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# Microplate based optical biosensor for L-Dopa using tyrosinase from *Amorphophallus campanulatus*

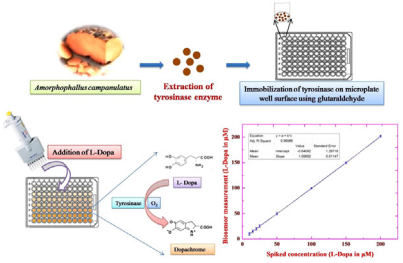
Amardeep Singh Saini, Jitendra Kumar, Jose Savio Melo *

*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India*

## HIGHLIGHTS

- Simple, rapid method of detection requiring small sample volume.
- First report on immobilization of tyrosinase from *A. campanulatus* on microplate.
- A wide linear range and low detection limit were obtained.
- Multiple sample analysis on single platform.
- Analysis of real sample (spiked blood plasma) was carried out.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Developing a biosensor which is capable of simultaneously monitoring L-Dopa levels in multiple samples besides requiring small reaction volume is of great value. The present study describes the detection of L-Dopa using tyrosinase enzyme extracted from *Amorphophallus campanulatus* and immobilized on the surface of the microplate wells. Among the different approaches used for immobilizing tyrosinase onto the microplate wells, glutaraldehyde treatment was found to be most effective. Besides enzyme activity, ESEM–EDS (environmental scanning electron microscope–energy dispersive system) and Atomic Force Microscopy (AFM) were also carried out to confirm the immobilization of tyrosinase enzyme onto the microplate well surface. This immobilized biocomponent was then integrated with an optical transducer for L-Dopa detection and it showed good reproducibility. The sensing property of the system was studied by measuring the initial rate of dopachrome formation at 475 nm. The calibration plot gave a linear range of detection from 10–1000 μM and the detection limit was calculated to be 3 μM. The immobilized biocomponent was stable for 41 days and was reused up to nine times. Spiked samples (blood plasma) were also analyzed using this biocomponent. This microplate based biosensor thus provides a convenient system for detection of multiple samples in a single run.



## 1. Introduction

L-Dopa is the precursor of various catecholamines and the immediate product of the rate limiting step in catecholamine biosynthesis. Hence, it occupies a prime position in functioning of the effector systems that use catecholamines. Measuring levels of L-Dopa in biological samples can thus provide important information relevant to diagnosis and treatment of several diseases. Plasma L-Dopa levels can help to detect a variety of disorders including tumors and inherited neurological diseases. Patients having neuroblastoma [1], malignant pheochromocytoma [2] and malignant melanoma [3] are reported to have high plasma L-Dopa levels. Also, patients receiving exogenous L-Dopa treatment are reported to have elevated plasma L-Dopa levels in the range of micrograms (milligrams) per liter [4]. Such high plasma levels are

* Corresponding author. Tel.: +91 22 25592760; fax: +91 22 25505151.
*E-mail addresses:* saini_amardeepsingh@yahoo.co.in (A.S. Saini), jkumar@barc.gov.in (J. Kumar), jsmelo@barc.gov.in (J.S. Melo).

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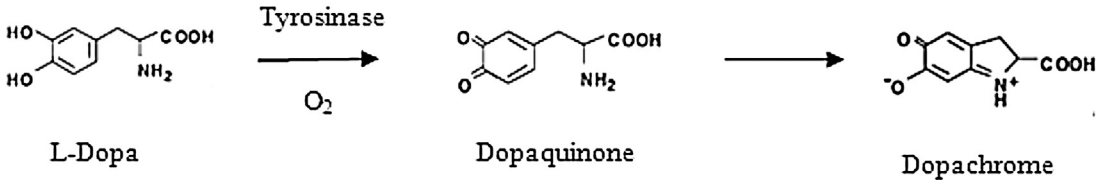
also observed during the pharmacokinetic studies of L-Dopa [5]. Thus, besides monitoring the levels of L-Dopa it is essential to have a detection system which is fast, sensitive and inexpensive.

Determination of L-Dopa has been carried out using various methods such as spectrophotometry [6], HPLC (high-performance liquid chromatography) [7], and electrochemical detection [8]. The development of biosensor for detection of L-Dopa has also attracted attention [9,10]. For this purpose generally, tyrosinase enzyme has been used as a biocomponent. Tyrosinase is a copper containing metalloprotein that utilizes molecular oxygen to catalyze the conversion of L-Dopa to dopachrome, which is a chromophoric product and can be detected with the help of an optical transducer.




