



ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(12):70-75
(<http://www.derpharmachemica.com/archive.html>)

Electro-oxidation and Determination of Sulfur-containing Amino Acid on Screen-printed Carbon Electrode Modified with NZ

Kavitha J¹, Mahalakshmi R²

¹Department of Chemistry, V.V. Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India

²Department of Chemistry, Post Graduate and Research, Thiagarajar College, Madurai, Tamilnadu, India

ABSTRACT

Nitromethane (1) upon treatment with Carbon disulfide (2) under ice cold condition in presence of dry methanol/Potassium hydroxide gave dipotassium salt of nitroketene dithioacetal (3). Further treatment of dipotassium salt (3) with transition metal zinc furnished NKDA-Zn complex (4). The structure of the synthesized complex was characterised using UV, IR, ¹H, ¹³C-NMR spectral, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) evidences. The electro-catalytic activity of the modified SPCE of NZ towards the detection of L-Met was carried out. The electrochemical behavior of the NZ/SPCE were examined using cyclic voltammetry, and differential pulse voltammetry (DPV).

Keywords: Nitroketene dithioacetals, Electro-catalytic activity, Electrochemical behavior, Voltammetry, Differential pulse voltammetry, Antibacterial activity

INTRODUCTION

In the chemistry of sulphur compounds nitroketene dithioacetal intermediates play vital role in the synthesis of several aliphatic and aromatic heterocycles [1-19]. Gompper and Schaefer started this chemistry of nitroketene dithioacetals in the year 1947. Recently, Rao et al. have reported several number of alkyl and benzyl derivatives of nitroketene dithioacetal [1-4]. The new metal ligand complex of NKDA-Zn was the as-synthesized NZ has high dispersible property in water. The modified SPCE of NZ has electro-catalytic activity towards the detection of L-Met. The electrochemical behavior of the NZ/SPCE were, examined using cyclic voltammetry and Differential Pulse Voltammetry (DPV). The antibacterial activity of the complex was evaluated using disc diffusion method.

MATERIALS AND METHODS

Analard grade chemicals and reagents were used in all the synthetic steps. The progress of product formation was monitored by TLC using hexane and ethyl acetate solvent system. Purification of all compounds carried out by column chromatography using silica gel 100-200 mesh. IR spectra were recorded in Shimadzu instrument as neat and KBr pellet. ¹H-NMR, ¹³C-NMR were recorded in Bruker-300 MHz NMR using Deuterated Chloroform (CDCl₃) as solvent.

Characterization

The crystalline structure of the NZ was characterized by Powder X-ray Diffraction (PXRD) by Xpert-Pro (PANalytical B.V., The Netherlands) diffractometer (Cu K α radiation, $\lambda=1.54 \text{ \AA}$). The morphology of the as-synthesized NZ was studied by Scanning Electron Microscopy (SEM) at room temperature (25°C) using SEM (Hitachi S-3000 H).

Fabrication of NZ/SPCE

As-synthesized NZ was firstly dispersed in water (0.05 mg/ml⁻¹) and keep in ultra-sonication treatment for 60 min. On surface of SPCE, the optimized the concentration of about 8 μ l of a dispersed solution was drop cast on the energetic surface of the SPCE followed by dried in an air oven at 30°C for 1 h.

EXPERIMENTAL SECTION

Synthesis of Dipotassium salt of 2-nitro-1,1-ethylene dithiolate (3)

To a well stirred and ice cooled solution of carbon disulphide (2) (23 ml, 0.41 mol) and nitromethane (1) (18 ml, 0.33 mol) solution of potassium hydroxide (39 g) in 95 ml of High Performance Liquid Chromatography (HPLC) grade methanol was added drop by drop for 40 min using a pressure equalizing funnel. The stirring was continued for 3 h at 0°C to -5°C. The reddish brown colour powder formed in the reaction mixture was quickly filtered and washed with dry methanol (3 \times 10 ml) followed by dry ether (3 \times 10 ml) to yield 38.0 g (55.2%) of reddish brown colour powder (3). The salt (3) was quickly transferred into brown colour sample bottle to avoid photodecomposition.

