Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

1. *Zno@Au core-shell characterization*

Three ZnO nanoparticles were randomly selected to be analysed by atomic force microscope (AFM). The respective height profiles are presented in supporting Figure 1, and the respective dimensions are presented in supporting Table 1.

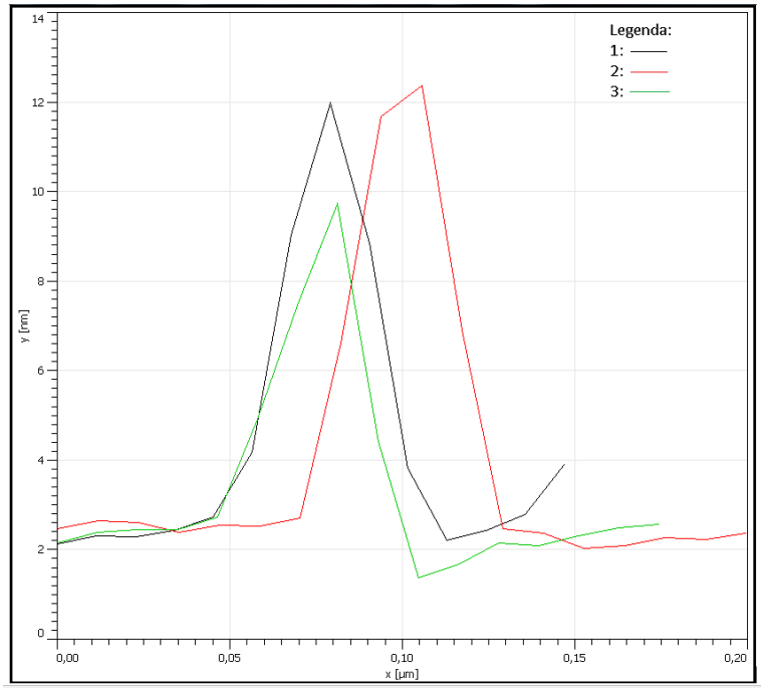

Figure 1. AFM profile lines of three selected ZnO nanoparticles.

To estimate particle size, it is necessary to calculate the difference between Y_2 and Y_1 values (supporting Table 1). According to the topographic images, the synthesis route resulted in symmetrical and well-distributed particles, for which the calculated values of nanoparticle dimensions correspond to 11 nm.

Table 1. Dimension data of ZnO nanoparticles.

n	X_1 (nm)	Y_1 (nm)	X_2 (nm)	Y_2 (nm)	$Y_2 - Y_1$ (nm)
1	195	89	201	100	11
2	141	123	154	134	11
3	110	196	120	207	11

Further characterization was carried out using three selected ZnO@Au core-shell nanoparticles. Their respective profile lines and dimension data are presented respectively in Figure 2 and Table 2.

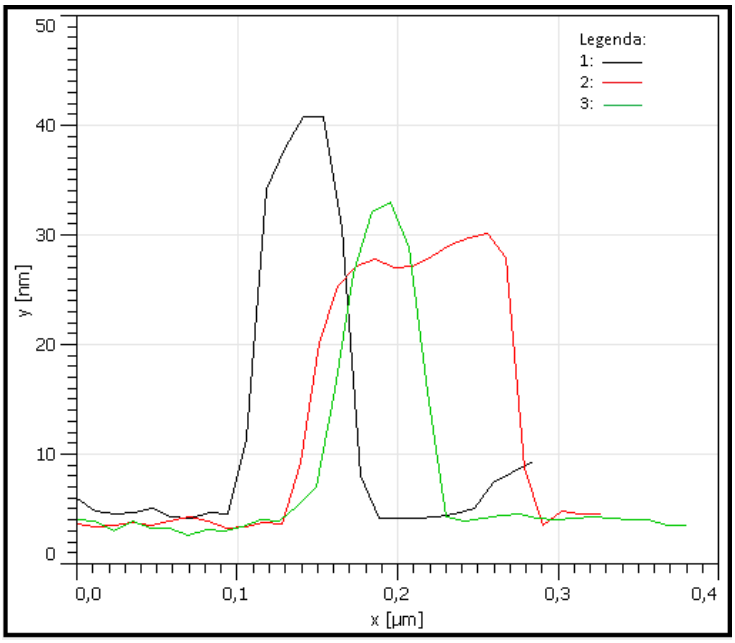

Figure 2. AFM profile lines of selected ZnO@Au core-shell.