L-Dopa Dopaquinone Dopachrome

In general, biosensors are gaining importance in various applications for the real time monitoring of analytes. A variety of approaches has been suggested for the development of such devices. The basic requirement of a biosensor is that the biological material should bring the physio-chemical changes in close proximity of a transducer [10,11]. Immobilization technology has played a major role in this direction [12,13]. A number of techniques have been developed in our laboratory for the immobilization of enzymes as well as cells on different support matrices for biosensor applications [10,14–18]. Fiorentino et al. [9] have reported the immobilization of mushroom tyrosinase in polyelectrolyte multilayer for developing an optical sensor. Also, Bertolino et al. [19] developed a microfluidic–enzymatic biosensor using immobilized tyrosinase, for electrochemical detection of pipemidic acid. Liu et al. [20] have made use of tyrosinase enzyme and immobilized it on modified core–shell magnetic nanoparticles for developing a biosensor in order to detect phenols. However, in all the above methods large number of samples cannot be analyzed at the same time, which is a limitation.

Currently, there is an interest in the development of biosensors for simultaneous analysis of multiple samples. In our earlier work, we have reported the use of microplate as a support for the immobilization of microbial cells and have used it for detecting multiple samples of pesticide [15,16].

In the present study, we have immobilized tyrosinase enzyme from *Amorphophallus* campanulatus onto the microplate well surface for the first time and have used it for developing an optical biosensor in order to detect L-Dopa. The analysis is based on the relationship between oxidation of L-Dopa and the amount of dopachrome formed; that was quantified by measuring the absorbance at 475 nm. The biosensor was calibrated using different concentrations of L-Dopa and its range of detection was measured. The analytical performance of the biosensor with respect to reproducibility, reusability and stability were evaluated. The proposed sensor was successfully applied for the determination of L-Dopa in spiked blood plasma samples. Thus, this microplate based biosensor has overcome the limitation of other sensors in the detection of multiple samples of L-Dopa on a single platform at one time.

## 2. Material and methods

### 2.1. Materials

3,4-Dihydroxy phenyl alanine (L-Dopa) was purchased from Sigma–Aldrich (USA, product number: D9628-25G), tyrosinase was extracted from corm of an elephant foot yam (*A. campanulatus*). Vacuum-gas plasma treated microplate (96 wells) made up of polystyrene was procured from BD Falcon (USA, product number: 353,072). Polyethyleneimine (PEI) (product number: P-3143) and glutaraldehyde (grade I, 25% in water, product number: G5882) were purchased from Sigma–Aldrich (USA). All other reagents used were of analytical grade.

### 2.2. Extraction of tyrosinase

Tyrosinase was extracted from corm of the tuber *A. campanulatus* [21] and then subjected to partial purification. For this, 2.6 kg of *A. campanulatus* was taken and homogenized using chilled phosphate buffer (0.1 M, pH 6). The homogenate was filtered using cheese cloth and the filtrate was then subjected to centrifugation at 2500 rpm for 7 min. The supernatant was further subjected to ammonium sulfate fractionation in the range of 20–60% saturation under cold conditions. The maximum tyrosinase activity was obtained at 60% saturation. The precipitate obtained was redissolved in fresh extraction buffer and dialyzed against phosphate buffer (10 mM, pH 6). The sample was then assayed for tyrosinase activity and total protein content by Folin's Lowry method. All the operations were carried out at 4 °C and the enzyme was stored at the same temperature. The enzymatic activity was calculated by measuring the formation of dopachrome at 475 nm. One unit of enzyme activity was defined as an increase in absorbance by 0.001 $min^{-1}$ at 475 nm at pH 6.0 and a temperature of 30 °C [10]. The extracted enzyme had a protein content of 1.2 mg $mL^{-1}$ and specific activity of 11,457 U $mg^{-1}$. The copper content of the enzyme sample was estimated using atomic absorption spectroscopy and was found to be $0.9 \pm 0.01$ μg $mg^{-1}$ of protein present.

### 2.3. Spectral study of dopachrome formation using free enzyme

For spectral study 200 μL of L-Dopa was added along with 40 μL of tyrosinase enzyme (78 U) to the microplate well. Spectrum was recorded in the range of 400–600 nm before adding the enzyme and also after 1, 3 and 5 min of enzyme addition.

### 2.4. Immobilization of tyrosinase on microplate well surface

Microplate (96 wells) made of polystyrene was used as a support for the immobilization of tyrosinase enzyme. Three different approaches were followed for immobilization of tyrosinase enzyme.

#### 2.4.1. Adsorption by directly loading enzyme onto microplate well surface

In this method, 40 μL of tyrosinase enzyme was added onto the microplate well surface and left for 1 h at 37 °C. The plate was then washed with phosphate buffer (0.1 M, pH 6.0) to remove any unbound enzyme and kept at 4 °C in the same buffer till further use.

