International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Sensitive Voltammetric Sensor for the Determination of L-Lysine Using Grapheme Nanoparticles Modified Carbon Paste Electrode

Dr. Mal Phebe Kingsley

NES Ratnam college of Arts, Science and Commerce (Autonomous), Bhandup (West), Mumbai-400078, India
Corresponding Author Email: *malphebekingsly[at]gmail.com*

Abstract: ***The fabrication of graphene nanoparticles and β- cyclodextrin modified carbon paste electrode (G-CD-CME) enabled facile electrochemical determination of Lysine (LYS) using differential pulse voltammetry. The graphene nanoparticles with β- cyclodextrin showed a synergistic effect by showing enhanced electrochemical signals in the rate of reduction of LYS by increasing the peak current with a corresponding decline of peak potential compared to carbon paste electrode which makes the reduction process thermodynamically more favorable. The modified electrode had a good sensitivity towards determination of LYS in a linear working range of 4.76 ×10^{-7} to 1.10 ×10^{-3} M with corresponding detection limits and 2.01 ×10^{-7} M LYS.***

Keywords: β- cyclodextrin, Graphene nanoparticles, Lysine, Differential pulse voltammetry

1. Introduction

Amino acids are classified into two general types: essential and non-essential amino acids. Essential amino acids are those that cannot be synthesized by the body on its own and thus need to be acquired through diet. Non-essential amino acids are those that your body can produce, specifically by the liver, without any outside help. If one of the essential amino acids is less than needed for an individual the utilization of other amino acids will be hindered and thus protein synthesis will be less than what it usually is, even in the presence of adequate total nitrogen intake ,protein deficiency has been shown to affect all of the body's organs and many of its systems, including the brain and brain function of infants and young children; the immune system, thus elevating risk of infection; gut mucosal function and permeability, which affects absorption and vulnerability to systemic disease; and kidney function The physical signs of protein deficiency include edema, failure to thrive in infants and children, poor musculature, dull skin, and thin and fragile hair. Biochemical changes reflecting protein deficiency include low serum albumin and low serum transferrin [1]. Determination of amino acids is important in various fields of research, particularly in food, soil, biotechnology and pharmaceutical industries. The need for essential amino acids, as well as disorders in the synthesis of amino acids that can cause a large number of illnesses, it is necessary to take good care of the quality of food and pharmaceutical preparations (regarding the amounts of amino acids) by means of which they are taken into the human body.

L-Lysine (LYS) is an amino acid found in the protein of foods such as beans, cheese, yogurt, meat, milk, brewer's yeast, wheat germ, and other animal proteins. Proteins derived from grains such as wheat and corn tend to be low in lysine content. The bioavailability of LYS is reduced with food preparation methods, such as heating foods in the presence of a reducing sugar (ie, fructose or glucose); heating foods in the presence of sucrose or yeast; and cooking in the absence of moisture at high temperatures. LYS is an essential amino acid in human nutrition because the body cannot produce it; therefore, it must be taken in either by diet or supplementation LYS has been studied for the prevention and treatment of herpes infections and cold sores. It also increases the intestinal absorption of calcium and eliminates its excretion by the kidney, suggesting that it might be helpful in osteoporosis. LYS has been investigated for its effects on increasing muscle mass, lowering glucose, and improving anxiety. Case reports suggest lysine may ameliorate angina. Lysine acetylsalicylate has been used to treat pain and to detoxify the body after heroin use. Lysine clonixinate has been used to treat migraine headaches and other painful conditions. LYS plays a major role in calcium absorption; building muscle protein; recovering from surgery or sports injuries; and the body's production of hormones, enzymes, and antibodies.[2].LYS measurement has received much attention because lysine is the limiting essential amino acid in many foodstuffs, and the ε -amino group in that amino acid facilitates the participation of LYS in degradation reactions affecting the proteins in foodstuffs during heat processing and storage.

The development of efficient analytical methodologies for the quantification of amino acids is very important due to the significance of these compounds in several fields. Different spectroscopic methods have been proposed such as chemilumi- nescence, fluorescence, spectrometry. In general, one of the main problems of these methodologies is the need for previous derivatization of the amino acids.

The detection of amino acids by direct electrochemical detection on bare electrodes has been the focus of attention by various researchers. Various electroanalytical methods and sensors for the detection of aminoacids has been at bare and modified electrodes. [3-5] Since, electrochemical method is a simple, fast and sensitive and used in the determination of aminoacids to real samples such as in pharmaceutical formulations, supplements and food products. [4,5] The use of these electrodes bears the advantage of disposability.

