

Glycerol-Octanohydroxamate-Clay Composite Film for Selective Voltammetric Analysis of Charged Species

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Abstract

A new sensitive, selective, and stable glycerol-octanohydroxamate-claymodified electrode (GOHATCME) has been developed for voltammetric analysis of charged species. The modified electrode was prepared by applying a mixture of glycerol, octanohydroxamate (OHAT), and montmorillonite clay at a glassy carbon electrode. Fourier Transform Infrared (FTIR) microscopy was employed to examine the morphological features of the clay composite film. It was observed that the addition of glycerol only prevented formation of cracks in the film and did not alter its features. Model analytes, dopamine (DA) and excess ascorbic acid (AA), were used to probe the film. A suite of experiments were performed to observe how DA and AA behave at the bare electrode (BE), glycerol-clay modified electrode (GCME), and GOHATCME. The linear range of DA calibration curve was 0.0025 – 0.25 mM, while that of AA was 0.050 – 0.75 mM. The GOHATCME completely excluded AA from the film. The detection limit of DA was 1.0 μ M.

Keywords: Montmorillonite clay, octanohydroxamate, dopamine, ascorbic acid, Voltammetry

1. Introduction

Voltammetric analysis is usually associated with some disadvantages, such as the co-existence of interfering species and electrode fouling by species that adsorb to the electrode surface. These disadvantages can be eliminated by modifying the electrode surface with a polymeric membrane. The modified electrodes, however, are also associated with a number of disadvantages such as reactant leaching, long response times due to slow diffusion of reactants, and instability of polymeric membranes.

This work aimed to eliminate the above mentioned limitations by employing glassy carbon electrode that is modified with a glycerol-octanohydroxamate-clay composite film (GOHATCME). The goal was to develop an anionic clay composite film, which can exclude interfering anionic species while detecting cationic analytes.

Standard Wyoming Montmorillonite (SWy-2) clay was the type of clay used in this project. Clays are naturally occurring and are abundant, nontoxic, much more stable, and cheaper than synthetic membranes. Montmorillonite clay film on an electrode surface provides important functions such as charge-exclusion, immobilization of species, preconcentration, catalysis of electrochemical reactions, and electron transfer enhancement [1-4].

Hydroxamates are very important O-O type chelating agents that have the ability of forming strong bonds with cations leading to the formation of strong monolayers [5-7]. Among the hydroxamates, octanohydroxamate (OHAT) is generally preferred for use as Cu and Fe collectors for metal ore flotation [5-7]. The chemical formula of OHAT is $\text{CH}_3(\text{CH}_2)_6\text{CONHO}^-$. Interactions of OHAT with clay is highly favorable

since SWy-2 is an Fe-bearing smectite clay mineral that contains about 2.3 mass percent lattice Fe (0.41 mol Fe/g clay) [8, 9].

Dopamine (DA) and ascorbic acid (AA) were chosen as model analytes to probe the clay films for the voltammetric analyses. DA is one of the naturally occurring catecholamine neurotransmitters in the central nervous system of mammals [10]. Neurotransmitters are naturally occurring chemicals that aid in the transmission of signals from one neuron to another across synapses. Neurotransmitters also play major roles in the stimulation of muscle fibers. DA release in the brain plays a key role in the regulations of pleasure and pain. Abnormal DA levels are associated with serious diseases such as Schizophrenia and Parkinson's [3, 10-12]. Development of a highly sensitive and selective electrochemical sensor for dopamine detection in clinical and biological samples is essential since there is increasing demand for more reliable, rapid and simple methods. Considering electrochemical detection, some interfering species co-exist in biological samples, of which AA is the major one. AA is an important nutrient and plays a vital role in human metabolism. It is a bioactive substance and exists in biological fluids such as blood and urine. It is also important for therapeutic purposes. Just as DA, AA levels in biological samples are associated with a number of abnormalities in the body [13, 14]. AA exists in relatively high concentrations in biological samples as compared to DA (10 times and over). The presence of high levels of AA in biological samples poses a problem for electrochemical DA detection as both species have similar oxidation potentials [15-17].

This work describes a new modified electrode that is applicable to selective analysis of biological/clinical samples, waste water treatments, and gel sensor systems. The GOHATCME has the advantage of low cost, preconcentration, charge-exclusion, and electrocatalytic activity. Preparation of clay composite films is simple and convenient since it does not require any special conditions. The GOHATCME was able to completely eliminate excess AA while preferentially detecting DA, due to the fact that the film and AA are both negatively charged whereas DA is positively charged at the physiological pH of 7.4.

2. Experimental

2.1 Chemicals and Clay: Ascorbic acid (Sigma-Aldrich), dopamine hydrochloride (Alfa Aesar), sodium octanohydroxamate (TCL Tokyo-Kasei), glycerol (Mallinckrodt Chemical Works), sodium phosphate monobasic (Acros Organics), and sodium phosphate dibasic (Fisher Scientific), were used as received. SWy-2 clay was obtained from the Source Clay Minerals Repository (Purdue University, East Lafayette, Indiana) and processed as described below. All chemicals and clays were prepared without further purification with 18 mΩ.cm³ nanopure deionized water (Barnsted EasyPure II, Thermo Scientific).

