A GNP-CYS-Nihcf (Ii) Graphite Composite Electrode for Determination of BHA

G. Sivasankari[a], K. Krishna Kumar[b] and S. Sriman Narayanan[b]*
[a]Department of Science and Humanity, Shri Sapthagiri Institute of Technology, Ocheri, Vellore.
[b]Department of Analytical chemistry, University of Madras, Guindy, Chennai.
***E-mail: sriman55@yahoo.com, Phone: +91-44-22202717, Fax: +91-44-22352494**

Abstract- **A surface modified Nickel hexacyanoferrate(II)electrode on L-cysteine-gold nano particle graphite wax composite was prepared for the determination of butylated hydroxyanisole (BHA) which is being used as an antioxidant in processed foods. This electrode has favorable properties such as low cost and simple preparation. The modified electrode was characterized by SEM, FTIR and cyclic voltammetric studies. The modified electrode showed good electrocatalytic activity towards the oxidation of BHA under optimal conditions and exhibited a linear response in the range from 9.9×10^{-6} to 1.03×10^{-3}M of BHA with a correlation coefficient of 0.999. The limit of detection was found to be 1.2×10^{-7}M. The analytical utility of the electrode for the determination of BHA in flow system was evaluated by hydrodynamic and chronoamperometric studies. The proposed sensor was also applied to the determination of BHA on spiked samples of biscuit.**

Keywords: GNP; nickel hexacyanoferrate; electro catalysis; BHA

I .Introduction:

The use of antioxidants in food and pharmaceutical industries is particularly important as they prevent the decomposition of organic compounds present in prepared products. Biological and toxicological aspects, detection, estimation and evaluation of antioxidants used as food additives have been described by Hudson (9). Along with the most common additives used as antioxidants phenolic compounds are generally added to food products in order to prevent oxidative degradation of fats and oils. Butylated hydroxyl anisole (BHA) is one of the most commonly employed antioxidant. While cooking or baking at higher temperatures it has the ability to remain active and so it is exclusively used in food products. Although it is widely used, excessive use of BHA can bring about some physical ailments such as allergy in adults and hyperactivity in children. Furthermore, a higher dose of BHA can cause tumors in lab animals (http://www.lactose.co). As BHA is comprehensively used as a food preservative and also toxic at higher amounts, it has become very essential to develop an accurate, reproducible and fast method for the determination of BHA. Different type of voltammetric procedures have been reported for the determination of BHA using carbon (4, 5), and Hg (24) as working electrodes. However, the detection limit was not sufficient for trace determination of this important compound and these methods were also found to be less reproducible due to the surface fouling. Wang and Freiha (26) described a method involving preconcentration of BHA at a carbon paste electrode prior to voltammetric measurement to improve detection limit. Ruiz and co-workers have been described the oxidation of BHA at a glassy carbon electrode based on flow injection method. (22).

There has been a considerable interest in recent years, in the development of chemically modified electrodes (CMEs) as they offer a lot of advantages over other electrochemical methods. CMEs improve the sensitivity and selectivity of electrochemical analysis by improving the kinetics of the reaction via an electrocatalytic process at the CME surface. Enhancing the selectivity and sensitivity of analysis is due to the function of the mediator which facilitates the charge transfer between the analyte and electrode. A number of modification strategies have been employed which are based on only a few approaches, such as covalent binding of the modifier to a specific surface site (15, 13) physical coating of the electrode surface with a polymer that contains the modifier grouping (1, 7), bulk modification of carbon materials (2, 12) and direct adsorption of the modifier on to the bare electrode surface (3. 14). We have reported that silver and cobalt hexacyanoferrate surface modified electrodes as suitable sensors for determination of BHA (6, 21)