This study aims to determine the detection limit LYS voltammetrically. The electrochemical studies are done using Cyclic Voltammetry, differential pulse voltammetery and Chronocoulometry.

2. Material and Methods

2.1 Chemicals

All chemicals were of Analytical grade and were used as received without further purification. L-Lysine, β-cyclodextrin, graphene powder (size < 0.2nm) were purchased from S.D. fine Chemicals, India.Polybion LC syrup,Mubvit L syrup, < Vitron schet, Health Vit Fitness L-Lysine Powder was purchased from medical store. Mineral oil was purchased from Fluka, India. Double distilled water was used for the preparation of aqueous solutions having a specific conductivity 0.4 -0.9 µS.

2.2 Instrumentation

All voltammetric measurements study have been performed on Eco Chemie, Electrochemical Work Station, model Autolab PGSTAT 30 using GPES software version 4.9005. A three electrode system employing an Ag/AgCl (3M KCl) and platinum electrode were used as reference and counter electrodes respectively. Graphene nanoparticles and β-cyclodextrin modified carbon paste electrode (G-CD-CME) was used as working electrodes. The pH measurements were performed using an ELICO LI 120 pH meter.

2.3 Preparation of carbon paste electrode (CPE) and Graphene nanoparticles and β- cyclodextrin modified carbon paste electrode (G-CD-CME)

CPE was prepared by mixing graphite with mineral oil at composition 70:30 (w/w) using a motor and pestle and was allowed to homogenize for 48 hours [6]. Graphene nanoparticles and β- cyclodextrin modified carbon paste electrode **(**G-CD-CME) was prepared by mixing mineral oil, graphite powder, graphene nanoparticles and β- cyclodextrin with various weight ratios (Table 1). The pastes were then packed into a Teflon micro tip (diameter 0.5mm) and a copper wire inserted into the paste established an electrical contact. A new surface was regenerated by pressing out an excess of paste out of the tip and polishing it against zero grade butter paper until the surface had a shiny appearance.

Table 1: Composition of various modified electrode with the weight ratios of graphite: graphene nanoparticles: β-cyclodextrin: mineral oil

Electrode	Graphite (mg)	Graphene nanoparticles (mg)	β- cyclodextrin (mg)	Mineral oil (mg)
G-CD-CME -1	60	2	8	30
G-CD-CME - 2	60	3	7	30
G-CD-CME - 3	60	4	6	30
G-CD-CME - 4 **(G-CD-CME)**	60	5	5	30
G-CD-CME - 5	60	6	4	30
G-CD-CME - 6	60	7	3	30
G-CD-CME - 7	60	8	2	30

2.4 Determination of L-Lysine

Differential pulse voltammetric (DPV) studies were carried out with appropriate quantity of the analyte (LYS) in 25mL standard volumetric flask and then making up to the mark with pH 4.0 Britton -Robinson buffer (BR). The solution was then transferred into an electrochemical cell and the measurements were carried out at 25 ± 0.2°C. N_2 gas purging was not required as oxygen did not interfere in the measurements. DPVs were recorded within the potential range − 0.9 V to − 0.1 V with a scan rate of 10 mVs^{-1} and modulation amplitude of 50 mV.

3. Results and Discussion

3.1 Effect of pH

Standard solutions of LYS (1×10^{-5} M) were used to find the optimum pH of the supporting electrolyte at CPE. The influence of pH on the oxidation peak current of LYS was investigated in the pH range of 2-10 employing Britton-Robinson (BR) buffer (0.04M) by DPV**.** The largest peak current was obtained at pH 4 for both the analyte (Fig 1(A)).The negative shift of Ep values with increasing pH and the slope of the Ea vs .pH curve indicate the involvement of proton transfer preceding the potential determining step [7]. The peak currents were found to increase in the beginning with the increase in pH showing maximum at pH 4.0 and decrease thereafter for both the molecules. This could be due to the fact that the reduction became kinetically less favorable due to repulsive electrostatic interactions with the surface of the electrode. Therefore this pH was selected as the optimum pH for further studies.