2.2 Clay Suspension: For every 15 g of the powdered clay, 500 mL of deionized water was added and stirred periodically for about 48 hours. The suspension was centrifuged at 5000 rpm (3214 rcf) for 30 minutes, using Eppendorf 5810R centrifuge. The supernatant was decanted and centrifuged again for 10 minutes. This process was repeated until no more clay particles settled at the bottom of the centrifuge tubes. The concentration of the resulting clay suspension was 7.54 mg/mL, as determined by gravimetric analysis, with particle size < 0.2 μm as described elsewhere [4].

2.3 Modified Electrodes: To prepare GCME, clay and glycerol were mixed in the ratio 95:5 by volume, and sonicated for two hours using Cole-Parmer 8891 sonicator. For GOHATCME, OHAT was added to the glycerol-clay mixture to make 40 mM (7.248 mg/mL) solution and sonicated for two hours. Mixtures were then left to stand overnight to achieve homogeneity. With the aid of a microliter syringe, 5 μL of mixture was smeared uniformly on a polished glassy carbon working electrode and left overnight to air dry.

2.4 Phosphate Buffer Solution (PBS): All solutions were prepared with 0.1 M phosphate buffer solution (PBS) in order to maintain solution pH of 7.40.

2.5 Fourier Transform Infrared (FTIR) Microscopy: The Perkin Elmer Spotlight 200i FTIR Microscopy System was used for simultaneous imaging and acquisition of IR spectra. Liquid nitrogen was used to maintain low and constant detector temperature, ensuring high quality and reproducible results. The films were prepared on FTIR base microscope slides and air-dried overnight. With each slide on the microscope stage of the system, images (400 μm x 400 μm) and IR spectra in the micro-ATR mode were obtained simultaneously.

2.6 Electrochemical Measurements: Electrochemical experiments were performed using CHI 660 Dpotentiostat (CH Instruments, New Jersey), with the bare or modified glassy carbon working electrode, an Ag/AgCl reference electrode, and a Pt counter electrode. The electrochemical cell was a 10-mL glass cell fitted with a 3-hole Teflon cap. All solutions were nitrogen purged (deoxygenated) for at least 10 minutes to eliminate oxygen interference and prevent the formation of oxide layer on the electrode surface. After each measurement (or set of measurements), the working electrode was polished using 0.05 micron alumina powder with polishing pad (CH Instruments) and sonicated. The electrochemical techniques used were cyclic voltammetry (CV) and differential-pulse voltammetry (DPV). Switching potentials of -0.4 V and 0.4 V with 50 mV/s scan rate were the parameters for CV. The parameters used for DPV were, potential step = 4 mV, amplitude = 50 mV, pulse width = 0.2 second, and pulse period of = second.

3. Results and Discussion

3.1 Effects of Glycerol Addition

Glycerol was added to the clay suspension with the aim of preventing the formation of cracks in the films. Glycerol was chosen because it is inert, nonvolatile, and very stable. Different volume percentages of glycerol, % (v/v), in the clay suspension were examined in order to determine the percent glycerol that would produce the highest sensitivity and not affect the features of the clay film. Cyclic voltammetric potential sweep of 50.0 μM DA between -0.40 V and 0.40 V were performed with the different GCMEs at 50 mV/s scan rate. Figure 1 shows the effect of the amount of glycerol on oxidation peak current (i_{pa}). The highest i_{pa} was observed with 5% (v/v) glycerol, which was used for all subsequent experiments.

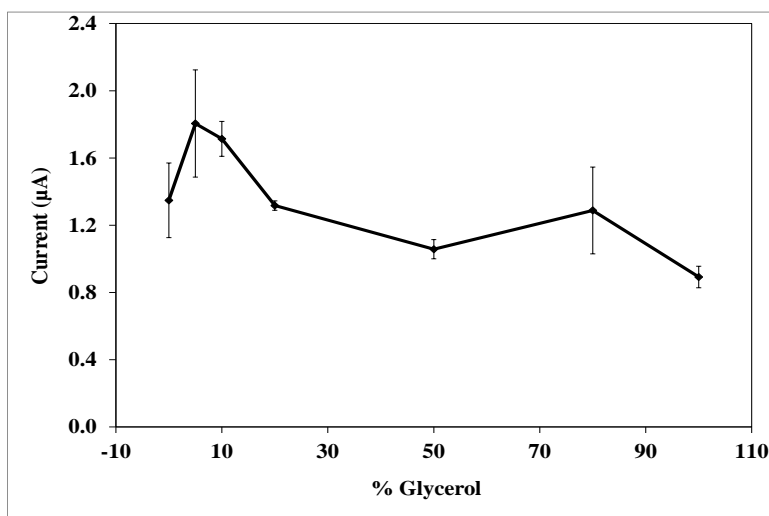


Figure 1. A graph of oxidative peak current versus volume percent glycerol in clay suspension used to prepare GCME. Peak currents are averages of three trials from CV at 50 mV/s with 50.0 μM DA in 0.10 M PBS (pH 7.4).

3.2 FTIR Microscopy

The FTIR microscope system was used to examine the morphological features of the clay films. Images of GCME/GOHATCME and their respective IR spectra (in the micro-ATR mode) were acquired simultaneously, as shown in Figure 2 below.

