



## ORIGINAL ARTICLE

**CTAB and 4-Nitroaniline Modified Montmorillonite: A Platform for Nanomolar Detection of Dopamine, Uric Acid and Ascorbic Acid**Pradyumna Goswami<sup>1,\*</sup><sup>1</sup>Assistant Professor (Chemistry), Government Ayurvedic College, Guwahati, 781014, Assam, India

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## ABSTRACT

In this work sodium montmorillonite clay (NaMMT) was organomodified with cationic surfactant molecule Cetyltrimethyl Ammonium Bromide (CTAB) and 4-Nitroaniline (4NA). Glassy carbon electrode (GCE) surface was now modified with that organomodified clay. The new electrode can now be designated as 4NACME. We demonstrated determination of Dopamine (DA) and Uric Acid (UA) in presence of high concentration of Ascorbic Acid (AA) in PBS of pH 7.4 using 4NACME. Modification of GCE offers substantial improvements in voltammetric sensitivity and selectivity towards the determination of DA and UA. It can inhibit the voltammetric response of AA while the redox reaction of DA is promoted. When a square wave voltammetric (SWV) technique was used, the peak separation between DAs and AAs was found to be 979 mV and between UAs and AAs was found to be 435mV. These results were again confirmed by cyclic voltammetric (CV) technique to establish the electrochemical reversibility of DA, UA and AA at 4NACME. From linear calibration plots the slopes ( $\mu\text{A/nM}$ ) and correlation coefficients are found to be 0.0043, 0.0041, 0.0009 and 0.9786, 0.9765, 0.9909 for DA, UA and AA respectively. The detection limits ( $3\sigma$ ) are 8nM, 14nM and 18nM for DA, UA and AA respectively. The modified electrode showed very good reproducibility with relative standard deviation < 3%.

**Keywords:** Montmorillonite; Electrochemistry; Sensing; Dopamine; Uric acid; Ascorbic acid

## INTRODUCTION

Montmorillonite (MMT) clay is widely used in various scientific fields due to its high cation exchange capacity, large specific surface area, good swelling capacity, high platelet aspect ratio and ease of surface modification<sup>1,2</sup>. Layered Smectite type MMT possesses a negative surface charge which is compensated by exchange of cations, such as Na<sup>+</sup> or Ca<sup>2+</sup>. However, surface active agents (surfactants) are widely used in recent researches field, particularly in electrochemical investigation. Due to the specific amphiphilic structure of surfactants, these molecules can be absorbed in the interfaces and surfaces. As organic cations exchange for exchangeable ions on the mineral surfaces, the cations are released into the solution. The organic cations may also enter into ion-exchange reactions with exchangeable cations between the layer surfaces of the clay and hence may be modified to make the clay strongly organophilic<sup>3</sup>. Here, NaMMT was organically modified with cationic surfactant cetyltrimethyl ammonium bromide (CTAB) to improve

NaMMT platelets separation<sup>1</sup>. The inorganic ions in the clay can be effectively replaced with organic cationic surfactant molecules through cation exchange reactions. This results in the expansion of the interlayer spacing, which leads to an increase in the basal spacing. Then 4-nitroaniline (4NA) was intercalated between the interlayer spaces. Now the GCE surface was modified with the 4NA-CTAB-NaMMT clay. In this paper we firstly explored the electrochemical feasibility of 4NA-CTAB-NaMMT clay modified GCE and then applied this modified electrode in detecting bioactive molecule dopamine (DA), uric acid (UA) and ascorbic acid (AA).

DA is one of the monoamine neurotransmitters in mammalian central nervous system whose deficiency may lead to serious diseases such as epilepsy, parkinsonism and senile dementia<sup>4-6</sup>. Moreover, abnormalities in UA levels are symptoms of several diseases such as hyperuricaemia and gout. Other diseases such as leukaemia and pneumonia are also associated with enhanced urate levels<sup>7</sup>. Similarly, AA is

one of the most important vitamins due to its antioxidant and pH regulator properties plays key role in health care, where its ability to increase high density lipoprotein production, down-regulate cholesterol, triglyceride synthesis, lower blood sugar and insulin requirements, thus reducing the risk of cardiovascular diseases, is well known<sup>2</sup>. Therefore, it is essential to develop simple and rapid methods for their determination in routine analysis.

