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Determination of Alfuzosin by Hybrid of Ionic Liquid-Graphene nano-composite Film Using Coulometric FFT Linear Sweep Voltammetry

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A novel electrochemical method was designed and developed for measurement of Alfuzosin based on combination of a new sensor with Coulometric FFT Linear sweep Voltammetry. The electrode was constructed by the pareparation of a matrix of ZrO_2 in graphene oxide and ionic liquid (1-butyl-3-methylimidazolium tetra fluoroborate) on glassy carbon electrode. The experimental conditions, such as pH, amount of nanoparticles and potential scan rate were optimized for the electrochemical measurement. The linearly concentrations ranges of Alfuzosin was from 2.0 –150 nM with a detection limit of 0.5×10^{-9} M. Moreover, the proposed sensor exhibits a good reproducibility and accuracy, the response time less than 10 s, high sensitivity with repeatability (R.S.D value of 2.6%) and long term stability (45 days).

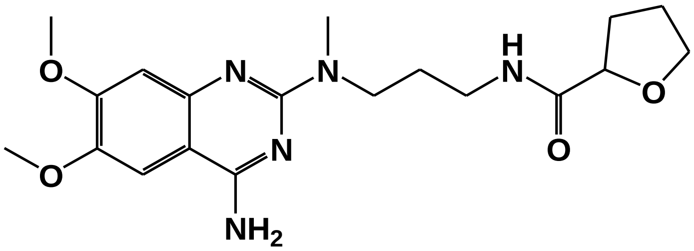
Keywords: FFT Linear sweep Voltammetry; Alfuzosin; ZrO_2 nanoparticles; Graphene nanosheet; ionic liquid

1. INTRODUCTION

Alfuzosin (ALF), N-{3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino] propyl} tetrahydro-2- furancarboxamide hydrochloride (Fig.1), is an antagonist of α_1 post-synaptic adrenergic receptors, showing some myorelaxant effects [1,2], which is used for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia and has been tried in the treatment of hypertension [3].

For routine clinical analysis a simple, sensitive, and rapid analytical method with high throughput analysis is necessary for quantization of the concentrations of alfuzosin in human plasma in order to support pharmacokinetic and bioequivalence studies.The most widely used methods for the determination of Alfuzosin involve high-performance liquid chromatography (HPLC) [4-6], mass spectrometry [7] LC-ESI-MS/MS [8], voltammetry [9] and chiral separation by using chiral columns [10] or chiral mobile phases [11] have been reported.

The presence of ionic liquid (IL) and Graphene nanosheets (GNS) in the matrix of the sensor provides an environment for the enhanced electrocatalytic activities. Under the optimal conditions, the sensor exhibited a linear response to Alfuzosin. To the best of our knowledge, this is the first application of Coulometric Fast Fourier Transform Linear Sweep Voltammetry (CFFTLSV) method [12-24]. Alfuzosin (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) has the following chemical structure.



Scheme1. Chemical structure of Alfuzosin

The fabrication of ZrO_2-Chitoason/GNS-IL/GCE involves two sequential stages. At first, the surface of a bare glassy carbon electrode was modified with GNS and IL then with ZrO_2 in Chitosan. The sensor was characterized by SEM and electrochemical impedance spectroscopy (EIS) methods.

2. MATERIALS AND METHODS

2.1. Reagents

The standard solutions were prepared with double distilled water when in use. Except as otherwise stated, 0.05 M NaH_2PO_4/Na_2HPO_4 buffer solution at pH 5.0 was used as the supporting electrolyte. Alfuzosin standard was supplied by Aqua Standard Europe. Chitosan (Chi) (85%) were purchased from Sigma–Aldrich. Graphene nanosheets purchased from Sinopharm Henan Bonzer Imp. All chemicals and solvents used were of analytical grade and were used as received. Double distilled water was used throughout the experiment. ZrO_2 nanoparticles were purchased from Beijing Chemical Reagent Company, China. The solution 3 mM $Fe(CN)_6^{4-}/^{3-}$ in 0.05 M KCl. 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIM-BF_4$, ionic liquid, IL) were of analytical grades from Merck Co.