The calculated value of the size of the ZnO@Au nanostructure is approximately 22 nm, (Table 2) which confirms the coating of ZnO nanoparticles with gold, producing core-shell nanoparticles.

Table 2. Dimension data of ZnO@Au core-shell.

n	X_1 (nm)	Y_1 (nm)	X_2 (nm)	Y_2 (nm)	$Y_2 - Y_1$ (nm)
1	79	72	89	94	22
2	100	121	117	143	22
3	196	205	210	228	23

2. *Optimization of experimental conditions*

Studies were carried out to optimize the immobilization conditions. Firstly, the influence of interaction time between the core-shell ZnO@Au and the enzyme tyrosinase was studied. For this, four different interaction times between core-shell and enzyme (1mU/mL) were tested: 20 min; 45 min; 2 hours and 24 hours. For each time, differential pulse voltammograms were obtained in 2500 µmol L^{-1} dopamine solution, in 0.1 mol L^{-1} phosphate buffer, pH 7.0. The results are shown in suplemmentary Figure 3A.

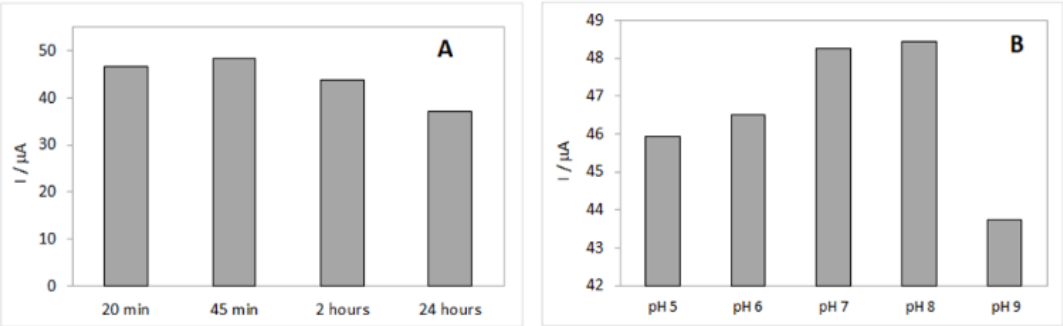

Figure 3. Influence of interaction time between the core-shell and the tyrosinase, (A) and washing pH (B) in biosensor performance in dopamine solution 2500 µmol L^{-1}, in 0.1 mol L^{-1} phosphate buffer, pH 7.0 at 25 mV pulse amplitude and 80 m s^{-1}.

Supplementary Figure 3A shows that the maximum current value was obtained when the enzyme was incubated with ZnO@Au nanoparticles for 45 minutes. When compared with the biosensor that was assembled using an interaction time of 20 minutes, the signal increase was only 3.5%, which is still significant for analysis at low analyte concentration. At longer interaction times, there was a decrease in the response of the biosensor. Increasing the incubation time to 2 and 24 hours would likely have resulted in a thicker biolayer, which impairs the transfer of electrons on the electrode surface, with a consequent decrease in current. Thus, the time of 45 minutes was selected for the subsequent experiments.

The effect of buffer pH used for washing the biosensors after incubating the enzyme solution with core-shell nanostructures was then studied. The pH of the wash buffer was increased in one unit, between 5 and 9, step by step. As the enzyme has basic amino acids within the structure, an increase in pH will allow the deprotonation of amino groups. Conversely, carboxyl groups will deprotonate to generate negative charges, thus affecting the immobilization process, which is based on electrostatic interactions. Differential pulse voltammograms were then obtained in 2500 µmol L^{-1} dopamine solution, in 0.1 mol L^{-1} phosphate buffer. Fig. 2B shows the values of current obtained for each pH of the wash buffer, highlighting that the highest response was recorded at pH 8.