In the present work, we report a new approach for the fabrication of a surface modified nickel hexacyanoferrate electrode using L-cysteine-GNP-graphite wax composite. L-cysteine was used as a cross linker to bind Ni^{2+} ion which was attached to $-NH_2$ group of L-cysteine, even though -COOH group is present. The metal ion was found to bind preferentially with $-NH_2$ group of cysteine which was confirmed by FTIR studies. Gold nanoparticles display four unique advantages over macro electrodes when used for electro analysis: they are enhancement of mass transport, catalysis, high surface area and control over electrode microenvironment (16). The surface modified NiHCF electrode was effectively used for the amperometric determination of BHA as it was found to have excellent electro catalytic activity for the oxidation of BHA. Differential pulse voltammetry (DPV) and flow injection techniques were also used for the determination of the analyte from commercially available biscuits samples spiked with BHA.

II. Experimental

a. Chemicals and reagents:

Graphite powder was purchased from Aldrich (1-2 µm). Butylated hydroxyanisole was obtained from Hi-media. Nickel chloride, Potassium ferrocyanide were obtained from Merck (Mumbai, India). All reagents were of analytical grade. Doubly distilled water was used for all electrochemical experiments. pH was varied using 0.1M HCl and 0.1M NaOH solutions. L-cysteine solution (20mM) was prepared by double distilled water and nickel solution (0.01M) was prepared by ethanol. Potassium ferrocyanide (0.02M) with 0.1M KNO_3 as the background electrolyte was used to derivatize the coordinated nickel ion. BHA stock solution was prepared by dissolving appropriate quantity in the minimum amount of methanol first and was then made up to 100ml using doubly distilled water. Doubly distilled water was used for further dilutions. Different types of biscuits containing no preservatives were purchased from a local market and these samples were used as model samples for analysis.

b. Apparatus

Fourier transform infrared (FTIR) spectra were recorded for L-cysteine adsorbed graphite powder and NiHCF complex using Bruker tensor 27. Scanning electron microscopy (SEM) images of the nanoparticles were recorded on a (Hitachi S-3400N). All the electrochemical measurements were carried out using a CHI electrochemical workstation (660 B) controlled by an IBM personal computer with standard three electrode configuration. The surface-modified NiHCF graphite wax composite electrode was used as working electrode, a platinum electrode as counter electrode and standard calomel electrode as the reference electrode.

c. Fabrication of GNP-NiHCF paraffin wax composite electrode

The citrate capped GNP were prepared using the literature procedure (10). The maximum absorption peak for the synthesized GNP was observed at the wavelength of 530 nm. Graphite powder (1 g) was added to 20mM of 125 ml synthesized gold nanoparticles solution and the mixture was stirred for two hours at 300 rpm in room temperature. The stirred mixture was centrifuged at 1450 rpm for 30 minutes and the residue was kept overnight for drying. A GNP graphite paraffin wax composite electrode was prepared by mixing 900mg of gold nanoparticles adsorbed graphite powder with paraffin wax in the ratio of 4:1. Gold nanoparticle graphite wax mixture was tightly packed in a glass tube of 3mm diameter. The electrode was removed gently from the tube after it turns hard. The electrode prepared in this ratio showed an excellent hardness and conductivity. This composite electrode was first dipped in 20 mM of L- cysteine solution for 2 hours. Then, this L-cysteine modified GNP composite electrode was dipped in 0.01 M Ethanolic solution of $NiCl_2$ for 3 minutes. The cysteine functionalized gold nanoparticles composite electrode has a greater affinity towards transition metal ion like Ni^{2+}. The Ni^{2+} ions coordinated to amine group was then derivatized using 0.02 M potassium ferrocyanide solution dissolved in 0.1 M KNO_3 by cycling in potential range of -0.2 to 1.0 V at the scan rate of 50 mV s^{-1} (21).

d. Analysis of commercial food samples

Commercial food sample consisting of biscuit was analyzed. The biscuit analyzed contained no preservatives. Thus, they had to be spiked for analysis. The sample was purchased in a local supermarket. The procedure of preparation was as follows. About 0.5 g of sample was weighed into a large tube. The

analyte was extracted with three 5 ml portions of ethyl acetate. The three extracts were mixed and filtered through a wattman filter paper. In case the filtrate was not clear, methanol was added, and the extracts shaken and filtered again.