The weak absorption band at 700 cm^{-1} due to C-S stretching of NKDA has been shifted in the metal complex showing that the metal ion is likely to coordinate to the ligand through this point. Some new medium bands around 3746 cm^{-1} and 3767 cm^{-1} in the complex, were tentatively assigned to (M-S) vibrations. Infrared spectral bands of ligand (NKDA) observed at 1170 cm^{-1} ($\nu\text{ N-O}$) and at 1410 cm^{-1} ($\nu\text{ N-CH=C}$) remained almost unchanged in position on complexation indicating absence of bonding through nitrogen. However, ($\nu\text{ C-S}$) of the ligand undergoes red shift of $70\text{--}80\text{ cm}^{-1}$ on complexation indicating bonding through sulphur in the complex. The systematic shift in C-S bonds of ligand clearly indicates formation of metal sulphur bond. The olefinic protons in $^1\text{H-NMR}$ were appeared in the range of $\sim 6.70\text{ ppm}$ for the complex. Similarly in $^{13}\text{C-NMR}$ the olefinic carbon atoms were appeared in the range of $\sim 146.1\text{ ppm}$ for the complex. The olefinic quaternary carbons were appeared in the range between $\sim 113\text{ ppm}$ for the complex. C, H, N elemental analysis data for the complex showed good agreement with the calculated values for the confirmation of molecular weight of the product.

PXRD measurements were carried out to examine the phase and structure of the as-synthesized sample. As shown in Figure 1A, the PXRD pattern of the as-synthesized NZ shows a broad line parallel to the amorphous nature appears at a 2θ range between $15\text{--}30^\circ$. The surface morphology of the NZ was observed by scanning electron microscopy. Figure 1B and 1C shows the different magnification of SEM images of a NZ is provided. From these images we confirmed that the as-synthesized NZ is in nanoparticle size.

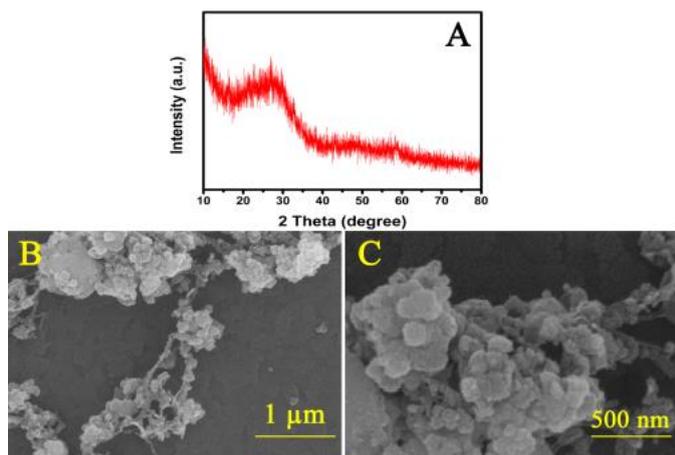


Figure 1: (A) PXRD, (B and C) SEM image of $(\text{Zn}(\text{NKDA})_3)$

Electro-oxidation of L-Met at NZ/SPCE

The electro-oxidation activity of the bare SPCE and modified SPCE was observed by Cyclic Voltammetry (CVs). Figure 2 shows the CV curves of different electrodes such as (a) bare SPCE presence of $200\text{ }\mu\text{M}$ L-Met, and (b) NZ/SPCE with $200\text{ }\mu\text{M}$ of L-Met in Phosphate Buffer Solution (PBS) (pH 7) at the scanning rate of 50 mV s^{-1} . There is no peak appeared when introduced SPCE into the electrochemical setup. In the addition of L-Met in bare SPCE a shows small difference in the CV curve with a small peak potential (1.228 V) at E_{pa} (anodic peak potential) with a small current. The well-marked anodic peak potential (E_{pa}) were detected at 1.196 V for the NZ/SPCE as shown in Figure 2. The results show that the electrochemical behavior of L-Met has an irreversible and oxidation-oxidation process. However relatively, NZ/SPCE is showed maximum electro-oxidation performance compared to bare SPCE in terms of high peak currents, and less over potential. Thus, NZ/SPCE has greater electro-oxidation ability to the oxidation of L-Met due to the perfect synergy between big surface area and high conductivity of the excellent electro-oxidation capability of NZ. The huge anodic peaks happen at peak potentials of 1.196 V due to $2e^-$ oxidation of the L-Met. The L-Met molecules were absorbed by the modified electrode and are oxidized at 1.196 V . The CV curves definitely authorizes that the NZ/SPCE is a good electrochemical detection of L-Met.

