Analytical Methods

Square wave adsorptive stripping voltammetric determination of anticancer drug nilutamide in biological fluids using cationic surfactant cetyltrimethylammonium bromide

Journal:	*Analytical Methods*
Manuscript ID	AY-TEC-07-2015-001811.R1
Article Type:	Paper
Date Submitted by the Author:	04-Sep-2015
Complete List of Authors:	Temerk, Yassien; Assiut University, Ibrahim, Hossieny; Assiut University, Farhan, Nasser; Assiut University,

Square wave adsorptive stripping voltammetric determination of anticancer drug nilutamide in biological fluids using cationic surfactant cetyltrimethylammonium bromide

Yassien Temerk*, Hossieny Ibrahim* and Nasser Farhan

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt

A sensitive square wave adsorptive stripping voltammetric method was described for the determination of anticancer drug nilutamide (NLM) in biological fluids based on the enhancement effect of cationic surfactant: cetyltrimethylammonium bromide (CTAB). In pH 6.0 Britton – Robinson (BR) as supporting electrolyte and in the presence of $1.45x10^{-4}$ mol L^{-1} CTAB, NLM yields a well-defined and sensitive reduction peak at the ZnO nanoparticles modified carbon paste electrode (ZnONPs/CPE). Various chemical and instrumental parameters affecting the monitored electroanalytical response were investigated and optimized for NLM determination. The electrochemical parameters such as surface concentration (Γ), electron transfer coefficient (α) and the standard rate constant (k_s) of NLM at the modified electrode were calculated. The achieved limits of detection and quantification were $3.21x10^{-9}$ mol L^{-1} and $1.07\ x10^{-8}$ mol L^{-1} by square wave adsorptive stripping voltammetry (SWAdSV), respectively. The applicability of the proposed method was successfully applied for the detection of NLM in blood and urine samples with good accuracy and precision.

*Corresponding author at: Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt. Tel.: +20 (88) 2080770; fax: +20 (88) 2080209.

E-mail address:Temerk44@yahoo.com

E-mail address:Hossienyomer@yahoo.com

1. Introduction

Nilutamide (scheme1) is a nonsteroidal anti-androgen drug primarily used in the treatment of prostate cancer. It blocks the action of androgens of adrenal and testicular origin, which stimulate the growth of normal and cancerous prostatic tissue.[1] Reports on the lung toxicity of drugs containing nitro aromatic groups explain their toxicity as resulting from ring oxidation and nitro reductive biotransformation pathway.[2] Acute liver failure, interstitial pneumonitis and mild elevations in serum enzymes have been reported during NLM therapy.[3] Due to the high toxicity of NLM the level control of this drug in dosage forms and in biological fluids is extremely important. The critical role of NLM in human emphasized its determination in biological fluids. In this context so far, two methods only have been reported for the determination of NLM including a micellar electrokinetic chromatography[4] and spectrophotometery.[5] Reviewing the literature revealed that no electrochemical methods were described for the determination of NLM. Hence, it is still of great significance to develop sensitive and simple voltammetric method for the determination of NLM. The development of electrochemical methodologies for monitoring the occurrence of the NLM is demanded since this drug is a concern human health.

In recent years, various chemically modified electrodes have been widely used in electrochemical applications as sensitive and selective electrodes for the determination of biomolecular and drugs.[6-16] Among different modifiers, nano crystalline materials have drawn a lot of attention in various areas due to their noticeable advantages such as large surface area, high thermal and chemical stability, tuneable porosity and biocompatibility.[17-20] Nanostructures modified electrodes have been adopted as a promising way to facilitate the direct electron transfer of biomolecules. These nanostructures include for example, nano-materials[21] and

nanostructures metal oxide[22]. Among oxides, ZnO nanostructure due to wide band gap (3.37 eV), large excitation binding energy (60 meV), non-toxicity and high electron communication features is preferred for development of sensor and biosensors for clinical diagnostics.[23] A further rather promising application is using ZnO nanostructures as materials in solar energy research.[24]

To our knowledge, the voltammetric determination of NLM in the presence of a long-chain cationic surfactant has not been reported. In this context surfactants with an amphiphilic character change the electrical properties of the electrode/solution interface and the electrochemical process of the other biologically active substances.[25]

It is well documented that modification of electrode surface by surfactants increases electron transfer between the electrode surface and analyte and also improve the detection limit.[26,27] The use of surfactant solution as modifiers can improve the sensitivity and selectivity of the voltammetric measurements. The experimental procedure for the modification of carbon based electrodes can be simplified if the modifying agent is added to the background electrolyte, which is termed in situ modification. The in situ modification has the advantage of shortening or eliminating the preparation steps before the analysis.

The main target of the present work is to develop a sensitive voltammetric method for the determination of NLM based on the enhancement effect of surfactants for the first time. To achieve this goal, low concentration of CTAB was added into the bulk solution to change the electrode/solution interface. The capability of the proposed SWAdSV was validated by the determination of NLM in human serum and urine samples.

2. Experimental

2.1. Instrumentation

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed using a Princeton Applied Research (PAR) Verstate 4 potentiostat/galvanostat (Princeton Applied Research, USA). The electrode system consisted of the carbon paste electrode (unmodified or modified) as working electrode, a Ag/AgCl (saturated KCl) reference electrode and a Pt wire auxiliary electrode. A PAR Model 305 stirrer was used for the SWV. For voltammetric measurements, the test solution was placed in a voltammetric cell (10 ml) and deoxygenated by bubbling nitrogen for 15 min to remove any oxygen to a level not interfering with the voltammetry to cathodic potentials. For cathodic stripping experiments an accumulation potential (E_{acc}) was applied for a certain accumulation time (t_a), while the solution was stirred at 400 rev / min. At the end of the accumulation period the stirrer was stopped and the solution was allowed to become quiescent for 15 s prior to the voltammetric scan. X-ray diffraction (XRD) was performed to characterize ZnO powder with (Model PW 1710 control unit Philips) diffractometer. The morphologies of CPE and ZnONPs/CPE were examined by scanning electron microscopy (SEM) (JEOL, JSM-5400 LV).