However, there are a number of problems with electrochemical methods due to the nature of the oxidative electrode reaction of dopamine. One of the primary problems is that the concentration of dopamine in the extracellular fluid of the caudate nucleus is extremely low (0.01-1  $\mu\text{M}$ ) for a healthy individual and in the nanomolar range for patients with Parkinson's disease,<sup>8-10</sup> while the concentrations of the main detection interferents, e.g., AA, are several orders of magnitude higher and the interferents undergo oxidation within the same potential window as dopamine.

It is generally believed that direct redox reactions of these species at the bare electrodes are irreversible and high over potentials are generally required for their amperometric detections. Moreover, bare electrodes often suffer from pronounced fouling effect due to the accumulation of oxidized products on electrode surface which results in rather poor selectivity and reproducibility. Oxidation of DA (forms dopamine-o-quinone) at the electrode surface in the presence of AA results in a homogeneous catalytic oxidation of AA. The regenerated DA returns to the electrode, resulting in an enhanced current<sup>11</sup>.

The successful route to overcome the problems of selectivity is to modify the electrode surface, because the modified electrode could decrease the over potential, improve the mass transfer velocity and effectively enrich the substance<sup>12,13</sup>. Various modified materials have been modified on various bare electrodes to solve DA detection problem<sup>14-20</sup>. However, some limitations with these modified electrodes are their poor reproducibility, low sensitivity, stability and high detection limit.

The present research is an attempt to study the effect of 4NA-CTAB-NaMMT clay modified GCE towards DA, UA and AA individually and in the mixture of the trio. It is supposed that in the presence of CTAB, the monomer surfactant molecule with cationic charge inside clay arranged on the anionic surface of the electrode makes the electrode oppositely charged (i.e. positive); so, AA, that has anionic charge, absorb to the electrode surface but DA (has cationic charge) repulsive from the electrode surface. Therefore, initially in the presence of CTAB inside clay, DA oxidation potential shifts to higher potential, AA potential shifts to the lower potential and UA potential lies between the two. Again, organization of cationic surfactant inside NaMMT clay makes the clay hard acid, while DA is apparently a hard base. According to Pearson's principle, hard acids prefer to

bind to hard bases, and soft acids prefer to bind to soft bases<sup>21</sup>. Furthermore, the presence of 4NA (electron donor species) inside clay material converts the analyte into a harder base resulting a strong complexing force between CME and DA. Thereby we can achieve better sensitivity.

Combining the interesting concepts mentioned above, we report here an improved voltammetric method for the determination of DA or UA in the presence of a high concentration of AA using clay-modified electrodes by square wave voltammetry (SWV). The clay modified electrode can largely widen the distance between the oxidation peaks of DA, UA and AA so that the interference for simultaneous determination of DA, UA and AA is largely decreased, because of cooperation of clay, surfactant and 4NA. This strategy offers an effective way for the simultaneous determination of a series of bio-substances.

## EXPERIMENT

### Reagents and chemicals

L-Ascorbic acid was purchased from Loba Chemie Pvt Ltd (India). Dopamine hydrochloride, Uric acid, 4-Nitroaniline and Cetyltrimethyl Ammonium Bromide were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Styrene was provided by Merck Schuchardt OHG (Germany). All reagents are of analytical grade and used without any further purification. Na-Montmorillonite clay was obtained from Prook County, Wyoming, USA. Double distilled water (prepared using Rieviera quartz double distillation apparatus) has been used for all electrochemical experiments. PBS of pH 7.0 and 7.5 were prepared by mixing the stock solutions of 0.05 M NaCl and 0.05 M  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  and then adjusting the pH with 0.05 M  $\text{H}_3\text{PO}_4$  or 0.05 M NaOH. Solutions of DA, UA and AA were prepared by simply dissolving the same in PBS of pH 7.4.

