



# Electrochemical sensing of dopamine via bio-assisted synthesized silver nanoparticles

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## Abstract

A simple, environment-friendly, cost-effective procedure is proposed for the synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Ziziphus mauritiana* (ZmL) as reducing as well as stabilizing agent and its application on glassy carbon electrode (GCE) for the detection of dopamine by cyclic voltammetry (CV). Results showed a substantial enhancement of peak current using Ag@GCE as compared to bare-GCE for the detection of neurotransmitter dopamine. The synthesized ZmL-AgNPs were characterized using ultraviolet–visible (UV–Vis) spectroscopy, Fourier transform infra-red (FT-IR) spectroscopy, X-ray diffraction (XRD) and atomic force microscopy (AFM). We investigated the electrochemical responses of both bare-GCE and Ag-assembled GCE using cyclic voltammetry to delineate the performance enhancement due to ZmL-AgNPs. The present ZmL-AgNP-assembled GCE displayed very high sensitivity and selectivity with excellent linear calibration range 10–100  $\mu\text{M}$  for detection of dopamine with a limit of detection and limit of quantification values 0.1 and 0.3  $\mu\text{M}$ , respectively. The sensor was successfully applied for dopamine detection in real urine samples.

**Keywords** Biosynthesis · Silver nanoparticles · Plant extract · Dopamine detection · Urine samples

## Introduction

Dopamine (DA) is a neurotransmitter that plays a key role in the functioning cardiovascular (CVD), central nervous systems of mammals (CNSM) and renal functions. Deficit in DA is associated with Parkinson's disease, schizophrenia and some other neuropsychiatric disorders such as seizures, attention deficit disorders, cognitive, palsies, uncontrolled

anger, migraine headaches, etc. For this reason, monitoring of the level of DA neurotransmitter in vivo is considered an important indication for diagnosis [1–3]. The quantification and sensing of DA play important role in monitoring, diagnoses, avoidance and management of various disorders of neurons. For this reason, monitoring the concentration of DA is a necessary technique in neurological diseases for clinical diagnosis. Recently, different methods have been

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reported for the determination of DA in pharmaceutical preparations and biological samples, such as electrochemistry [4, 5], chemiluminescence [6, 7], spectrofluorimetry [8], high-performance liquid chromatography (HPLC) [9] with different detectors and mass spectrometry [10–12]. Most of these techniques are sophisticated, sensitive, expensive, time consuming and require complicated sample preparation. Therefore, the development of new, fast, sensitive and practical method for the detection of DA still remains a challenge. Due to this, simple, cost-effective, economical and useful sensor for the determination of DA is required. Moreover, as compared to advanced techniques for the analysis of DA, electrochemical cyclic voltammetry (CV) is efficient, simple and sensitive method using different metal nanoparticles (MNPs) which generated a lot of interest in the last few decades. Different routes for the synthesis of silver nanoparticles have been used using different chemicals, which are very toxic and detrimental to the environment. According to green chemistry rules; a green strategy has been adopted for the synthesis of AgNPs by using plant extract of *Ziziphus mauritiana* (*Zm*) leaves. Synthesis of silver nanoparticles using plant extract as capping/reducing agents becomes a key focus due to their single step with excellent plasmonic activity and eco-friendly nature [1]. The resource (such as leaves, fruits, flowers, vegetables, etc.) of the plant extract is known to manipulate the characteristics of the NPs [13]. *Zm* leaves are the first time to be used as capping/reducing agents for the synthesis of AgNPs and applied as an electrochemical sensor for DA detection. This plant is considered the most widespread fruit tree and is found in pastoral areas of Sindh, Pakistan. *Zm* plant is known to contain several bioactive phytochemicals such as phenolic acids, amino acids, carbohydrates, and vitamin A and ascorbic acid [14, 15]. The most important phytochemicals responsible to be used as stabilize or capping agent such as carboxylic acids, flavones, terpenoids, ketones, aldehydes, phenolic acids and amides were also reported and identified by Fourier transform infrared (FT-IR) spectroscopic studies [16–20]. The present work is based on the synthesis of simplistic and stable *Ziziphus mauritiana* leaf-based silver nanoparticles (*ZmL*-AgNPs). The formation and characterization of *ZmL*-AgNPs were confirmed by UV/visible spectrophotometer, FT-IR, XRD and AFM techniques. Finally, the sensitivity of the assembled AgNPs@GCE for dopamine detection was checked via differential pulse voltammetry (DPV) technique.