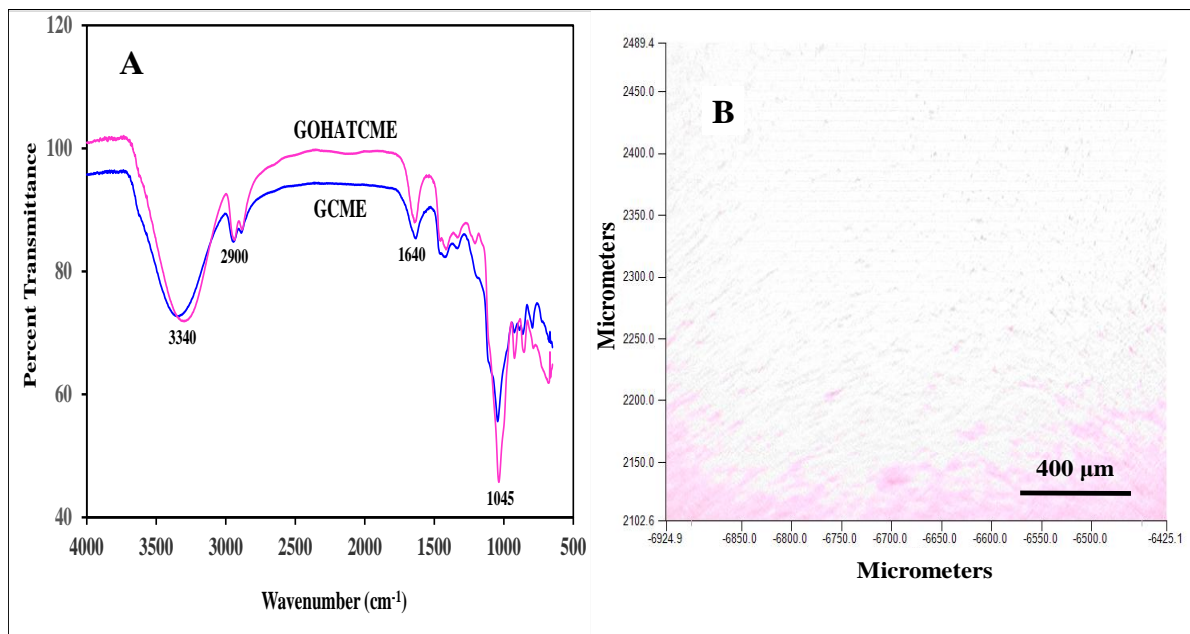


Figure 2. (A) FTIR microscopy spectra in the ATR mode of GCME and GOHATCME; (B) FTIR microscopy image of GOHATCME.

Figure 2A shows the IR micro-ATR spectra of GCME and GOHATCME. The SWy-2 clay is a hydrous aluminosilicate layered material that consists of structural OH groups, tetrahedral silicates (Si-O) and octahedral aluminates (Al-O and Al-OH) [3, 4]. The broad band centered around 3340 cm^{-1} is due to hydrogen bonding of the structural OH groups in the clay. The weak band around 2900 cm^{-1} is a result of Si-H where Si is sp^3 hybridized. The Si=O stretching band was observed at 1640 cm^{-1} . The strong stretching band at 1045 cm^{-1} is a result of Si-O stretch from the silicates. The Si-O/Si=O bands were a little shifted to lower wavenumbers compared to the normal C-O/C=O bands, consistent with the fact that Si is heavier than C. Also, the C=O stretching band is typically much stronger than the C-O band, but the opposite was observed in the clay film where the Si=O band was rather weaker than the Si-O band. This is due to interactions of glycerol with the clay. Comparing the two spectra, it can be seen that addition of OHAT did not cause significant changes in the bands. There were only slight shifts and the bands. Si-O stretching band around 1045 cm^{-1} became stronger, due to adsorption of the OHAT onto the clay layer surfaces. It must be noted that glycerol and OHAT exhibit similar characteristic bands as clay so they all coincide at these major bands described above.

3.3 System Performance

The performance of the system was tested with 50.0 μM DA and 250.0 μM AA at GCME. The efficiency of the modified electrode relies on intercalation, partitioning, retention, and stability of analytes in the clay film. Based on these conditions, the uptake of DA and AA were monitored using CV scans. For each analyte, the GCME was immersed in the solution with immediate start of CV scans. Successive scans were obtained, one immediately following the other, until the oxidative peak current (i_{pa}) leveled off. With the use of DA, the i_{pa} increased with successive scans and maximized after 3 minutes, indicating time-dependent intercalation into the clay film. The i_{pa} then remained constant, which indicated adequate intercalation, retention, and stability of DA in the clay film (figure not shown). The i_{pa} reaching a maximum and remaining constant after 3 minutes suggested that equilibration occurred between the DA in the film and the DA remaining in the bulk solution.

With the use of AA, on the other hand, i_{pa} immediately maximized (first scan) and slightly reduced before remaining fairly constant after 3 minutes (figure not shown), implying that AA did not undergo time-dependent intercalation into the clay film. As will be discussed in detail later in the text, both the AA and the clay platelets are negatively charged, hence AA could not penetrate the interlamellar spaces of the clay film. In addition, the AA species were not effectively retained in the clay film but rather diffused freely along the edges of the clay platelets.

Since the peak currents did not reduce with time but remained constant after three minutes, it can be concluded that leaching did not take place. Also, higher response was observed with DA than with AA. At the given pH, the former is cationic and hence penetrated the clay film to a greater extent than the latter, which bears the same negative charge as the clay platelets.