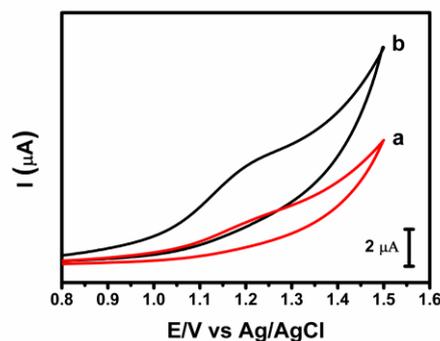


Figure 2: CVs response of (a) bare GCE with $200\text{ }\mu\text{M}$ L-Met (b) NZ/SPCE with $200\text{ }\mu\text{M}$ L-Met in 0.05 M PBS (pH 7)

Effect of concentrations and scan rates

Figure 3A, shows CV responses of NZ/SPCE in PBS containing different concentrations of L-Met at a scanning rate of 50 mV s^{-1} . When the absence of L-Met (a), the NZ/SPCE does not show any discernible peaks, whereas, a well-defined oxidation peak was observed in the presence of $100\text{ }\mu\text{M}$ of L-Met. Additionally, the oxidation peaks current of the L-Met increases with increasing the L-Met concentrations from 100 to $1000\text{ }\mu\text{M}$ of L-Met (a-j). The results reveals that the excellent electro-oxidation behavior of L-Met at NZ/SPCE and can be used for sensitive determination of L-Met. Figure 3B demonstrates the plot between oxidation peak current and the concentration of L-Met, which may be communicated by a linear regression equation as $E_{\text{pa}}(\text{V}) = 16.009x - 1.3572$, $R^2 = 0.996$.

Figure 3C shows that the effect of scanning rate of electro-oxidation responses of NZ/SPCE in PBS (pH 7) for the determination of L-Met. The CV curves observed the oxidation peaks currents are gradually increased when increasing the anodic peaks from 20-200 mVs^{-1} . When the oxidation peaks current (I_{pa}) increases the scan rate also increases linearly. Additionally, the inset linear plot among oxidation peak current versus the square root of the scan rates has displayed a relationship which suggesting that the oxidation process of L-Met appeared at the NZ/SPCE is a diffusion controlled electron transfer process (Figure 3D). From the results, we approve that the observed CV reports are due to the determination of L-Met diffused on the surface of the NZ/SPCE. Figure 3D demonstrates the plot between oxidation peak current and the square root of scan rate, which may be communicated by a linear regression equation as $E_{pa}(\text{V})=0.7243x - 3.4794$, $R^2=0.9906$. The above results are clearly confirming that the process is a diffusion controlled irreversible process.

$$\text{Limit of Detection (LOD)}=3S_b/b \quad (1)$$

The LOD is calculated by using the above formula, slope of the straight line of the electrochemical analytical curve and SD of the mean value for seven Voltammogram of the blank (S_b).

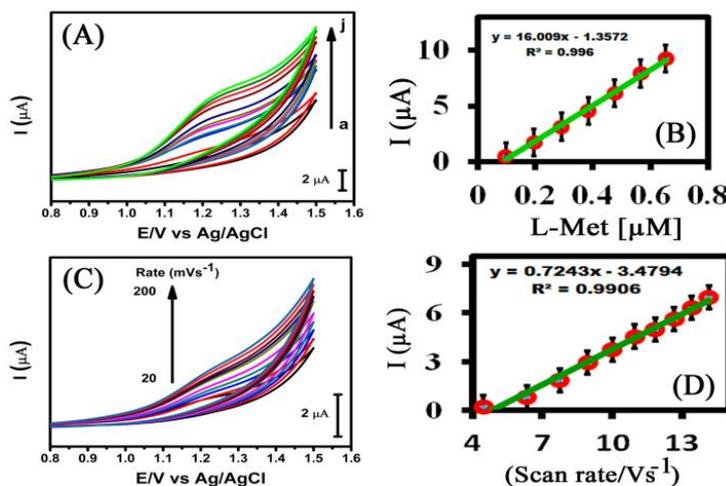


Figure 3: (A) CVs of NZ/SPCE in PBS (pH 7) in the presence of L-Met with different concentrations (a-j=100-1000 μM L-Met) at a scan rate of 50 mV s^{-1} , (B) The plot between oxidation peak current and the concentration of L-Met, (C) CVs obtained at NZ/SPCE in PBS (pH 7) containing 200 μM L-Met at different scan rates (20-200 mV s^{-1}) and (D) The plot between oxidation peak current, and the square root of scan rate

Electrochemical oxidation of L-Met

The electrochemical oxidation of L-Met was done at NZ/SPCE by DPV [20,21]. The DPV technique is a more sensitive method for the detection of L-Met than CV. The DPV responses of the NZ/SPCE with the additions of various concentrations of L-Met (6-8347 μM) into the PBS (pH 7). The sensitivity was calculated by using the slope of the calibration plot as shown in the Figure 4. The calculated sensitivity was about 2.8002 (± 0.002) $\mu\text{A } \mu\text{M}^{-1} \text{cm}^{-2}$ and LOD is 50.1 μM . A linear relationship between L-Met concentration vs peak current (I_{pa}) was obtained using 6-8347 μM of L-Met, and a linear regression values and correlation coefficient as $I_{pa}(\text{V})=0.1562x - 0.1016$ and $R^2=0.993$ respectively. The detection limit was 50.1 μM observed from the slope of the calibration plot, as shown in the Figure 4.