3. Selectivity studies

To verify the biosensor selectivity to dopamine in the presence of substances commonly found in physiological urine samples, five biosensors were constructed, and all were applied to a sample that was spiked with 250 µmol L^{-1} of dopamine in 0.1 mol L^{-1} phosphate buffer, pH 7.0. The dopamine response was analysed with each of the putative interferents glucose, ascorbic acid and sodium oxalate (all at 250 µmol L^{-1}) and with a mixture of all three interferents. The study was evaluated using differential pulse voltammograms. Supplementary Figure 4 presents a comparison of current readings obtained in each case, taking the signal obtained for dopamine (250 µmol L^{-1}) as 100%.

As seen in Supplementary Figure 4, the dopamine response in the presence of the studied interferents did not significantly change the signal. The device showed high selectivity,

presenting a difference of less than 7% in the presence of the studied interferents. This result is extremely relevant because ascorbic acid is a potential interferent in urine samples. Supplementary Figure 4 presents a comparison of the current obtained in each case, taking the signal obtained for dopamine (250 µmol L^{-1}) as 100%.

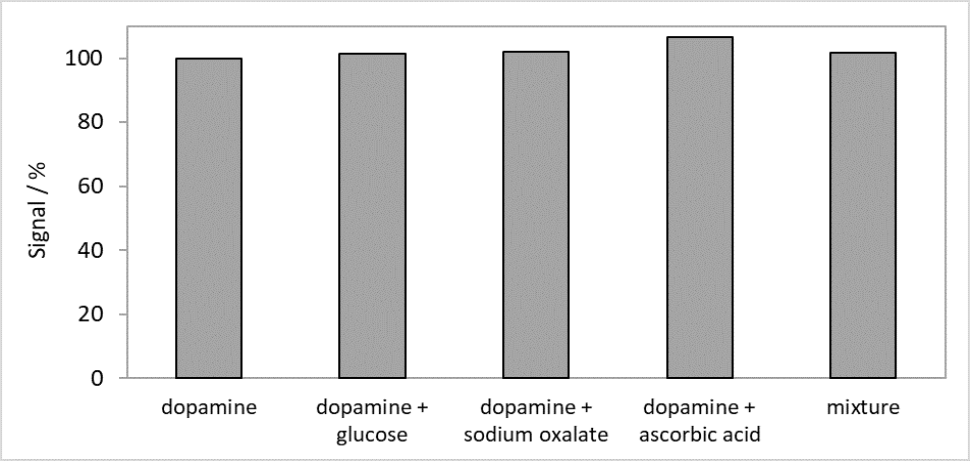

Figure 4. Current variation obtained with the proposed biosensor in the presence of putative interferents in 250 µmol L^{-1} dopamine solution, in 0.1 mol L^{-1} phosphate buffer, pH 7.0 at 25 mV pulse amplitude and 80 mV s^{-1}.

4. Biosensor stability

A study was carried out to evaluate the stability of the proposed device, testing the reusability of the biosensor without significant loss of response. For this purpose, a biosensor was constructed under optimized conditions. The device was used daily to measure its response to 250 µmol L^{-1} dopamine in 0.1 mol L^{-1} phosphate buffer, pH 7.0. After each measurement, the dopamine sample was removed by capillarity using clean tissue paper. The device was washed with buffer and stored at 4 °C until the next day. The resulting values of current determined on different days are shown in Supplementary Figure 5.