#### 2.4.2. Adsorption followed by glutaraldehyde treatment

In the second method, 40 μL of tyrosinase enzyme was added in the microplate well and left for 1 h at 37 °C. After this, 1% glutaraldehyde was added and the plate was incubated at 37 °C for

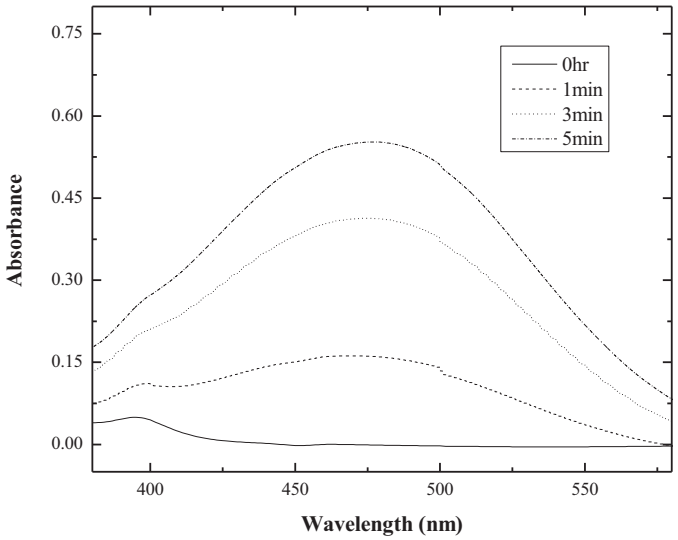



**Fig. 1.** Wavelength scan showing formation of dopachrome at different time intervals.

another 30 min. After incubation it was washed with phosphate buffer (0.1 M, pH 6.0) and kept in the same buffer at 4 °C till further studies.

#### 2.4.3. Adsorption followed by PEI treatment

In the third approach, 40 μL of tyrosinase enzyme was added onto the microplate well and left for 1 h at 37 °C. After this, 1% PEI was added and the plate was incubated at 37 °C for another 30 min. After incubation; it was washed with phosphate buffer (0.1 M, pH 6.0) and kept at 4 °C in the same buffer till further use.

### 2.5. ESEM–EDS study of tyrosinase immobilized on microplate

Scanning electron microscope (Quanta 200 ESEM, FEI, USA) was employed to observe the change in surface structure of the microplate well after immobilization of tyrosinase enzyme. ESEM was carried out by mounting pieces of microplate well onto the stub, and micrographs of the wells (blank well and immobilized) were studied. EDS spectra was also recorded for the respective samples.

### 2.6. Determination of kinetic constants

The kinetic constants $K_m$ and $V_{max}$ were determined for both free and immobilized tyrosinase enzyme using L-Dopa as the substrate (in the concentration range of 1–6 mM). These constants were calculated using Lineweaver–Burk plot according to the following equation:

$$v^{-1} = \left[\frac{K_m}{V_{max}}\right][S]^{-1}{V_{max}}^{-1} \quad (1)$$

where $S$ is the substrate concentration, $v$ and $V_{max}$ represent the initial and maximum rate of the reaction, respectively and $K_m$ is the Michaelis constant representing the substrate concentration at which the reaction rate is half of $V_{max}$. The slope and y-intercept of the straight line obtained from the Lineweaver–Burk plot gave the value of $K_m/V_{max}$ and ${V_{max}}^{-1}$, respectively. The values of $K_m$ and $V_{max}$ calculated from the Lineweaver–Burk plot ($n = 3$) for both free and immobilized enzyme are reported as mean ± S.D.

### 2.7. Transducer and operating system

Multi detection microplate reader (MDMR) from Bioteck (Synergy™ HT) was used in the present study for absorbance measurements. A xenon flash light source in conjunction with the photomultiplier tube (PMT) detector helped to make time-resolved measurements. Wavelength range in this reader can be selected from 200–999 nm with an increment of 1 nm. The transducer used here was optical and the microplate reader was completely controlled via KC4™ PC software for all operations. L-Dopa was added into the enzyme bound wells and the absorbance was measured at 475 nm. The absorbance difference between the initial and final reading corresponds to the amount of dopachrome formed.

### 2.8. AFM characterization of tyrosinase immobilized well surface

For AFM study, the inner bottom surface of the well was carefully cut and removed. On this surface, tyrosinase enzyme was immobilized as discussed in Section 2.4.2. The well surface without the enzyme served as the control. The imaging was performed using Nanosurf Easyscan 2 AFM that was controlled with the help of SPM control software provided with it. The normal spring constant value of the cantilever was 0.2 N $m^{-1}$ and a constant force of 20 nN was applied. The image size used for studying was around 50 μm in both X and Y horizontal directions for all samples. For measurement, different areas of control and enzyme immobilized well surfaces were visualized under the same conditions. The average root mean square roughness ($R_q$) of the well surface was calculated using the topographical data obtained from AFM micrographs. The $R_q$ values were determined with the help of software, by taking an average of five different locations from the AFM micrographs for control as well as enzyme immobilized well surfaces.