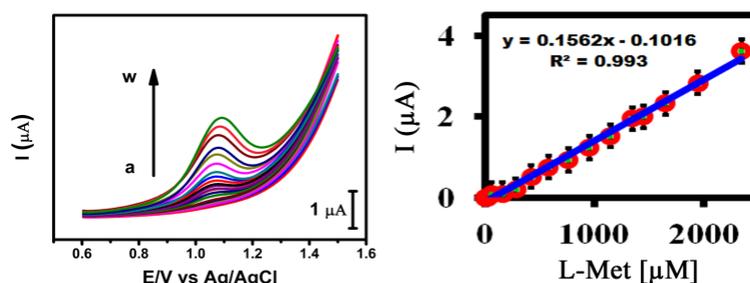


Figure 4: DPVs of NZ/SPCE towards increasing concentration of L-Met in PBS (pH 7) (a to w=6-8347 μM), and calibration plot

Stability, reproducibility and repeatability

In order to examine storage stability of the NZ/SPCE, its electro-catalytic answer towards L-Met (100 μM) is checked up to one week of the storage period, the catalytic current slightly decreased, while 94.62% of the initial response current was engaged which exposes good storage stability of the film. Additionally, the electrode displays considerable repeatability with Relative Standard Deviation (RSD) of 3.51% for five repetitive measurements carried out using single electrode and it exhibits considerable reproducibility with RSD of 3.21% for five independent measurements carried out in five different electrodes.

Antimicrobial activity study

The antibacterial activity for the complex NZ was determined by disc-diffusion method. The antibacterial activity studies in nutrient agar medium for the following organisms *Escherichia coli*, *Proteus sp.*, *Serratia marcescens*, *Pseudomonas aureginosa*, *Citrobactor sp.*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Micrococcus*, *Staphylococcus aureus* and *Staphylococcus viridians* were carried out.

Preparation of nutrient agar medium

Exactly 1 g of peptone, 0.5 g of beef extract and 0.5 g of sodium chloride were weighed and transferred into conical flask and dissolved in 100ml of distilled water after the pH range was checked for 7.0-7.2 finally added 1.5 g of agar into the conical flask. It was closely packed with cotton plug and placed in an autoclave for 15 min for sterilization. 100 ml of nutrient agar medium was poured into the petri plates and allowed for solidification without any disturbance. The test organism was lawned on the surface of the agar medium using a cotton swap. The concentration of 100 µg of the complex was loaded on the empty discs using micropipette. The loaded sterile disc was placed in the culture lawned medium and incubated in the inverted position for 16-18 h at 35°C. 100 µg of chloramphenicol loaded discs were used as standard drugs. The results were reported in the Table 1.

Table 1: Antibacterial activity of nitroketene dithioacetal-zinc complex

Name of the culture	Zone of inhibition in mm for complex NZ	Standard
<i>Escherichia coli</i>	19	24
<i>Proteus sp.</i>	20	19
<i>Serratia marcescens</i>	14	20
<i>Pseudomonas aeruginosa</i>	17	22
<i>Citrobactor spp.</i>	12	21
<i>Klebsiella pneumonia</i>	13	18
<i>Bacillus subtilis</i>	19	27
<i>Micrococcus</i>	17	26
<i>Staphylococcus aureus</i>	21	31
<i>Streptococcus viridans</i>	20	29

The antibacterial activity studies for the complex NZ was reported for the first time in this report following Disc-Diffusion method in nutrient agar medium. The complex was screened against *E. coli*, *Proteus sp.*, *S. marcescens*, *P. aureginosa*, *Citrobactor sp.*, *K. pneumonia*, *B. subfills*, *Micrococcus*, *S. aureus* and *S. viridians*. The complex showed good antibacterial activity.

