RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Table of content/Graphical abstract
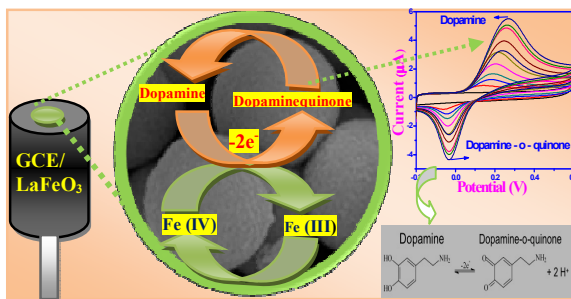
Modified glassy carbon electrode with LaFeO$_3$ microspheres constructed of nanospheres having high electrocatalytic ability towards detection of neurotransmitter compound dopamine.

Detection of neurotransmitter compound dopamine by modified glassy carbon electrode with self-assembled perovskite LaFeO₃ microspheres constructed of nanospheres

S. Thirumalairajan[a, b, *], K. Girija[b, c], Valmor R. Masterlo[a], V. Ganesh[d] and N. Ponpandian[b]

[a] Instituto de Física de São Carlos (IFSC), University de São Paulo, CP 369, 13560-970 São Carlos, SP, Brazil

[b] Department of Nanoscience and Technology, Bharathiar University, Coimbatore 641 046, India

[c] Department of Science and Humanities, Dr. N.G.P. Institute of Technology, Coimbatore 641 048, India

[d] Electrodics and Electro Catalysis Division, Central Electrochemical Research Institute (CSIR-CECRI), Karaikudi 630 006, India

[*] Corresponding author: Phone: +55 (16) 33739828. Fax: +55 (16) 33739824

E-mail address: sthirumalairajan@gmail.com, (S. Thirumalairajan)

1

Abstract

In this paper we report the detection of neurotransmitter compound dopamine by LaFeO$_3$ microspheres constructed of nanospheres modified electrode. The morphology, structure and composition of the prepared nanostructure were characterized using SEM, TEM, XRD and XPS respectively and the electrocatalytic properties were investigated using cyclicvoltammetry and amperometric studies. The modified electrodes presented remarkable increase in the efficiency of the electrocatalytic oxidation of dopamine. The biosensor exhibited high sensitivity at a low detection limit of 59 nM and wide linear range from $2x10^{-8}$ to $1.6x10^{-6}$ M (R=0.9983). More importantly, the sensor effectively avoids the interference of ascorbic acid and uric acid. A possible electrocatalytic mechanism has been proposed, and the LaFeO$_3$ microspheres constructed of nanospheres are highly promising for the detection of neurotransmitter compound dopamine because of their high selectivity, fast response and good sensitivity.

Keywords: dopamine, selectivity, sensitivity, LaFeO$_3$, nanospheres, XPS

2

1. Introduction

Electroanalytical methods have been used during the past decade to investigate the role of neurotransmitters in the brain due to their electro-active nature.[1] Dopamine (DA), ascorbic acid (AA) and uric acid (UA) is a problem of critical importance not only in the field of diagnostic and pathological research, but also for biomedical chemistry and neurochemistry. Particularly, DA is an important catecholamine neurotransmitter in the mammalian central nervous system, and their loss in neurons result in disease such as Parkinson. Dopamine is found in high amounts (50 nmol/g) in caudate nucleus (a region of the brain). However, the concentration of dopamine in the extracellular fluid of the caudate nucleus is extremely low for a healthy individual and even lower or it is completely depleted for persons affected with Parkinson's disease.[2, 3] Selective and sensitive determination of dopamine has been a long-standing goal, and the electrochemical technique is the most advantageous way in the determination of dopamine. A major problem in dopamine detection is the interference of AA and UA, which is also present in biological fluids at very high concentration than dopamine level.[4] Moreover, electrode surface can be easily fouled by the product of ascorbic acid and uric acid oxidation which results in poor selectivity and sensitivity in the determination of dopamine, reported in our previous publication.[5]