III. Result and discussion

a. SEM characterization of the surface

Fig.1 shows the SEM images of (a) bare graphite wax composite electrode (b) GNP absorbed graphite wax composite electrode in the range from 6-9nm and (c) NiHCF –GNP- graphite wax composite electrode. The figure clearly suggests the formation of NiHCF particles on the electrode surface and that the particle size ranges from 150-200 nm.

b. FTIR characterization of the surface

Fig.2 shows the FTIR spectra of (a) pure cysteine, (b) cysteine adsorbed GNP graphite powder and (c) GNP-Cys-NiHCF. The absorbance peaks at 1139 cm^{-1},1572 cm^{-1} and 2552 cm^{-1}, in Fig 2a which can respectively be ascribed to NH_3^+ rocking, $-COO^-$ asymmetric stretching, and SH stretching vibrations.[23].

The peak of -SH was changed between the cysteine and cysteine bound GNP, indicating that the -SH group on cysteine is involved in binding to GNP. (Fig.2b). The peak of $–COO^-$ asymmetric stretch on cysteine did not change significantly compared with that on Au-cysteine, indicating that the $–COO^-$ was not effectively involved in Ni binding. Fig.2c shows that the peak of NH_3^+ rocking shifted from 1139 cm^{-1} of the original cysteine spectrum to 1241cm^{-1} for the cysteine-bound GNP-Ni complex. The absence of the characteristic absorption peak of S–H groups around 2550 cm^{-1}demonstrates the formation of GNP-cysteine-Ni complex by the reaction between GNP-cysteine and Ni.
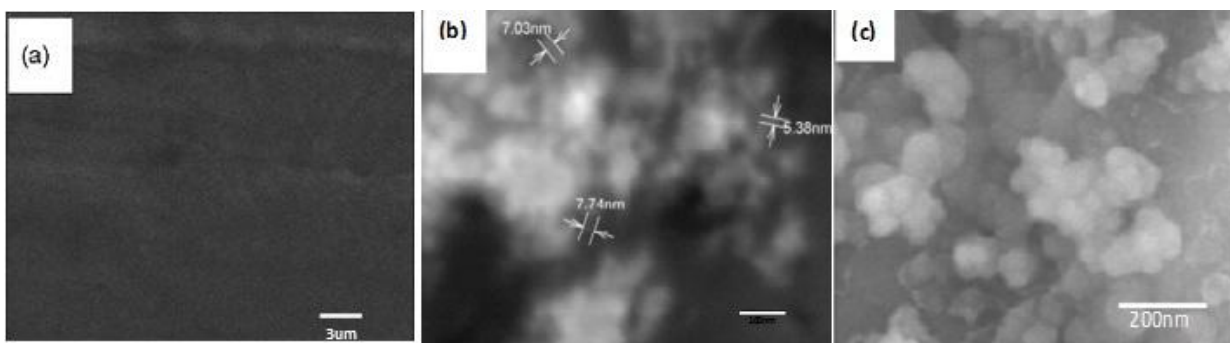

Fig. 1. The SEM images (A) represent as a bare electrode, (B) presence of GNP on graphite matrix (C) NiHCF particles on the modified electrode.
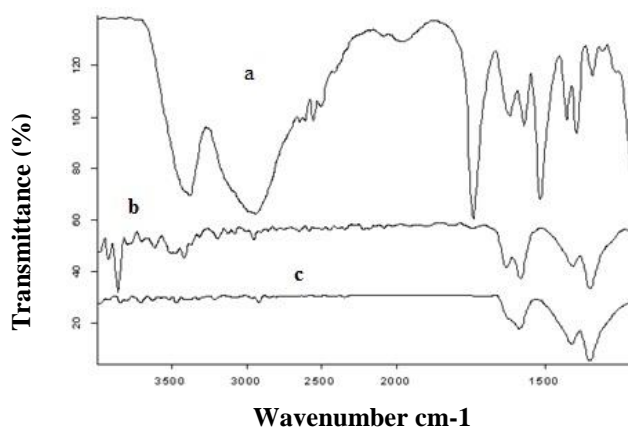