## Materials and methods

All chemicals were purchased from Sigma-Aldrich unless otherwise stated and used without further purification: silver nitrate ( $\text{AgNO}_3$ ), dopamine, hydrochloric acid (HCl), sodium hydroxide (NaOH), glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ), uric acid

( $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$ ), ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), Nafion ( $\text{C}_7\text{HF}_{13}\text{O}_5\text{S}$ ,  $\text{C}_2\text{F}_4$  0.1 M, 5%), ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ), leaf extract of *Ziziphus Mauritiana* (*ZmL*), potassium salt (KCL), and phosphate buffer (0.1 M of pH 7.4) used as active electrolyte in this study. Milli-Q water was used in all the experiments. All glassware used in the production of nanoparticles was cleaned with distilled water and ethanol and then dried in an oven at 50 °C before use.

## Characterization techniques for *ZmL*-AgNPs

Synthesized *ZmL*-AgNPs were characterized using (UV–visible spectrophotometer, Model-Perkin Elmer 365), (X-ray diffraction, model Bruker D-8), (Atomic force microscopy model-Agilent 5500, Santa Clara, USA) and (Fourier Transform infrared spectrophotometer model-Nicolet 5700 of Thermo, Madison, USA) while (electrochemical measurements were recorded using a (model 760 E-bi-potentiostat CH Instruments, TX and USA).

## Extraction method

25 g of fresh *ZmL* was added in 100 mL of Mili-Q water and allowed to boil at 100 °C for 15 min; solution was cooled at room temperature. The extract was filtered by Whatman no. 1 filter paper to get a clear solution. The filtrate was stored at 4 °C for further synthesis of nanoparticles.

## Synthetic protocol

*ZmL*-AgNP synthesis was carried out by following mentioned procedure: 3.5 mL of leaf extract solution was mixed into 22.5 mL of 1 mM silver nitrate solution. Then the mixture was shaken at 130 rpm on electric or mechanical shaker machine for up to 30 min until brown color was observed; it indicated the formation of AgNPs. Further, the complete reduction of (silver ions  $\text{Ag}^+$ ) was carried out within 24 h; in addition, the stability of synthesized *ZmL*-AgNPs was monitored for up to 2 weeks and it shows that particles have outstanding stability.

## Modification of electrode

Briefly, electrode modification was achieved by clean; rinse and thoroughly polished GCEs were treated in sonicated bath filled with de-ionized water and ethanol to achieve a proper surface before modification. To modify the GCE's surface "simple drop-casting methodology" was used with an exact amount 3  $\mu\text{L}$ . 0.2 g  $\text{mL}^{-1}$  from the suspension of ethanol of functionalized *ZmL*-AgNPs. Under the  $\text{N}_2$  purging system modified layer was then dried and by casting Nafion membrane to make sure complete adherence of NPs

to the modified layer. The devised electrode is named *ZmL*-AgNPs@GCE/Naf throughout the manuscript [21].

### Electrochemical detection of dopamine

The electrochemical detection of dopamine was achieved using differential cyclic voltammetry as primary mode of quantification. The experimental system consisted of three-electrode system with Ag/AgCl as a reference and Pt wire as a counter electrode [22]. The measurements were performed using *ZmL*-AgNP-GCE as working electrode at room temperature, within the potential window of 0.15–0.55 V. Sensitivity of the sensor was  $10 \times 10^{-6} \text{ AV}^{-1}$ . The phosphate buffer was taken as an active electrolyte solution with conc. 0.1 M at 7.4 pH.

### Sample preparation for characterization

#### Fourier transform infrared spectroscopy (FT-IR)

Solution of synthesized *ZmL*-AgNPs was poured in the Petri dish and dried on pre-heated water bath at 100 °C. The silver nanoparticles were dried and washed with methanol and deionized water three times to remove organic and inorganic impurities. The substance was scratched, gathered and handled in KBr pellets for FT-IR contemplates within the range of 400–4000  $\text{cm}^{-1}$ .