2.2. The sensor fabrication

A glassy carbon electrode, GCE, (3 mm in diameter) were polished well with 1.0, 0.3 and 0.05 µm alumina slurry and then it was washed thoroughly with doubly distilled water. The electrodes were successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and then allowed to dry at room temperature. The fabrication of ZrO_2-Chi/GNS-IL/GCE electrode involves two sequential stages. First, deposition of mix 8 mg GNS and 3%IL on GCE, and then 1.0 mg ZrO_2 was dispersed in 1.0 mL 0.5 wt.% chitosan solution with ultrasonication to form ZrO_2–Chi mixed solution. Then, 5.0 µL of the mixed solution was dropped on the surface of the pretreated GNS-IL/GCE and dried in silica gel desiccators, the ZrO_2-Chi/ GNS-IL/GCE was obtained. The sensor was rinsed throughout with doubly distilled water. When not in use, the fabricated electrode was stored in pH 5.0 PBS at 4 °C in a refrigerator.

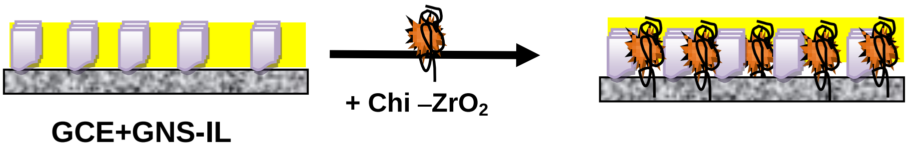


Figure 2. Schematic figures of electrode surface preparation

2.3. Instrumentation

A homemade potentiostat was used for CFFTLSV voltammetric measurements. The potentiostat was connected to a PC equipped with an analog to digital data acquisition board (PCL-818H, Advantech Co.). During the experiments the computer were dictated by the condition for the data acquisition requirements electrochemical software was developed using Delphi 6.0. The program was used to generate an analog waveform and acquire current readings. The potential waveform was repeatedly applied to the working electrode and then the data was acquired, and stored by the software. Also, the program was able to process and plot the data in real time.

EIS measurements were performed in 3 mM $K_3Fe(CN)_6$ in 0.05 M KCl. A stock solution of 2 mM ALF was firstly prepared, and then an aliquot was diluted to the appropriate concentration. Before each measurement, the three-electrode system was installed in a blank solution, and CFFTLSV voltammetry scan from 0 to 900 mV (*vs.* SCE) was recorded.

3. RESULTS AND DISCUSSION

3.1. Morphology characterization of the sensor

In order to characterize the modified surface of the electrode, the surface morphologies of ZrO_2-Chi/GNS-IL/GCE was examined by SEM method. Fig. 3A shows SEM image of the senor surface. In this image, it can be seen that the ZrO_2 nanoparticles tend to form clusters, and the nanoparticle ZrO_2 were dispersed among carbon nanosheets. The diameter of the particles is around 80-100 nm. Also, the graphene sheet has lateral dimensions from a few hundred nanometers to several micrometers, and a thickness of about 10 nm. In fact, via π–π interaction between the surface of GNS and the double bond in ALF molecules could be a better situation for accumulation of the analyte, as well as sensitivity for the sensor [25].