Fig. 2 FTIR spectra of (a) pure cysteine (b) cysteine adsorbed GNP graphite powder and (c) GNP-Cys-NiHCF modified electrode.

c. Electrochemical behaviour of the modified electrode

Cyclic voltammetry was used to study the electrochemical behaviour of NiHCF modified electrode. The result have been obtained with the NiHCF modified electrode in 0.1 M NaNO$_3$ at a scan rate of 20mVs^{-1}. Redox peaks were observed at 0.366V and 0.29V corresponding to the anodic and cathodic reactions respectively, and the formal potential was found to be 0.328 V. Different electrolytes were used to test the performance of the modified electrode. In the redox reaction of the metal hexacyanoferrates, the transfer of an electron is always accompanied by the simultaneous motion of a counter cation in and out of the cage structure in order to maintain the charge balance. In the reduction a counter cation insertion into the film and its exclusion upon oxidation have been verified by Kulesza et al (11). Thus the size of the counter cation along with its mobility plays a key role in determining the voltammetric response of the NiHCF modified electrode. The cyclic voltammetric responses of the NiHCF modified electrode in solution of 0.1 M LiCl, NaNO$_3$, KNO$_3$, NH$_4$Cl, KCl and NaCl has been studied.(Fig not shown) It was clear that the higher peak currents along with lower formal potential for NiHCF modified electrode could be achieved in presence of Na$^+$ ions. Anions were found not to alter the electrochemical behavior of NiHCF, and hence 0.1 M NaNO$_3$ was chosen as the suitable supporting electrolyte for further experiments.

The effect of scan rate on the electrochemical behaviour of the modified electrode was studied in 0.1M NaNO$_3$. Fig.3 shows the cyclic voltammogram of the NiHCF modified electrode at various scan rate such as 10, 20, 30 upto 150 mV. On varying the scan rate from 10mV-150mV/s, the peak-to-peak separation varied from 0.047 to 0.18V. Peak-to-peak separation must be zero for the ideal behaviour of the modified electrode. All the modified electrodes have shown higher peak separation and larger peak width, and the non-ideal behaviour of the electrodes which are attributed the following reasons: (a) distribution of species with different E^0 values (25). (b) repulsive interactions between ions in the lattice and (c) difference in spatial distribution of redox centers. Moreover, it can be seen from the inset of Fig.3A that the peak currents (Ip$_a$ and Ip$_c$) have a linear relation with the square root of the scan rate corresponding to a diffusion- controlled reversible reaction and display the nature of a surface wave. Owing to the various reasons associated for increase in   E$_p$ at higher scan rates, Laviron derived a general expression where the limitation is attributed to charge transfer kinetics (17). Based on this Laviron equation the electron transfer rate constant (K$_s$) was calculated to be 0.766 s^{-1}(Fig.3B).

The performance of the modified electrode was also studied under different pH conditions in the range 2-9. The pH of the background electrolyte was varied using HCl and NaOH. It was found that the peak current did not vary much in the pH range 2-6 but a maximum response in current was obtained at pH 7 (Fig 3C). At pH greater 7 there is a decrease in current response. The possible reason could be the hydroxylation of NiHCF (18), the hydroxylated moiety formed is electrochemically inactive and gets dissolved into the background electrolyte which results in a decrease in current response at pH greater than 7. Hence a neutral pH was chosen for further experiments.

d. Electrocatalytic oxidation of BHA

In order to assess the electrocatalytic activity of the NiHCF modified composite electrode towards the oxidation of BHA, cyclic voltammograms were recorded in the presence and absence of BHA in 0.1 M NaNO$_3$. As shown in Fig.4 upon the addition of 7.6x10^{-5}M BHA, there was a dramatic enhancement in the anodic peak current at the modified electrode whereas a slight increase in the anodic current was observed at the bare electrode. Also, the anodic potential for oxidation of BHA at the NiHCF modified electrode is at 0.53 V, whereas at the bare electrode BHA undergoes oxidation about 0.65 V. The decrease in overpotential and enhancement of anodic peak current for BHA oxidation indicates the strong electrocatalytic effect of the NiHCF modified electrode.