#### X-ray diffractometry (XRD)

For XRD sample solid *ZmL*-AgNPs were obtained by following the same procedure as mentioned above for FTIR. Dried material of silver nanoparticles was further dissolved in acetone. After that, it was sonicated for homogenous suspension. Moreover, it was spiked on a cover slip which was placed in the center of Petri dish. Finally, the petri dish was shifted on a pre-heated water bath at 100 °C. Dried well-coated disk with silver nanoparticles was processed for XRD to record X-ray diffraction patterns at  $2\theta$  degree in the range of 20°–80°.

#### Atomic force microscopy (AFM)

AFM images of both liquid and dried *ZmL*-AgNPs can be used; but in this study, liquid sample was applied on a cleaved clean mica sheet and proceed for AFM study (without any pre-treatment before analysis).

### Real sample preparation

Especially, urine samples collected from 15 kidney failure patients of LUMDL by following the sampling manual of the international sampling standard for urine. Immediately,

liquid samples were stored at 20 °C before analysis. 0.1 g of active carbon was added to 5 mL of sample for discoloring. The sample was centrifuged using a vortex-mixture for 20 s and separated by filter paper. In the last sample, filtrate yield was diluted in (0.1 M 100  $\text{mL}^{-1}$ ) NaOH solution and the solution was mixed using sonication procedure until dilution completed.

## Results and discussion

### UV-Vis spectroscopy

The bio-reduction of *ZmL*-AgNPs was evaluated by UV-Vis spectroscopy. This technique is very important for the analysis of metal nanoparticles and provides in-depth information about the optical properties. UV-visible spectroscopy results confirmed the existence of Ag because of the particular peak in the region of 400–430 nm it depends upon the type of capping agent [24]. The *ZmL*-AgNPs showed the characteristic peak of surface plasmon resonance absorption band at 426 nm is also reported by [28–30]. This confirms the formation of nanoparticles and synthesized particles was stable up to 2 weeks as shown in Fig. 1a.

### Fourier transform infrared spectroscopy (FT-IR)

FTIR analysis was performed to identify the major functional groups present in *Zm* and their possible association in the preparation and stabilization of *ZmL*-AgNP pellets. FTIR spectrum (Fig. 2a) shows extremely strong vertices and strips varying at 3304, 2917, 1728, 1610  $\text{cm}^{-1}$  may be related to the amide groups of proteins released by herbal or aromatic compounds of carbon and carbon double bonds. There are clear changes in peaks seen in Fig. 2b after the

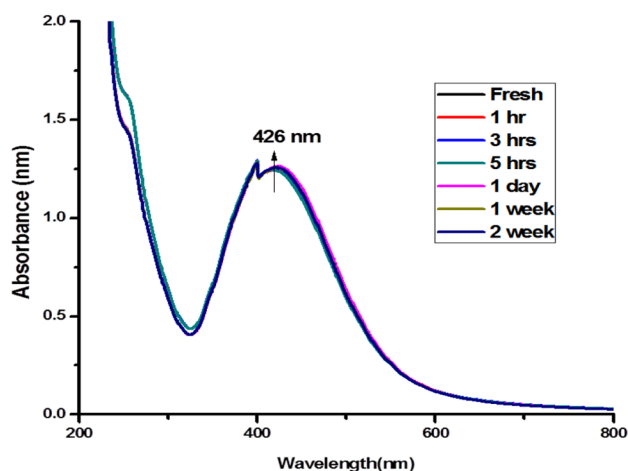
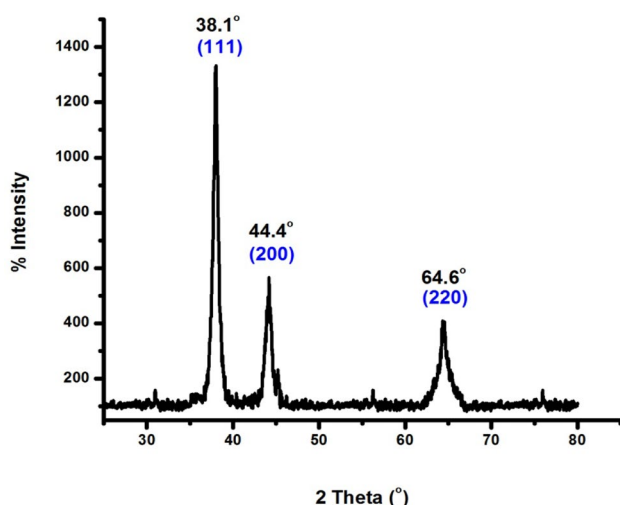
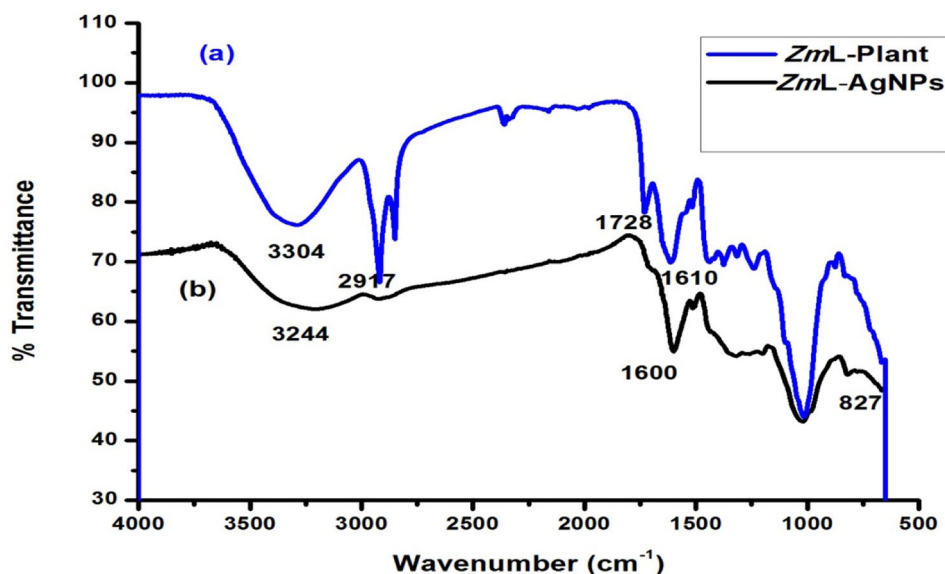