2.2. Chemicals and reagents

Nilutamide, zinc nitrate hydrate, and CTAB were purchased from Sigma- Aldrich chemicals (St. Louis, MO, USA). Paraffin oil and graphite powder were obtained from Merck Company (Darmstadt, Germany). High- quality de-ionized water was used to prepare the solutions. A Stock solution of 10^{-3} mol L^{-1} NLM was prepared by dissolving an appropriate amount of the compound in ethanol and then it was stored in the dark at 4 °C. Other diluted solutions were prepared by diluting of the stock solution. CTAB was dissolved in distilled water to prepare 0.1M stock solution. The supporting electrolyte was Britton – Robinson (BR) buffer prepared by adding an appropriate amount of sodium hydroxide (0.4 mol L^{-1}) to an orthophosphoric acid,

boric acid and acetic acid mixture (0.08 mol L^{-1}). All chemicals were of reagent grade (Merck, Darmstadt, Germany). The pH values of the buffer solutions were measured with a Jenway Model 3310 pH meter.

2.3. Preparation and characterization of ZnO nanoparticles

The zinc oxide nanoparticles (ZnONPs) were prepared by thermal decomposition method. First two grams of zinc nitrate were taken and fired in a furnace for 4 hours at 500 °C. It was further ground in a mortar to make a fine powder of ZnONPs. The ZnONPs were characterized by XRD. A definite line broadening of the XRD peaks indicates that the prepared material consists of particles in the nanoscale range (Fig.S1, ESI). All the diffraction peaks can be indexed to the ZnO hexagonal wurzite structure (JPCDS card number: 04-008-8196). The average particle size of the sample was found to be 20.52 nm which is derived from the full width at half-maximum (FWHM) of more intense peak corresponding to 101 plane located at 36.34° using Scherrer's formula.[28]

2.4. Preparation of the modified and unmodified electrodes

The bare carbon paste electrode (CPE) was prepared by hand mixing of 70% graphite powder with 30% paraffin oil to produce a homogenous carbon paste. The paste was packed firmly into the cavity and smoothed on a weighing paper. The electrical contact was achieved via a copper wire. The modified ZnONPs/CPE was fabricated using the same procedure, except the introduction of various amounts of ZnONPs into the unmodified carbon paste.

2.5. Urine and serum pretreatment

Urine and serum samples of healthy volunteers were stored frozen until assay. Urine samples were centrifuged and filtered before use. In order to precipitate the proteins, 0.5 ml blood serum was added into 2 ml methanol, and then centrifuged for 20 min at

4000 rpm using tabletop high speed centrifuge TDZ4A-WS. The clear supernatant layer was filtered through 0.45 μm milipore filter to obtain a protein-free spiked human serum sample.

3. Results and Discussion

3.1. Morphological characterization of ZnONPs/CPE

Fig. 1 compares the morphological features of CPE and ZnONPs/CPE using SEM. Significant differences in the surface structure of two electrodes were observed. Fig. 1B confirms that the surface of CPE was well covered by ZnONPs. As seen in Fig. 1B, the SEM image shows CPE sheets in the presence of ZnONPs are densely and almost uniformly dispersed, indicating successfully deposition of the ZnONPs on the CPE surface. Moreover, some pores are also observed. This porous structure could significantly increase the effective electrode surface area, which play an important role in enhancing electro-conductivity and facilitate the diffusion of analyte into the surface of the electrode. In this context it was found that nanoporous materials possess the large surface area and the uniform pores can provide more catalytic sites via its catalytic mesostructure or the loading of a large amount of catalyst, hence making possible high sensitivity detection.[29,30] Moreover the great porosity and uniform structure facilitate the fast transport of the target analytes to active sites in the nanopores.[31] So, the morphology of the ZnONPs /CPE would be beneficial to its electrochemical performance.

3.2. Cyclic voltammetric characterization of modified electrodes

First, to appraise the electrochemical behavior of ZnONPs/CPE, potassium ferrocyanide solution was used as a probe. Fig. 2 displays the cyclic voltammograms of bare CPE (a) and ZnONPs /CPE (b) in solution of 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ redox probe at scan rate of 100 mVs^{-1}. As the CPE , the peak-to-peak potential separation

(ΔE_P) was 306 mV , corresponding to an irreversible electron transfer process while at the ZnONPs /CPE , the ΔE_P was decreased to 184 mV, indicating a quasireversible electron transfer process, Furthermore the peak current of $[Fe(CN)_6]^{3-/4-}$ was increased at ZnONPs/CPE compared to the bare electrode. The results indicated that the presence of ZnONPs in the carbon paste had great improvement on the electrochemical response, which was partly due to the excellent characteristics of ZnONPs such as a good electrical conductivity, high chemically stability and high surface area.

Cyclic voltammetry of $[Fe(CN)_6]^{3-/4-}$ at ZnONPs /CPE in the presence of CTAB is interesting (Fig. 2, curve 3). A well defined redox of $[Fe(CN)_6]^{3-/4-}$ with great enhancement was observed in the presence of $1.45x10^{-4}$ mol L^{-1} CTAB. Furthermore, the peak-to-peak separation (ΔE_P) decreases to 108 mV and the ratio of anodic to cathodic current is 0.985≈1 indicating that $[Fe(CN)_6]^{3-/4-}$ undergoes a reversible redox reaction. On the basis of this observation, it is clear that the presence of CTAB exerts a significant adsorption on the electrode surface leading to decrease of over potential in the process and an enhancement of the peak current.

The active surface area of modified electrode was estimated according to the slope of the I_p versus $\nu^{1/2}$ plot for a known concentration of $[Fe(CN)_6]^{3-/4-}$ based on the Randles-Sevcik equation:[32]

$$I_p = 2.69x10^5 n^{3/2} A D^{1/2} \nu^{1/2} C_o$$

Where I_p refers to the cathodic peak current, n is the electron number, A is the electroactive surface area of electrode, D is diffusion coefficient ($7.60x10^{-6}$ cm^2s^{-1}), C_o is the concentration of $[Fe(CN)_6]^{3-/4-}$ and ν is the scan rate. The electroactive surface area of both CPE and ZnONPs/CPE in the presence of CTAB was calculated. The area of CPE (0.073 cm^2) is less than the modified electrode area (0.127 cm^2)

which the increase of the electroactive surface area in modified electrode exhibited the influence of ZnONPs as an effective modifier that provide a large surface and facilitate the electron transfer between the electrode and the solution.

3.3. Electrochemical behavior of NLM at modified and unmodified electrodes

Square wave voltammetry was utilized to study the electrochemical behavior of NLM in BR buffer of pH6.0 at the surface of the bare CPE and modified ZnONPs/CPE in the presence of $1.45x10^{-4}$ mol L^{-1} CTAB (Fig.S2, ESI). The electrochemical reduction of NLM shows a single cathodic peak in the potential range 0 to –1.0 V. This cathodic peak is attributed to the reduction of $–NO_2$ group at C-4 on the NLM molecule (scheme 1). It could be observed that the reduction peak current of NLM at the modified electrode significantly enhanced and it was almost 2.7 times larger than unmodified electrode.