It is, therefore, without a doubt that clay should exhibit better response characteristics than synthetic cation-exchange polymers that are commonly used as sensing films. Based on these findings, electrochemical scans of all experiments were performed after 3 minutes of soaking the modified electrode in the analyte solution.

3.4 Analysis at BE and GCME

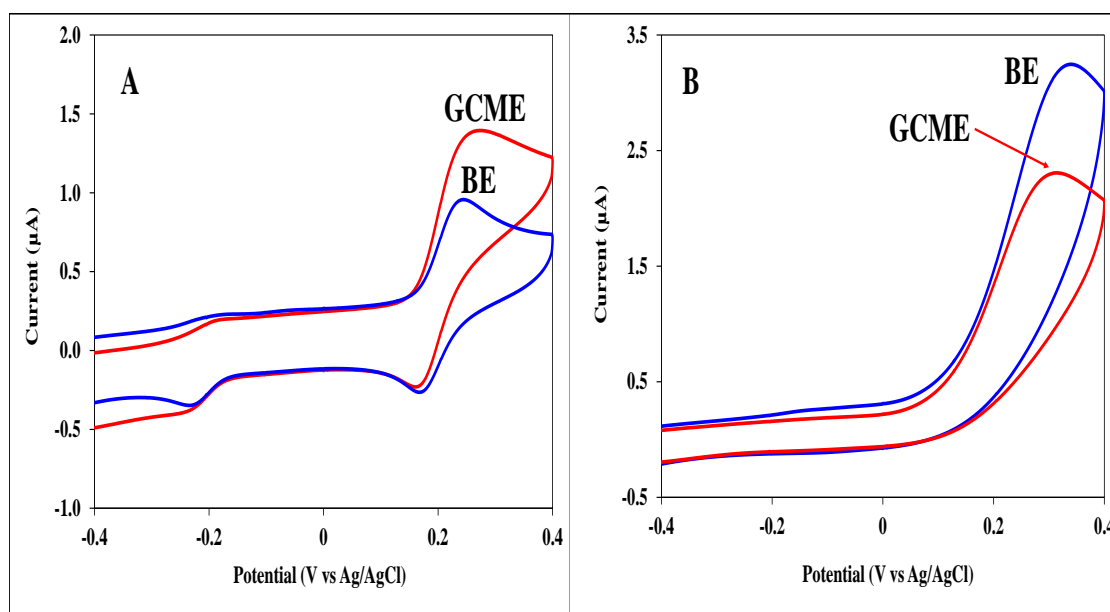


Figure 3. Cyclic voltammograms at 50 mV/s for (A) 25.0 μM DA and (B) 250.0 μM AA in 0.1 M PBS (pH 7.4) at BE and GCME.

Figures 3 and 4 are CVs that compare the irreversible oxidation of DA and AA at the BE to those at the GCME. The DA oxidation peak potentials at BE and GCME were around 0.25 V and 0.26 V versus Ag/AgCl, respectively (Figure 3A). Similarly, the AA oxidation peak potentials at BE and GCME were around 0.33 V and 0.32 V, respectively, as shown in Figure 3B. For a mixture of DA and AA in solution (Figure 4), a single oxidation peak was observed around 0.27 V and 0.23 V at the BE and GCME, respectively. For the mixture, a slightly less positive potential was seen at the GCME compared to the BE, suggesting a catalytic effect of the clay film.

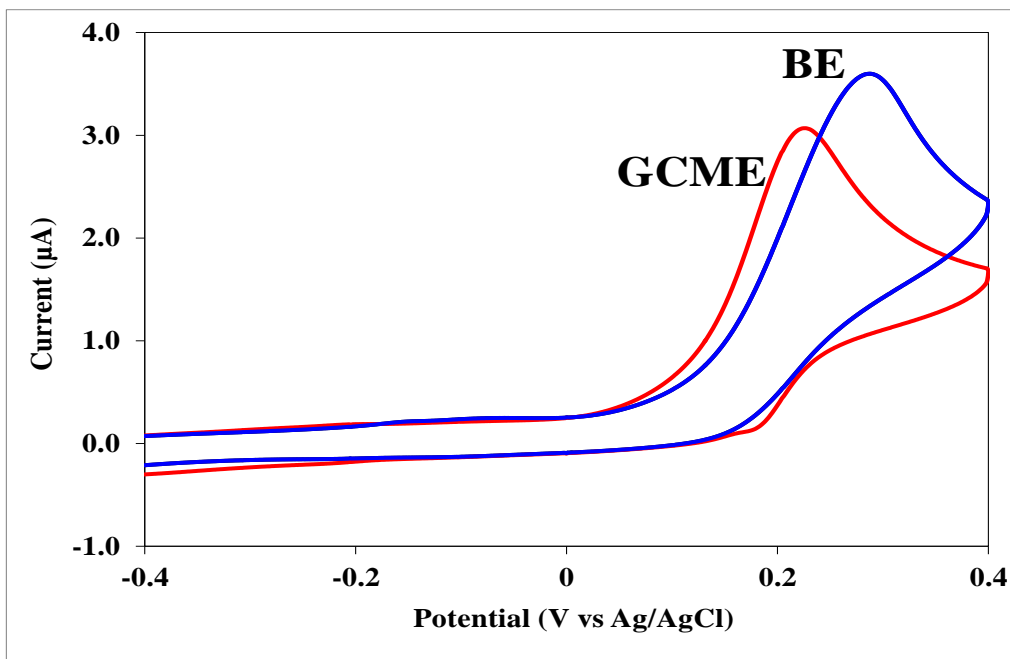


Figure 4. Cyclic voltammograms at 50 mV/s for a mixture of 25.0 μM DA and 250.0 μM AA in 0.1 M PBS (pH 7.4) at BE and GCME.