Fig. 1 a Stability of *ZmL*-AgNPs



**Fig. 2** a FTIR spectrum of leaf extract of *Ziziphus mauritiana* b *ZmL*-AgNPs



**Fig. 3** XRD pattern of synthesized *ZmL*-AgNPs

formation of NPs with shift from 3304 to 3244  $\text{cm}^{-1}$ , while 2917 and 1728  $\text{cm}^{-1}$  totally disappeared, with slight shifting in 1610–1600  $\text{cm}^{-1}$  was observed which are related to the C=O bond of the carbonyl group and the long-term vortices of the amide also occurs in this range and new peak appeared around 500  $\text{cm}^{-1}$ , which is not present in spectrum a but present in b; it confirms the formation of *ZmL*-AgNPs.

### X-ray diffractometry (XRD)

The crystalline nature of *ZmL*-AgNPs was further confirmed from X-ray diffraction (XRD) analysis as shown in Fig. 3. The typical XRD patterns of *Ziziphus Mauritiana* reduced silver nanoparticles show that the number of Bragg reflections at  $2\theta$  values was observed at 38.1°, 44.4° and 64.6°,

respectively. These results illustrate that the silver nanoparticles formed in this present synthesis are crystalline in nature. It was compared and confirmed by JCPDS File No. 04–0783. Moreover, the most intensive peak located at  $2\theta = 38.1$  corresponding to the diffractions of spherical nanoparticles crystallized in the FCC structure with basal (111) lattice plane [27].

### Atomic force microscopy (AFM)

Morphology and topography of *ZmL*-AgNPs were studied by atomic force microscopy (AFM). In Fig. 4a, it was noticed that *ZmL*-AgNPs are spherical shape with small size in the range of 5–55.5 nm and Fig. 4b shows histogram (size distribution) of silver nanoparticles.

### Electrode study

The cyclic voltammetry (CV) was used as a primary mode for describing the electrochemical behavior of the modified glassy carbon electrode through silver nanoparticles. Cyclic voltammetry response for dopamine shows oxidation at modified electrode, silver nanoparticles modified electrodes in the absence and presence of dopamine solution of 1 mM in phosphate buffer solution of pH 7.4 was performed 50  $\text{mVs}^{-1}$  as shown in Fig. 5. The silver nanoparticle-modified electrode shows a prominent peak at 0.8 V with a high value of anodic peak current; however, there is an obvious peak appeared for unmodified glassy carbon electrode with small anodic peak current. For bare electrode, negligible or very little peak was observed; this indicates that silver nanostructures exhibit good conductivity along with excellent electrocatalytic properties for the oxidation of dopamine. This is a novel approach for the fabrication of biosensors based