This significant improvement of current at the modified electrode revealed the existence of synergistic effects of ZnO nanoparticles and the cationic surfactant. On the basis of these observations, it is clear that the ZnONPs exerts a significant catalytic effect on the electrochemical reduction of NLM and on the otherhand increased the available surface area of the electrode. High conductivity of ZnO nanoparticles is due to the presence of oxygen vacancies, shallow zinc interstitial, hydrogen impurity and other donor type point defects.[33,34] The role of cationic surfactant can be related to the adsorption of CTAB molecules on the surface of modified electrode which can facilitate the electron transfer of NLM and can significantly improve the sensitivity of NLM. In this context, it is well established that the surfactants can be adsorbed on the solid surfaces to form the surfactant film, which may alter the overvoltage of the electrode and influence on the electron transfer.[35] In the present case, hydrophobic NLM is induced to the electrode surface

by CTAB. As a result, peak current of NLM is increased and the sensitivity is enhanced.

The influence of potential scan rate on the reduction of NLM at ZnONPs/CPE in the presence of $1.45x10^{-4}$ mol L^{-1} CTAB was studied by cyclic voltammetry. CVs for $7.40x10^{-5}$ M of NLM in BR buffer solution of pH 6.0 with scan rates ranging from 50 to 600 mVs^{-1} were investigated (Fig. 3). A linear relationship between the reduction peak current (I_P) and the scan rate (ν) showed predominantly adsorption process. The equation is noted in BR buffer of pH 6: I_{Pa} (μA) = –0.74 + 0.085 ν (R^2 = 0.992), indicating the electrode process of NLM was adsorption-controlled.

For a totally irreversible, the relation between E_P and ν can be expressed by the Laviron's equation:[36]

$$E_P = E° – (2.3RT/\alpha nF) [\log (RTk_s/ \alpha nF) – \log\nu]$$

Where E° (V) is the formal potential, α is the transfer coefficient, k_s (s^{-1}) is the electrochemical rate constant and other symbols have their usual meaning. αn values can be obtained from the slope of the linear plot of E_P with respect to logν. The α value was calculated from the difference between peak potential (E_P) and half wave potential ($E_{P/2}$) according to the equation given below for the electrode process.[37]

$$\Delta E = E_P – E_{P/2} = (47.7/\alpha)\ mV$$

The calculated value of α is 0.52. So, the number of electrons (n) transferred in the electroreduction of NLM was calculated to be ≈ 4. Thus, we conclude that NLM in BR buffer undergoes four electrons irreversible reduction reaction. In this context the nitro group present in NLM is one of the strongest of the common electron-withdrawing groups. As voltammetric studies indicated that NLM is 4ē, $4H^+$ transfer process, the nitro group at C-4 on the NLM molecule can be reduced to hydroxylamine derivative (scheme 1).

Also, the value of k_s can be determined from the intercept of the straight line of E_P vs. logν. The values of k_s were calculated equal to 0.028 and 0.011 for the modified and unmodified electrode, respectively. The large value of heterogeneous charge transfer rate constant at the modified electrode in comparison at CPE may be attributed to the good electrical conductivity as well as the increase of the electrode area in the presence of ZnONPs.

The surface concentration of electroactive species, Γ, can be calculated from the slope of I_P vs. scan rate of equation:[38]

$$I_p = n^2F^2\nu A\ \Gamma/4RT$$

Where n is the number of electrons transferred, F (C/mol) is the Faraday's constant, A (cm^2) is the area of the electrode, Γ is the surface concentration of the electroactive substance, NLM, and ν (Vs^{-1}) is the scan rate. The surface concentration (Γ) of NLM on the surface of modified electrode was estimated to be $9.25x10^{-10}$ mol cm^{-2}, which was larger than $1.01x10^{-10}$ mol cm^{-2} on CPE. These values imply that the presence of ZnONPs increase the surface area of the electrode, which in turn increase the Γ of NLM.

3.4. Optimization of the amount of modifier in the electrode

The voltammetric signals of $7.40x10^{-5}$ mol L^{-1} NLM were affected by the composition of the paste. It was observed that the sensitivity of the sensor first rapidly increases with increasing the ZnONPs content in the paste up to about 20% and then decreases with the higher loadings (Fig. 4). This is because the sites for adsorption increased with the increase of ZnO nanoparticles percentage in modified electrode, while the excess of ZnO nanoparticles increased the resistance of the electrode. Hence ZnONPs (20% w/w) modified carbon paste electrode were used throughout the work.

3.5. Effect of surfactant

The effect of different types of surfactants including CTAB, sodium dodecyl sulfate (SDS) and Triton X-100 (TX-100) was investigated. It is observed that the addition of CTAB to the NLM enhanced the peak current and the limit of detection is found to be lower while SDS and TX-100 did not play a significant role on the electrode process. This indicates that the reduction of NLM becomes easier in presence of micellar system formed by CTAB. The effect of CTAB concentration on the cathodic peak current of NLM is shown in Fig.5. The cathodic peak current of 7.40×10^{-5} mol L^{-1} NLM increases steadily in the beginning with increase in concentration of CTAB and reaches a maximum at 1.45×10^{-4} mol L^{-1} CTAB and after that decreases continuously. It may be interpreted that at 1.45×10^{-4} mol L^{-1} CTAB the adsorption behavior changes from monomer adsorption to monolayer adsorption with increase in concentration of CTAB at the electrode surface.[39] However, the peak current decreases with further increase in CTAB concentration, it may be due to the inhibition of electron transfer by aggregates of micelles. Another reason for decrease in peak current is the increase of hydrophobicity of CTAB micelles that might decrease the electron transfer rate constant[40] and result in the decrease of peak current at high CTAB concentrations. Therefore, concentration of 1.45×10^{-4} mol L^{-1} CTAB is chosen at optimum one.

3.6. Influence of pH

The influence of pH on the reduction of NLM in BR buffer containing 1.45×10^{-4} mol L^{-1} CTAB at ZnONPs/CPE was investigated. As can be seen in Fig. 6, the reduction peak current of NLM increased with increasing pH value until it reaches 6.0 and then decreases when the pH increases further. Thus, pH 6.0 was deemed optimum. Considering the sensitivity for determination of NLM, pH 6.0 is chosen for the subsequent analytical experiments. Moreover, the peak potential of reduction of NLM shifted negatively and linearly as the solution pH increased from pH2 to pH10 and

that it obeys the following equation: $E_P(V) = -0.279-0.065pH$. The slope value of the E_P/pH plot was 65mV/pH. This value indicated that an equal number of electrons and protons were involved in the electrochemical reduction process of NLM based on the Nernst Equation.