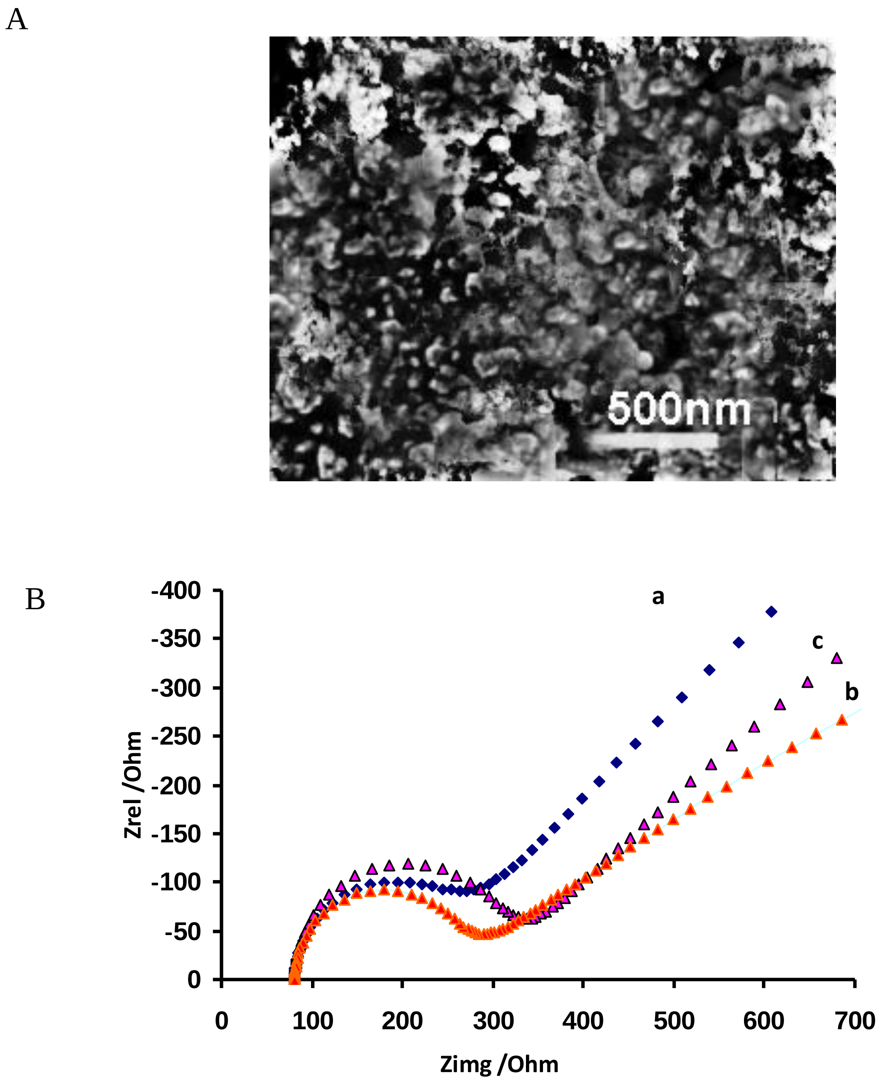


Figure 3. A) SEM images of Chi-ZrO_2/GNS-IL. B) EIS plots of modified electrode in 3 mM $K_3Fe(CN)_6$ with 0.05 M KCl: (a) bare GCE (b) GNS-IL/GCE and (c) Chi-ZrO_2/GNS-IL/GCE.

The EIS method, which is a power tool to probe the features of the surface of the sensor, was employed to monitor the modifying process of electrode. Fig. 3B demonstrates EIS curves for the bear GC, Chi-ZrO_2/GNS-IL/GC and GNS-IL/GC electrode in 0.05 M KCl solution containing 3 mM $[Fe(CN)_6]^{3-}/^{4-}$, respectively. The results indicated that the R_{ct} (charge transfer impedance) of GNSIL/GCE (curve b) was lower than that was obtained for the bare GC electrode. This could be attributed to the better conductivity of GNS-IL.

Also, the value of Z_{img} for Chi-ZrO_2/GNS-IL/GC electrode (curve c) is larger than that of the bare GC electrode, suggesting that the layer of Chi-ZrO_2 could coat on the electrode surface, which is an indication of lower accumulation of $[Fe(CN)_6]^{3-/4-}$, on the electrode surface. In this plot, the semicircle portion, observed at higher frequencies, corresponds to electron-transfer-limited process, whereas the linear part is characteristic of the lower frequencies range and represents the diffusion-limited electron-transfer process, where the modified Chi-ZrO_2/GNS-IL/GCE present a better slope, which is indication of faster electron transfer.