(a)

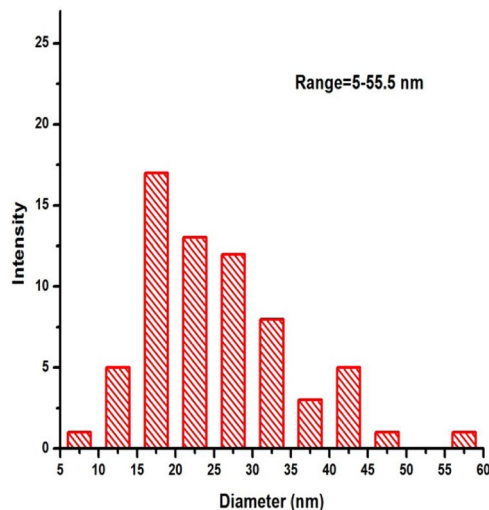
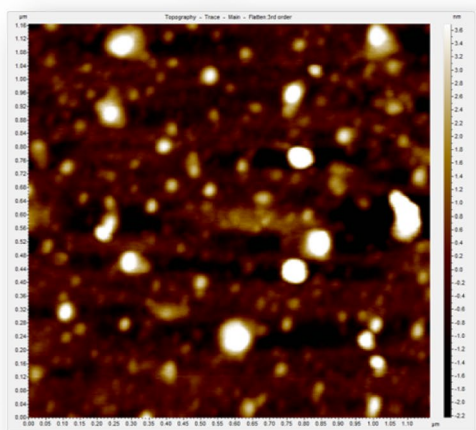


Fig. 4 a AFM image of *ZmL*-AgNPs. b Histogram of *ZmL*-AgNPs

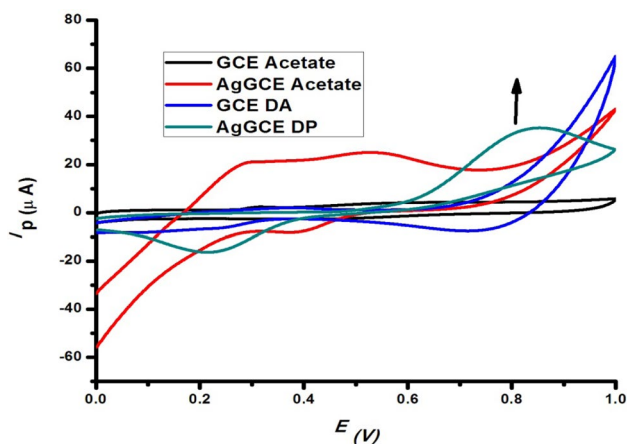


Fig. 5 Cyclic voltammetry study at various electrodes

on AgNPs and can be used for the development of sensitive sensor devices. The improved conductivity and high surface to volume ratio of *ZmL*-AgNPs modified electrode showed favorable oxidation of dopamine at low potentials.

**Scan rate study**

The effect of scan rate at *ZmL*-AgNPs@GCE modified electrode was investigated in 1 mM dopamine as depicted in Fig. 6. This study has revealed that the oxidation peak potential and peak current possessed linear relationship for the scan rates from 200 to 500 mVs<sup>-1</sup> as shown in Fig. 6a. This signifies that the oxidation of dopamine at *ZmL*-AgNPs@GCE-modified electrode is diffusion controlled. Cyclic voltammograms recorded with *ZmL*-AgNP nanostructures

modified GCE in 0.1 M phosphate buffer 7.4 pH in the existence of 1 mM dopamine at scan rates 10–100 mVs<sup>-1</sup> Fig. 6b shows the plot of *I*<sub>p</sub> vs. square root of scan rate.