3.7. Effect of accumulation time and potential

It was significant to fix the accumulation potential and the accumulation time when adsorption studies were intended. Both conditions could affect the amount of adsorption of NLM on the electrode surface. Bearing this in mind, the effect of accumulation potential and time on SWAdSV signals was studied. When accumulation potential was varied from +0.1 V to -0.2V vs. Ag/AgCl, the peak current increased because the applied potential is near the reduction potential of NLM and therefore the NLM molecules tend to accumulate at the electrode surface. But at potential higher than 0.0 V vs. Ag/AgCl the peak current dramatically decreased because by applying the potentials that are higher or about the reduction potential of NLM, the drug molecule do not have any time to accumulate at the surface. Therefore the accumulation efficiency decreased and the electrochemical signals were decreased consequently. Hence, a potential of 0.0 V vs. Ag/AgCl was applied as the accumulation potential.

Also the influence of accumulation time ranging from 0.0 to 300s on the electroreduction of NLM was investigated. The peak current increased gradually as accumulation time increased from 0 to180s. However, with further increasing in accumulation time beyond 180s, the peak current tended to be almost stable illustrating that adsorptive equilibrium of NLM molecules on the electrode surface was achieved. Therefore, the optimal accumulation time of 180s was chosen in stripping analysis of NLM.

3.8. Optimization of the SWAdSV parameters

The peak current obtained in SWAdSV is dependent on various instrumental parameters such as frequency (f), scan increment (ΔE_s) and pulse amplitude (E_a). Although the square wave voltammetric peak magnitude of 7.4×10^{-5} mol L^{-1} NLM in BR buffer of pH6 was almost directly proportional to each f, ΔE_s and E_a, however better developed and symmetrical voltammetric peaks were obtained under the following pulse parameters: f = 50Hz, ΔE_s = 8 mV and E_a = 50 mV. These parameters reflect voltammograms of high sensitivity and best peak morphology.

3.9. Stability and reproducibility of the sensor

The reproducibility of ZnONPs/CPE was investigated using SWAdSV measurements for 6.92×10^{-8} mol L^{-1} NLM in BR buffer solution containing 1.45×10^{-4} mol L^{-1} CTAB. The relative standard deviation (RSD %) for seven successive assays was 0.99%. When using five different electrodes, the RSD % for five measurements was 1.5 %. The stability of the modified electrode has been also investigated by measuring the current response of 3.92×10^{-8} mol L^{-1} NLM every few days. The modified electrode retained 98% of its initial peak current response. These results indicated that ZnONPs/CPE had a good stability and repeatability for the detection of NLM.

3.10. SWAdSV determination of NLM concentration

As a highly sensitive and low detection limit electrochemical method, SWAdSV was performed to investigate the relationship between the reduction peak current and the concentration of NLM under the optimal conditions. The SWAdSV responses for different concentrations of NLM in BR solution of pH6.0 are illustrated in Fig. 7A.

As shown in Fig. 7B, the reduction peak current has a good relationship with the NLM concentration in the range from 7.60×10^{-8} mol L^{-1} to 3.05×10^{-7} mol L^{-1}. The regression equation was I_p (μA) = 4.26 + 6.56 $\times 10^7$ C(M) (R =0.997). Limits of

detection (LOD) and quantitation (LOQ) of bulk NLM were estimated using the expression: kSD_a / b where k = 3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept and b is the slope of the calibration plot. LOD and LOQ of 3.21 $x10^{-9}$ and 1.07 $x10^{-8}$ mol L^{-1} NLM, respectively, were achieved by SWAdSV (Table 1). The obtained limit of detection for NLM is significantly lower than the previously reported chromatographic ($8.20x10^{-8}$ mol L^{-1})[4] and spectrophotometric ($7.12x10^{-6}$ mol L^{-1})[5] methods. The low detection limit can be attributed to the synergistic effect of ZnONPs and CTAB, in which ZnONPs provide a large specific surface area to increase the loading amount of NLM whereas cationic surfactant possesses efficient enhancement in the sensitivity of NLM. Meanwhile, the electron transfer on the electrode surface can be accelerated and the electrochemical signal is amplified due to the outstanding electric conductivity of ZnONPs. Therefore, sensitive detection of NLM was achieved using the proposed SWAdSV method.

The robustness of the optimized SWAdSV method for assay of NLM was examined by evaluating the influence of small variation in some of the most important operational parameters (pH5.8–6.2) , accumulation potential E_{acc} (+0.1–0.0V) and accumulation time (150–180s). The obtained percentage recoveries and relative standard deviation (97.53% to 98.76%) indicated insignificant effect within the studied range of variation of optimum operational conditions, and consequently the optimized SWAdSV method was considered reliable for assay of bulk NLM and it could be considered robust.

In order to evaluate the accuracy and precision of the proposed SWAdSV method, analysis of NLM in a bulk form was carried out over one day (intra – day assay) and for seven days (inter- day assay). The results obtained were presented in Table 2. The

results demonstrated that values were within the acceptable range and the developed SWAdSV method was both accurate and precise for the sensitive detection of NLM.

In order to evaluate the selectivity of the method for determination of NLM, the influence of potentially interfering substance on the determination of this compound was investigated. The tolerance limit of interfering species was considered as the maximum concentration that gave a relation error less than ± 5.0 % at a concentration level of $1.16x10^{-7}$ mol L^{-1} NLM. Ascorbic acid, oxalic acid, glutaric acid, uric acid, glycine, glucose, starch, Cu (II), Fe(II), Mg(II) and Ca(II) have no effect on the I_p of NLM up to 200 fold excess. The inclusion of CTAB in the supporting electrolyte was found to convenient for selective determination of low levels of NLM in the sample.

3.11. Assay of NLM in spiked human serum

The developed SWAdSV method was applied for the determination of NLM spiked in human serum samples (Fig.S3, ESI). No potentially interfering compounds such as amino acids (cystine, cysteine, serine and alanine) and biogenic amines (histamine, tryamine and tryptamine) which may present in biological samples occurred in the potential range where the analytical peak appeared. The variation of the peak current (I_P/μA) versus concentration of NLM was linear within the range $7.81x\ 10^{-8}$ mol L^{-1} to $5.40\ x\ 10^{-7}$ mol L^{-1}; its corresponding regression equation was: I_p (μA) = 1.78 + $4.71x10^7$ C(M) (R =0.990). LOD and LOQ values were calculated and summarized in Table 1. The observed recovery (97.36% to 101.83%) indicates that the proposed SWAdSV method had good accuracy and great potential for practical analysis of NLM in real clinical samples. The results obtained for intra – day and inter – day precision and accuracy were presented in Table 2. As can be seen, the RSD values of measurements were not greater than 1.83% and 2.63% for intra – day and inter – day

determinations, respectively. Both the intra-day and inter- day reproducibilities of the voltammetric method were fairly good.