Controlling the morphology of a nanomaterial is a crucial parameter in modifying its properties. Over the past few years, tremendous effort has been taken to control the size and shape of perovskite materials and a variety of morphologies have been reported in the case of perovskite $LaFeO_3$.[6-8] Nanostructures of perovskite $LaFeO_3$ are highly attractive and attention has been paid to due to their low band gap energy (~2.1 eV), good catalytic, optical and magnetic properties which are useful for applications in visible-light photocatalyst, gas sensor, magnetic data storage, photovoltaic cells, solid oxide fuel cells, and most recently as a biosensor.[9, 10] $LaFeO_3$ nanostructures have been synthesized using a variety of wet chemical

techniques such as, hydrothermal, sol-gel, co-precipitation, combustion, sonochemical

synthesis etc.[11-13] However, reflux condensation process is a facile dominant tool for the

synthesis of anisotropic nanoscale material. Significant advantages of this method over other

wet chemical techniques are the possibility to control the size to achieve different

morphology at low temperature. The process is less complicated and cost effective. Our

research group is interested in exploring the underlying connection of soft template, such as

citric acid, CTAB and urea with different morphology using wet chemical process.[14]

In the present work, we report for the first time the facile synthesis of $LaFeO_3$

microspheres constructed of nanospheres via one-step wet chemical route and their

characterizations. Further, modified glassy carbon electrode with $LaFeO_3$ microspheres for

dopamine detection is also reported.

2. Experimental section

2.1 Preparation of $LaFeO_3$ microspheres constructed of nanospheres

$LaFeO_3$ microspheres were prepared using analytical-grade lanthanum nitrate

hexahydrate $(La(NO_3)_3.6H_2O)$ and potassium ferric cyanide $K_3[Fe(CN)_6]$ as starting materials

and polyethylene glycol (PEG, MW 200) as surfactant. In a typical synthesis, starting

materials were dissolved in 30 ml double distilled water under magnetic stirring followed by

addition of PEG. The molar amount of PEG surfactant added was equal to the total molar

amount of metal nitrate. The solution was refluxed with continuous stirring at 90 °C for 12 h

in a three-necked refluxing pot. After the reaction setup was cooled down to room

temperature naturally, the green coloured precipitate obtained was washed repeatedly with

ethanol and distilled water to remove unwanted ions, followed by drying under 80 °C,

calcination at 800 °C for 2h to obtain pure $LaFeO_3$ samples.

2.2 Characterization and property measurements

The structure and purity of the prepared nanostructures were determined by XRD - Bruker D8 Advance X-ray diffractometer using Cu-Kα radiation (λ=0.15418 nm). The thermal analysis was determined using TG/DTA (SDT Q 600 V20) from RT to 1000 °C under N_2 atmosphere. XPS measurement was performed using ESCA + Omicron UK XPS system with Mg Kα source of photon energy 1486.6 eV. Scanning electron microscopy (JEOL JSM-6380LV) and transmission electron microscopy (JEM) 2100 F performed with an acceleration voltage of 200 kV by placing the powder on a copper grid was utilized to observe the morphology and size of the prepared samples. N_2 adsorption/desorption was determined by BET measurements using Micromeritics ASAP 2020 nitrogen adsorption apparatus and the pore size distribution by BJH method.

2.3 Electrochemical measurement and Fabrication of modified galssy carbon electrode

Electrochemical measurements were performed (EG & G Instrument model 6310 work station) in a conventional two compartment three electrode cell with mirror polished 0.07 cm^{-2} glassy carbon (GC) as the working electrode, Pt wire as the counter electrode and 3M KCl Ag/AgCl as the reference electrode. All the measurements were carried out in phosphate buffer solution (pH = 7.2) under N_2 atmosphere at RT. The GC electrode was polished to mirror like surface with 0.05 μM alumina powder and sonicated in double distilled water for 10 min. 100 mg of $LaFeO_3$ nanospheres was dispersed in 10 mL anhydrous alcohol and ultrasonicated for 30 min. 10 μL of alcoholic dispersed $LaFeO_3$ from the above solution was dropped onto the GC surface and air dried at ambient temperature.

5

3. Results and discussion

3.1 Morphological analysis of LaFeO₃ microspheres constructed of nanospheres

The morphology of the synthesized samples examined using SEM images (Fig. 1(a))
exhibit microsphere like morphology with size between 0.5 to 1.5 μm. No other morphology
was observed indicating high yield of microspheres. The higher magnification image in Fig.
1(b) clearly reveals that individual LaFeO₃ microspheres are constructed of numerous
nanospheres with an average uniform size ~ 60 nm (Fig. 1(c)). This value is in close
agreement with the crystalline size determined from XRD discussed in later section. The
TEM image (Fig. 1(d)) taken at a particular area of LaFeO₃ microsphere shows several
anisotropically directed nanospheres self-assembled to form microstructures due to local
supersaturation.[15] Further, these microspheres are stable without breaking into scattered
individual nanospheres even at high temperature for long period of time, which confirms that
these building units are tightly connected with each other to construct the entire LaFeO₃
microspheres.