**Quantification of dopamine**

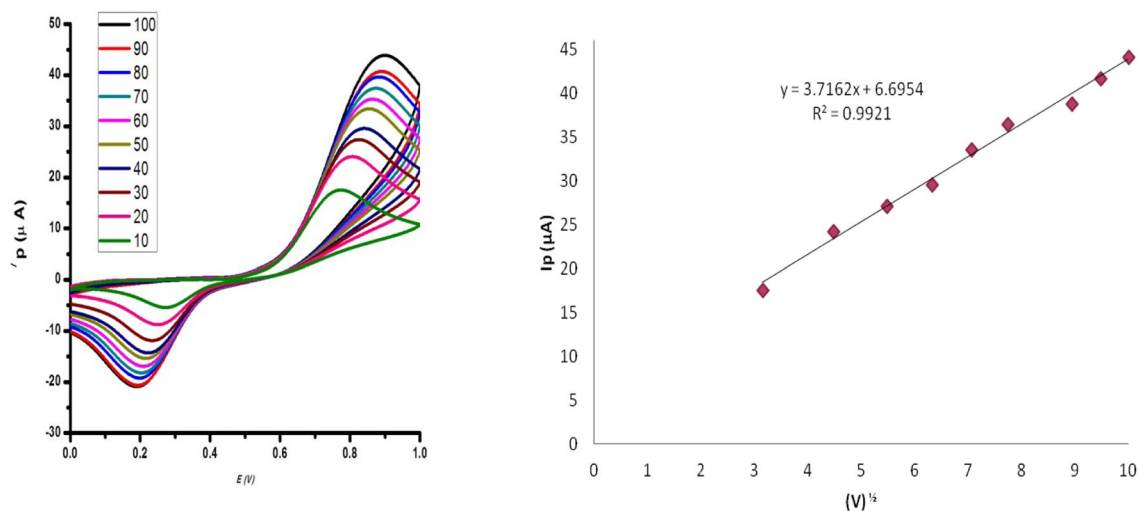
Quantification of dopamine was carried out through DPV technique to check the sensitivity of the prepared *ZmL*-AgNP-assembled-GCE. Different concentrations were spiked in the range of 10–100 μM and the corresponding variation of the peak anodic current *I*<sub>pa</sub> was recorded as shown in Fig. 7a. The graph shows a linear increase in *I*<sub>pa</sub> with the increase in the DA concentration with a correlation coefficient *R*<sup>2</sup> = 0.992 shown in Fig. 7b. The limit of detection (LOD) 0.1 μM and limit of quantification (LOQ) is 0.37 μM at *ZmL*-AgNP-assembled-GCE.

**Reproducibility of sensor**

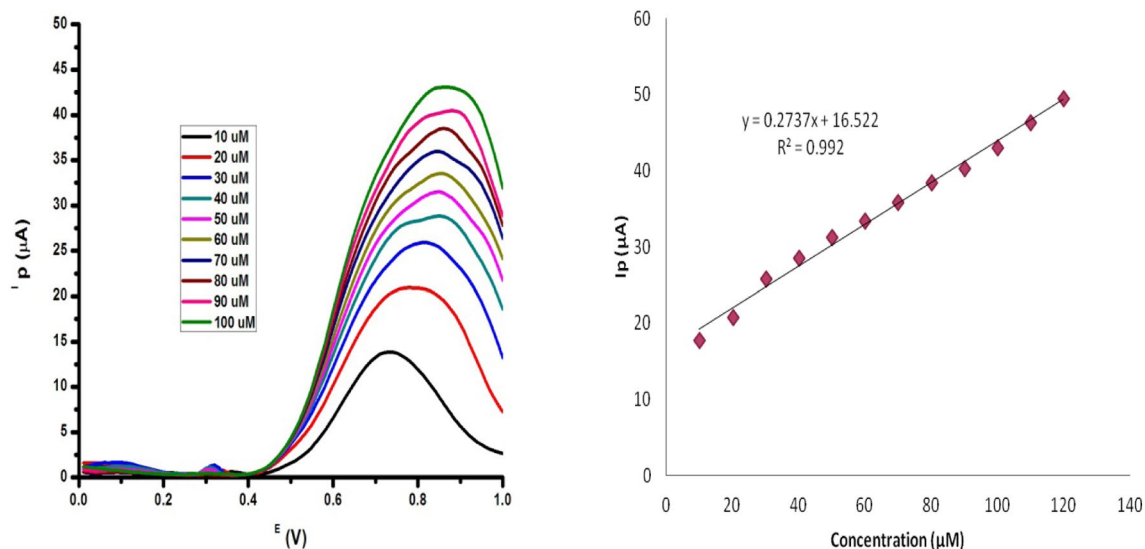
The reproducibility of the DA sensor was investigated with 5 repeated runs indicating that the developed sensor inherits good reproducibility for sensing of dopamine as shown in Fig. 8. It means we can use a single modified electrode for several measurements with any appreciable loss in its activity for DA detection.