Calibration curves of both DA and AA at BE and GCME were developed as shown in Figures 5 and 6, respectively. The linear relationship between the i_{pa} and the DA concentration was within a concentration range of 2.5 μM and 250.0 μM (Figure 5), while that of AA was within a concentration range of 50.0 μM and 750.0 μM (Figure 6), at the chosen instrument sensitivity scale (A/V) of 1×10^{-6} . The detection limits of DA and AA were found to be 1.0 μM and 10.0 μM , respectively. The respective linear regression equations for DA at BE and GCME were $i_{\text{pa}} = 0.0245C_{\text{DA}} + 0.0218$ ($R^2 = 0.9997$) and $i_{\text{pa}} = 0.0319C_{\text{DA}} + 0.0596$ ($R^2 = 0.9961$). Similarly, the respective linear regression equations for AA at BE and GCME were $i_{\text{pa}} = 0.0068C_{\text{AA}} + 0.0177$ ($R^2 = 0.9631$) and $i_{\text{pa}} = 0.0052C_{\text{AA}} + 0.1138$ ($R^2 = 0.9985$). The coefficient of determination, R^2 , in each case was very strong ($0.8 < R^2 < 1$), an indication that peak currents are highly correlated with the concentrations. This implies that the regression models will provide highly accurate predictive concentration values.

There was an enhancement of the i_{pa} of DA at the GCME compared to that at the BE (Figure 3A). Also from Figure 5, it was observed that at all concentrations of DA, the peak currents at GCME were higher than those at BE. This shows a strong electrocatalytic effect by the clay film for DA detection. On the other hand, the peak currents at GCME were lower than the peak currents at BE, for all concentrations of AA (Figures 3B and 6). This is evidenced by the fact that at pH of 7.4, DA exists in the cationic form ($\text{pK}_{\text{a}1} = 8.93$ and $\text{pK}_{\text{a}2} =$

10.41) while AA exists in its anionic form ($pK_{a1} = 4.10$ and $pK_{a2} = 11.34$) [10,17-20]. Most clay layer surfaces are negatively charged so are expected to readily attract the cationic DA into the interlayers and repel the anionic AA, preventing it from entering the film [21]. This enhanced DA detection while diminishing AA detection. In addition, DA structure consists of both amino group and phenol group, therefore classified as a hard base. The amino group is protonated at pH 7.4 while the phenol group (the hard base) usually remains neutral. Recall that SWy-2 is an Fe-bearing smectite clay mineral that contains about 2.3 mass percent lattice Fe (0.41 mol Fe/ g clay) [8, 9]. Since Fe^{3+} is also a hard acid, strong interaction between DA and structural Fe^{3+} in the clay interlayers occurs [7].

However, the GCME could not efficiently exclude AA. This called for the incorporation of OHAT, which was expected to adsorb and form a monolayer, increasing the net negative charge on the clay layer surfaces and completely excluding any AA or any anionic species present.