### 2.9. Calibration studies using tyrosinase immobilized microplate

Tyrosinase immobilized microplate was integrated with the optical microplate reader. It was calibrated using different concentrations (10–6000 μM) of L-Dopa and the absorbance was monitored at 475 nm due to the formation of dopachrome ($\varepsilon = 3700\ M^{-1}\ cm^{-1}$) [9]. All experiments were carried out with phosphate buffer (0.1 M, pH 6.0) at room temperature.

### 2.10. Analysis of spiked plasma sample with biosensor

For real sample analysis, blood plasma samples were spiked with L-Dopa at different concentrations of 10–200 μM. These samples were then quantified for L-Dopa detection by using the tyrosinase based microplate biosensor.

## 3. Results and discussion

### 3.1. Spectral changes during dopachrome formation

Spectral changes were recorded by performing wavelength scan in the range from 400–600 nm, for 5 min to observe the changes taking place due to the formation of dopachrome. The time period of 5 min was selected to study the formation of dopachrome as the response was linear within this time period Fig. S1. It was observed that formation of dopachrome (orange color) is accompanied by changes in the spectrum at 475 nm (Fig. 1) and this was the basis for the development of an optical biosensor by immobilizing tyrosinase enzyme onto the inner surface of the microplate wells for detection of L-Dopa.

### 3.2. Optimization of method for the immobilization of tyrosinase

Immobilization of the enzyme is a key aspect in the development of a biosensor. For immobilization of biomaterials, various techniques such as layer-by-layer (LbL) approach [22], adsorption followed by cross-linking [12], covalent attachment [23] etc. can be used. Fiorentino et al. [9] have made use of the LbL approach and reported the immobilization of tyrosinase films on an optical quartz support by alternating the enzyme layer with the polymer (poly(dimethyl diallyl ammonium chloride)) layer. In another study, Bertolino et al. [19] have immobilized tyrosinase enzyme on 3-aminopropyl-modified controlled pore glass using glutaraldehyde and used it for developing a microfluidic enzymatic sensor for determination of pipemidic acid in pharmaceutical samples. In our previous studies, glucose oxidase and microbial cells were immobilized by adsorption onto the surface of onion membrane and microplate respectively; followed by glutaraldehyde cross-linking and used in biosensor applications [15,24]. In the present study, polystyrene microplates that were used for the immobilization of tyrosinase enzyme were vacuum gas plasma treated. In the process of vacuum gas plasma treatment, highly energetic oxygen ions are generated, which then oxidizes and gets grafted onto the surface of polystyrene chains [25]. The main advantages of microplate based assays are the use of small sample volumes and simultaneous analysis of multiple samples on a single platform. The response time was only 5 min for the measurement of multiple samples. This is the first report where tyrosinase from *A. campanulatus* has been immobilized onto polystyrene microplate and used for optical biosensor application.

Three different approaches were followed for immobilizing tyrosinase onto the inner surface of microplate wells namely (i) adsorption (ii) adsorption followed by 1% PEI treatment (iii) adsorption followed by 1% glutaraldehyde treatment. The results obtained from these different methods are reported in Table S1. In the first method, $74.5 \pm 3.3$ U of tyrosinase was adsorbed onto the well surface. Out of the three approaches this method gave maximum enzyme activity, but in the subsequent reaction leaching of the enzyme was observed. In the second