**Sensor stability**

Repeatability and stability of the DA sensor were checked by analyzing 5 similarly modified *ZmL*-AgNP-assembled glassy carbon electrodes for sensing of 10 μM DA solution at pH 7.4. According to procedure each electrode was



**Fig. 6** **a** The effect of scan rate at *ZmL*-AgNP-modified electrode was investigated in 1 mM dopamine. **b** The plot of  $I_p$  vs. square root of scan rate



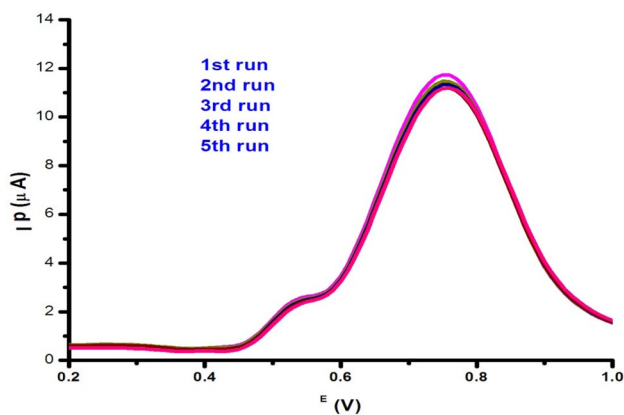
**Fig. 7** **a** Concentration was varied in the range of 10–100  $\mu\text{M}$  and the corresponding variation of the peak anodic current  $I_{pa}$  was recorded. **b** Graph shows a linear increase in  $I_{pa}$  with the increase in the DA concentration with a correlation coefficient value is 0.992

checked for its performance for 3 alternate days with 3 replicates and compared with a standard value of DA taken from the calibration plot. Table 1 presents the recorded data.

The data suggest that all the electrodes perform in a range of 98.7–102% recovery with respect to the original concentration of 10  $\mu\text{M}$  DA taken as 100%. Thus the repeatability of each similarly modified electrode at several days will not affect the overall stability. The little bit of variation is due to human error for depositing the accurate quantity of *ZmL*-AgNPs over the surface of GCE.

### Selectivity of sensor

Developing a selective dopamine biosensor was a challenging task due to the coexistence of competing agents such as glucose, uric acid and ascorbic acid in physiological fluids. This may cause interference during the electrochemical detection of dopamine due to their closely related oxidation potential. To demonstrate the selectivity and suitability of silver nanostructures for the prevention of interferences from these species, an amperometric technique was used as shown in Fig. 9 and Table 2. The experiment was performed by taking a concentration of 1 mM



**Fig. 8** Reproducibility of sensor for the developed sensor for DA detection

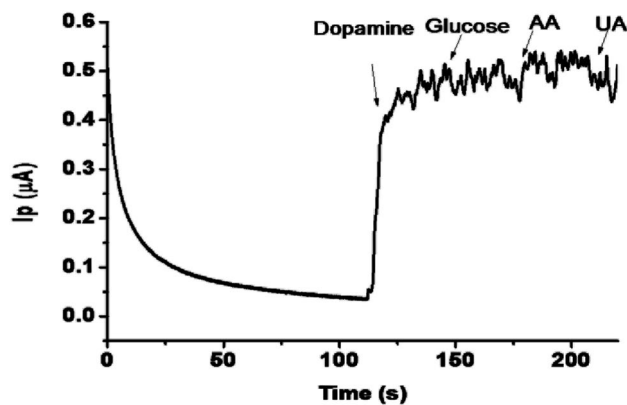
dopamine along with 10 mM each of ascorbic acid, uric acid, and glucose in PBS of pH of 7.4 under stirred conditions. It can be viewed that an increase in current potential was observed when dopamine was added and negligible change in current was found after the addition of glucose, uric acid and ascorbic acid. this indicates that the prepared silver nanoparticles have an excellent electro-catalytic property for dopamine and relatively poor response for other co-existing interference species in physiological fluids. Hence by looking at the results of selectivity it was safe to say that the purposed dopamine biosensor can be used as a potential candidate for the determination of dopamine with high selectivity.