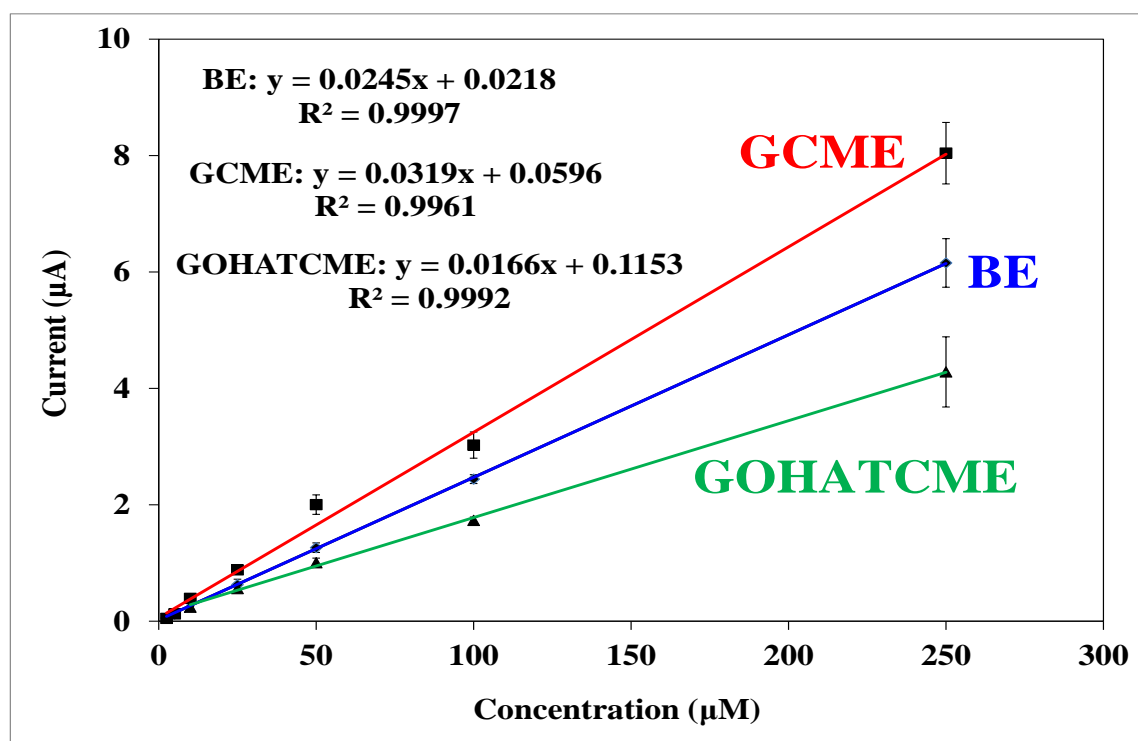


Figure 5. Calibration curves by CV at 50 mV/s for DA in 0.1 M PBS (pH 7.4) at BE, GCME, and GOHATCME.

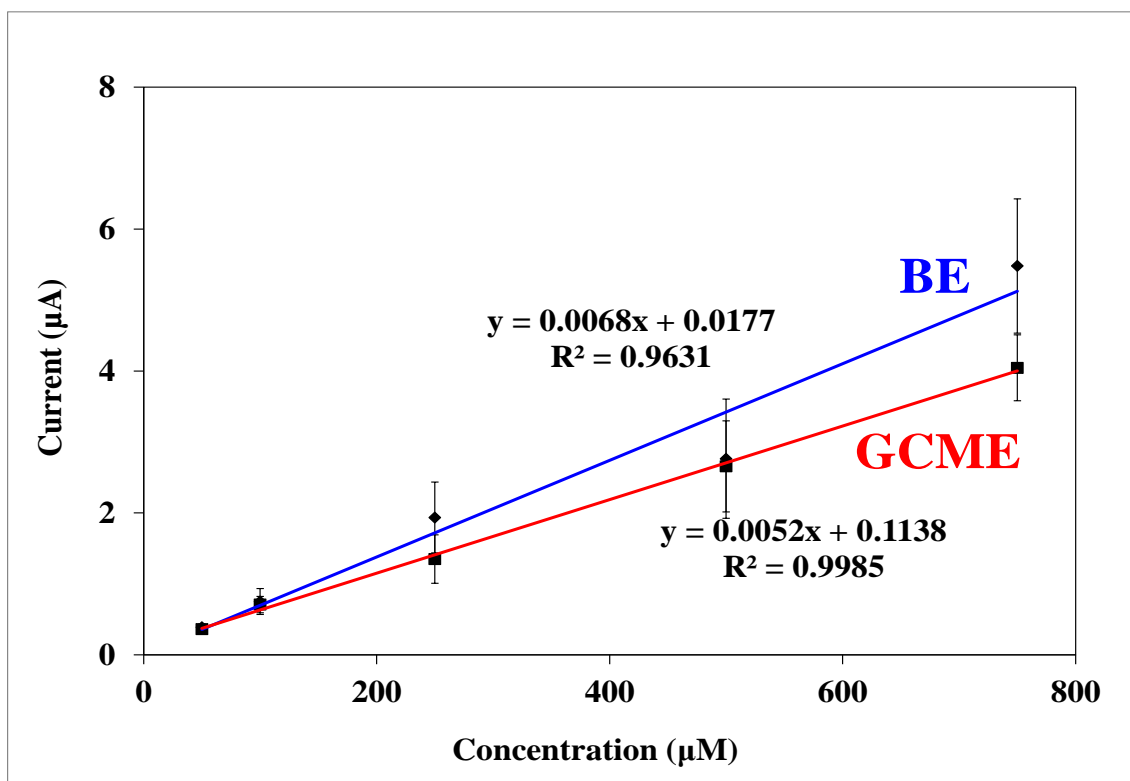


Figure 6. Calibration curves by CV at 50 mV/s for AA in 0.1 M PBS (pH 7.4) at BE and GCME.

3.5 Analysis at GOHATCME

DA and AA samples were then analyzed at the GOHATCME. Based on the linear range of the calibration curves discussed in section 3.4 above, 25.0µM DA and 250.0µM AA were used, unless otherwise stated. Different amounts of OHAT were first used to make the GOHATCME in order to determine the minimum amount required to completely exclude the AA. With 3.624 mg/mL (20.0 mM) OHAT, i_{pa} of AA was relatively lower than that at GCME. The i_{pa} reduced further when 5.436 mg/mL (30.0 mM) was used. The i_{pa} completely disappeared with the use of 7.248 mg/mL (40.0 mM) OHAT, hence all subsequent GOHATCME films were prepared with the addition of 7.248 mg/mL OHAT (figures not shown). This is in agreement with published reports, which show that high adsorption and complete monolayer formation occur when the OHAT content is comparable to the mineral content in the suspension [5-7]. Recall that the concentration of the clay suspension was 7.54 mg/mL, very close to the 7.248 mg/mL (40.0 mM) of OHAT.

Figures 7A and 7B compare the DPV scan of DA and AA at BE and GOHATCME, respectively. It was observed that i_{pa} of AA was produced at the BE but no AA peaks were seen at the GOHATCME. This was true for all trials. This implies that the GOHATCME efficiently discriminated against AA detection.