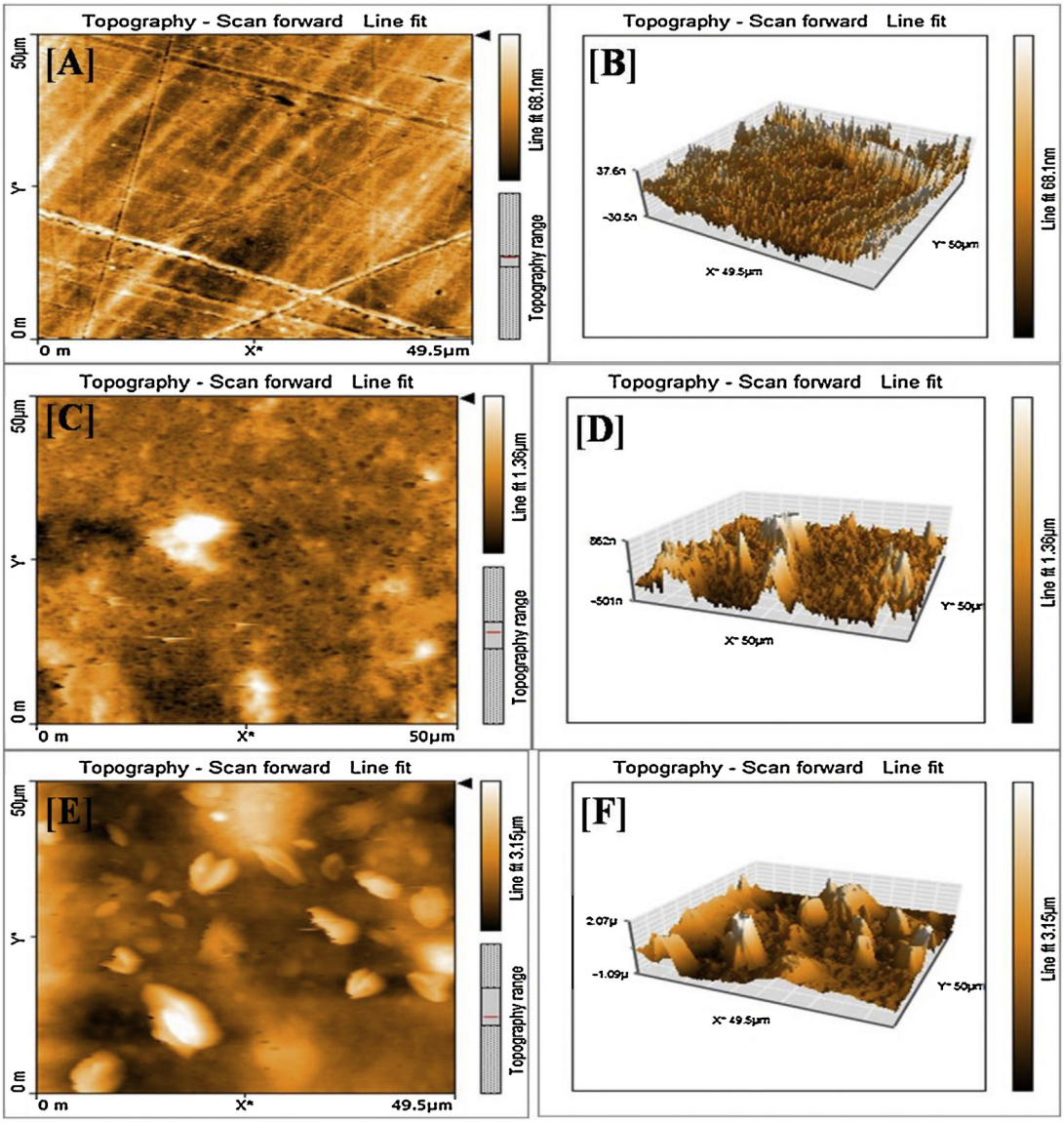



**Fig. 2.** AFM topographic micrographs of: plain well surface [A] tyrosinase immobilized without glutaraldehyde treatment well surface [C] and tyrosinase immobilized with glutaraldehyde treatment well surface [E]. 3D view of: plain well surface [B] and tyrosinase immobilized without glutaraldehyde treatment well surface [D] and tyrosinase immobilized with glutaraldehyde treatment well surface [F].

method, 30.1 ± 3.0 U of tyrosinase enzyme was adsorbed onto the microplate well surface. In earlier studies from our laboratory we have shown the successful use of PEI for adhesion of cells/enzyme on various supports like glass [26], rice husk [27], and jute fabric [28]. However in contrast to those studies, when tyrosinase was immobilized onto microplate surface using 1% PEI, a decrease in enzyme activity was observed. This can be due to the interaction between PEI and copper based active site of tyrosinase. Conformational changes in the active site of the enzyme due to such interactions can lead to decrease in enzyme activity. Such results have also been observed by Zhang and Rochefort [29] while investigating the role of PEI on laccase enzyme. Also, with PEI treatment, drop in enzyme activity was observed during subsequent use. In the third method, 48.9 ± 3.8 U of tyrosinase was adsorbed following 1% glutaraldehyde treatment. Here, no leaching of enzyme was observed in the subsequent use. This was due to glutaraldehyde treatment that helps in cross-linking and binding the enzyme to the surface and to each other; thereby increasing the stability of the biocomponent. However, a decrease in enzyme activity was observed as compared to the first method since some of the $-NH_2$ groups present in the active site of the enzyme might have been involved in cross-linking with glutaraldehyde. Due to this glutaraldehyde treatment, the activity of the enzyme has decreased. However, in the subsequent reuse, no loss in activity was observed which suggests that the treatment with glutaraldehyde can be a potential approach for immobilizing tyrosinase onto the microplate well surface, making it beneficial for reuse. Thus, out of the three methods used for immobilization, glutaraldehyde was chosen as the best method.