The NiHCF present at the electrode surface gets oxidised at its oxidation potential. This oxidised form of the mediator in turn oxidizes BHA to t- butyl quinone (TBQ) and gets reduced. The reduced form of NiHCF again gets oxidised at the electrode surface producing the oxidation current at the same time. Thus an enhanced anodic current was observed for every addition of BHA. The quantification of BHA was carried out by measuring the catalytic current as a function of BHA concentration. The linear regression equation of catalytic current versus concentration of BHA is obtained from the experimental data and is given as Ip (A) =2.42 [BHA] (M) + 2.87   A. The catalytic current was found to increase linearly with the concentration of BHA in the range from 9.9x10^{-6} M to 1.03x10^{-3}M with a correlation coefficient of 0.

999.(Fig 4a) Thus the sensor could be used to determine the concentration of BHA in the above range. The detection limit was found to be 1.2×10^{-7} M.
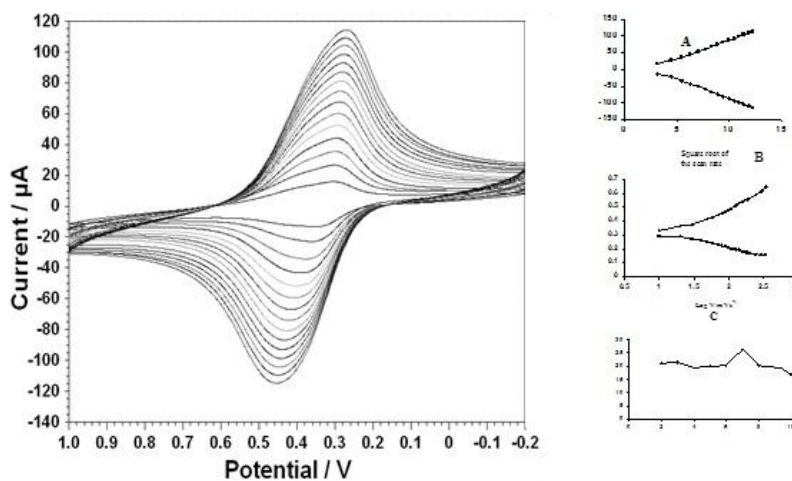

Fig. 3.cyclic voltammogram of the NiHCF modified electrode at different scan rates in 0.1M $NaNO_3$ (pH 7.0). The scan rates from inside to outer are 10-150 mVs^{-1} with increments of 10 from 10 mV^{-1} , (3A) dependence of peak current I_{pa} and I_{pc} on square root of scan rate( ), (3B) variation of peak potential vs. logarithm of scan rates (log). (3C) effect of pH on catalytic current of the NiHCF modified electrode with 1.6×10^{-5} M of BHA in 0.1M $NaNO_3$.
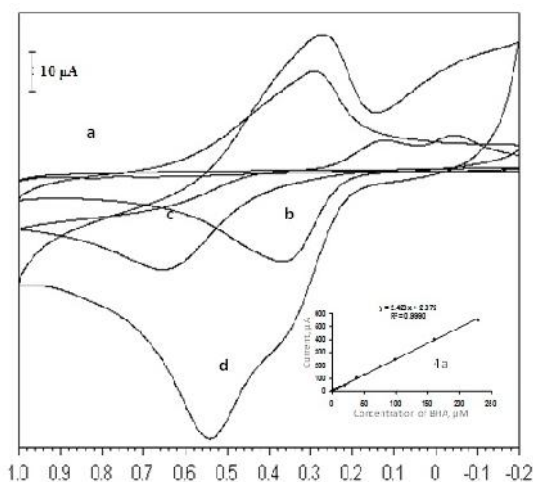