The HRTEM image of nanospheres constructed by LaFeO₃ microspheres is shown in
Fig. 1(e). The orderly and clear lattice fringes parallel to each other show that the building
blocks of the microspheres are well crystallized, and the interplanar distance between
adjacent lattice plane is 0.27 nm corresponding to the d-spacing of (121) LaFeO₃ plane.
The corresponding SAED pattern (Fig. 1(f)) indicates the high crystalline nature is due to the
major diffraction spots corresponding to (121), (220) and (202). No diffraction spots were
attributed to the secondary phase or impurity. Thus by correlating the outcome from TEM
and HRTEM images it is concluded that the observed results are in good agreement with
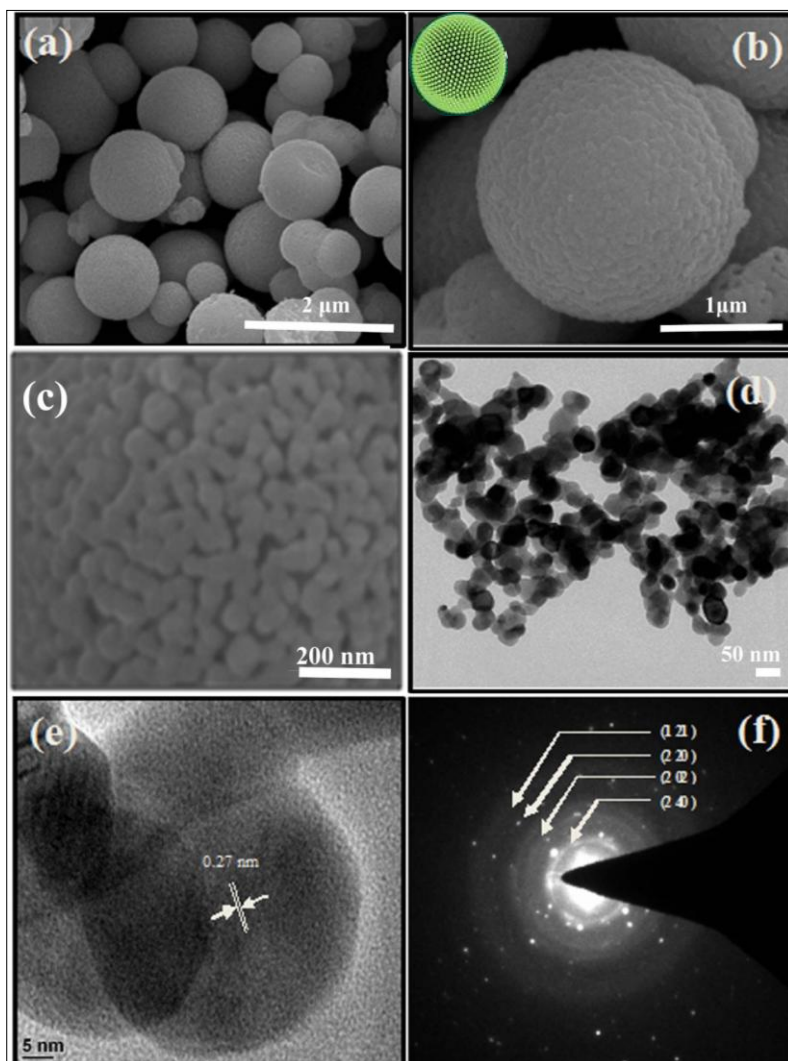SEM and XRD.

Fig. 1 SEM image (a) low magnification, (b and c) high magnification (inset - schematic illustration of microspheres), (d) TEM image, (e) HRTEM and (f) SAED pattern of microsphere constructed by LaFeO$_3$ nanospheres

3.2 Formation of LaFeO$_3$ microspheres

When the surfactant PEG was added to the precursor solution containing (La(NO$_3$)$_3$.6H$_2$O) and K$_3$[Fe(CN)$_6$], the metal ions could be easily absorbed on the surface of the non-ionic PEG surfactant because of strong interaction between activated oxygen in the PEG molecular chains and the metal ions. Because of the long-chain structure and flexibility of PEG, the La3d and Fe2p-PEG complex forms a network structure of polymer and due to hydrogen-bonding effect the complex forms spherical aggregates in water which act as nucleation centers for the formation of LaFeO$_3$ nanospheres.[16] These nucleate and mineralize

on the surface of $LaFeO_3$ aggregates forming the microspheres constructed of $LaFeO_3$ nanospheres.