Mixtures of DA and AA were also analyzed, as shown in the DPV scans in Figures 7A and 7B. At the BE, relatively high i_{pa} was observed indicating the oxidation contribution from both DA and AA. On the other hand, the GOHATCME exhibited i_{pa} that was very much comparable to that of DA alone and much lower than the corresponding peak current at BE, confirming the efficient exclusion of AA. However, the peak current shifted positive, indicative of the fact the presence of both OHAT and AA hindered the intercalation of DA and diminished the catalytic effect of clay on DA.

It is believed that OHAT adsorbs to the clay through chemisorption, forming negatively charged

monolayers on the surfaces and interlayers of the clay. Adsorption of hydroxamates by chemisorption through the lattice metal cations would leave a highly negative net charge on the clay platelets, leading to enhanced exclusion of AA. This implies that the negatively charged monolayer was able to efficiently prevent the AA (also negatively charged) from entering the clay film. Also, high adsorption occurs at pH values close to the pK_a of the OHAT (~ 8). At system pH of 7.4, high OHAT adsorption and negatively charged monolayer formation is expected. The result is a complete negatively charged monolayer of the GOHATCME, which is highly favorable for the exclusion of negatively charged AA [5, 6].

Calibration curve for DA at the GOHATCME was also developed using CV (Figure 5). The linear relationship between the peak current and the DA concentration was within a concentration range of 10.0 μM and 250.0 μM . The detection limit of DA at the GOHATCME was 5.0 μM , slightly higher than that at the GCME (1.0 μM). This is due to the fact that the presence of OHAT monolayer diminished the catalytic effect by the clay on DA. The linear regression equation for DA at the GOHATCME was $i_{pa} = 0.0166x + 0.1153$ ($R^2 = 0.9992$). Similar to BE and GCME, the coefficient of determination (R^2) was very strong, which is an indication that the peak currents are highly correlated with the concentrations. This also implies that the regression models will provide highly accurate predictive concentration values.

3.6 Effect of Scan Rate at GOHATCME

The effect of scan rate was investigated with 100 μM DA at GOHATCME using CV. Scan rates used were 10, 25, 50, 75, and 100 mV/s. A graph of i_{pa} versus scan rate (v) showed a linear relationship, indicative of surface-controlled process (figures not shown). Also, a graph of $\log i_{pa}$ vs $\log v$ gave a slope of 0.30, suggesting a diffusion-controlled process since it is less than 0.53 (the theoretical value or upper limit for purely diffusion controlled processes) [10]. The linear relationship observed between $\log i_{pa}$ and $\log v$ also suggests that the oxidation of DA at GOHATCME is fast with high electrocatalytic efficiency [18]. Anodic peak potential (E_{pa}) changed only slightly with increasing v and there was no linear relationship. The average E_{pa} values (from three trials) at 10, 25, 50, 75, and 100 mV/s were 0.300, 0.313, 0.315, 0.327, and 0.330 V, respectively. These results revealed that the process is simultaneously dominated by both adsorption and diffusion [10], consistent with the fact that OHAT underwent chemisorption on the clay layer surfaces and DA underwent diffusion and intercalation into the clay interlayers.

3.7 Stability of GOHATCME:

Film stability is one of the important factors to consider when developing modified electrodes. As such, stability and durability of the GOHATCME were investigated. Films were left uncovered for one, two, three, and four weeks. Afterwards, the films were scanned with DA daily for five days and then with AA on the sixth day. Reproducible results were obtained for the five days of scanning with DA using the various films. Amazingly, the films were still able to effectively exclude AA despite leaving them for weeks and five times of scanning with DA, indicating a highly stable and effective GOHATCME.