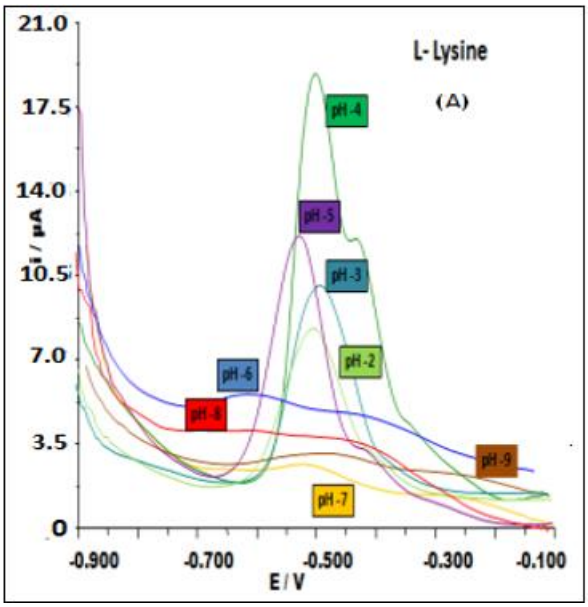

Figure 1A: pH study by Differential pulse voltammetry for reduction of 1×10^{-5} M LYS at CPE *vs.* Ag/AgCl; in 0.04M BRbuffer (pH 4); scan rate 100mV/s at 25^0C

Equal number of protons and electrons were involved in the reduction. Further various buffers such as Britton-Robinson (BR), Phosphate (Phos), Citrate (Cit), Acetate (Act) and Potassium hydrogen phthalate (KHP) buffer were used at pH 4.0. The concentration of the buffers was taken as 0.1M except for BR buffer where concentration was 0.04M. Amongst all the buffers used, BR buffer gave the best response in terms of peak current and peak shape for LYS**.** Thus BR buffer was chosen for further experiments.

3.2 Effect of surface modification and Optimization of the amount of the modifier

The effect of modifier for the oxidation of LYS has been studied using DPV. Fig. 2A represent the DPV's for LYS (1×10^{-5} M) at CPE and G-CD-CME. It is observed from this figure that the modification of the CPE with graphene nanoparticles and β- cyclodextrin enhanced the LYS signal by 3.6 times with a concurrent shift in E_p and from − 0.49 V to − 0.534 V for LYS towards more negative side as compared to CPE. This is due to the loading of graphene nanoparticles and β- cyclodextrin acting as a promoter by increasing the electro-active surface area.

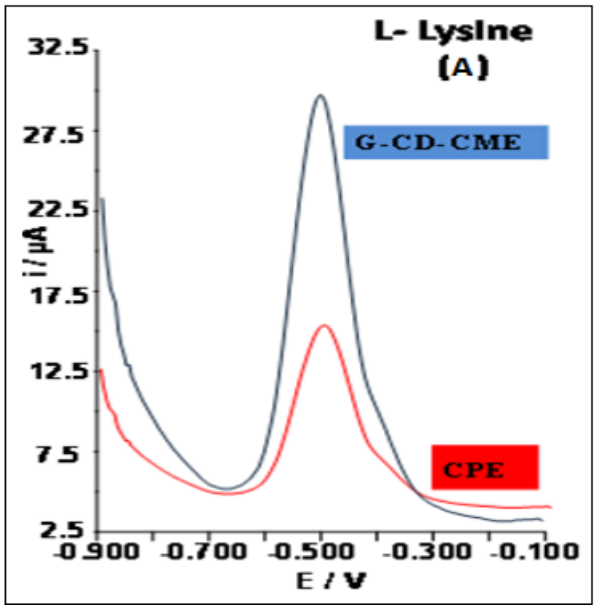

Figure 2 A: Differential pulse voltammetry for reduction, of 1×10^{-5} M at CPE and G-CD-CME *vs.* Ag/AgCl in 0.04M BR buffer (pH 4); scan rate 100mV/s at 25^0C

Further the electrochemical response in terms of the influence on the amount of graphene nanoparticles and β-cyclodextrin added to CPE was studied. The peak current increased with increase in the percentage weight of nanoparticles till the ratio of composition (w/w) for graphite: graphene nanoparticles: β- cyclodextrin: mineral oil was 60:5:5:30. Beyond this composition there was a gradual decrease in peak current. This can be due to the increase in resistance of the electrode to electron transfer. The presence of excessive nanoparticles in the modified electrode may obstruct the diffusion process of the oxidation reaction products away from the electrode surface [8].