### 3.3. ESEM–EDS study of microplate well surface

ESEM study was carried out in order to observe the surface changes in the wells of the microplate due to immobilization of tyrosinase enzyme. Here, both test (tyrosinase immobilized) as well as blank wells of the microplate were imaged as shown in Fig. S2. An aggregated smear was observed on the micrographs of test (immobilized) wells (Fig. S2[B–D]) which was absent in case of blank well surface (Fig. S2[A]). The presence of such aggregated smear onto the surface of microplate well suggests the immobilization of tyrosinase on the well surface. Also, the EDS spectrum of the plain well surface reveals that the surface is made up of carbon as the major component (Fig. S3[A]). After the immobilization of tyrosinase enzyme, additional peaks of nitrogen (N), oxygen (O), and sulfur (S) appeared in the spectra that confirm the presence of enzyme on the well surface (Fig. S3[B–D]). The peaks of sodium (Na) and phosphorus (P) were due to the presence of buffer in the system in which the enzyme was present. The presence of chlorine (Cl) in Fig. 3[D] is due to HCl that was used to adjust the pH of PEI solution to 7.0 during its preparation.

### 3.4. Kinetic parameters

The $K_m$ value for immobilized enzyme was 4.9 ± 0.15 mM, which is 3.5 times higher than that of free enzyme (1.4 ± 0.05 mM). Paranjpe et al. [21] have also observed similar $K_m$ value of 1.05 mM for the same enzyme in the free form, using L-Dopa as the substrate.

Studies with L-Dopa as a substrate have shown that mushroom tyrosinases have $K_m$ values in the range of about 1–10 mM [30–33]. In general, the $K_m$ of an immobilized enzyme is different from that of the free enzyme due to diffusional limitations, steric effects and ionic strength [34]. The change in affinity of the enzyme to the substrate is probably caused either by the modifications in the enzyme introduced by the immobilization procedure or by the lower accessibility of the substrate to the active site of immobilized enzyme [35]. Various functional groups of the reactive amino acids in the protein act as nucleophiles and interact with glutaraldehyde, which can lead to modifications in the enzyme [36]. The decrease in $V_{max}$ from 0.63 ± 0.03 μmol $min^{-1}$ to 0.5 ± 0.05 μmol $min^{-1}$ can be attributed to the alterations of the microenvironment upon immobilization of the enzyme onto the microplate well surface. The immobilized enzyme is located in an environment that is different from that when it is in free solution. Also, there is a partitioning of substrate between the solution and the support; hence, the substrate concentration in the neighborhood of the enzyme may be significantly different from that in the bulk solution. So, the $V_{max}$ of the immobilized enzyme is lower than that of free enzyme [37–39]. Similar decrease in $V_{max}$ was also observed by Xu et al. [40] while studying the kinetic parameters of cross linked tyrosinase aggregates.

### 3.5. AFM characterization

AFM was used to observe the topographical changes in the well surface before and after immobilization of tyrosinase enzyme. It provides the ideal means for characterization of immobilized enzyme on the biosensor surface [41]. The roughness of the sample surface is an important feature that may give information about the surface changes. In the control sample the $R_q$ was observed to be 11.2 ± 1.6 nm and after adsorption of the enzyme onto the well surface, the value increased to 182.7 ± 5.6 nm. Further, when glutaraldehyde treatment was carried out, the $R_q$ value was calculated to be 580 ± 22.2 nm. Increase in the $R_q$ value suggests that the roughness of the surface has increased due to the deposition of enzyme–glutaraldehyde layer. Tyrosinase immobilized surface shows a prominent difference as compared to the control surface (Fig. 2A). The surface of the well which has tyrosinase adsorbed on it (Fig. 2C) shows the deposition of an enzyme layer. The blank area that was present in the control sample (Fig. 2B) were filled, after adsorption of the enzyme onto the surface (Fig. 2D and F). Also, when glutaraldehyde was used for the treatment purpose, there was a non-homogenous surface that was observed on the well surface (Fig. 2E). Similar observations have been reported by Saal et al. [42] while studying the immobilization of glucose oxidase onto mica carrier.