3.12. Assay of NLM in spiked human urine

In order to evaluate the validity and practical applicability, the proposed SWAdSV method was applied for the assay of NLM spiked in human urine samples (Fig.S4, ESI). The well-defined reduction peak of NLM was observed at Ca. –0.49V. The voltammograms clearly depict that the peak current increases significantly for the peak at Ca. –0.49V, thereby confirming that it corresponds to the reduction of NLM. The analytical results are summarized in Table 1. The satisfactory recovery of NLM in human urine samples indicates that the proposed method had great potential in the practical sample analysis. Repeatability and reproducibility of the proposed SWAdSV for assay of NLM in urine samples were examined by intra-day and inter-day assay (Table 2). The obtained results indicate the precision for assay of NLM in urine samples.

Conclusions

The development of a method for the determination of NLM was described. The results obtained in the paper demonstrated the synergistic effect of CTAB and ZnONPs modified carbon paste electrode on the first voltammetric determination of NLM. The proposed SWAdSV method was very sensitive, free of common interferences with the molecule of interest and had a nanomolar detection limit. The developed voltammetric method is superior to previously reported methods in respect to limit of detection. LOD of 3.21×10^{-9} mol L^{-1} NLM was achieved by SWAdSV, whereas the corresponding limits using the chromatographic and spectrophotometric methods were 8.20×10^{-8} mol L^{-1} and 7.12×10^{-6} mol L^{-1}, respectively. The low

detection limit can be attributed to the synergistic effect of ZnONPs and CTAB, in which ZnONPs provide a large specific surface area to increase the loading amount of NLM whereas cationic surfactant possesses efficient enhancement in the sensitivity of NLM. Moreover the developed SWAdSV procedure was successfully applied for determination of NLM in biological fluids with good accuracy and precision. The method could be adopted for the pharmacokinetic studies as well as for quality control laboratories. The determination of drug in presence of surfactants provides new medium for study of interaction of drugs with surfactants.

Acknowledgement

Y.M. Temerk is grateful to the Alexander von Humboldt Stiftung, Germany for a financial support of this investigation.

References

1. M. G. Harris, S. G. Coleman and D. Faulds, *Drugs Aging*, 1993, 3, 9-25

2. G. L. Kedderis and G. T. Miwa, *Drug Metabolism Reviews*, 1988, 19, 33–62.

3. A. R. Babu, R. M. Borkar, G. Raju, B. Raju and R. Srinivas, *Biomed. Chromatogr.*, 2014, 28, 788-793.

4. J. Znaleziona, V. Maier, J. Petr, J. Chrastina and J. Ševčík, *Chromatographia*, 2011, 74, 151-155.

5. M. M. Carvalho, D. S. M. Ribeiro, J. L. M. Santos and J.A.V. Prior, *New J. Chem.*, 2014, 38, 2856-2864.

6. L. Zheng and J. F. Song, *Anal. Biochem.*, 2009, 391, 56–63.

7. H. Beitollahi, M. M. Ardakani, B. Ganjipour and H. Naeimi, *Biosens. Bioelectron.*, 2008, 24, 362-368.

8. M. R. Ganjali, N. M. Kazami, F. Faridbod, S. Khoee and P. Norouzi, *J. Hazard. Mater.*, 2010, 173, 415-419.

9. M. M. Ardakani, Z. Akrami, H. Kazemian and H.R. Zare, *Int. J. Electrochem. Sci.*, 2009, 4, 308 – 319.

10. M. M. Ardakani, M. A. Karimi, S. M. Mirdehghan, M. M. Zare and R. Mazidi, *Sens. Actuators B*, 2008, 132, 52-59.

11. Z. Taleat, M. M. Ardakani, H. Naeimi, H. Beitollahi, M. Nejati, and H.R. Zare, *Anal. Sci.*, 2008, 24, 1039-1044.

12. M. M. Ardakani, H. Rajabi, H. Beitollahi, B. B. F. Mirjalili, A. Akbari and N. Taghavinia, *Int. J. Electrochem. Sci.*, 2010, 5, 147 – 157.

13. R. N. Goyal, V. K. Gupta, N. Bachheti, R. A.Sharma, *Electroanalysis*, 2008, 7, 757 – 764.

14. R. N. Goyal, V. K. Gupta, N. Bachheti, *Analytica Chimica Acta*, 2007, 597, 82–89.

15. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Talanta*, 2008, 76, 662–668.

16. R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *Analytical Biochemistry*, 2010, 407, 79–88.

17. N. Zhou, J. Wang, T. Chen, Z. Yu and G. Li, *Anal. Chem.*, 2006, 78, 5227–5230.

18. Y. C. Liu, C. C. Yu and K. H. Yang, *Electrochem. Commun.*, 2006, 8, 1163-1167.

19. F. N. Crespilho, M. E. Ghica, M. Florescu, F. C. Nart, O. N. Oliveira and C. M. A. Brett, *Electrochem. Commun.*, 2006, 8, 1665-1670.

20. L. Agüí, J. Manso, P. Y. Sedeño and J. M. Pingarrón, *Sens. Actuators B*, 2006, 113, 272-280.

21. T. Tangkuaram, C. Ponchio, T. Kangkasomboon, P. Katikawong and W. Veerasai, *Biosens. Bioelectron.*, 2007, 22, 2071-2078.

22. S. J. Bao, C. M. Li, J. F. Zang, X. Q. Cui, Y. Qiao and J. Gu, *Adv. Funct. Mater.*, 2008, 18, 591–599.

23. F. Zhiyong and J. G. Lu, *J. Nanosci. Nanotechnol.*, 2005, 5, 1561-1573.

24. L. S. Mende and J. L. MacManus-Dris, *Materials Today*, 2007, 10, 40-48.

25. M. P. Char, E. Niranjana, B. E. K. Swamy, B. S. Sherigara and K. V. Pai, *Int. J. Electrochem. Sci.*, 2008, 3, 588 – 596.