3.3 Structural, thermal, composition and surface area analysis of $LaFeO_3$ microspheres

The crystal phase of $LaFeO_3$ microspheres was investigated by XRD. As shown in Fig. 2(a), it is clearly evident that all the diffraction peaks are consistent with the standard data for bulk $LaFeO_3$ crystals (JCPDS 37-1493) which has orthorhombic perovskite structure with lattice constant $a = 5.658$, $b = 7.855$ and $c = 5.689$ Å. No characteristic peaks arising from reactants, impurities, La_2O_3/ Fe_2O_3 or other phases were detected. The strong and narrow diffraction peaks observed indicate high crystallinity of the $LaFeO_3$ samples. The average crystallite size was found to be 65 nm using the Scherrer's formula.[17]

Fig. 2(b) represents the TG/DTA data obtained for $LaFeO_3$ microspheres conducted at a heating rate of 20 °C/min. TGA process accompanying 1.9 % weight loss observed at ca. RT to 380 °C is attributed to the evaporation of absorbed water, also evident from the exothermic peak at 370 °C in the DTA curve. A weight loss of about 2% occurs in the range 380 to 510 °C and the corresponding DTA peak appears at 500 °C which can be assigned to the decomposition of nitrates and other organic impurities.[18] A weight loss of 1.8 % in the range 510 to 760 °C is due to the complete decomposition of oxycarbonates and the corresponding DTA peak at 590 °C can be possibly attributed to the gradual crystallization of $LaFeO_3$. At higher temperature no obvious weight loss is observed indicating there is no additional phase or structural change in $LaFeO_3$. Therefore, in order to obtain the $LaFeO_3$ samples with high purity, 800 °C was chosen as the calcination temperature.
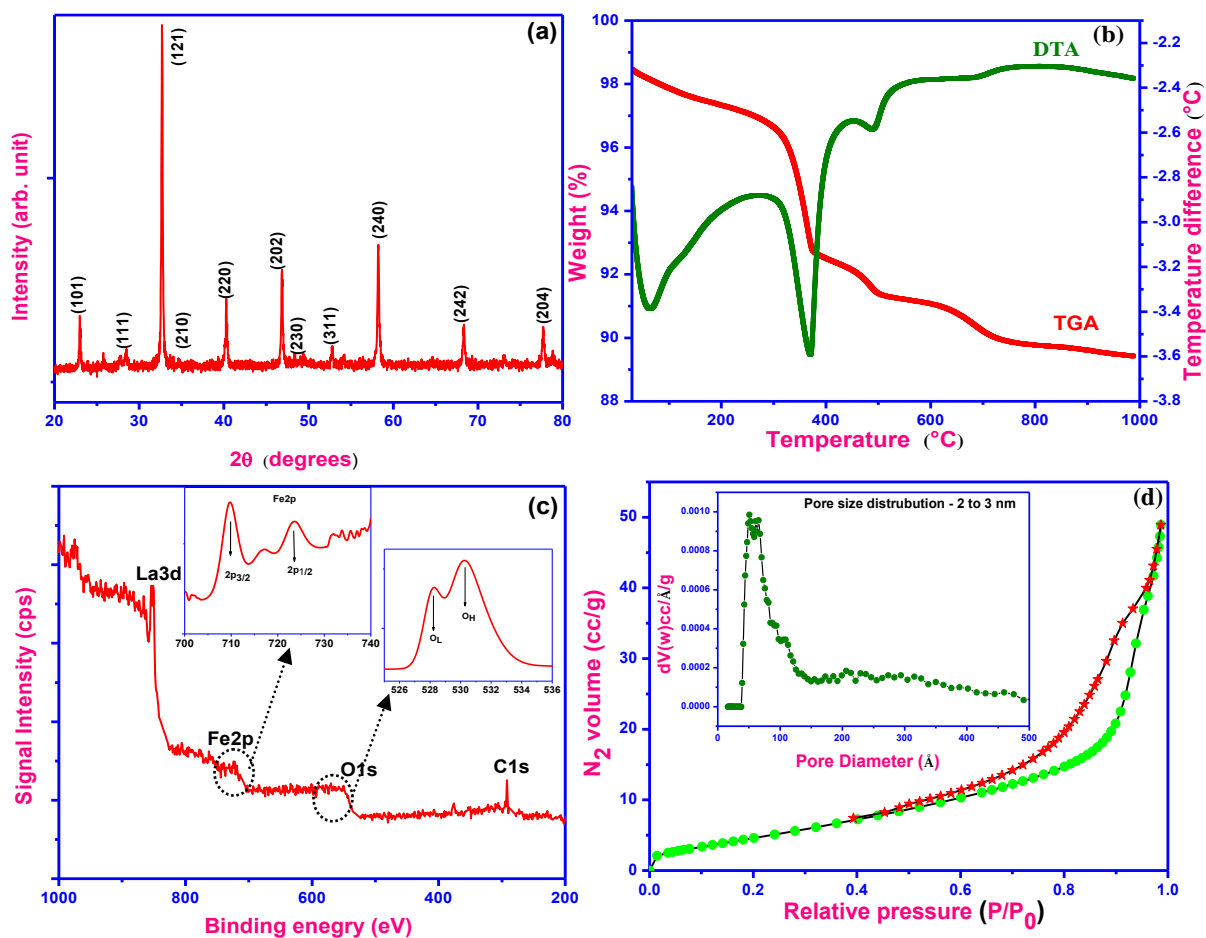
8