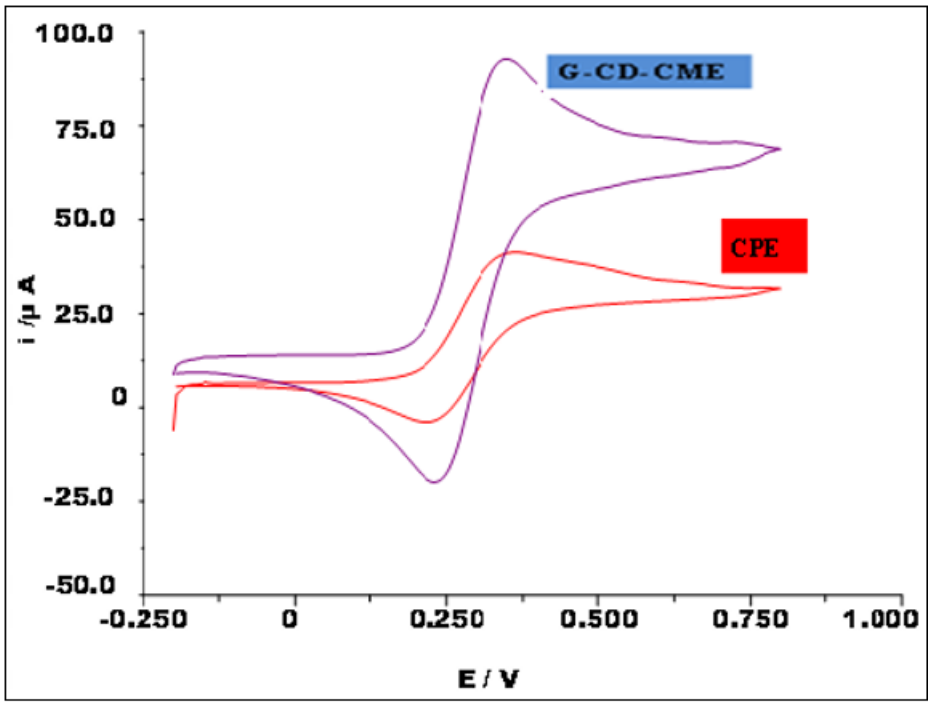

Figure 3: Cyclic voltammetry of 6mM $K_3Fe(CN)_6$ in 0.1M KNO_3 at CPE and G-CD-CME

The surface area of the two electrodes CPE and G-CD-CME of the same nominal bore size were found out using 6mM of mixture of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ system in 0.1N KNO_3.The surface area of both the electrodes CPE and G-CD-CME were calculated using Randles-Sevcik [9] equation and were found to be 0.0159 cm^2 and 0.0662 cm^2 respectively

3.3. Chronocoulometry (CC)

Chronocoulometry was employed to determine the kinetics and mechanism of electro-oxidation reaction of LYS (1.0×10^{-5}M) at CPE and G-CD-CME. Double-potential step chronocoulometry after point to point background subtraction was performed for LYS. The plot of charge (Q) *vs*. the square root of time ($t^{1/2}$) showed a linear relationship for both the molecules. From the slope and intercept of the above plot the diffusion coefficient (D_{coeff}) and Q_{ads} of LYS were estimated respectively which is given by Anson equation [10]. The calculated parameters are tabulated in Table 2. The increase in the value of slope and charge due to adsorption (Q_{ads}) for G-CD-CME indicate greater accumulation of LYS on its surface rather than on CPE. The surface coverage (Γ^0) for the two electrodes is calculated from equation 8, where n is the number of electrons, F is the Faraday's constant (96485 C/mol) and A is the area of the electrode surface (cm^2).

Table 2: Chronocoulometry of LYS (1×10^{-5} M)

Molecule	Electrode	Slope (10^{-4} μC/$s^{-1/2}$)	Intercept Q_{ads} (10^{-6} μC)	Surface coverage (10^{-9} mol/cm^2)	Diffusion Coefficient (10^{-6} cm^2/sec)
LYS	CPE	2.04	3.39	1.45	2.43
	G-CD-CME	3.83	48.2	5.89	82.26

$$Q_{ads} = nFA\,\Gamma^0 \qquad (8)$$

It is observed from the Table 2 that the surface coverage in the case of G-CD-CME is greater than for CPE indicating the sensitivity of the LYS towards the modified electrode.