26. B. J. Sanghavi and A. K. Srivastava, *Electrochim. Acta*, 2010, 55,

27. N. Chowdappa, B. E. Kumara Swamy, E. Niranjana and B.S. Sherigara, *Int. J. Electrochem.Sci.*, 2009, 4, 425-434.

28. B. D. Cullity, Elements of X-Ray Diffraction, Addison-Wesley, Reading, Mass, USA, 3rd edition ,1967).

29. Z.H. Dai, S.Q. Liu, H.X. Ju, H.Y. Chen, *Biosens. Bioelectron*., 2004, 19, 861-867.

30. Z. Dai, H. Ju, *Trends in Analytical Chemistry*, 2012, 39, 149-162.

31. S. Wu, H. Wu, Y.Y. Liu, H.X. Ju, *Chem. Mater*., 2008, 20, 1397-1403.

32. R. N. Goyal, V. K. Gupta and S. Chatterjee, *Sens. Actuators B*, 2010, 149, 252–258.

33. S. K. Arya, S. Saha, J. E. R. Vick, V. Gupta, S. Bhansali and S.P. Singh, *Anal. Chim. Acta*, 2012, 737, 1-21.

34. V. Gupta, *Thin Solid Films*, 2010, 519, 1141-1144.

35. R. M. Kotkar and A. K. Srivastava, *Anal. Sci.*, 2008, 24, 1093-1098.

36. E. Laviron, *J. Electroanal. Chem.*, 1979, 101, 19-28.

37. A. J. Bard and L. R. Faulkner, Elecrochemical Methods Fundamentals and Applications, Wiley, New York, 2nd edn ,2004.

38. E. Laviron, *J. Electroanal. Chem.*, 1979, 100, 263-270.

39. R. Jain and J. A. Rather, *Colloids Surf. B*, 2011, 83, 340-346.

40. M. Ortiz, C. Muñoz, C. Z. Urzúa and A. Á. Lueje, *Talanta*, 2010, 82, 398-403.

Figure Captions

Scheme1. The proposed mechanism for electroreduction of NLM at ZnONPs/CPE

Figure 1. SEM images of (A) CPE and (B) ZnONPs/CPE.

Figure 2. Cyclic voltammograms of 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl obtained at (1) CPE, (2) ZnONPs/CPE and (3) modified ZnONPs/CPE in the presence of $1.45x10^{-4}$ mol L^{-1} CTAB. Scan rate 100 mVs^{-1}.

Figure 3. (A) Cyclic voltammograms 7.40 x 10^{-5} mol L^{-1} NLM obtained at modified ZnONPs /CPE in the presence of 1.45 x 10^{-4} mol L^{-1} CTAB; at various scan rates; (1) 50, (2) 100, (3) 200, (4) 300, (5) 400, (6) 500 and (7) 600 mVs^{-1} in BR buffer of pH 6.0. (B) Dependence of E_P on lnν. (C) Dependence of I_p on ν.

Figure 4. Plot of peak current of 7.40 x 10^{-5} mol L^{-1} NLM in BR, pH6 using SWV versus the percentage of ZnO nanoparticles in the paste. Accumulation potential, 0.0 V; scan increment, 8 mV; frequency, 50 Hz and pulse height, 50 mVpp and accumulation time, 60 s.

Figure 5. Plot of peak current of 7.40 x 10^{-5} mol L^{-1} NLM in BR, pH6 using SWV versus the concentration of CTAB. Other conditions as in Fig.4

Figure 6. The effect of pH on the reduction peak current and peak potential of 7.40 x 10^{-5} mol L^{-1} NLM using SWV. Other conditions as in Fig.4

Figure 7. (A) SW voltammograms of NLM at modified ZnONPs/CPE in the presence of 1.45 x 10^{-4} mol L^{-1} CTAB in BR solution of pH 6.0. [NLM]: (1) Blank: (2) 3.92 x 10^{-8}, (3) 7.81 x 10^{-8}, (4) 1.16 x 10^{-7}, (5) 1.55 x 10^{-7}, (6) 1.93 x 10^{-7}, (7) 2.49 x 10^{-7}, (8) 3.05 x 10^{-7} and (9) 3.60 x 10^{-7} mol L^{-1}. Accumulation time, 180 s. Other conditions as in Fig.4

(B) Calibration plot of I_P (μA) vs. [NLM] in BR solution of pH 6.0.

