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Electroanalysis of Unconventional (Herbal) Drugs Using Surface Modified Electrodes in Conjunction with Cyclic Voltammetry

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Abstract: *In this paper we report on the use of surface modified electrodes in conjunction with cyclic voltammetry to study the redox profiles of quinine in unconventional drugs-herbal medicine. The unconventional drugs used in the study include cinchona bark and malbet. The results show quinine in cinchona is redox active with the oxidation and reduction potentials on bare carbon graphite electrode occurring at 0.495V and 0.345V respectively. The oxidation/reduction potential on bentonite modified electrode occurred at 0.450V/ (0.345V and -0.030V). It was observed that, when in contact with the bentonite over along period, quinine in cinchona is physi-sorbed. The redox process of the quinine extract is diffusion limited. In the case of malbet the quinine in Alstonia scholaris was detected . The other natural products in malbet appear not to be electroactive. The oxidation and reduction peak potential for quinine in alstonia scholaris appeared at 0.540V and 0.225V in 1M H_2SO_4 and 0.525V and 0.330 V in HCl.The malbet appears to ingress into bentonite lattice and is pre-concentrated in the bentonite hence, giving enhanced redox signals.The electrochemical signal obtained in malbet is attributed to quinine in Alstonia scholaris.*

Key Words: *Cinchona, Malbet, unconventional (herbal) drug, surface modified electrode*

I. Introduction:

A drug may be defined as any substance that brings about a biological response through its chemical action on a biological system. The drug molecule normally interacts with a specific molecule in the biological system that plays a regulatory role. The targets sites of drugs include lipids, proteins (enzymes and receptors), and deoxyribonucleic acid (DNA). When this occurs the shape of the substrates active site changes which can lead to activation or deactivation of the enzyme. A drug can also act on receptors by opening ion channels or closing them.

For effective drug action, the drug molecule must have the appropriate size, electrical charge, shape, and atomic composition. A useful drug must have the necessary properties to be transported from its site of administration to its site of action. A practical drug should be inactivated or excreted from the body at a reasonable rate so that its action will be of appropriate duration. A drug may be solid at room temperature, liquid, or gaseous. The molecular size of drugs can be very large such as cataplasm t-pA, a protein of MW 59,050. However the vast majority of drugs have MW between 100 and 1000.

Malaria is an infectious disease caused by a one-celled parasite known as *Plasmodium* which is transmitted to humans by the bite of the female Anopheles mosquito.

Unconventional drugs have been used in the past to treat malaria due to microorganisms such as bacteria, fungi or parasites developing resistance to the traditional antimalarials i.e.,unaffected by the drug.

The evolution and spread of multidrug-resistant *Plasmodium falciparum* malaria have prompted the need to use combinations of antimalarial drugs and herbal medicines.

Quinine

Quinine is derived from the bark of the cinchona tree, a traditional remedy for intermittent fevers from South America.

Family: Rubiaceae
Genus: Cinchona
Species: officinalis, ledgeriana, succirubra, calisaya
Synonyms: ***Quinaquina officinalis, Quinaquina lancifolia, Quinaquina coccinea***
Common names: Quinine bark, quina, quinine, kinakina, China bark, cinchona bark, yellow cinchona, red cinchona, Peruvian bark, Jesuit's bark, quina-quina, calisaya bark, fever tree
Parts Used: Bark, wood.

The alkaloid quinine was purified from the bark in 1820, and it has been used in its chloride or sulphate form in the treatment and prevention of malaria since that time. Cinchona trees remain the only practical source of quinine (1-16)

The structure of quinine is given in figure 1.

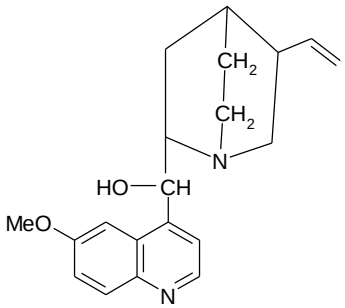

Fig. 1: Structure of quinine

In this analysis the cinchona bark used originated from the Republic of Rwanda. The natural product is dark brownish in color (see figure 2).

Fig. 2: Picture of cinchona bark

The density of this material when powdered completely is 0.476g/ml.
The quinine bark is partially soluble in water giving a value of 0.095g/l.