Fig. 2 (a) XRD pattern, (b) TG/ DTA curves, (c) XPS survey spectrum (inset - high resolution spectra of Fe and O) and (d) N_2 adsorption-desorption isotherm of microspheres constructed of $LaFeO_3$ nanospheres

The elemental makeup and the oxidation state of $LaFeO_3$ microspheres were studied using XPS survey spectrum (Fig. 2(c)). No peaks other than La(3d), Fe(2p), O(1s) and C(1s) were observed indicating the synthesized $LaFeO_3$ microspheres are of high purity. All the peaks were calibrated using C1s (284.6 eV) as the reference. La peaks were observed at 845.8 eV which corresponds to spin - orbit splitting of $3d_{5/2}$ and $3d_{3/2}$ of La^{3+} ions in oxide form. The peaks at 719.8 eV correspond to $2p_{3/2}$ and Fe $2p_{1/2}$ which is consistent with Fe^{3+} ions in oxide form.[19] The binding energy at 525.8 eV of O(1s) XPS signal is due to the contribution of La–O and Fe–O in $LaFeO_3$ crystal lattice. From the relative intensities of the XPS spectra, the atomic ratio was calculated as 1:1:3 between La, Fe and O.

9

N$_2$ adsorption-desorption isotherm (Fig. 2 (d)) of LaFeO$_3$ microspheres exhibit a type-IV isotherm with a hysteretic loop in the range 0.6–1.0 P/P$_o$ indicating the presence of mesoporosity. The BJH pore diameter distribution (inset in Fig. 2(d)) shows a pronounced peak, confirming a high degree of uniformity of the pores. The specific BET surface area is 95.80 m^{2}g^{-1} and the total pore volume is 0.105 cm^3 g^{-1} respectively. The generated mesoporosity in the material is owed to the inter-nanosphere space. The large surface area and pore volume indicates the LaFeO$_3$ microspheres would possess a fascinating adsorbing ability to analytes in biosensing.

3.4 Electrocatalytic properties of modified electrode with LaFeO$_3$ microspheres

Recently, research in the development of perovskite oxide nanostructures has emphasized its application in biosensing. The present study confirms the LaFeO$_3$ microspheres modified GC electrode can sense dopamine (DA), ascorbic acid (AA) and uric acid (UA). The electrocatalytic mechanism of modified electrode with LaFeO$_3$ microspheres (Fig. 3 (a)) for dopamine biosensing involves the electrochemical oxidation of Fe (III) producing Fe (IV) complex on the surface of electrode followed by the electron transfer of dopamine and consequently regeneration of Fe(III) in the complex. The oxidation of dopamine to dopaminequinone by liberating two hydrogen can be catalyzed by the Fe (IV)/(III) redox couple in the alkaline medium which is also confirmed from the oxidation and reduction peaks in Fig. 3(b). The modified electrode exhibited high electrocatalytic activity towards dopamine oxidation which improves the reversibility and enhances the electron transfer kinetics. The incorporation of Fe (IV) complex in the LaFeO$_3$ microspheres helped to improve the dopamine electrocatalytic activity.