### Instrumental and Experimental set-up

#### Electrochemistry

CHI 600B Electrochemical Analyzer (USA) with a three-electrode cell assembly was used for electrochemical studies. Electrochemical experiments were carried out under a blanket of Nitrogen gas after passing the gas through the solution for 10 minutes. The electrode potential values were reported with respect to the normal hydrogen electrode, NHE. The working electrode is Glassy Carbon (GC) disc, reference electrode is Ag-AgCl (3M NaCl) and Sodium Nitrate (0.1M) is the supporting electrolyte. In the Osteryoung Square Wave Voltammetry (OSWV) experiments, the square wave amplitude is 25 mV, the frequency is 15 Hz and the potential height for base staircase wave front is 4 mV. Prior to every experiment the GC electrode was polished firmly on micro-cloth using fine 0.05  $\mu\text{M}$  alumina powders followed by sonication for 2-3 minutes in double distilled water and then rinsed thoroughly with water.

### Modification of NaMMT

5 g of the NaMMT was dissolved in 500 mL hot water to obtain a uniform dispersion. 2 g of CTAB was dissolved in 200 mL double distilled water. The two solutions were mixed together and stirred vigorously for 6 h at 60°C using a hot plate magnetic stirrer. The solution was centrifuged using high speed REMI 24 centrifuge at 6000 rpm for 5 min. The modified MMT suspension was washed repeatedly using double distilled water and filtrated, until no precipitate appeared when the filtrate was titrated with a 0.1 mol/L AgNO<sub>3</sub> solution. The filtered cakes were dried in a vacuum oven at 80°C for 12 h, and ground using mortar to obtain fine powder of the organo-modified nanoclay<sup>1</sup>. Then, a CTAB-NaMMT clay suspension was prepared by taking 0.135g clay in 40 ml MeOH. Added 0.9g (6 mmol) 4NA to the suspension and stirred well for 2 hours. Allowed another 12 hours to diffuse 4NA. Centrifuge the mixture at 6000 rpm for 5 min, washed repeatedly with MeOH and dried in oven at 50°C. The intercalated clay is now designated as 4NA-CTAB-NaMMT. The modified clay was then characterized by XRD, FTIR and TGA (**Supplementary data**).

### Preparation of 4NACME

A suspension of the modified clay was prepared by mixing 0.1g of the clay in 10mL dichloromethane. 1.0 μL of the suspension was placed on the tip of a pre-cleaned GC electrode surface using a Hamilton micro-syringe and allowed to dry under nitrogen environment. 1.0 μL of a styrene solution (prepared by dissolving 0.1 g styrene in 10 mL of dichloromethane) was dropped over the above modified electrode and again dried under the blanket of nitrogen for 10 minutes. The modified electrode now has been designated as 4NACME.

## RESULTS AND DISCUSSION

### Advantages of 4NACME

The advantage of the combination of clay with CTAB and 4NA was first demonstrated by SWV and then by CV recorded in PBS of pH 7.4 containing 360nM UA. The SWV current responses were found to increase from  $4.217 \times 10^{-8}$  A to  $2.764 \times 10^{-6}$  A with the combination of trio. Similarly in CV experiment we also got two distinct reduction and oxidation peak having potential  $E_{p1} = -0.281$  V,  $E_{p2} = 0.171$  V ( $E_{1/2} = -0.055$  V) and current  $I_{p1} = 1.94 \times 10^{-5}$  A,  $I_{p2} = -1.527 \times 10^{-5}$  A ( $I_{pc}/I_{pa} = 1.27$ ) when scanning between 1.0 and -0.8V. But such distinct peaks were not observed without combination of clay with CTAB and 4NA. The obviously increase in UA response at modified electrode with clay is a good indication of the excellent accumulation ability with the combination of clay with CTAB and 4NA. Similar improvements of peak current were also observed in case of DA and AA also (results not shown).

Additional reason for the combination of clay with CTAB and 4NA is such that even though AA is a major concern for DA and UA detection, in real samples there are also other interferents. The cover up of 4-nitroaniline with clay is primarily designated to protect the surface from many surface-active compounds.

### Effect of pH on the 4NACME

It is clear that DA sensor in physiological application must have high sensitivity at pH 7.4. Therefore, we investigated the effect of pH on the modified electrode. The experimental results showed that both peak current and peak potential of the 4NACME changed with the pH value of the bulk solution (**Supplementary data**).