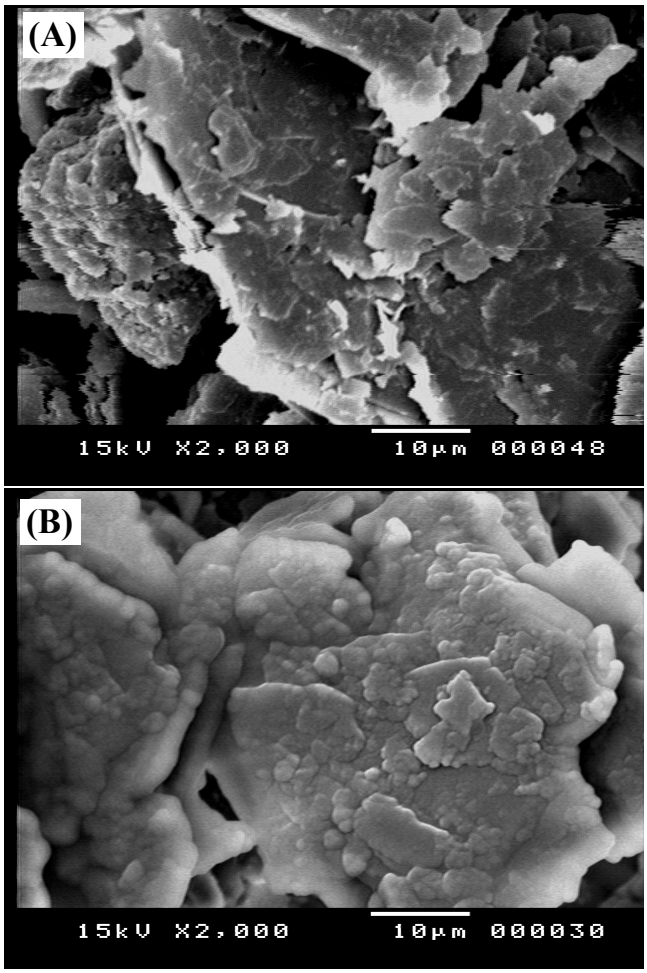


Fig.1

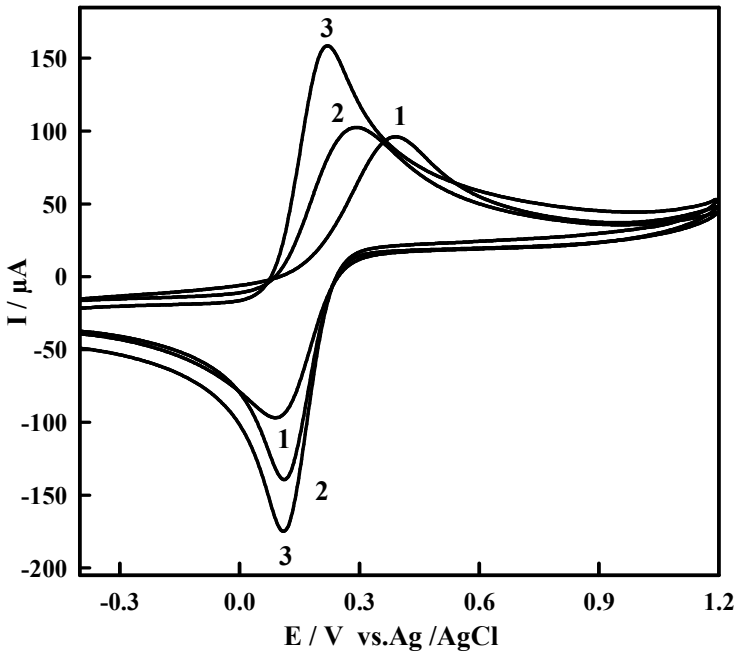

3
2
1
150
100
50
0
-50
-100
-150
-200
I / μA
1
2
3
-0.3
0.0
0.3
0.6
0.9
1.2
E / V vs.Ag /AgCl