Malbet is a trade name of herbal antimalarial drug manufactured by Solar Herbo PVT. Ltd in India.
Analysis has been a major challenge to most quality laboratories given that these herbal medicines are a cocktail of various plant materials with different therapeutic effect. The various components are the following: Holarrhena antidysenterica, Picrorrhiza kurroa, Tricosanthes dioica, Piper nigrum, Laghusutshekhar(Generic preparation),Tribhuvankirti (Generic preparation) and Alstonia scholaris, an alkaloid which acts as quinine in malaria fever.
This has compounded the analysis in that the group. To analyze these quantitatively has been a major problem given that there are very many functional groups. Therefore surface modified electrodes were used to investigate these systems based on the following premises:

i. The electrochemical technique will specifically identify the quinine redox group
ii. The surface modification especially where a host matrix such as bentonite has been used will preconcentrate the functional groups hence enhancing the electrochemical signal.

Experimental section:

The cinchona bark containing quinine and Malbet drug were used as received without any further chemical processing.

All acids H_2SO_4 and HCl (Aldrich) were used as received without further purification. All solutions were prepared using de-ionized water. The clay montmorillonite, bentonite (sourced from Athi River Mining Company Ltd., Kenya) was purified as described in reference (17-21)
In generating the cyclic potential scans, we used an electrochemical unit comprised of a Princeton Applied Research (PAR) model 173 potentiostat/galvanostat, a logarithmic current converter model 369 that controlled the current, a PAR model 175 universal programmer. The output signal was fed into a PAR RE 0089 X-Y recorder.

Results and Discussion:

i) Electrochemical Analysis of Cinchona bark:

The solution of the cinchona bark was obtained by dissolving 1g of the bark of quinine in 1M H_2SO_4 for a period of 24 hours. The potential of the bare carbon working electrode was then cycled from -0.2V to 0.9V

at 20mV/sec. The resultant cyclic voltammetric response is shown in figure 3. The oxidative/ reductive peaks appear at 0.495V/0.345V.

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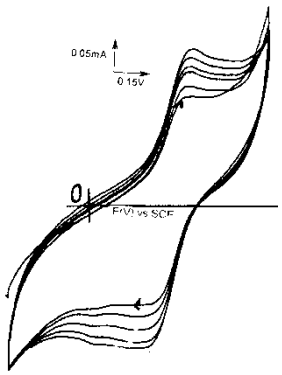

Fig. 3: Cyclic voltammetric response obtained when bare carbon working electrode potential was cycled within the potential range -0.4V to 0.90V at 20mV/sec in a solution containing 1 g of quinine bark in 1M H_2SO_4

The cyclic votammogram is quasi reversible with broad peaks. It is observed that the quinine bark redox profile is basically the same as that of quinine found in conventional quinine containing antimalarials.
Cinchona bark was ground to a fine powder and 10g was weighed and put in 50ml of ethanol. The solution was covered to avoid evaporation. Extraction was allowed to take place. The solution was then filtrated and the filtrate was mixed with bentonite. The different mixtures were allowed contact hours ranging from 24 hours to 96 hours. This allowed maximum adsorption of the quinine ethanol extract by bentonite. The dry mixture was then used to modify the electrode surface. The cyclic voltammetric responses obtained for the various samples ranging from 24 hours to 96 hours were obtained by cycling the potential of the modified electrode from -0.2V to 0.95V at a scan rate of 20mV/sec in 1M HCl and 1M H_2SO_4 (figure 4).

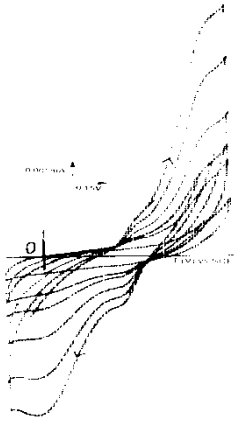

Fig.4 : CV response when quinine filtrate/bentonite modified electrodes was cycled in 1M H_2SO_4. Potential range: -0.2V to 0.95V. Scan rate of 20mV/sec

The cyclic voltammetric response in 1M H_2SO_4 (see figure 3), yielded poorly defined oxidation and reduction shoulders at 0.450V and 0.345V/-0.030V. The poor redox response can be attributed to the fact that, quinine from cinchona bark is probably not able to ingress into the bentonite matrix (tetrahedral or octahedral sites). This is purely from steric considerations.

When the sample was left to stand for a period of 96 hours, the cyclic voltammetric responses obtained were much better defined (see figure 4).