By comparing both the curve it may be concluded that within pH range 7-8 the peak current reaches maximum value with minimum and consistent  $E_p$  value. But when the pH of the immediate environment was changed to strong basic condition of pH 9 and 10, the peak potential shifted negatively a lot from the consistent value of -0.096 to approximately -0.25V. Hence it is proved that the 4NACME showed the highest sensitivity at the lowest oxidation potential value in physiological environment (pH 7.4). It is promising as a sensor for real in vivo measurement of DA. The pH dependency of the peak potential indicates that protonation/deprotonation is taking part in the charge transfer process.

### Electrochemistry of DA, UA and AA at 4NACME

Square wave voltammogram (SWV) of the 4NACME in PBS of pH 7.4 is demonstrated in **supplementary data**.

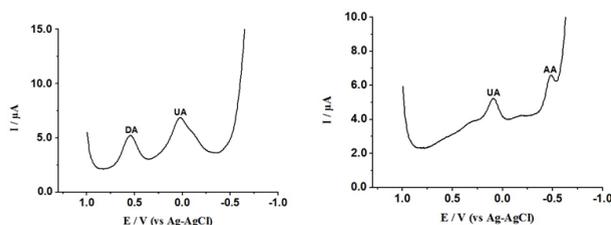
It shows an oxidation peak at potential -0.076V with peak current  $1.925 \times 10^{-6}$  A. Cyclic voltammogram of the modified electrode also shows a pair of redox peaks with redox potential value  $-0.090 \text{ V} \pm 0.005 \text{ V}$  (not shown). The redox peak currents found to increase linearly with scan rates (**Supplementary inset**). This linearity tells the electrochemical reversibility of the modified electrode.

SWV of 4NACME were also conducted for 200nM DA, 360nM UA and 960nM AA separately in PBS of pH 7.4 with 6 s preconcentration time at open circuit (**Supplementary data**).

As shown in **supplementary data** the peak potential for DA was found at 0.620V with peak current  $0.879 \times 10^{-6}$  A, peak potential of UA was found at +0.024V with peak current  $2.764 \times 10^{-6}$  A and peak potential of AA was found at -0.320V with peak current  $1.073 \times 10^{-6}$  A.

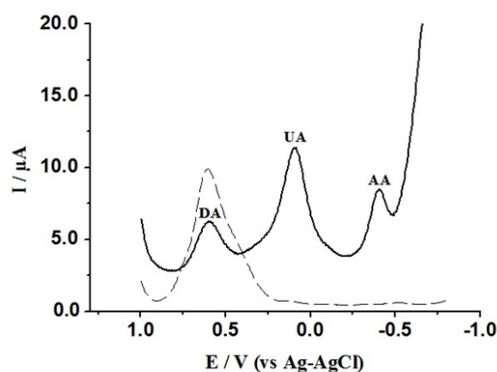
It can now be expected that the problem for the electrochemical detection of DA due to overlapped voltammetric response of coexisted species AA and UA can be solved with the 4NACME. We tried to record the SWV response of 200 nM DA in presence of 360 nM UA and 360 nM UA in presence of 960 nM AA (Figure 1). Two peaks were observed with peak potential 0.595 V and 0.020 V which

were confirmed to be for DA and UA by comparing with individual DA & UA peak as shown in **supplementary data**. Similarly in Figure 1 also two peaks observed at 0.034V and at -0.380V were confirmed for UA and AA respectively by comparing with individual UA & AA peaks as shown in **supplementary data**.



**Fig. 1:** Square wave voltammogram of a mixture of 200nM DA and 360nM UA and a mixture of 360nM UA and 960nM AA at 4NACME in PBS of pH 7.4

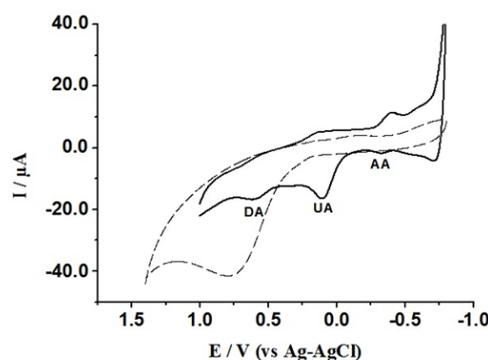
It is well known that AA widely coexists with DA and UA in biological sample. Therefore, eliminating AA interference is an important target for any DA and UA analytical method. A comparison on the performance of the 4NACME to a mixture of 200nM DA, 360nM UA and 960nM AA in PBS of pH 7.4; over the bare GCE are shown in Figure 2.