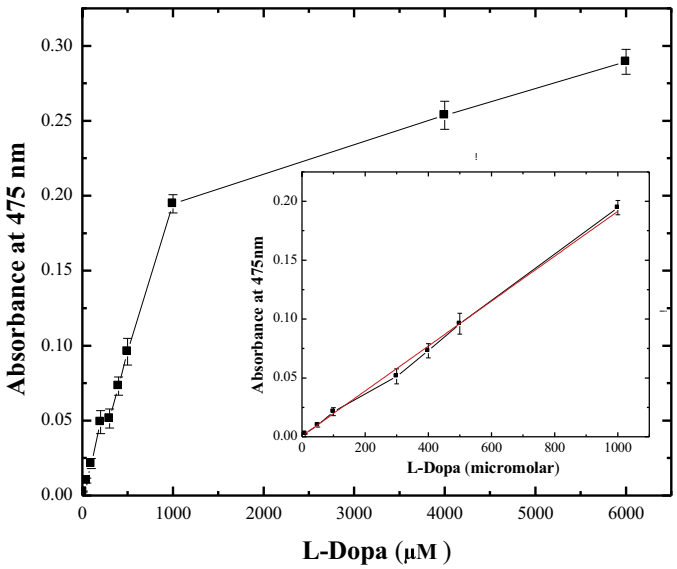



**Fig. 3.** Calibration plot of the biosensor using tyrosinase immobilized microplate using L-Dopa (10–6000 μM). (Inset showing linearity range between 10–1000 μM with linear regression equation of $y = 0.00019x + 0.00074$, $R^2 = 0.99816$ where $y$ represents the absorbance at 475 nm and $x$ is the concentration of substrate.)

Thus, immobilization of tyrosinase onto the microplate surface was characterized with the help of AFM micrographs and the enzymatic assays that were performed.

### 3.6. Calibration of biosensor and detection range

Biosensor was calibrated using different concentrations (10–6000 μM) of L-Dopa and absorbance was measured. As observed in Fig. 3, the graph is linear in the range from 10 to 1000 μM with a linear regression equation $y = 0.00019x + 0.00074$ ($R^2 = 0.99$) where $y$ represents the absorbance at 475 nm and $x$ represents the substrate concentration. Therefore 10–1000 μM was considered as the detection range of the biosensor which was comparable with the earlier reports [7,9,43,44]. Detection range of the present biosensor was equivalent to the high throughput spectroelectrochemical detection method used for other phenolic compounds like dopamine [45]. L-Dopa level in plasma samples were reported as 500–1000 μM during pharmacokinetic studies [5]. The proposed biosensor can thus be used in monitoring the plasma levels of L-Dopa in such cases. A detection limit of 3 μM was obtained with S/N = 3 in response to the blank sample. The detection limit in this study was lower than that reported by Fiorentino et al. [9] and was comparable to Tembe et al. [10].

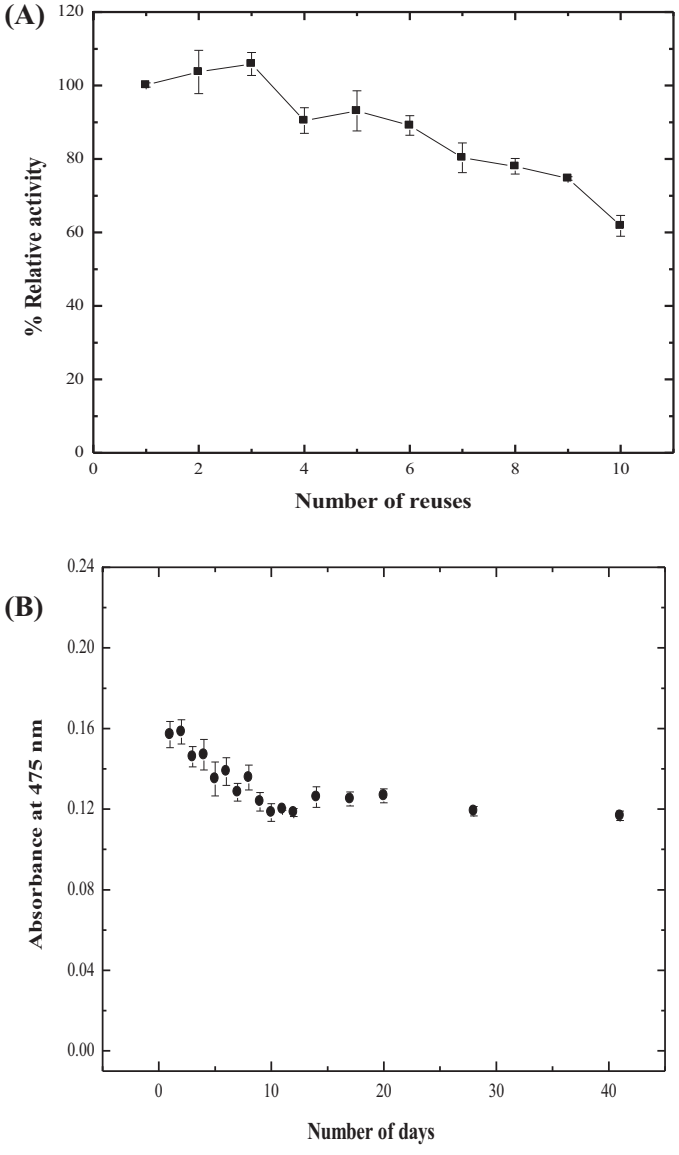



**Fig. 4.** (A) Reusability of the wells containing tyrosinase immobilized onto the inner surface of the microplate. (B) Stability of tyrosinase immobilized microplate based biosensor at 4 °C.