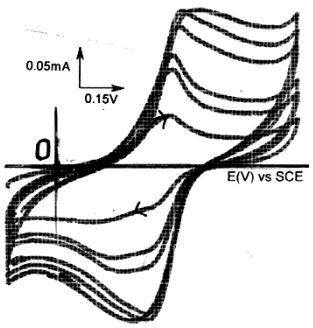

Fig.5: CV responses when quinine filtrate/bentonite modified electrode was cycled in solution containing bark of quinine in 1M H_2SO_4 solution. Potential range: -0.2V to 0.95V. Scan rate of 20mV/sec

The oxidative peak potential in 1M H_2SO_4 occurred at 0.465V and a reduction peak at 0.330V. The efficiency of the redox process computed from the correlation coefficient of the plot of anodic versus cathodic peak current gave an efficiency of 98%. See table 1.

Table 1: i_{pa} and i_{pc} versus time for cinchona bark filtrate

Time(min)	3.78	5.68	7.58	11.38	15.22	19	22.81	26.62	32.33
$i_{pa(mA)}$	0.17	0.19	0.21	0.24	0.265	0.29	0.31	0.33	0.35
$i_{pc(mA)}$			0.19	0.21	0.235	0.255	0.275	0.295	0.315

The redox properties of quinine appear to be enhanced for the case where the bentonite was in contact with quinine extract for a long period of time (96hours). It is possible that due to the long

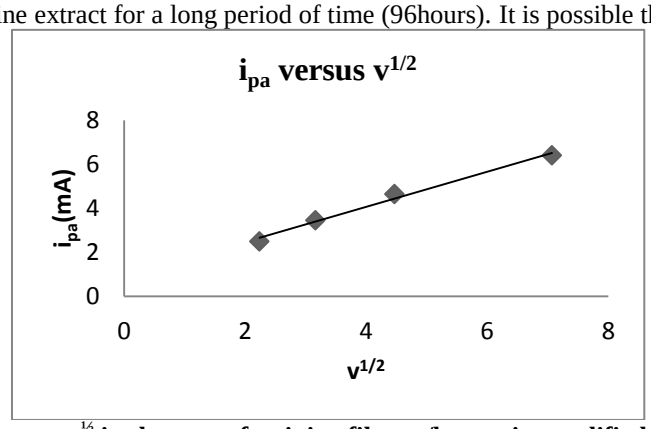

Fig. 6: Plot of i_{pa}versus $v^{½}$ in the case of quinine filtrate/bentonite modified electrode contact period, physi-sorption of quinine occurs.

The redox process of quinine is diffusion limited. This is confirmed by the linear plot obtained from i_{pa}versus $v^{½}$.

In the next set of experiments, 20 mls of the quinine filtrate was mixed with 20ml 1M H_2SO_4. (1:1 vol/vol). The bare carbon electrode potential was then cycled from -0.2V to 0.95V at 20mV/sec.

The profile of the cyclic voltammogram is similar to those observed earlier except that, there were two oxidative centers at 0.450V and at 0.540V which fused into one well defined oxidative peak on continued potential cycling at 0.5325V. The reduction peak was a single well defined peak occurring at 0.330V. We observe that, the oxidation potential shifts positively and reduction towards negative potential, suggesting that on continued cycling the quinine becomes more difficult to oxidize but easy to reduce. The improvement in the quinine extract redox process could be purely the result of increased concentration of quinine in the mixture. It is also possible that there could be follow up reactions associated with the system, which compliments the quinine redox activity, but this remains purely speculative.

The redox process is diffusion limited, yielding a linear plot for i_{pa} versus $v^{½}$.

In the next set of experiments 10g of crushed quinine bark were dissolved in 8.3ml of concentrated HCl and allowed to react for 48hours. This mixture was topped up to 100ml with water to make 1M HCl. The solution was subjected to electrochemical analysis whereby the potential was cycled from -0.2V to 0.95V. The resultant cyclic voltammogram resembled that obtained in H_2SO_4 except that, the oxidation peak is broad and misshapened with two shoulders appearing at 0.480V and 0.675V with the corresponding reduction peaks occurring at 0.075V and 0.345V. The poor redox response is probably due the low $[H^+]$, since HCl is a monoprotic acid.

The quinine filtrate was mixed with bentonite forming a slurry, which was in turn used for electrode modification. The redox profile of the quinine improved with the oxidation peak characterized with a diffusion tail peak occurring at approximately 0.345V. Thus, with bentonite matrix, there was significant improvement in electron transfer kinetics leading to sharp and well defined peaks. This improvement in the redox profile results from preconcentration of the quinine in the bentonite host matrix. The scan rate dependence studies yield a linear plot in the case of i_{pa} versus v confirming that the process is the surface attached species (see figure 6), unlike previously where the processes were diffusion limited.