**Fig. 2:** Square wave voltammogram of a mixture of 200nM DA, 360nM UA and 960nM AA at bare GCE (dashed line) and 4NACME (solid line) in PBS of pH 7.4

As evident from the Figure 2, in case of 4NACME three separated peaks were observed at potential value +0.604, +0.060 and -0.375V with peak current value  $2.81 \times 10^{-6}$ A,  $6.049 \times 10^{-6}$ A and  $2.622 \times 10^{-6}$ A respectively ( $I_{UA}/I_{DA} = 2.15$ ,  $I_{UA}/I_{AA} = 2.30$ ). The peaks at +0.604V, +0.060V and -0.375V was confirmed to be for DA, UA and AA by comparing their individual peaks (**figures in supplementary data**). After modification of the bare GCE, the oxidation potential for UA and AA shifted to negative potential and three well-resolved anodic peaks were present at the 4NACME. These results were again confirmed by taking CV of 4NACME in PBS of pH 7.4 containing mixture

of 200nM DA, 360nM UA and 960nM AA (Figure 3). It is well known that the reversibility of the electrochemical reaction of DA, UA and AA at bare GCE is slow, indicating the sluggish electron transfer kinetics of these compounds at bare GCE, which may be related to the electrode fouling caused by the deposition of these compounds and their oxidation products on the electrode surface<sup>22</sup>.

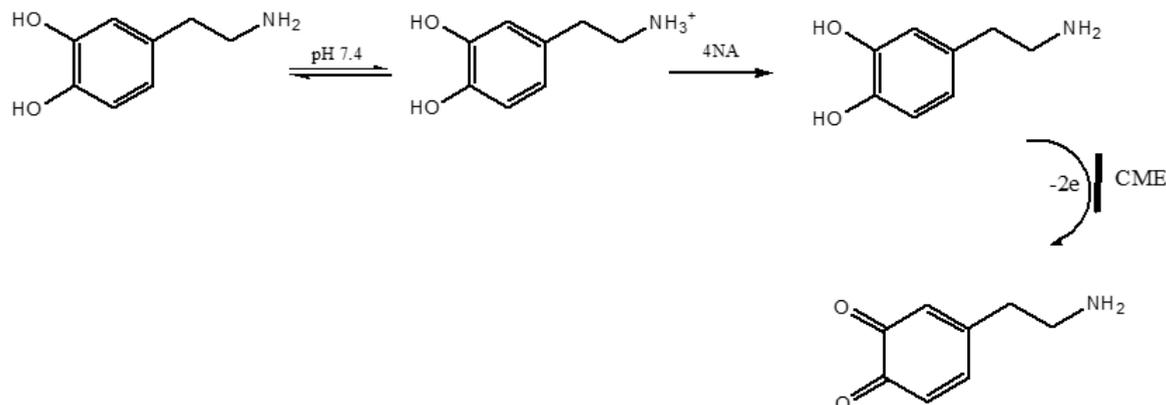


**Fig. 3:** Cyclic voltammogram of a mixture of 200nM DA, 360nM UA and 960nM AA at bare GCE (dashed line) and 4NACME (solid line) in PBS of pH 7.4 (Ag-AgCl is the reference electrode, scan rate:  $0.1 \text{ Vs}^{-1}$ )

In Figure 3 three couples are observed at  $E_{1/2}$  values +0.600V, +0.045V and -0.365V which were confirmed to be for DA, UA and AA respectively by comparing their individual peaks (**supplementary data**). The anodic and cathodic peak potentials of DA appear at 0.613V and 0.587V respectively; with potential peak separation 0.026V which indicate the reversibility of DA at 4NACME (It also indicates the involvement of two electrons in DA oxidation at 4NACME). The peak potentials for oxidation of UA occur at 0.073V and 0.017V with peak separation value 0.056V which again indicate the reversibility of the reaction. Similarly for AA oxidation at 4NACME surface we observe anodic and cathodic peak potentials at -0.328V and -0.401V respectively with peak separation value 0.073V indicating reversibility.