Fig.4.Cyclic voltammograms in 0.1 M $NaNO_3$ (pH 7.0)at a scan rate of 20 mVs^{-1} (a) bare electrode in the absence of BHA (b) modified electrode in the absence of BHA(c) bare composite electrode in the presence of 7.6×10^{-5} M BHA (c) (d) NiHCF modified electrode in the presence of 7.6×10^{-5} M BHA.Fig.4B.calibration graph for BHA measurement. Fig.4C. calibration graph of BHA measurement in DPV.

e. Amperometric determination of BHA

Hydrodynamic voltammetry was performed to study the electrocatalytic response of NiHCF modified composite electrodes under dynamic conditions and to optimize the operating potential for amperometric measurements. In hydrodynamic voltammetric studies, the potential of the working electrode was increased gradually in stirred solution, and the resulting steady state current was measured and plotted against the applied potential. The plot of current versus applied potential in the presence of 4.76×10^{-5} M BHA at the

bare and modified electrodes is shown in fig.5. Almost negligible response is observed with the bare electrode while a significant increase in current was observed with the modified electrode. The current increases from 0.2 V and approaches to a maximum value at of 0.5V. Hence a potential greater than or equal to 0.5V can be applied for electrocatalytic oxidation of BHA in flow systems.

The amperometric response of the NiHCF- modified electrode for the electrocatalytic oxidation of BHA was studied. The current-time response obtained for the NiHCF modified electrode in a stirred solution (300rpm) for successive increments of 4.14×10^{-5}M BHA is shown in fig.5a. The current response increases with increasing concentration of BHA. The plot of catalytic current versus BHA concentration is shown in fig 5b. A good linear response was obtained in the range 9.9×10^{-6} - 1.03×10^{-3}M BHA with the correlation coefficient of 0.999. The response of the modified electrode on continual usage was checked by repeating the experiments for 6 times using the same amount of BHA which showed 1.9% variation.

f. Real sample analysis

In order to confirm the applicability of the proposed electrode, we used the electrode for the determination of BHA in two different spiked biscuit samples, and the results are shown in Table 1. The values obtained are an average of five replicate measurements and the statistical treatment of the data indicates that the method is precise and reliable.
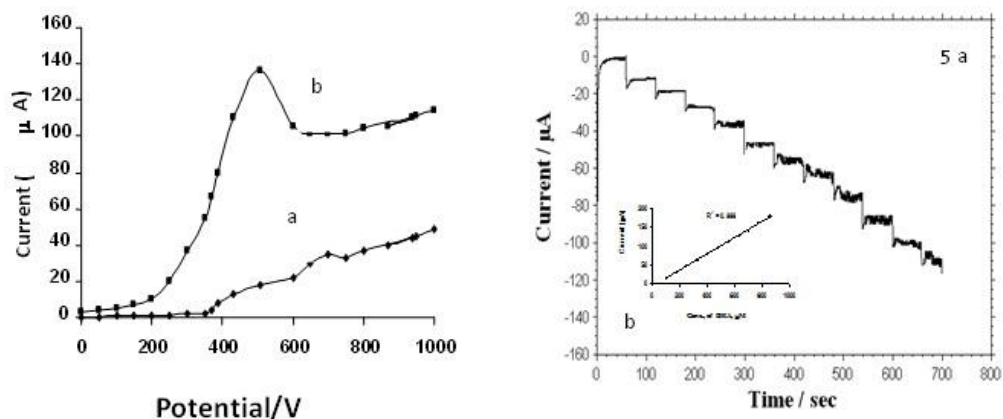

Fig. 5 Hydrodynamic voltammograms obtained with (a) bare and (b) NiHCF modified electrode. Fig.5a. Chronoamperometric response at NiHCF modified electrode for each addition of 4.14×10^{-5}M BHA to 0.1 M $NaNO_3$ (pH 7.0) stirring rate: 300 rpm; potential: 0.5 V. Fig.5b.Calibration graph for BHA measurement.