In Fig. 3(b)(1) we observe that modified GCE has no redox peaks for DA, AA and UA and the oxidation peak potentials are close to each other. The cathodic peak at -60 mV

and anodic peak at 140 mV appears for 2 μM DA (Fig. 3(b)(2)), leading to a peak potential

separation (ΔE) of about 200 mV for modified GCE. Dopamine oxidation to

dopaminequinone results in the oxidation peak and the vice-versa leads to the appearance of
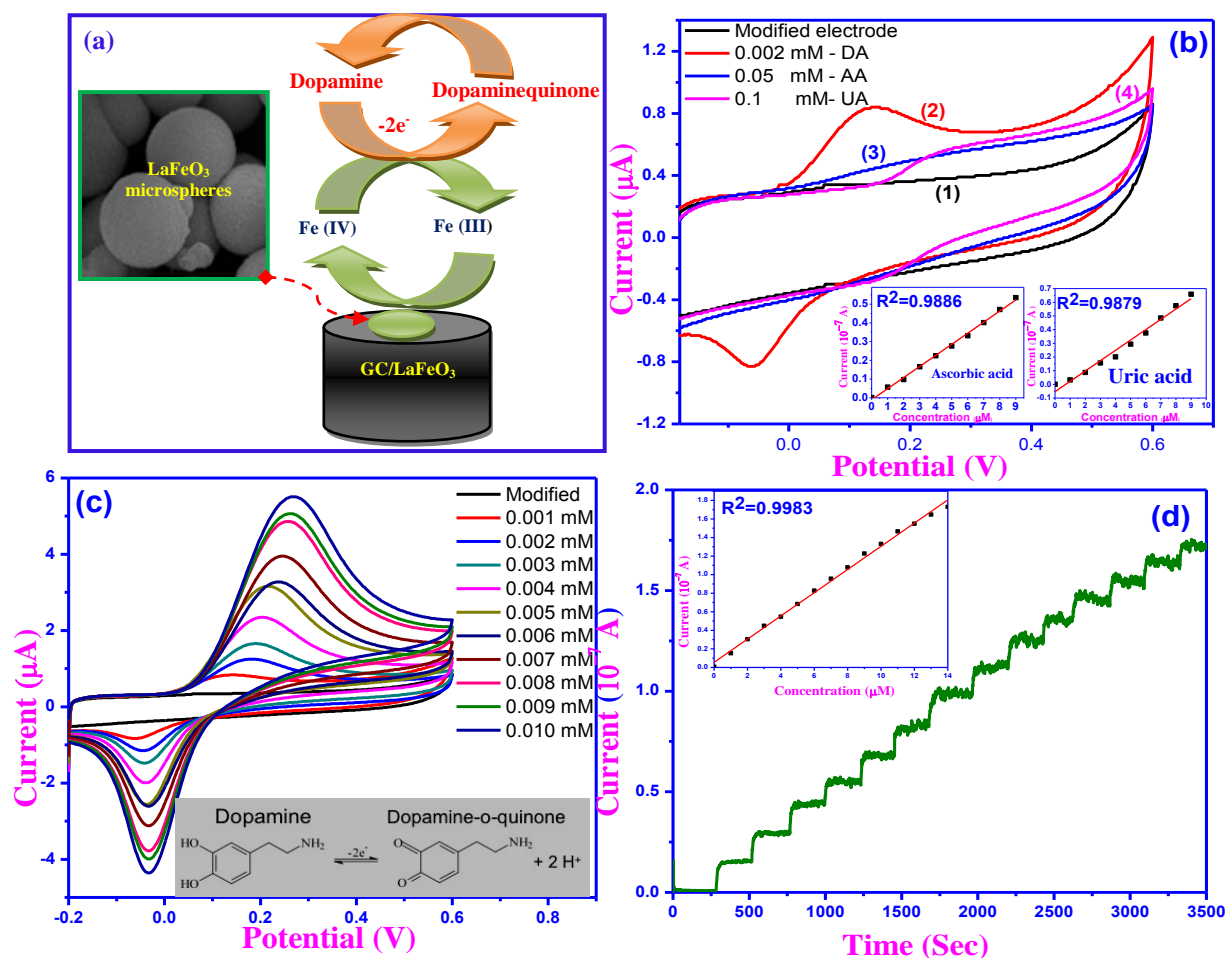
reduction peak.