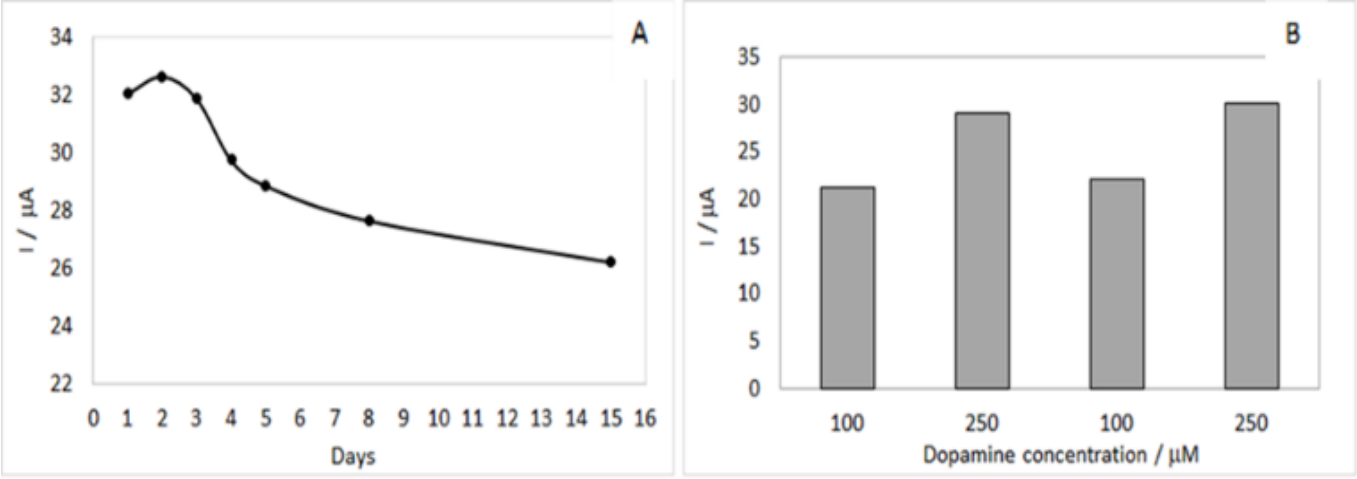

Figure 5. Measured current values using the same biosensor during 15 days (A) and in two different dopamine concentrations (B), in 0.1 mol L^{-1} phosphate buffer, pH 7.0 at 25 mV pulse amplitude and 80 mV s^{-1}.

The results show that after 15 days, the biosensor performance decreases by approximately 20%, which is considered significant since it is intended to determine low concentrations of dopamine in the samples. During the first five days, the biosensor response varied around 11%. Thus, it is believed that this is the limit interval for the effective reusability of the biosensor. However, since it is not known whether the cause was due to storage or continuous use, the miniaturized biosensor can be regarded as a disposable device, as it is in most cases.

In addition, biosensors involving the determination of catecholamines can adsorb oxidized products on the electrode surface, generating unreliable responses. Thus, the same biosensor was tested at two different concentrations of dopamine at high and low values, (250 µmol L^{-1}) and (100 µmol L^{-1}) within the linear range. Four consecutive measurements were performed with the same device at the latter concentrations. Supplementary Figure B shows the results obtained.

It was demonstrated that the results at different concentrations varied around 4%. Thus, it is verified that the measurements in the presence of high values of dopamine do not influence the behaviour of the biosensor. These data indicate that the biosensor does not adsorb oxidized products on the surface and, therefore it has no memory effect.

5. Application of the proposed biosensor in a synthetic urine sample

To check the accuracy and selectivity of the biosensor in samples containing other substances along with the analyte, it was applied in synthetic urine spiked with 250 µmol L^{-1} of dopamine. This study was carried out in triplicate using differential pulse voltammetry and the results are presented in supplementary Table 3.

Table 3. A determined current (mA) was obtained using the developed biosensor in synthetic urine spiked with 250 µmol L^{-1} of dopamine.

Measurement	Current / µA
1	29.602
2	30.045
3	30.182
Mean	29.943 ± 0.3

The mean value obtained was 29.943 µA which when substituted in the calibration curve provided an estimated concentration of 259.43 µmol L^{-1} of dopamine, with a relative error (%) of approximately 3.8%. The estimated and actual concentrations of dopamine are in close agreement, confirming that the biosensor can measure dopamine levels in samples containing interferents such as urine.