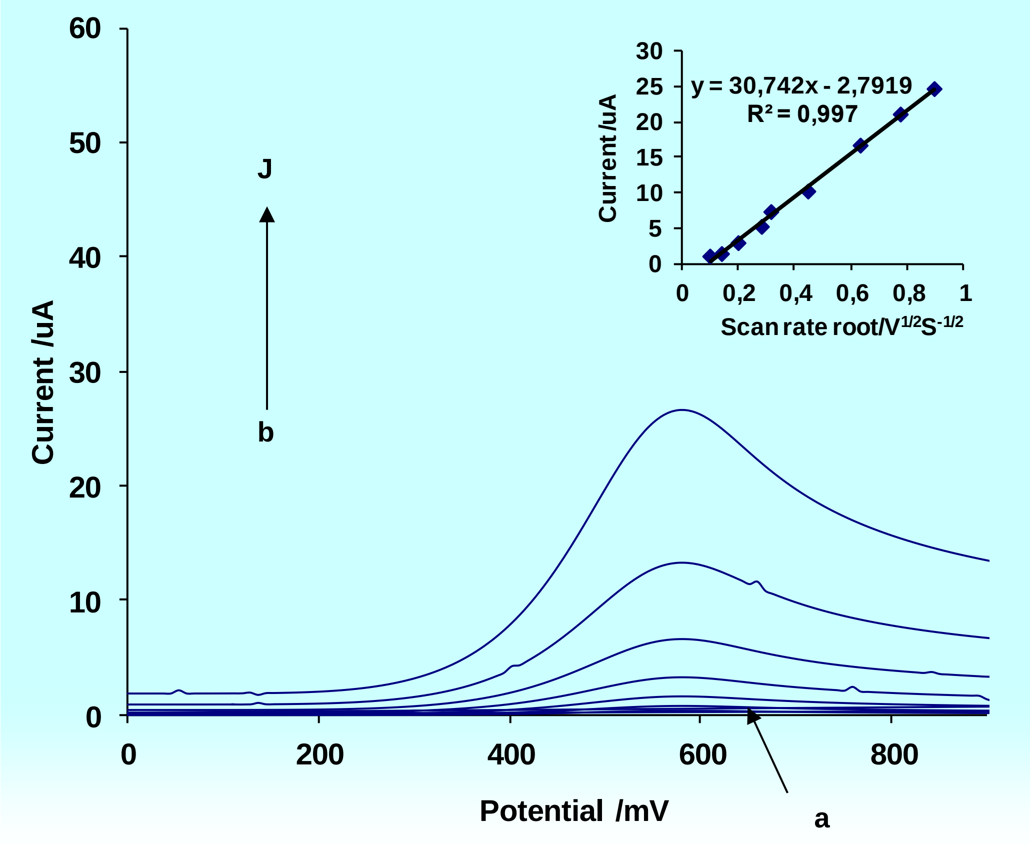


Figure 4. Typical Linear sweep voltammograms of 2 μM Alfuzosin in 0.05 M PBS pH 5.0 at the bare GCE, Chi-ZrO_2/GNS-IL/GCE; scan rates 10 to 800 mV/s

Fig. 4 showed the linear sweep voltammograms of ALF on Chi-ZrO_2/GNS-IL/GC electrode in 0.05 M phosphate buffer solution at pH 5.0. Curve a in the figure shows that at the bare GCE ALF shows very weak redox peaks, which point to a lower reaction rate of ALF on the GCE surface. In contrast, the curves b to j (in range of 0 to 900 mV/s) show a well-defined oxidation peak on the modified electrode, in which anodic a peak potentials is about 540 mV (*vs.* SCE). The modification could enhance the value of the electron-transfer rate, and, may also provide conditions for higher accumulation capacity and catalytic ability of the sensor surface to contribute in the oxidation reaction of ALF.

The inset in Fig. 4 demonstrates the measured current peaks of the Chi-ZrO_2/GNS-IL/GCE in 0.05 M PBS at different scan rates. The peak current was grown in the scan rates in the range from 10 to 800 mV/ s, proportionally. The measurements show that there is a fine linear relationship between the peak current and root of scan rate, with equations as i_p = 30.742 $v^{1/2}$ -2.7919 (R^2=0.997), This results, suggest that the reaction is quasi-reversible diffusion-controlled electron transfer process.

Fig. 5 illustrates the recorded CFFTLSV voltammograms of the Chi-ZrO_2/GNS-IL/GCE in potential range of 0 to 900 mV and in time window (260 s)., In this graph, the time axis represents the time of experiment was take placed, The potential axis on this graph represents potential range that applied to the working electrode during each potential ramp.

In CFFTLSV method charge passing *(Q)* through the sensor was calculated during the potential ramp, for determination of ALF signal. The sensor responses in form of *ΔQ* are calculated based on the integration of the current changes in a selected potential range at the recorded LSVs. The sensor response was calculated based on the following equation;