During the initial cycling the peak was double humped. Unlike in the previous case, the double hump was at 0.675V and the reduction peak at 0.075V. These transient peaks are probably associated with chemical

products which are expelled from the clay montmorillonite matrix as a result of repulsive forces attributable to metal cations in the O_h sites.

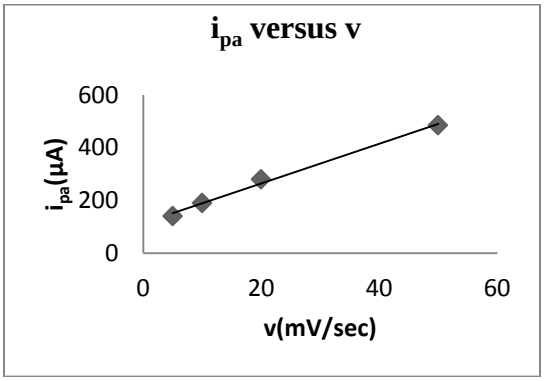

Fig. 7: i_{pa}versus v when bentonite/ quinine filtrate modified electrode was cycled in 1M H_2SO_4 from -0.2V to 0.95V

This result is significant in that, it shows that the bentonite host matrix immobilizes the quinine on the electrode surface hence increasing its accessibility for electrochemical analysis.

Electrochemical Analysis of Malbet:

0.3g of the malbet tablet was weighed and dissolved in different solutions containing 1M HCl and 1M H_2SO_4. The potential of the bare carbon electrode was then cycled from -0.4V to 0.9V at a scan rate of 20mV/sec. The resultant cyclic voltammetric responses are shown in figures 8a and 8b.

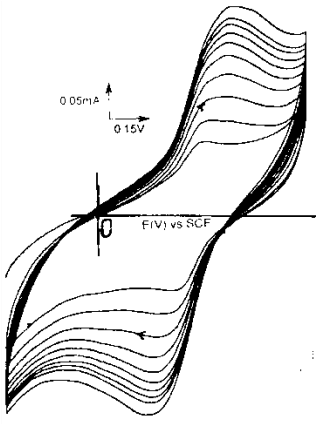

Fig. 8a

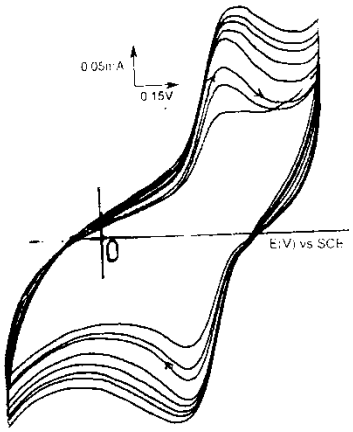

Fig. 8b

Fig. 8a and 8b: CV responses obtained when bare carbon electrode was cycled in a solution containing 0.3g malbet in 1M H_2SO_4 and 1M HCl respectively. Potential range: -0.4V to 0.9V. Scan rate of 20mV/sec

We observe well defined quasi reversible peaks occurring at 0.540V/0.225V and 0.525V/0.330V for the oxidation/reduction peaks in 1M H_2SO_4 and 1M HCl respectively. The oxidation and reduction peaks increased with continuous cycling and a plot of i_{pc}versus i_{pa} for 1M H_2SO_4 and 1M HCl yielded an efficiency of 97% and 72.7% respectively (see figures 5.130a and 5.130b). The differences in the redox efficiency can be attributed to differences in the anion types and $[H^+]$.

This analysis which was carried out on bare carbon working electrode confirms the prescence of quinine in malbet. The oxidation /reduction profile is similar to that observed in the case of conventional quinine anti malarial and cinchona. It is interesting to note the quinine in alstonia scholaris has been detected. The other plant components do not appear to have redox active

Bentonite modified working electrode:

When the surface of the carbon electrode was modified using bentonite as a host matrix, we observed that the oxidative peak is much sharper and with the peak potential occurring at 0.480V, a shift of 45mV towards negative potential and a reduction peak potential occurring at 150mV representing a shift of 150mV towards negative potential.