Determination of BHA in spiked commercial biscuits samples:

Samples	Concentration of BHA (µM)		Recovery (%)
	Added	Found[a]	
Sample I	30	29.20± 0.70	98.4
	40	39.81± 0.49	99.5
	50	49.42± 0.60	98.84
Sample II	30	29.9 ± 0.50	99.6
	40	39.68 ± 0.48	99.2
	50	49.20 ± 0.58	98.4

[a] Average of four replicate experiments ± R.S.D

g. Interferences

The effect of possible interferences present in the samples were studied. The main substance that could interfere in the determination of BHA is ascorbic acid which was found to alter the current signal of BHA significantly and hence such an influence of ascorbic acid could be eliminated by coating the surface of the sensor with nafion as reported elsewhere (19)

3.1.7 Stability of the modified electrode

The stability of the NiHCF modified electrode was evaluated by subjecting the modified electrode to potential cycling of 100 times in the potential range $0 - 1.0$ V at a scan rate of 20 mV/s in 0.1 M $NaNO_3$. The electrode remained stable on continuous cycling even up to 100 cycles. Also, the electrode was found to be reproducible after the analyte oxidation indicating that the surface fouling was not observed with the modified electrode.

IV. Conclusion

The graphite–wax electrode modified with nickel hexacyanoferrate was demonstrated as an excellent amperometric sensor for electrochemical determination of BHA. Studies under dynamic conditions confirmed its promising application in flow systems. Linear response was observed over the range from $9.9x10^{-6}$ to $1.03x10^{-3}$M BHA with the correlation coefficient of 0.9990. The higher sensitivity of 2.42µM/µA was observed and the detection limit was found to be about1.2×10^{-7} M. Studies on the stability of the electrode showed that the electrode exhibited remarkable stability.

Acknowledgements

Reference

1. Albery. W. J, Hillman.A.R, Annual Reports on the progress of chemistry section c, Vol.78, physical chemistry, Royal society of chemistry, London, pp. 377, 1981.
2. Alegret.S, Cespedes. F, Fabregas. E.M, Monoz.J, Carbon- pollumer biocomposites or amperometric sensing. Journal of Biosens. Bioelectron Vol. 11, pp. 35-44, 1996.
3. Anson. F.C, Ohsaka. T, Saveant.J.M, Kinetics of electron transfer cross reactions with in redox polymers coatings protonated plylysine copolymer with incorporated electroactive anions. Journal of Am. Chem. Soc. Vol. 105, pp. 4883-4890, 1983.
4. Atuma .S.S, Lindquist. J, Voltammetric determination of tocopherols by use of a newly developed carbon paste electrode. Journal of Analyst Vol. 98, pp. 886-896, 1973.
5. Cozzi. D, Raspi. G. Nucci. L, A cell with solid microelectrode with periodical renewal of the diffusion layer. Journal of Electroanal. Chem, Vol. 12, pp. 36-44, 1966.
6. Jayasri.D, Sriman Narayanan.S, Electrocatalytic oxidation and amperometric determination of BHA at graphite-wax composite electrode with silver hexacyanoferrate as electrocatalyst. Journal of Sensors and Actuators, B, Vol. 119, pp. 135-142, 2006.
7. Glazier. A, Campbell.E.R, Campbell. W.H. Construction and characterization of nitrate reductase-based amperometric electrode and nitrate assay of fertilizers and drinking water. Journal of Anal. Chem, Vol. 70, pp. 1511-1515, 1998.
8. http://www.lactose.co.uk./milkallergy/foodadditives 300.html
9. Hudson.B.J.F, Food antioxidants, London:Elsevier. pp.5-7, 1990.
10. Kenneth R. Brown, Daniel G. Walter, and Michael J. Natan, Seeding of Colloidal Au Nanoparticle Solutions. 2. Improved Control of Particle Size and Shape Chem. Mater., 12 (2), pp 306–313, 2000
11. Kulesza.P.J, Malik.M.A, Berrettioni.M Giorgetti. M, Zamponi. S Schmide. R, Marassi.R, Synthesis and characterization of Nanostructured Cobalt hexacyanoferrate.Journal of physical.chem. B Vol. 102, pp. 1870, 1998.