$$\Delta Q_n = Q_n - Q_0 \quad (1)$$

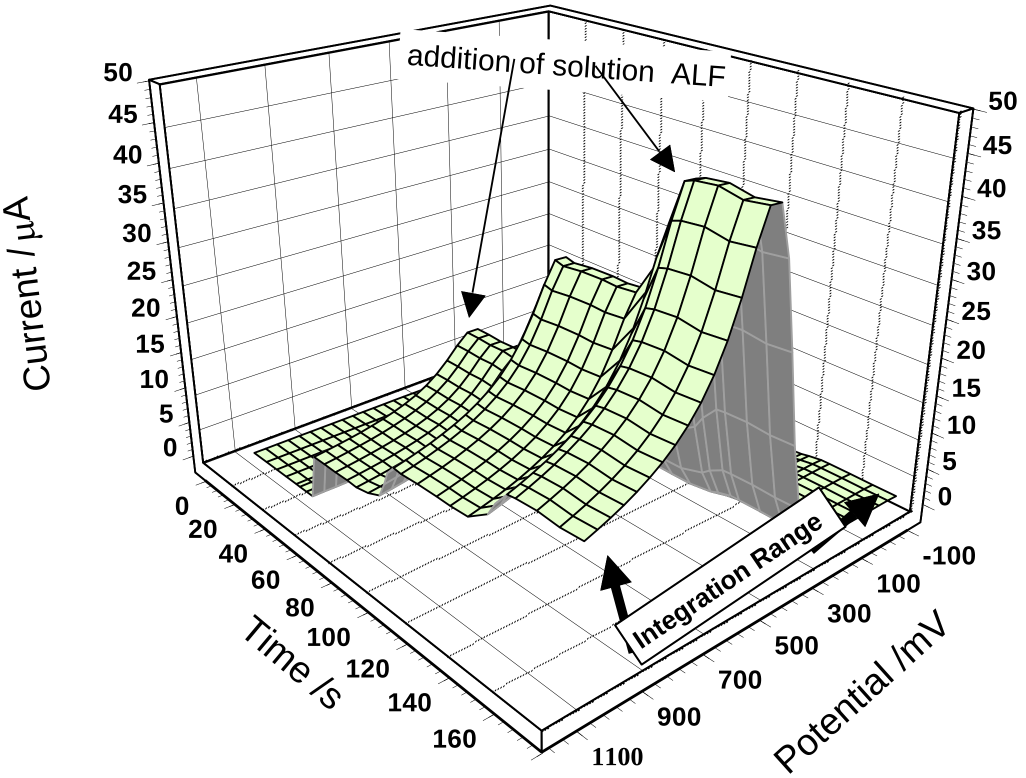


Figure 5. a) CFFTLSV voltammograms of the Chi-ZrO_2/GNS-IL/GCE sensor in absent and present of 1.0, 2.0., and 3.0 ×10^{-6} M Alfuzosin in PB solution at pH 5.0 in the potential range of 0 to 900 mV at potential scan rate 800 mV/s.

where n is number of scan (n>0), Q_n is the electrical charge obtained by integration of n^{th} LS voltammogram curve between 0 and 900 mV, and Q_0 represents the value of Q in the absence of the analyte.

Furthermore, the results indicate that with increasing the concentration of ALF the spiked standard sample solution ΔQ increases proportionally. The figure shows that after addition of 1.0×10^{-6} M ALF in the PBS a signal appears at potential 590 mV. However, as mentioned above the excessive accumulation of ALF on the surface of Chi-ZrO_2/GNS-IL/GCE can enhance of direct electron transfer between the active sites of the sensor and ALF.

3.2. Optimization of scan rate

Due to this fact that the response of the detector is diffusion controlled, it can be expected that the sensitivity of the measurements to be depend on the potential scan rate. So, the influence of scan rates on the sensitivity of the detector response to addition of solutions having a concentration of 2.0×10^{-7} M of ALF was investigated, at scan rates 200 to 2000 mV/s).

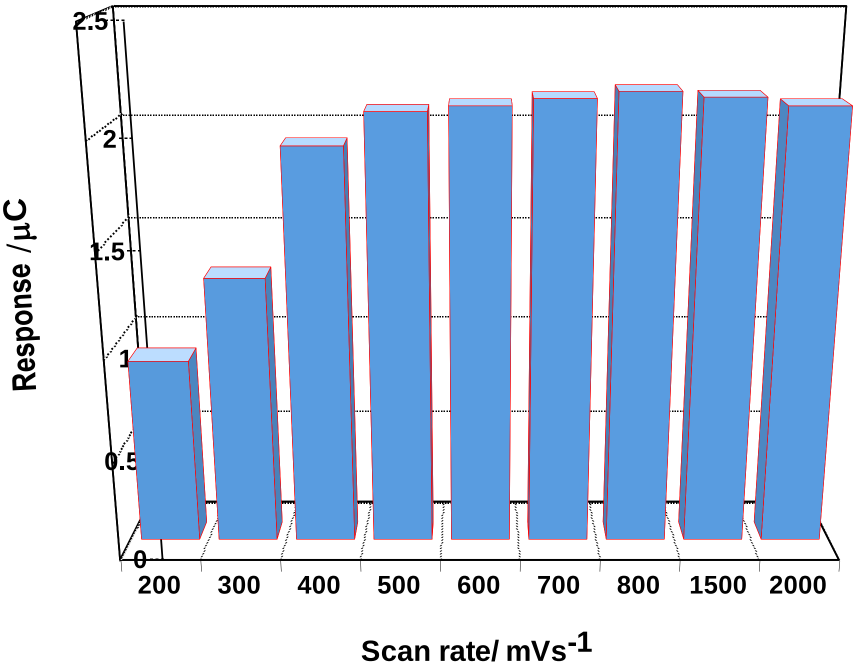


Figure 6. The effect of the scan rate on the response of the Chi-ZrO_2/GNS-IL/GCE in 2.0×10^{-7} M Alfuzosin in 0.05 M PB solution in the potential range of 0 to 900 mV.