Fig. 3 (a) Schematic illustration of electrocatalytic mechanism for dopamine oxidation at LaFeO₃ microspheres modified GCE, (b) CV recorded for low concentration of DA, high concentration of AA and UA (inset - current Vs concentration for UA and AA), (c) different concentration of DA (1 μM to 10 μM) and (d) amperometric i-t curve for the determination of DA by LaFeO₃ microspheres modified electrode (inset - current Vs concentration for DA)

In case of 50 μM AA and 100 μM UA broader oxidation peak at 188 mV (AA, Fig. 3(b) (3))

and 235 mV (UA, Fig. 3(b) (4)) were observed. The low oxidation peak separation of 48, 47

11

and 95 mV noticed for DA-AA, UA-AA and UA-DA are considered to be insufficient for

simultaneous determination of these species. The concentration of DA is found to be 25 times

and 50 times lesser for AA and UA. Finally, the negative surface of $LaFeO_3$ microspheres

attracts the cation DA and simultaneously repels AA and UA anions, which is clearly evident

from the redox peak at −50 mV. Therefore, the $LaFeO_3$ microspheres exhibit strong

electrocatalytic activity in response to dopamine.

The dopamine peak current was found to increase with dopamine concentration in the

range 1.5×10^{-7} to 5.6×10^{-6} M as represented by the linear curve with a correlation

coefficient of 0.9979 as shown in Fig. 3(d) (inset) and the sensitivity analysis was studied

using amperometric technique.[20] The current response for the addition of each 100 nM is

presented in Fig. 3d. The steady state current response was attained within 5 s with a sample

interval of 180 s. The dependence of response current on the concentration of DA was linear

with a correlation coefficient of 0.9983 as shown in the inset of Fig. 3(c). In the present

report, the low detection limit of 59 nM at S/N = 3 for dopamine concentration range 2×10^{-8}

to 1.6×10^{-6} M was obtained for the $LaFeO_3$ modified electrode confirming high selectivity

and good sensitivity towards DA. The fabricated $LaFeO_3$ modified electrode has been

compared with other reported modified electrodes to examine its superiority and the results

are shown in Table 1.

Table: 1Comparison of $LaFeO_3$ dopamine sensor with other reported sensors

Electrode	pH	Linear concentration	Detection limit (M)	Ref
MCPE	4.0	$2.6 \times 10^{-4} - 1.2 \times 10^{-3}$	2.5×10^{-5}	[21]
Ru–red/NaY/CPE	4.8	$1.2 \times 10^{-4} - 1.0 \times 10^{-2}$	8.5×10^{-5}	[22]
Ionic liquid carbon	6.8	$2.6 \times 10^{-6} - 1.5 \times 10^{-3}$	---	[23]
GNP-MEA-NIHCF	7.0	$8.2 \times 10^{-7} - 2.5 \times 10^{-3}$	53×10^{-8}	[24]
WO3·H2O-GCE	7.2	$1.0 \times 10^{-7} - 1.0 \times 10^{-6}$	12×10^{-8}	[25]
PEDOT-SWNT	7.0	$2.0 \times 10^{-5} - 1.0 \times 10^{-1}$	10×10^{-8}	[26]
$LaFeO_3$	7.2	$2 \times 10^{-8} - 1.6 \times 10^{-6}$	59×10^{-9}	this work

The reproducibility of LaFeO$_3$ microspheres modified electrode was evaluated from 0 - 10 μM concentration in the linear range 3.6 x 10^{-7} to 4.3 x 10^{-6} M by CV measurements. The relative standard deviation of LaFeO$_3$ biosensor at 1 μM response for 5 successive measurements was 2.7 % indicating good reproducibility. The stability of biosensors was studied by comparing the CV peak current at an interval of 4 h. The decrease in the cathodic peak current was less than 3.4 % indicating good stability. Moreover, the biosensor was able to retain 97.6 % of their initial response after one week of aging effect suggesting good long-term stability.

4. Conclusion

In summary, we report for the first time the use of reflux condensation process to form LaFeO$_3$ microspheres constructed of nanospheres. The modified GCE showed excellent electrocatalytic properties with high selectivity and good sensitivity to DA detection with a wide linear range and without interference from at least superior orders-of magnitude of AA and UA than DA. Therefore, the results highlight the promising use of LaFeO$_3$ microspheres in the construction of new dopamine biosensor.
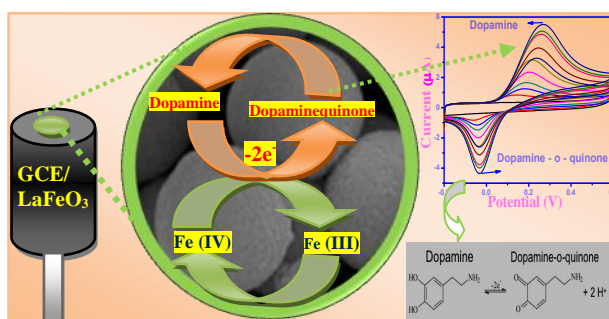
Acknowledgment