Since these reductions in oxidation potential do not occur in the case of bare carbon working electrode, it can be attributed to pro-concentration of malbet in the bentonite lattice. This not only enhances the

electrochemical signal, but also can lead to a reduction in Gibbs free energy resulting from entropic effects as a result of the realignment of the redox functional groups in the bentonite host matrix. This is not surprising given the selective and complexation properties of clay montmorillonite.

Extraction of Malbet Components Using Ethanol

0.3g of malbet was placed in 20ml of ethanol and allowed to stay for 24hours. The extract was then put in a solution of 1M H_2SO_4 and the potential of the bare carbon cycled from -0.4V to 0.9V at 20mV/sec.

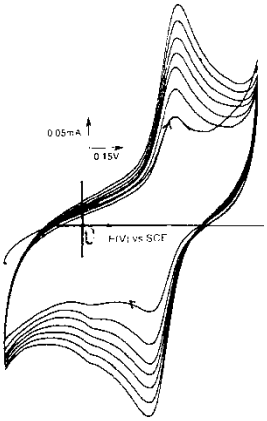

Fig. 9: CV response when bare carbon electrode was cycled in a solution containing malbet ethanol extract and 1M H_2SO_4. Potential range: -0.4V to 0.9V. Scan rate of 20mV/sec

The resultant cyclic voltammogram yielded very sharp oxidation/reduction peaks occurring at 0.465V and 0.315V and a shoulder with drifting potential occurring at 0.0V and 0.030V. The oxidative and reductive peaks increased with each cycle with the redox efficiency being 99%. This enhanced electrochemical signal is attributed to the efficient extraction of the constituents by ethanol.

The same analysis was repeated but this time using bentonite modified electrode. We obtained a broad poorly defined cyclic voltammogram (see figure 10).

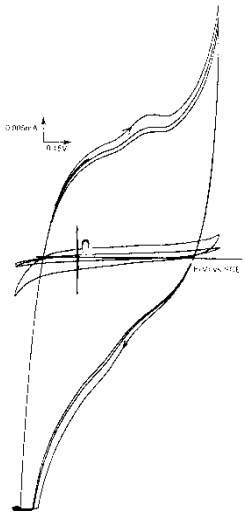

Fig. 10: CV response when bentonite modified electrode was cycled in a solution containing malbet ethanol extract and 1M H_2SO_4. Potential range: -0.4V to 0.9V. Scan rate of 20mV/sec

This is probably due to the fact that ethanol interferes with the hydration in the basal layer of the clay montmorillonite. This in turn affects the electrochemical kinetics of the redox active functional groups. This assertion is confirmed by the observation that, when 0.3g of malbet is placed in 20ml of water and left to stay for 24hours and then the potential of the working electrode cycled in 1M H_2SO_4 electrolyte media. The cyclic voltammetric response is characterized by sharp and well defined oxidative and reductive peaks occurring at 0.480V and 0.120V respectively.

Effect of pH on Malbet Redox Activity

Malbet/ bentonite electrode potential was cycled from -0.4V to 0.9V in various solutions containing 0.1M, 0.2M, 0.5M, 1M and 1.5M H_2SO_4. The resultant cyclic voltammetric response yielded sharp oxidative and reductive peaks with the oxidative peak potentials shifting at a rate of 58.77mV/pH unit (see figure 10).

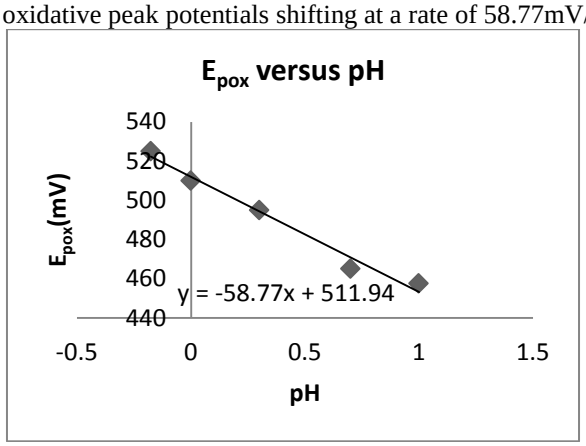

Fig. 11: Oxidative peak potential (E_{pa}) versus solution pH

This suggests a $1\bar{e}/1H^+$redox process. This is in agreement with earlier proposed model for quinine oxidation/reduction (ref).