Fig.2

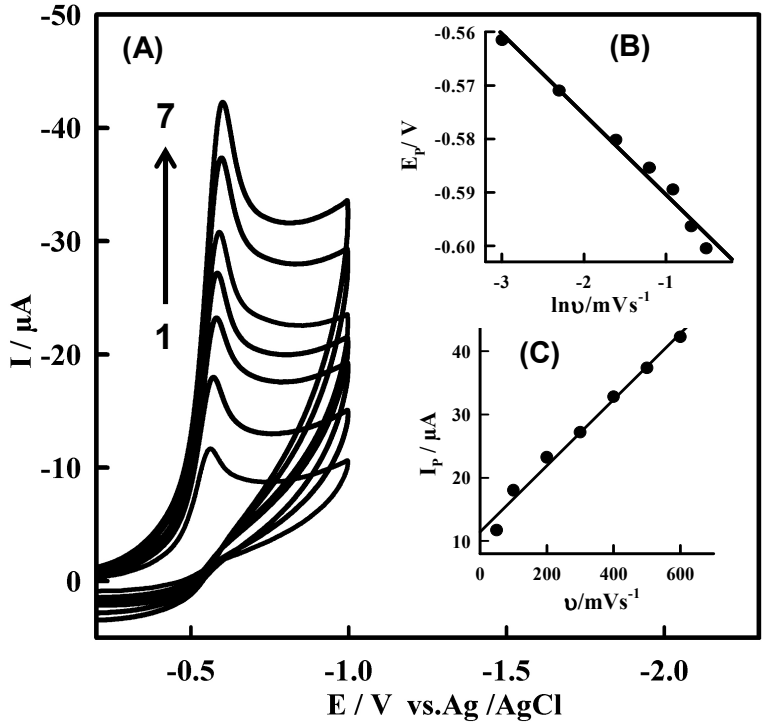


Fig.3

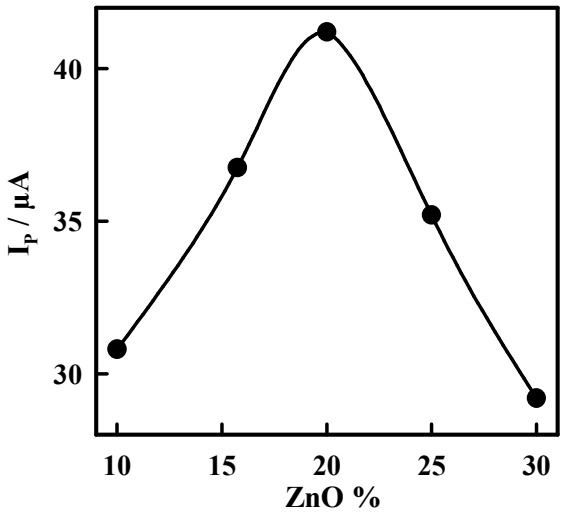


Fig4

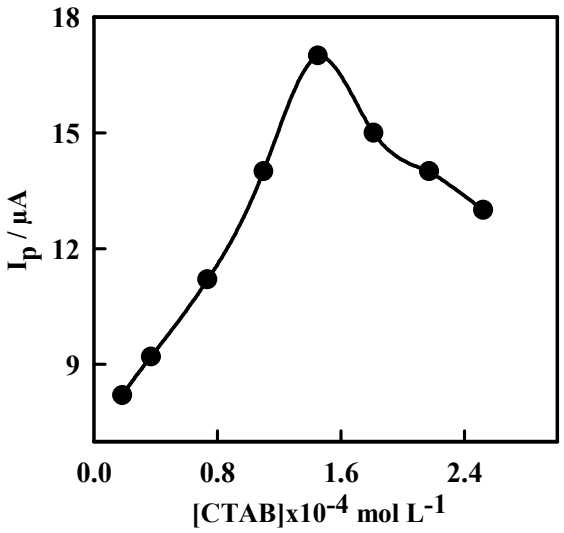


Fig.5

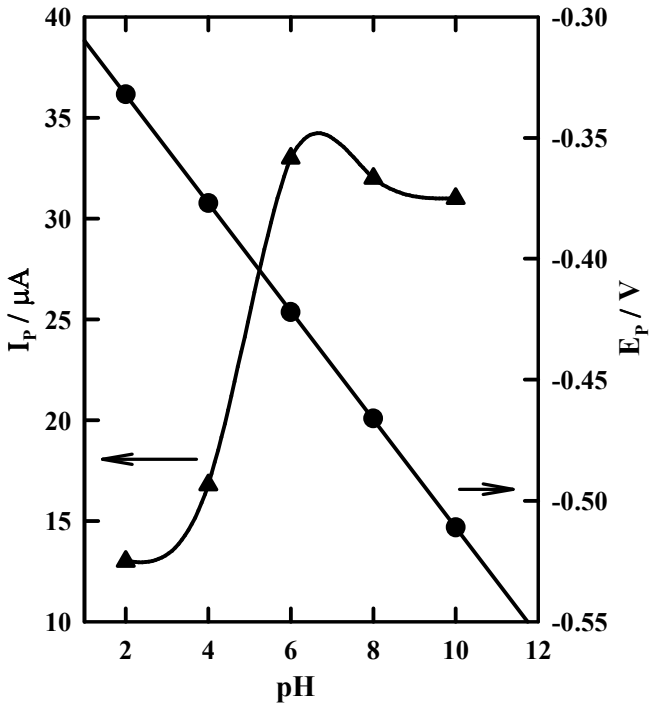


Fig.6

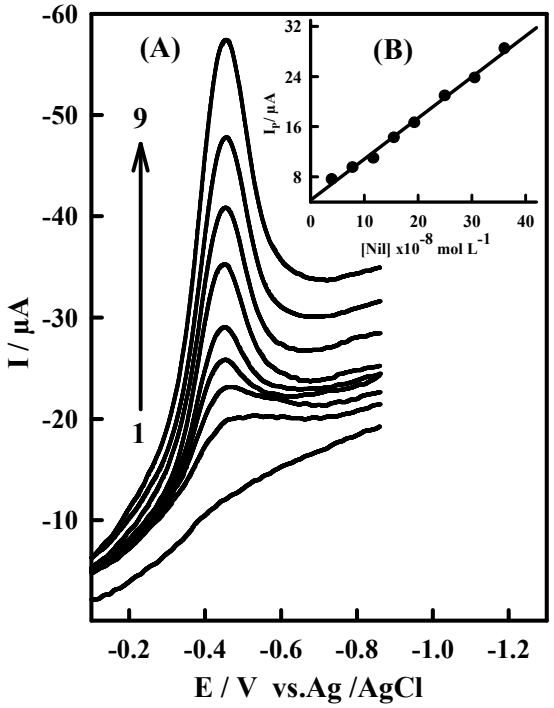

(A)
(B)
9
1
I / µA
E / V vs.Ag /AgCl
-60
-50
-40
-30
-20
-10
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
32
24
16
8
0
10
20
30
40
I_p / µA
[Nil] x10^{-8} mol L^{-1}

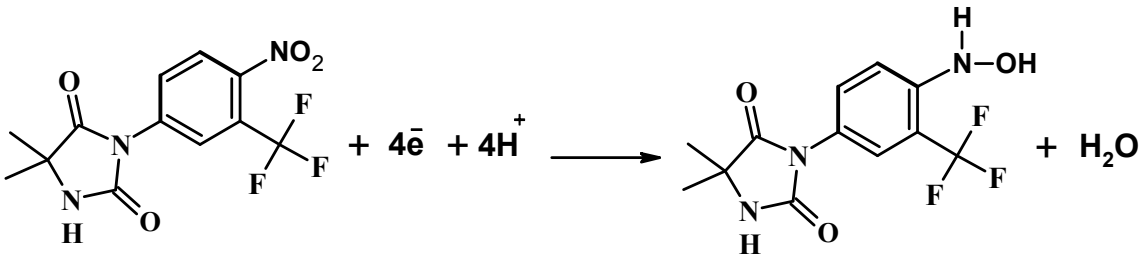
Fig.7

Table1. Characteristics of the calibration curves for determination of NLM in bulk solution and in human serum and urine samples using SWV at ZnONPs/CPE in the presence of CTAB.

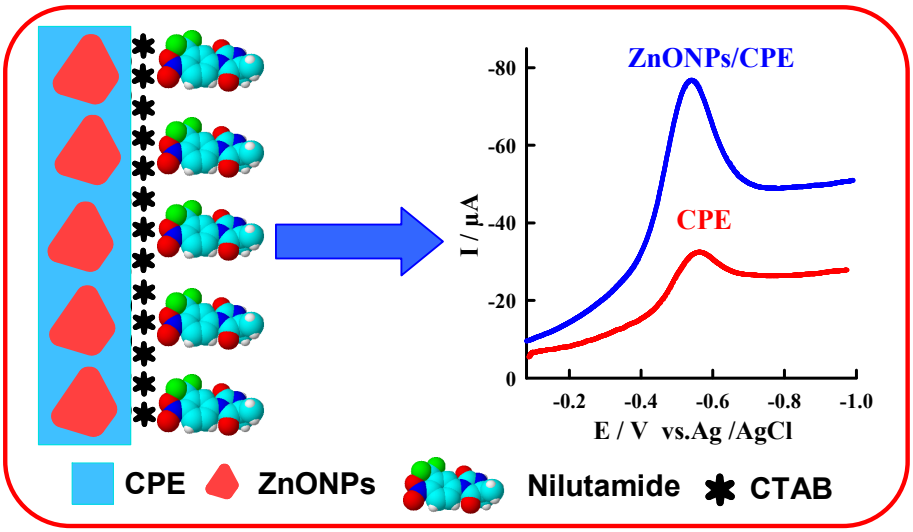
Linearity (M)	Straight line equation I_P (μA) = a+bC	Regression coefficient (R)	LOD (M)	LOQ (M)
Bulk form				
$7.60x10^{-8}$ - $3.60x10^{-7}$	$I_P = 4.26 + 6.56 x10^7C$	0.997	$3.21x10^{-9}$	$1.07x10^{-8}$
Serum				
$7.81x10^{-8}$ – $3.96x10^{-7}$	$I_P = 1.78 + 4.71x10^7C$	0.990	$9.32x10^{-9}$	$3.10x10^{-8}$
Urine				
$7.81x10^{-8}$ - $5.40x10^{-7}$	$I_P= 2.90 + 6.36x10^7C$	0.998	$7.40x10^{-9}$	$2.46x10^{-8}$

Table 2. Precision (intra and inter day) and accuracy for assay of NLM

Added (10^{-8} M)	Found (10^{-8}M)	Precision RSD %	Accuracy Bias (%)	Recovery (%)
		Intra – day		
Bulk form				
3.92	3.90	0.99	-0.51	100.51
Serum sample				
11.1	10.90	1.80	-1.83	101.83
Urine sample				
15.50	15.0	3.22	-3.33	103.33
		Inter – day		
Bulk form				
3.92	3.89	0.99	-0.76	100.76
Serum sample				
11.1	11.40	2.70	2.63	97.36
Urine sample				
15.50	15.90	2.58	2.51	98.72



Scheme1



A sensitive square wave adsorptive stripping voltammetric method was described for the determination of anticancer drug nilutamide (NLM) based on the enhancement effect of cationic surfactant: cetyltrimethylammonium bromide (CTAB). The electrochemical reduction of NLM in the presence of CTAB at ZnONPs/CPE was successfully applied for the determination of NLM in human biological fluids.