3.4. Determination of LYS by Differential Pulse Voltammetry (DPV):

Based on the above findings, an analytical procedure is worked out for determining LYS by DPV. Fig.3 is the voltammograms of LYS. The linear working range (LWR), empirical limits of detection (LOD) (S/N=3), linear regression equation (LRE) and correlation coefficient (r) were determined and are presented in Table 3.

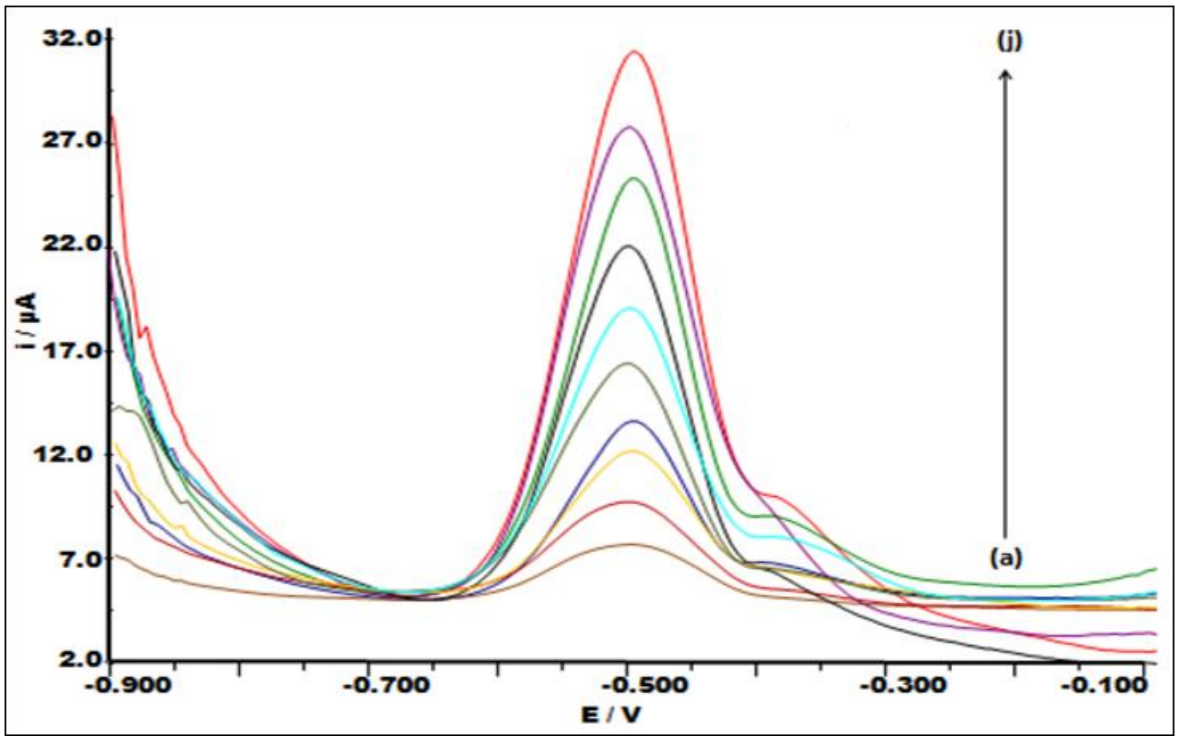

Figure 3: DPV curves obtained at G-CD-CME for LYS at different concentrations a) 2.0 b) 3.0 c) 4.5 d) 5.8 e).6.1 f) 7.2 g) 8.5 h) 9.0 i) 12.0 j) 25.0 µM:scan rate 10mV/s in 0.04M BR (pH 4.0): pulse amplitude 50mV.

Table 3: Analytical parameters for electrochemical determination of 1×10^{-5} M LYS at G-CD-CME in pH 4.0 BR buffer (0.04M)

Molecule	LOD	%RSD	LWR	LRE	r
Statistical data for individual molecule					
LYS	2.01×10^{-7} M	2.61	4.76×10^{-7} M to 1.10×10^{-3} M	I_p (µA) = 0.043 (0.1µM) + 0.928	0.990

3.5 Validation studies, interference studies and analytical applications:

For validation of the proposed method, various parameters such as repeatability, reproducibility, precision and accuracy of the analysis were obtained by performing five replicate measurements for LYS (1×10^{-5} M) over intraday assay (single day, n = 5) and inter-day assay (for a period of 1 week). Satisfactory mean percentage recoveries (%R) and relative standard deviations (% RSD) were obtained and are presented in Table 4. The recoveries obtained confirmed high precision and accuracy of the proposed method

