five determinations

Conclusions

In this study, a carbon nanosphere-modified electrode was prepared and it was applied for the detection of L-serine and L-phenylalanine using CV, DPV, SWV and LSV. The prepared electrode was found to have excellent electrocatalytic activity towards both the amino acids' detection. In both the cases, the oxidation peak current was linearly proportional to its concentration over the range from 10^{-4} to $10^{-6}\,M$ (1–100 μM) with a very low detection limit (correlation coefficient of 0.99783 for L-serine and 0.99618 for L-phenylalanine). This modified sensor showed excellent sensitivity, selectivity and strong stability. This paper also demonstrates the suitability of CN electrode for fast analysis of L-serine and L-phenylalanine in standard solutions as well as in commercial pharmaceutical products.

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Conflict of interest The authors declare that they have no competing interests.

Author contributions SD has been involved in experimental works. SD has also made substantial contributions to the analysis and interpretation of data. SD and MS carried out the manuscript preparation. MS has given final approval of the version to be published.

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