As it is shown in the Figure 6, the detector exhibits the maximum sensitivity at 800 mV/s of scan rate. The effects of the sweep rate on the detection performance can be taken into consideration from different aspects, such as the speed in data acquisition during the current sampling, which may due to limitation in analog-to-digital broad, second and limitations in kinetic factors of rate of electrochemical processes.

3.3. Optimization of pH

The pH effect on the sensor performance was investigated by measuring the electrode response to 2.5×10^{-7} M ALF in various pHs. The pH was change at a definite range in order to determine the optimum value, in which the sensitivity of the sensor is the highest. In figure 1 was shown that the structure of ALF contains NH groups, which could the measurement be influenced by the pH of solution. Due to this reason determination of the optimum pH is one of the important parameter in solution. Fig. 7 shows the results of examination of pH on the detection method sensitivity. The CFFTLSV measurements were recorded at scan rate of 800 mV/s. The sensor response increases gradually from pH 3.0 to 5.0 and after achieving the maximum, the response decreases, indicating that the optimum pH 5.0 can be used for the detection of ALF.

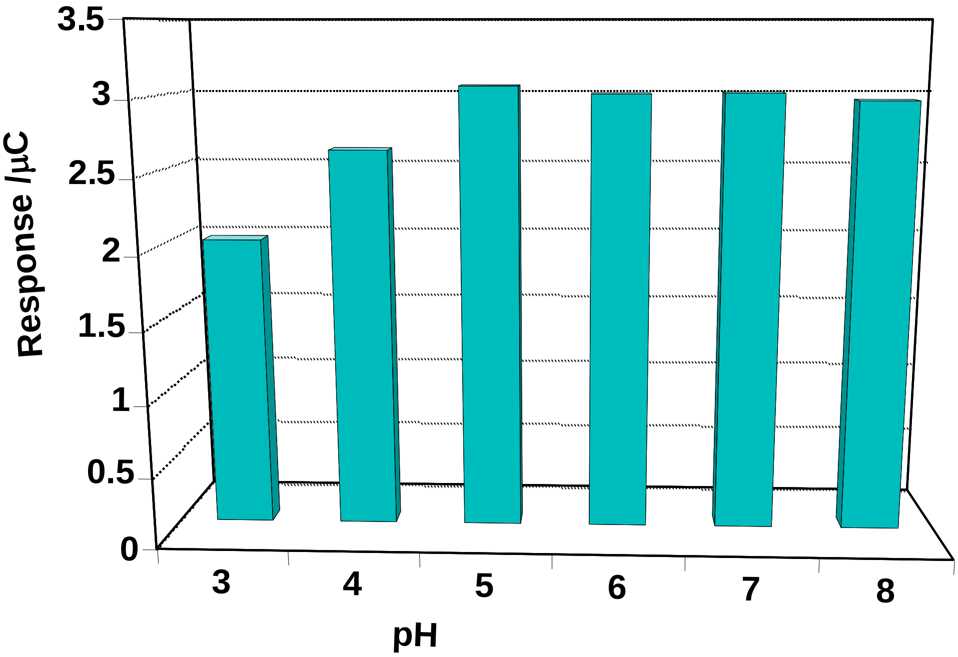


Figure 7. The effect of pH concentration on the sensor response to 2.5×10^{-7} M Alfuzosin in 0.05 M PB solution in the potential range of 0 to 900 mV at potential scan rate 800 mV/s

3.4. Optimization of amount of nanoparticles

Fig. 8 shows the effect amount of ZrO_2 and GNS on the sensor response to 5.0×10^{-7} M ALF in 0.05 M PBS at pH=5.0. As shown in figure, for ZrO_2 NPs the value of ΔQ increase with the volume of the ZrO_2–Chi mixed solution up to 5.0 µL on the eletrode. On the other hand, at the higher volume of the solution, the value of ΔQ decreases. This could be due to the increase of the resistance of the surface electrode by the adding more chitosan solution. Moreover, the figure demonstrates that the change of the sensor response with the weight of GNS in the content of the modifier at the surface. In these measurements, various weights of GNS (5 to 20 mg) in constant volume of IL were tested. The graph indicates that the value of *ΔQ* increases with the adding GNS up to 8 mg and then decreases slightly.