12. Martorell.E, Cespedes.F, Fabregas.F.M, Alegret. S, Amperometric determination of pesticide using a biosensor based on a polished graphite-epoxy biocomposite. Journal of Anal. Chim. Acta, Vol. 290, pp. 343-348,1994.
13. Merz.A., in: Steckhan .E (Ed.,), Topics in current chemistry,Vol.152, Springer-verlag, Berlin, p.51, 1993.
14. Mortimer.R.J. Electrochromic materials, Chem. Soc. Rev. Vol. 26, pp. 147-156, 1997.
15. Murray. R.W. in: Bard. A. J.(Ed.,), Journal of Electroanalytical chemistry, Vol.13, Marcel Dekkar, New York, pp. 191, 1984.
16. Pong B K, Hendry Elim I, Chong J X , Ji W, Bernhart Tront L and Lee J Y, New insights on the nanoparticle growth mechanism in the citrate reduction of gold (III) salt; formation of the Au nano wire intermediate and its non-linear optical properties. Journal of Phys. Chem. C, Vol. 111, pp. 6281-6287, 2007.
17. Pournaghi- Azhar. M.H, Razmi-Nerbi.H, Volatammetric behaviour of aluminum electrode modified with nickel and nickel hexacyanoferrate films, prepared by electroless deposition. Journal of Electroanalytical chemistry, Vol. 456(1-2), pp. 83-90, 1988.
18. Ravisankar.D, Sriman narayanan.S, Russ. Amperometric sensor for hydrazine determination based on mechanically immobilized NiHCF modified electrode. Journal of Electro. Chem. Vol. 37, pp.1149-1153, 2001.
19. D. Ravisankaran, N.Uehera, T.Kato. A metal dispersd sol-gel biocomposite amperometric glucose biosensor, Biosens. Bioelectron. Vol. 18, pp. 721-728, 2003.
20. Richard prabagar. S.J, Sriman Narayanan.S, Surface modification of a amine functionalized graphite for preparation of cobalt hexacyanoferrate (CoHCF) modified electrode an amperometric sensor for determination of butylated hydroxyl anisole (BHA). Journal of Anal Bioanal Chem, Vol. 386: pp. 2107-2115, 2006.
21. Ruiz.M.A. Garcia-Moreno.E, Pingarron.J.M, Determination of tert-butyl hydroxyl anisole and tert-butylhydroxy tolune by flow injection with ampoermetric detection.Journal of Electroanalysis, Vol. 11, pp. 470-474, 1999.
22. Shindo H and Brown TL, Infrared spectra of complexes of Lcysteine and related compounds with zinc (II), cadmium (II), mercury (II), and lead(II). J Amer Chem Soc, Vol 87, pp. 1904 – 1908, 1965.
23. Smith.L.I, Spillane, L.J, Kolthoff. I. M., Chemistry of vitamin EXXXV. The behaviour of tocopherols at the dropping-mercury electrode. Journal of Am.Chem.Soc. Vol. 64, pp. 447-451, 1942.
24. Siperko. L.M. Kuwana. T, Electrochemical and spectroscopic studies of metal hexacyanometalate films, Journal of Electrochem. Soc, Vol. 130, pp. 396-402, 1983.
25. Wang. J, Freina. B.A., Preconcentration and differential pulse voltammetry of BHA at a carbon paste electrode. Journal of Anal. Chim. Acta. Vol. 154, pp. 87-94, 1983.