Table 4: Precision and Bias of assay for standard LYS solution by DPV (n =5)

Molecule	Concentration taken (mol L^{-1})	Mean concentration found (mol L^{-1})	Mean recovery %	Bias %	Precision % RSD
LYS	**Intra day**				
	1.0×10^{-5}	0.989×10^{-5}	98.9	1.10	2.23
	Inter day				
	1.0×10^{-5}	0.995×10^{-5}	99.5	0.50	1.49

In order to further extend the validity of the proposed method, verification of the matrix effect on LYS determinations by DPV was studied. The influence on the peak heights of some interferents commonly present, some of them which form the major components of multivitamin pharmaceutical preparations were evaluated. Under optimal experimental conditions the tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than ± 5.0% at a concentration of l 1×10^{-5} M LYS. Five replicates of each experimental set were performed. The results showed tolerance limit of 200 fold of glucose, 100 fold for citric acid and thiamine hydrochloride, 150 fold for tartaric acid and nicotinamide, 500 fold for riboflavin and 50 fold for cyanocobalamine showing that the present modified electrode was highly selective towards LYS in the presence of common physiological interferents.

The validity of the G-CD-CME electrode was verified in the determination of LYS in various pharmaceutical preparations by standard addition method.

Table 5: Assay of LYS in pharmaceutical preparations (n =5)

Pharmaceutical preparation	Furosemide	
	Amount of drug in the sample (mg)	Amount of drug obtained in the proposed method (mg) ± RSD
Syrup		
Polybion LC Syrup	375.0	375 ± 2.73
Mubivit L syrup	250.0	251.5 ± 1.79
Powder /Sachet		
Vitrion Sachet	10mg	10.1 ± 3.2
HealthVit Fitness L-Lysine Powder	1mg	1.05 ± 2.17

DPV technique using scan rate 10mV/s in 0.04M BR (pH 4.0): pulse amplitude 50mV were performed to record the peaks. Amount of LYS present in syrup and powder are given in Table 5.

4. Conclusion

Graphene nanoparticles and β- cyclodextrin modified carbon paste electrode was developed as an effective and highly sensitive electrode for determination of L-Lysine by differential pulse voltammetry. This electrode gave a considerable enhancement in sensitivity, improved detection limit, reproducibility and repeatability thereby being a promising electrode for the determination of trace amounts of Lysine in pharmaceutical and clinical preparations. Further, due to reliability and stability of the modified electrode it offers a good possibility to be used in quality control laboratories for identification and quantification of samples.

References

[1] Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Institute of Medicine's *Food and Nutrition Board. usda.gov* (2006)
[2] Gaby AR, Natural remedies for Herpes simplex, *Altern Med Rev*. 11 (2006) 93–101.
[3] Majlinda Vasjari, Arben Merkoc, John P. Hart, and Salvador Alegret Electrochemical Sensors, *Microchim Acta* 150 (2005) 233-238
[4] Guillermina L. Luque, Nancy F. Ferreyra, Gustavo A. Rivas, Electrochemical sensor for amino acids and albumin based on composites containing carbon nanotubes and copper microparticles, *Talanta* 71 (2007) 1282–1287
[5] H. Heli, N. Sattarahmady and M. Hajjizadeh, Electrocatalytic oxidation and electrochemical detection of guanine, L-arginine and L-lysine at a copper nanoparticles-modified electrode, *Anal. Methods*, 6 (2014) 6981-6989.
[6] V. Karel, S.Ivan, M.Radovan, Carbon paste electrodes in electroanalytical chemistry, *J. Serb.Chem.Soc.* 74 (10) (2009)1021–1033.
[7] P. Zuman and L-Meites, Progress in Polarography, Vol 3, Wiley, New York, 1972, p.85.
[8] S. Qu, J.Wang, J.Kong, P.Yang, G. Chen, Magnetic loading of carbon nanotube/nano-Fe_3O_4 composite for electrochemical sensing, Talanta. 71 (2007) 1096-1102
[9] Joseph Wang, Analytical Electro Chemistry, second ed., Wiley-VCH, New York,2001
[10] Allen J. Bard, Larry R. Faulkner, Electrochemical Methods Fundamentals and Applications, second ed., John Wiley & Sons, New York,2001.