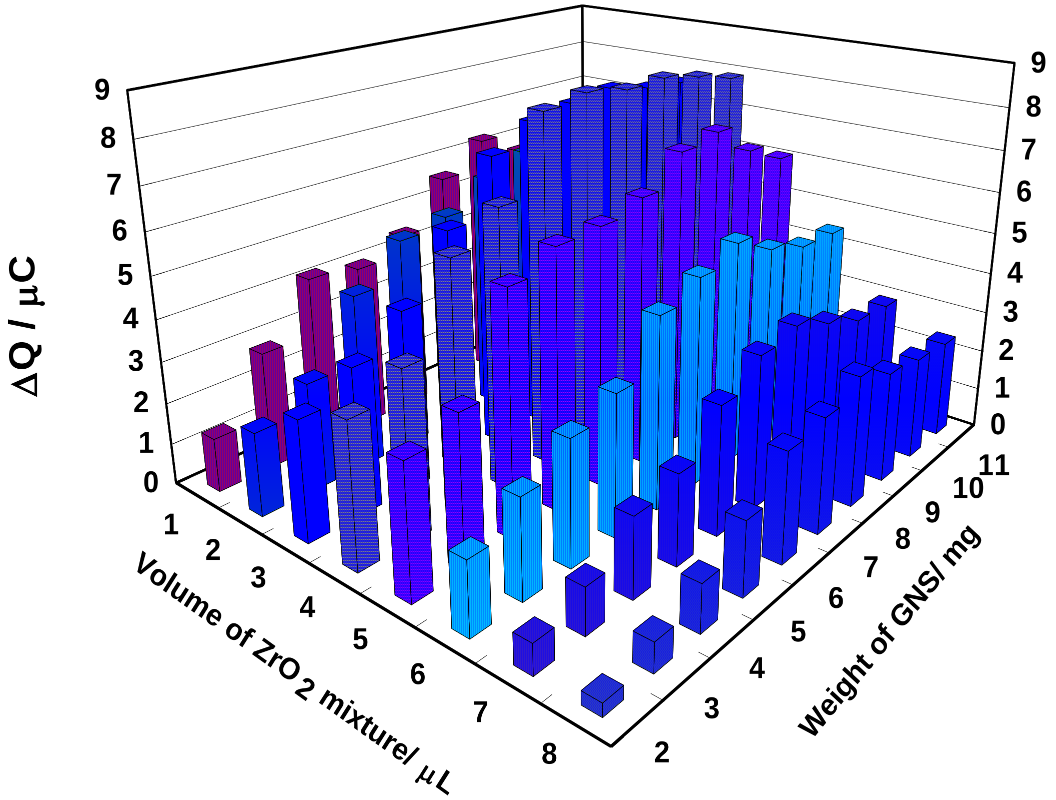


Figure 8. The effect of amount of ZrO_2 and GNS on the sensor response to5.0×10^{-7} M of Alfuzosin in 0.05 M PBS at pH=5.0

3.5. Calibration curve

Fig. 9A shows response of the sensor to addition of spiking standard solutions of 50 nM of ALF. As mentioned above the sensor response is expressed in µC, which is calculated by integrating of currents in a slected potential. For this reason, the magnitude of the sensor response depends on the choice of the potential range for integration, and the best range for this operation experimentally was found 0 to 900 mV. Under optimal conditions in the determination of the presence of ALF, the response time, which is defined as the time when 95% of the steady-state response is reached was about 10 s with CFFTLSV method (see Fig. 9A).

Fig.9B illustrates a typical *ΔQ* response [26-29] of the Chi-ZrO_2/GNS-IL/GCE to a set of standard solutions of ALF (from 2.0 to 200.0 nM in 0.05 M PB solution, pH5.0. In this figure, each point represents the integrated signal for 3 consecutive additions of the Alfuzosin standard solutions.

In general, Fig. 9B shows the analyte response has a linear dynamic range of 2 to 150 nM, with correlation coefficient of R^2=0.998 values. The estimated detection limit based on signal to noise ratio (S/N=3), was found 0.5 ±0.01 nM. A long-term storage stability of the ZrO_2-Chi /GNS-IL/GCE sensor was tested for 45 days, and it was seen that the sensitivity retained 97.5%.