13

References

1 P.A. Prakash, U. Yogeswaran, and S.M. Chen, *Sensor,* 2009, **9**, 1821-1844.

2 G.S. Wilson, and R. Gifford, *Biosens. Bioelectron*., 2005, **20**, 2388-2403.

3 A. M. Bertorello, J. F. Hopfield, A. Aperia, and P. Greengard, *Nature*, 1990, **347**, 386-388.

4 P. Zhang, F. H. Wu, G. C. Zhao, and X. W. Wei, *Bioelectrochemistry,* 2005, **67**, 109-114.

5 S. Thirumalairajan, K. Girija, V. Ganesh, D. Mangalaraj, C. Viswanathan, and N. Ponpandian, *Cryst. Growth De*s., 2013, **13(1)**, 291-302.

6 H. Su, L. Jing, K. Shi, C. Yao, and H. Fu, *J. Nanopart. Res.,* 2010, **12**, 967-974.

7 G. Wang, J. Sun, W. Zhang, S. Jiao, and B. Fang, *Microchim. Acta.,* 2009, **164**, 357-362.

8 D. Kuang, A. Xu, Y. Fang, H. Q. Liu, and D. Fenske, *Adv. Mater.,* 2003, **15,** 747-1750.

9 R. de la Rica, C. Pejoux, C. F. Sanchez, A. Baldi, and H. Matsui, *Small,* 2010, **6**, 1092-1095

10 M. Law, L. E. Greene, J. C. Johnson, and P. D. Yang, *Nat. Mater.,* 2005, **4**, 455-459.

11 K. Girija, S. Thirumalairajan, A. K. Patra, D. Mangalaraj, N. Ponpandian, and C. Viswanathan, *Current Appl. Phys.,* 2013, **13** (4) 652- 658.

12 X. Li, C. Tang, M. Ai, L. Dong, and Z. Xu, *Chem. Mater,.* 2010, **22,** 4879-4889.

13 J. Chandradass, and K. H. Kim, *Mater. Chem. Phys.,* 2010, **122,** 329-332.

14 S. Thirumalairajan, K. Girija, Neha Y. Helber, D. Mangalaraj, C. Viswanathan, and N. Ponpandian, *RSC Adv.,* 2013, **3**, 7549-7561.

15 Y. Cheng, Y. S. Wang, Y. H. Zheng, and Y. Qin, *J. Phys. Chem., B* 2005, **109**, 11548-551.

16 J. Park, V. Privman, and E. Matijevic, *J. Phys. Chem., B* 2001, **105**, 11630–11635.

17 P. Sharma, J. Yeo, D. M. Kim, and C. H. Cho, *J. Mater. Chem.,* 2012, **22**, 2838–2843.

18 M. Lebid, and M. Omari, *Arab. J. Sci. Eng*., 2014, **39**, 147–152.

19 H. Su, L. Jing, K. Shi, C. Yao, and H. Fu, *J. Nanopart. Res.,* 2010, **12**, 967–974.

20 S. Comby, E. M. Surender, and T. Gunnlaugsson, *Inorg. Chem.* 2014, **53 (4)**, 1867-1879.

21 M.F.S. Teixeira, M.F. Bergamini, and N. Bocchi, *Talanta,* 2004, **63**, 1083-1088.

22 X.X. Yan, D.W. Pang, Z.X. Lu, and H.J. Tong, *J. Electroanal. Chem.,* 2004, 569, 47.

23 A. Safavi, N. Maleki, O. Moradlou, and F. Tajabadi, *Anal. Biochem.*2006, **359**, 224 -229.

24 P. Prabhu, R. Suresh Babu and S. SrimanNarayanan, *Sens. Actuators B*, 2011,*156*,606-614

25 V. Hariharan, S. Radhakrishnan, M. Parthibavarman, R. Dhilipkumar, C. Sekar, *Talanta*,

   2011, **85**, 2166-2174.

26 J. Mathiyarasu, L. Nyholm, *Electroanalysis* 2010, **22**, 449–454.

Table of content/Graphical abstract

Modified glassy carbon electrode with LaFeO$_3$ microspheres constructed of nanospheres having high electrocatalytic ability towards detection of neurotransmitter compound dopamine.