CONCLUSION

In conclusion, NZ was prepared and characterized by X-Ray Diffraction (XRD), SEM which showed that the agglomeration in structure. The as-synthesized NZ has high dispersible property in water. The modified SPCE of NZ has electro-catalytic activity towards the detection of L-Met. The electrochemical behavior of the NZ/SPCE were, examined using cyclic voltammetry, and differential pulse voltammetry (DPV). From the electrochemical study, the limit of detection, sensitivity, and linear range are 50.1 µM, 2.8002 (± 0.002) µA µM⁻¹ cm⁻² and 6-8347 µM. The assembled sensor exhibits a tremendous electro-catalytic performance with a low limit of detection and good sensitivity property. The antibacterial activities for the series of the synthesized complex.

NZ was screened for the first time with ten different types of bacteria. Chloramphenicol was used as reference standard. From the result it is found and reported that the new complex of NKDA with Zinc showed good antibacterial activity [22].

ACKNOWLEDGEMENT

The authors sincerely thanks to the management of V.V. Vanniaperumal College for providing necessary facilities and SASIKUMAR RAGU, C/O Prof. Shen-Ming Chen, Ph.D., Research Scholar, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei, Taiwan - 106, (Republic of China) for the valuable permission to record the spectral evidences and to carry out the Electro-oxidation and determination of sulfur-containing amino acid on Screen-Printed Carbon Electrode modified with NZ. The authors thank Dr. L.Sakthikumar, M.Sc., M.Phil., Ph.D., Assistant professor, Department of Chemistry, Saiva Bhanu Kshatriya College for his constant encouragement.

REFERENCES

- [1] H.S.P. Rao, L. Sakthikumar, S. Shreedevi, *Sulfur Lett.*, **2002**, 25, 207-218.
- [2] H.S.P. Rao, L. Sakthikumar, S. Vanitha, S. Sivakumar, *Tetrahedron Lett.*, **2003**, 44, 4701-4704.
- [3] S.S. Raj, H. Surya Prakash Rao, L. Sakthikumar, H.K. Fun, *Acta Cryst.*, **2000**, 56, 1113-1114.
- [4] T.I. Reddy, *Tetrahedron Lett.*, **1993**, 49, 2101-2108.
- [5] A.C.H. Hsu, P. Osci-Gyimah, R.W. Joseph, B.C. Lange, *Eur. Pat.*, **1997**, 126, 263848.
- [6] H.S.P. Rao, S. Sivakumar, *J. Org. Chem.*, **2005**, 70(11), 4524-4527.
- [7] R. Alan Aitken, *Progress in Heterocyclic Chemistry.*, **2005**, 16, 272-282.
- [8] H.S.P. Rao, S. Sivakumar, *J. Org. Chem.*, **2006**, 71, 8715-8723.
- [9] S.K. Richardson, A.R. Howell, *Synthesis.*, **2007**, 18, 2755-2778.
- [10] H.S.P. Rao, S. Sivakumar, *Beilstein J. Org. Chem.*, **2007**, 3, 31.
- [11] H.S.P. Rao, K. Vasantham, *J. Org. Chem.*, **2009**, 74, 6847-6850.
- [12] H.S.P. Rao, K. Vasantham, *J. Chem. Sci.*, **2011**, 123(4), 411-420.
- [13] C. Li, X.Y. Xu, J.Y. Li, Q.F. Ye, Z. Li, *J. Labelled Comp. Radiopharm.*, **2011**, 54(13), 775-779.
- [14] C.W. Yang, Y.X. Bai, N.T. Zhang, C.C. Zeng, L.M. Hu, H.Y. Tian, *Tetrahedron.*, **2012**, 68(49), 10201-10208.
- [15] L. Pan, X. Bi, Q. Liu, *Chem. Soc. Rev.*, **2013**, 42(3), 1251-1286.
- [16] H.S.P. Rao, *Indian J. Chem. Sect. B.*, **2008**, 47, 272-275.
- [17] L. Sakthikumar, E. Anjukam, S. Dileepkumar, *Adv. Pharmacol. Toxicol.*, **2008.**, 9(1), 127-129.
- [18] P. Ramesh, K. Bhaskar., *Der Pharma Chemica.*, **2013**, 5(4), 191-197.

- [19] D. Ravi Sankar Reddy, J. Risy Namratha, *Der Pharma Chemica.*, **2013**, 5(1), 235-240.
[20] S. Ragu, S. M. Chen, P. Ranganathan, S. P. Rwei, *Int. J. Electrochem. Sci.*, **2016**, 11, 9133-9144.
[21] R. Karthik, R. Sasikumar, S. M. Chen, J. Vinoth Kumar, A. Elangovan, V. Muthuraj, P. Muthukrishnan, F.M.A. Al-Hamaid, M. Ajmal Ali,
[22] M.S. Elshikh, *J. Colloid. Interface. Sci.*, **2017**, 487, 289-296.