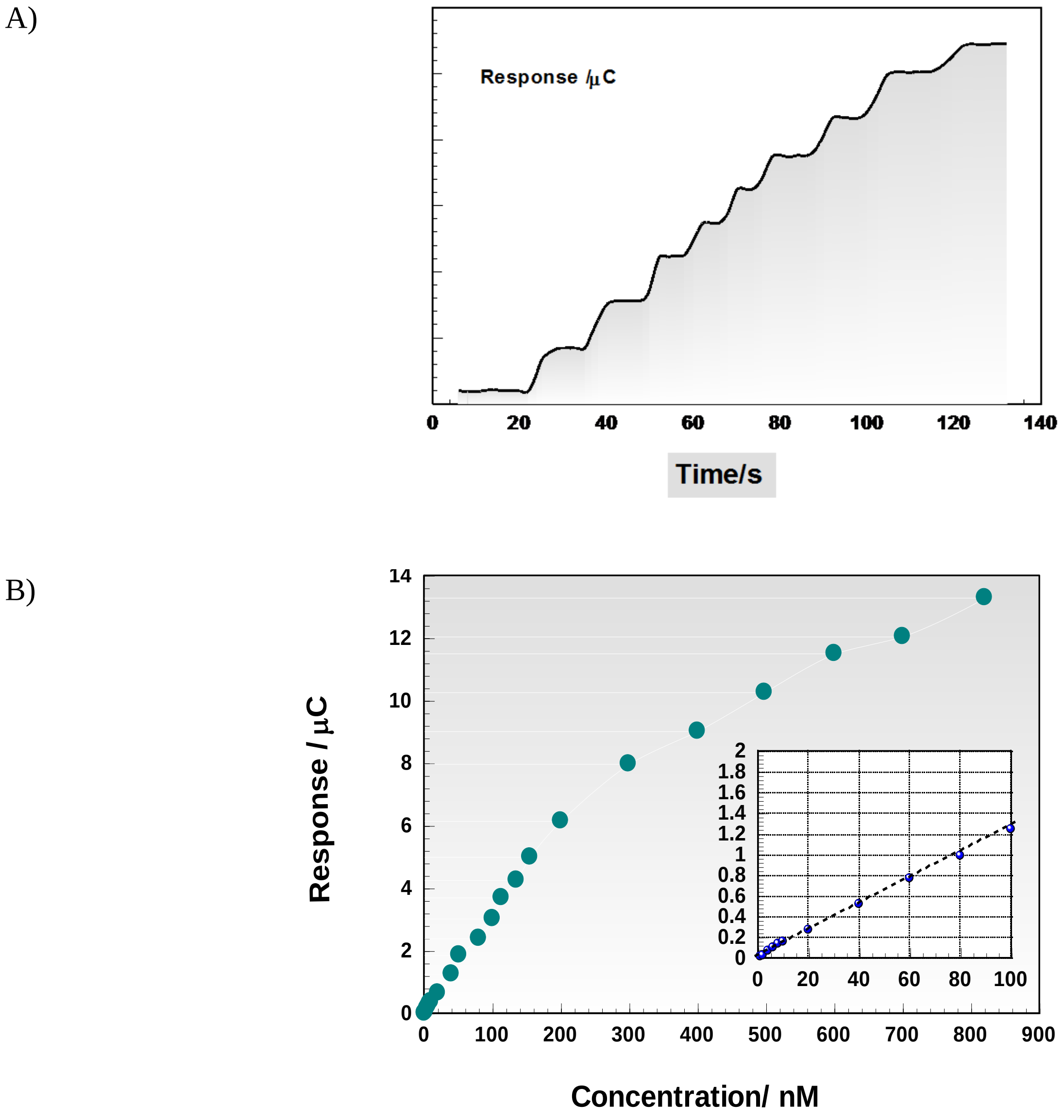


Figure 9. Response of the Chi-ZrO_2/GNS-IL/GC electrode to Alfuzosin upon the following concentrations: A) 2 to 800 nM n PB solution, pH 5.0 B) The calibration curve for Alfuzosin determination

4. CONCLUSIONS

In this work, the new sensor used here had been fabricated by modifying the GC electrode surface with Chi-ZrO_2/GNS-IL. We have successfully constructed a novel and high-performance platform for the coulometric determination of ALF, which is highly sensitive. Such sensitivity is attributed to the synergy of GNS and ZrO_2 nanoparticles on the oxidation of ALF and application of CFFTLSV method. It can be suggested that the sensor has excellent responses because of the large surface area and fast electron transfer of graphene nanoparticles. Using CFFTLSV method creates reproducible signals with response time less than 10 s and detection limit of 0.5 nM. The sensitivity retained 97.5% of initial sensitivity. In addition, the electrochemical method reveals some other excellent characteristics such as wide linear range, low detection limit and relatively a long-term stability (for 45 days). Also the proposed sensor has easy fabrication and low cost. These results can provide a new strategy for using sensor and can be applied in practice.

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