It is noted that most of the above-published papers are given in Table 3. Medicinal plants were used by [23, 24] synthesized silver nanoparticles (AgNPs) applied for antimicrobial applications. Moreover, [25] developed a sensor for the detection of dopamine but silver nanoparticles are not much efficient hence used graphene oxide as a supportive material to enhance the sensitivity of electrochemical sensor. Moreover [26] used surfactant (SDS) to improve the stability of synthesized silver nanoparticles. However, it is reported for the first time a single step, facile, bio-assisted, fast and easy method to synthesize AgNPs via leaf extract of *Ziziphus mauritiana* plant without any supportive material in synthesis as well as in application.

**Table 1** Data for proving stability and repeatability of the sensor via DA recovery

| Electrode # | Day 1 (DA in μM) | Day 3 (DA in μM) | Day 5 (DA in μM) | % Recovery |
|-------------|------------------|------------------|------------------|------------|
| 1           | 9.9 ± 0.3        | 10.1 ± 0.1       | 9.8 ± 0.2        | 99.3       |
| 2           | 10.4 ± 0.4       | 10.1 ± 0.6       | 10.0 ± 0.3       | 101.7      |
| 3           | 9.9 ± 0.2        | 9.9 ± 0.4        | 10.1 ± 0.2       | 99.7       |
| 4           | 9.9 ± 0.2        | 9.9 ± 0.5        | 9.8 ± 0.4        | 98.7       |
| 5           | 10.2 ± 0.4       | 10.1 ± 0.4       | 10.3 ± 0.4       | 102.0      |

± standard deviation



**Fig. 9** Interference study

**Table 2** Interferences are well under the acceptable limit and the results verify the suitability of modified GCE for DA determination selectively

| Interfering ions | Percent interference% |
|------------------|-----------------------|
| Uric acid        | 0.4                   |
| Ascorbic acid    | 2.1                   |
| Glucose          | 1.3                   |

### Application on real samples

Urine samples were 10 times diluted with buffer before spiking and mean percent recovery values were found out for the 3 replicates by spiking dopamine solution into the samples. The percent recovery values lied in the range of 99.4–100.5% that distinctively corroborates the appropriateness of the proposed sensor for the analysis of dopamine from urine samples as shown in Table 4.

### Conclusion

The present study reports an easy one-pot synthesis of *ZmL*-AgNPs from silver nitrate salt and leaf extract of *Ziziphus mauritiana* which are used for the first time as reducing as well as stabilizing agents. It was characterized via sophisticated techniques such as UV–Vis spectroscopy, FTIR spectroscopy and AFM imaging. *ZmL*-AgNPs show good

**Table 3** The comparative data of other reports especially based on LOD of DA

| Probe                                  | Application            | (LOD $\mu\text{M}$ ) | References   |
|--|------------------------|----------------------|--------------|
| <i>Camellia sinensis</i> -AgNPs        | Antimicrobial activity | –                    | [23]         |
| <i>Ziziphus nummularia</i> -AgNPs      | Antimicrobial activity | –                    | [24]         |
| Graphene oxide-AgNPs                   | Electrochemical        | 0.2                  | [25]         |
| Banyan leaf-AgNPs + SDS                | Electrochemical        | 0.08                 | [26]         |
| <i>Ziziphus mauritiana</i> (ZmL-AgNPs) | Electrochemical        | 0.1                  | Current work |

**Table 4** Application of developed sensor on urine samples

| Samples | Spiked ( $\mu\text{M}$ ) | Found ( $\mu\text{M}$ ) | $\pm$ SD | Percent recovery |
|---------|--------------------------|-------------------------|----------|------------------|
| Urine-1 | 20                       | 19.9                    | 0.01     | 100.5            |
| Urine-2 | 50                       | 50.1                    | 0.20     | 100.2            |
| Urine-3 | 90                       | 89.5                    | 0.01     | 99.4             |

SD standard deviation,  $n = \text{no. of replicates}$   $n = 3$

stability with small particle size in the range of 5–55.5 nm. ZmL-AgNPs show good sensing properties for detection of DA with good linear response in the concentration range of 10–100  $\mu\text{M}$ . The time response of the sensor was found to be three min with a detection limit of 0.1  $\mu\text{M}$  as well. This proposed sensor may offer sensitive and low-cost strategy for the detection of dopamine from biological samples.

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## Declarations

**Conflict of interest** All authors have no disagreement and opposing interests regarding the submitted research work.

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