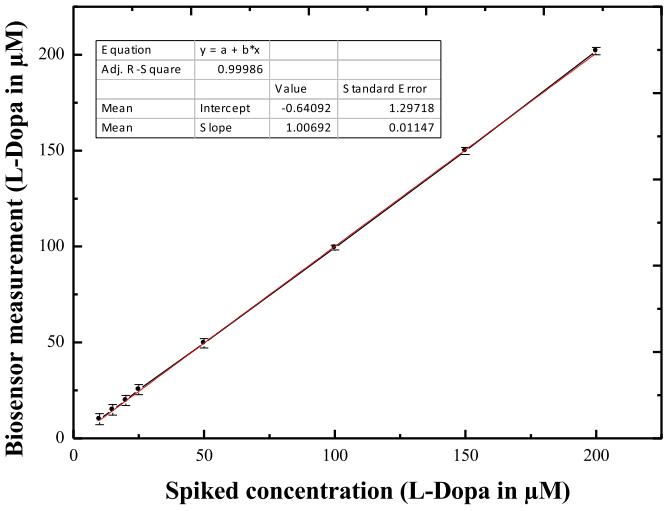



**Fig. 5.** Graph showing correlation between spiked concentrations of L-Dopa in blood plasma sample vs concentration of L-Dopa measured with the developed biosensor.

### 3.7. Reusability, reproducibility and stability study of the enzyme immobilized microplate well

For better application of any biosensor, reusability is one of the most important factors. It was observed that immobilization in the presence of glutaraldehyde helped in cross-linking and binding the enzyme to the well surface and also to each other. Thus, in the presence of glutaraldehyde there was less leaching of the enzyme which allowed the reuse of the immobilized biocomponent. It was also observed that 75% of enzyme activity was retained after 9 repeated reactions (Fig. 4A). The storage stability of the immobilized biocomponent was also studied. The stability of the enzyme was observed at regular intervals by measuring dopachrome formation at 475 nm. Tyrosinase immobilized microplate biosensor showed a stability of 41 days with a retention of 75% activity when stored at 4 °C (Fig. 4B). The low relative standard deviation value of 0.012 ($n = 4$) demonstrated high reproducibility and uniformity of analysis.

### 3.8. Analysis of real sample

The practical applicability of the proposed biosensor was examined using real sample. Blood plasma sample was spiked with different concentrations of L-Dopa (10, 15, 20, 25, 50, 100, 150 and 200 μM) and then subjected to quantification using tyrosinase immobilized microplate biosensor. In all, there were 10 replicates for each of the eight different concentrations studied. Thus, multiple spiked plasma samples were analyzed at the same time by measuring the absorbance at 475 nm. Table S2 shows that the recovery results were satisfactory and the proposed biosensor can be used successfully for determination of L-Dopa in blood plasma samples. As shown in Fig. 5, the values of L-Dopa concentrations obtained by using the biosensor showed good correlation with that of known spiked concentration values of L-Dopa. The straight line fit gave a linear regression equation of $y = 1.0056x + 0.392$

($R^2$ = 0.9995), which demonstrated the feasibility of the developed biosensor for L-Dopa detection.

## 4. Conclusion

In the present study, we describe the immobilization of tyrosinase enzyme extracted from *A. campanulatus* onto 96 well microplate made of polystyrene, for the construction of an optical biosensor to detect L-Dopa. Immobilization of tyrosinase onto the inner surface of the wells was confirmed by ESEM–EDS, AFM and by measuring the enzymatic activity of the immobilized biocomponent. Tyrosinase immobilized microplate was associated directly with optical transducer microplate reader having the advantage of detecting multiple samples at the same time. The detection range of this biosensor was estimated to be 10–1000 μM and the detection limit was 3 μM. The immobilized biocomponent could be reused up to nine reactions and was stable for 41 days. The developed tyrosinase based sensor showed good reproducibility. Spiked blood plasma samples were also analyzed for L-Dopa by using the developed optical biosensor. Present study is a good alternative to other analytical methods and reports an innovative concept wherein microplate was used as an immobilizing matrix and applied for L-Dopa detection with reusable enzymatic biocomponent.

## Acknowledgement

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.08.016.

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