II. Conclusion:

It has been shown from the results presented that, chemical modification of the electrode surface can allow electrochemical assessment of unconventional drugs, as long as they contain redox active moieties. These redox active groups in herbal medicine can be marked and electrochemically profiled quantitatively. The electrochemically determined quantity of the redox active moiety can be then be correlated to the quality of the unconventional drug.

References:

[1]. Acton N. and Roth R.J., (1992), On the conversion of dihydroartemisinic acid into artemisinin. *J. Org. Chem.* **57**, 3610-3614.
[2]. Avery, M. A., S. Mehrotra, J. D. Bonk, J. A. Vroman, D. K. Goins, and R. Miller, **(**1996). Structure-activity relationships of the antimalarial agent artemisinin. 4. Effect of substitution at C-3. *J. Med. Chem*. 39, 2900-2906.
[3]. Bachi, M. D., Korshin E. E., Hoos R., and Szpilman A. M., (2000). Synthesis and reactions of antimalarial bicyclic peroxides. *J. Heterocycl. Chem*. 37:639-646.
[4]. Bakhshi, H. B., T. Gordi, and M. Ashton. (1997). In-vitro interaction of artemisinin with intact human erythrocytes, erythrocyte ghosts, haemoglobin and carbonic anhydrase. *J. Pharm. Pharmacol***. 49**:223-226.
[5]. Benoit-Vical F., Robert A. and Meunier B., (2000), In vitro and in vivo potentiation of artemisinin and synthetic endoperoxyde antimalarial drugs by metalloporphyrins. *Antimicrob. Agents Chemother*. **44**, 2836-2841.
[6]. Berman, P. A., and P. A. Adams, 1997. Artemisinin enhances heme-catalysed oxidation of lipid membranes, *Free Radic. Biol. Med.* 22:1283-1288.
[7]. Bloland P.B., (2001), Drug Resistance in Malaria, Malaria Epidemiology Branch, Centers for Disease Control and Prevention WHO/CDS/CDS/DRS.**4**, 27
[8]. Blumberg P.M. and Strominger J. L., (1974), Interaction of Penicillin with the bacterial cell: Penicillin – binding proteins and penicillin – sensitive enzyme, Bact. Rev. **38**, 291.
[9]. Bunnag, D., T. Kanda, J. Karbwang, K. Thimasarn, S. Pungpak, and T. Harinasuta, (1995), Artemether-mefloquine combination in multidrug resistant falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* **89**:213–215.
[10]. China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, (1982). Metabolism and pharmacokinetics of qinghaosu and its derivatives. *J. Traditional Chinese Med*. **2,** 25-30.
[11]. Cumming, J.N., Ploypradith P. and Posner G.H. (1997), Antimalarial activity of artemisinin (qinghaosu) and related trioxanes: mechanism(s) of action. *Advances in Pharmacology* (San Diego) **37**, 253
[12]. Graham L.P., (1997), An Introduction to Medicinal Chemistry, Oxford University Press.
[13]. Trape J. F, Pison M. P, Preziosi M. P, (1998), Impact of chloroquine resistance on malaria mortality. C R Acad Sci III, **321**, 689-697.
[14]. Triggs J.K., Mbrana H., Chambo O., Watkins E. and Curtis C.F., (1997), Resistance to pyrimethamine/ sulfadoxine in Plasmodium falciparum in 12 villages in North East Tanzania and a test of chlorproguanil/dapsone. *Acta Trop*., **63**, 185-189.
[15]. WHO (Roll Back Malaria), (1985).
[16]. WHO, (2001). The use of antimalarial drugs. Report of an Informal consultation. WHO/CDS/RBM33, pp:141.
[17]. Arnold B.B. and Murphy G.W., (1965), Studies on the electrochemistry of carbon and chemically modified carbon surfaces, *J. Phys. Chem.* **65**, 135-136.
[18]. Bakhshi A.K. and Rattan P., (1997), Electrical conducting polymers: an emerging technology, *Curr.Sc.* **73**, 648
[19]. Bard A.J. and Rudzinski W.E., (1986), Clay modified electrodes, Part VI: aluminium and silicon pillared clay modified electrodes, *J.Electroanal. Chem*. **199**, 323-340
[20]. Bard A.J. and Villemure G., (1990), Part 9: Electrochemical studies of the electroactive fraction of adsorbed species in reduced and preadsorbed clay films, *J. Electroanal. Chem*. **282**, 107-121.
[21]. Chow E. J. and Gooding J. (2006)**,** Peptide modified electrodes as electrochemical metal ion sensors, *Electroanalysis* **18**, 1437 – 1448.