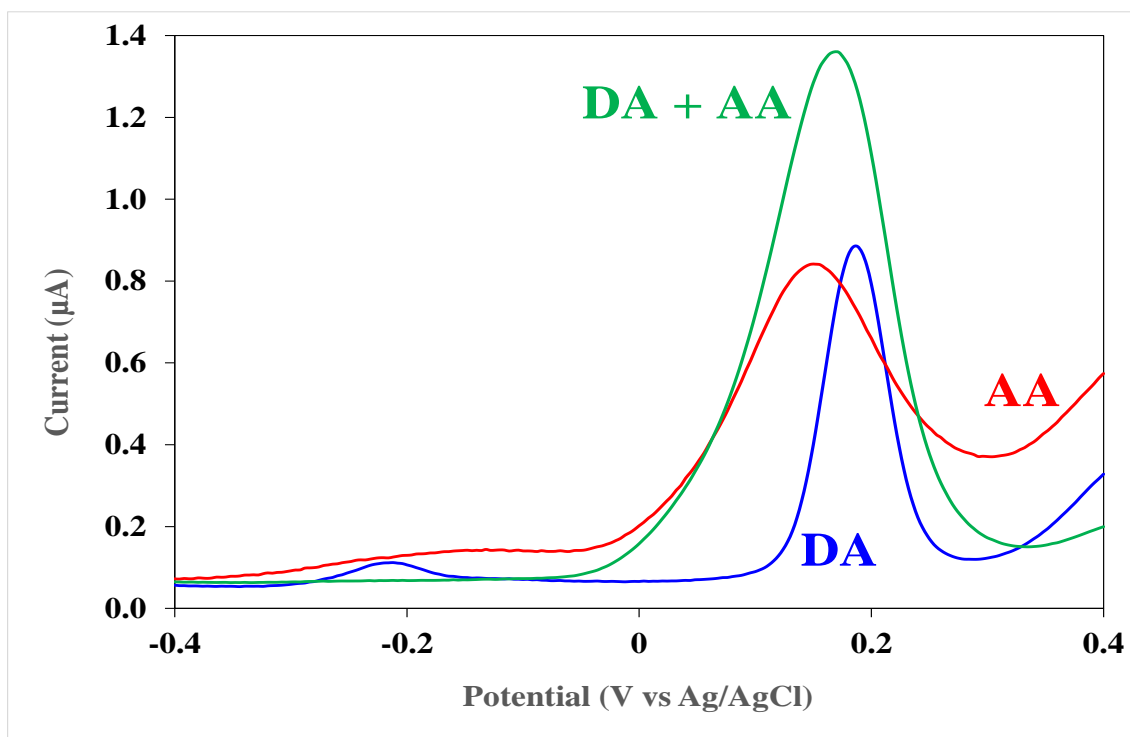


Figure 7A. Differential-Pulse voltammograms for 25.0 μM DA, 250.0 μM AA, and 25.0 μM DA + 250.0 μM AA, in 0.1 M PBS (pH 7.4) at BE.

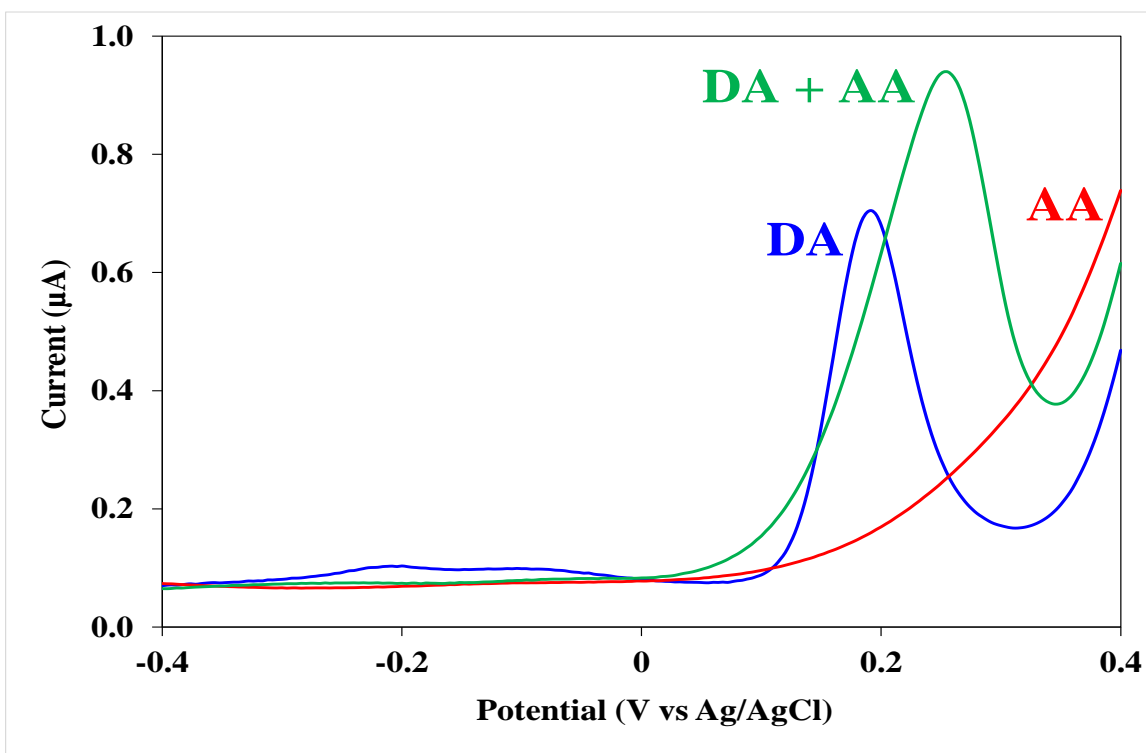


Figure 7B. Differential-Pulse voltammograms for 25.0 μM DA, 250.0 μM AA, and 25.0 μM DA + 250.0 μM AA, in 0.1 M PBS (pH 7.4) at GOHATCME.

4. Conclusion

This work describes a new sensitive, selective, and stable clay composite modified electrode (GOHATCME) for selective voltammetric analysis of charged species. Cationic DA in the presence of excess anionic AA were used as model analytes to probe the GOHATCME films at glassy carbon electrodes. This work presents significant advantages due to preconcentration, charge-exclusion, and electrocatalytic features of clay. Additional advantages include low cost, fast detection, low detection limits, and high precision. It was demonstrated that the GOHATCME modified electrode has the ability to selectively detect DA while excluding excess AA at physiological pH of 7.4. Calibration curves showed strong coefficients of determination (R^2), indicating that peak currents were highly correlated with concentrations. The problems of AA interference, electrode fouling, analyte leaching, and membrane instability were eliminated by this film. The use of only clay could not solve the problem of selectivity, hence the need to incorporate OHAT into the clay interlayers. The OHAT protected the modified electrode from anionic species. The modified electrode proved to be reproducible, durable and stable. On the whole, the DPV technique is a good choice with regards to background discrimination.

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