The reversibility of electrochemical reaction of DA, UA and AA was also demonstrated by plotting peak current versus scan rate in their respective individual CV experiments (results not shown). These results demonstrate that 4NACME not only catalyses the oxidation of DA, UA and AA, but also dramatically enlarge the peak separation among DA, UA and AA in their mixture. The increase in separation of peak potentials, and the enhanced sensitivity, allows simultaneous determination of DA, UA and AA.

Linear calibration plots are obtained over the 20-200nM, 20-360nM and 40-960nM concentration of DA, UA and AA respectively in PBS of pH 7.4. The slopes ( $\mu\text{A/nM}$ ) and correlation coefficients are found to be 0.0043, 0.0041, 0.0009 and 0.9786, 0.9765, 0.9909 for DA, UA and AA respectively (**Supplementary data**).



Scheme 1:

The detection limits ( $3\sigma$ ) are 8nM, 14nM and 18nM for DA, UA and AA respectively. Note that these detection limits are very low as compared to many other methods. To characterize the reproducibility of the 4NACME, repetitive measurement-regeneration cycles were carried out in a PBS of pH 7.4 containing 200nM DA, 360nM UA, 960 nM AA. The modified electrode showed very good reproducibility with relative standard deviation  $< 3\%$ .

We have tried to understand the mechanism of oxidation of DA, UA and AA on 4NACME surface. There are different possible interactions that involved in the oxidation of ascorbate anion, DA and UA at the 4NACME in PBS of pH 7.4. It is supposed that in the presence of CTAB, the monomer surfactant molecule with cationic charge inside clay arranged on the anionic surface of the electrode makes the electrode oppositely charged (i.e. positive). The voltammetric response of ascorbate anion at the CTAB induced clay film has been due to the electrostatic interaction between the ascorbate anions and the cationic fixed sites of CTAB induced clay film. The amino group of DA is expected to be charged positively at pH 7.4. The slow catalytic effect of the clay film towards the DA molecule is attributed to the electrostatic repulsion between the organomodified clay film and positively charged DA in PBS of pH 7.4. Therefore, initially in the presence of CTAB inside clay, DA oxidation potential shifts to higher potential, AA potential shifts to the lower potential and UA potential lies between the two. However, in the present study, organomodified clay film also affects the electrochemical oxidation of DA with a significant sensitivity. One possible explanation for this is that the effect generated due to the electrostatic repulsion might have compensated according to the mechanism shown in Scheme 1.

Organization of cationic surfactant inside NaMMT clay makes the clay hard acid, while DA is apparently a hard base.

According to Pearson's principle, hard acids prefer to bind to hard bases, and soft acids prefer to bind to soft bases<sup>21</sup>. Furthermore, DA possesses a functional group of  $-\text{NH}_3^+$  at pH 7.4. Presence of 4NA inside clay causes the deprotonation of  $-\text{NH}_3^+$ . Evidently,  $-\text{NH}_2$  is more nucleophilic than  $-\text{NH}_3^+$ . DA can therefore turn into a harder base with the presence of 4-nitroaniline and hence achieving an even stronger binding force between the 4NACME and DA. Thereby we can achieve better sensitivity.

## CONCLUSION

In this work we have organically modified NaMMT clay with cationic surfactant CTAB and organic molecule 4NA. This modification was characterized by XRD, FTIR and TGA. GCE surface was now covered with that organomodified clay. The new electrode can now be designated as 4NACME. We demonstrated the selective and sensitive determination of DA and UA in presence of high concentration of AA in PBS of pH 7.4 using 4NACME. A novel approach for the utilization of cationic surfactant and 4NA inside layered smectite type MMT in electroanalytical applications is described in this work. The modified electrode can stop the homogeneous catalytic oxidation of AA during the oxidation of DA and UA in a mixture of the trio. The 4NACME showed exceptional stability and relatively lower detection limit for the determination of DA compared to several surfaces mentioned in the literature<sup>17,18</sup>.

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