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Utilization of sodium montmorillonite clay for enhanced electrochemical sensing of amlodipine

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Nanosize surface of sodium montmorillonite has been prepared via sonication and deposited on glassy carbon electrodes for use as working electrode in a highly sensitive electrochemical biosensor for the detection of trace amounts of the calcium channel blocking drug, amlodipine. The cyclic voltammetric behaviour of amlodipine is studied in the *p*H range of 1.0−13.0. In alkali medium (*p*H 13.0) the sensor shows good response. Cyclic voltammograms show one oxidation peak and one broad reduction peak, which may be due to the oxidation of secondary amino group and reduction of chlorine and nitro groups respectively. Plots of log peak current and potential when correlated with log scan rate, indicate irreversible electron diffusion controlled redox reaction. The optimum conditions have been established by differential pulse stripping voltammetry. The anodic peak current is linear with concentration of the analyte at optimum conditions; the detection limit has been determined to be 0.01 µg/mL. A simple, sensitive and time-saving differential pulse stripping voltammetric procedure has been developed for estimation of amlodipine in its formulations as tablets.

Keywords: Electroanalytical chemistry, Electrochemistry, Sensors, Clays, Sodium montmorillonite, Cyclic voltammetry, Differential pulse stripping voltammetry, Amlodipine

For several decades now, the utilization of sodium montmorillonite (NaMM) has been popular in electrochemical studies. NaMM is smectite clay with high chemical stability, good adsorption and good penetrability, thus making it suitable as effective clay modified glassy carbon electrode for electrochemical studies. Electrochemical behavior of small redox substances incorporated into the clay modified membrane has been studied extensively[1]. Montmorillonite modified electrodes have been widely employed for the determination of trace analysis of pollutants[2-5].

Calcium, an essential chemical in the process of muscle contraction, is directly involved in the electrochemical reactions that allow muscle cells to contract as part of a regular heartbeat. Calcium is vital for maintaining good health, as it is involved in many every day cellular processes, including gene regulation, memory and cell death. However, excessive levels of calcium can lead to arrhythmias, as well as hypertrophy, apoptosis and cardiac remodeling. By virtue of their ability to increase nitric oxide (NO) production, it is believed that calcium channel blockers (CCBs) may be involved in the inhibition of platelet aggregation[6]. Amlodipine besylate is a dihydropyridine derivative with calcium antagonist activity[7]. It is used mainly as an anti-hypertensive and anti-anginal agent. The main metabolic pathway is by the oxidation of dihydropyridine ring to its pyridine analog[8]. Chromatographic techniques such as GC[9], LC[10] and HPTLC[11] have been applied for estimation of amlodipine besylate in tablets and biological fluids. Amlodipine besylate has also been determined by spectrophotometric methods[12]. Chromatographic methods offer a high degree of selectivity but need sample clean-up and relatively heavy instrumentation. A differential pulse stripping voltammetric method[13] has been described for the determination of amlodipine besylate at *p*H 5.5. The polarographic and voltammetric behavior of some calcium antagonist drugs at the dropping mercury and glassy carbon electrodes have also been reported[14].

Thus, the determination of calcium channel blocker such as amlodipine, with enhanced sensitivity and utilization of natural materials assumes significance. The present study is aimed at the electrochemical behavior and assay of amlodipine at a NaMM clay modified glassy carbon electrode.

Experimental

Electroanalytical studies were carried out on a CH Instruments electrochemical workstation (model 760C). The calcium channel blocker drug, amlodipine, was received from Sri International Pharmaceuticals, Mumbai, India and used as such.

The stock solutions were made up in methanol/doubly distilled TKA-LAB purified water (80:20). In aqueous (80:20) methanol media of Britton Robinson buffer tablet (4.0, 7.0, 9.2), KCl and H_2SO_4 (0.1 mol dm^{-3}) were used as supporting electrolyte for the analysis. Montmorillonite KSF was purchased from Acros Organics, Belgium.

Purging and blanketing of nitrogen were carried out for the analyte solution placed in the electrochemical cell of 15 mL capacity for 25 min under stirring prior to recording the voltammograms. In order to get reproducible results, the glassy carbon electrode was pretreated in two ways: (a) mechanical polishing over a velvet micro-cloth with an alumina suspension, and, (b) electrochemical treatment by applying a potential of 1.5 V for 2 s.

For preparation of the sodium montmorillonite modified glassy carbon electrode, the montmorillonite clay (5 g) was washed with Ultrapure water and dried at ambient temperature. Mixtures of the washed montmorillonite KSF clay samples (1 g) and sodium chloride (5 mL, 0.5 *M*) were ultrasonicated for 4 h and then kept for 1 h. The clay was centrifuged, washed thoroughly with water and dialyzed to remove the salt before being dried. Ultrasonication of about 0.1 g of sodium montmorillonite in 10 mL water and 100 mg of carboxy methylcellulose (CMC) was done for 1 h. Sodium montmorillonite colloid (SMC) was prepared from the purified montmorillonite powder as described previously[5]. An aliquot of SMC (120 µL) was mixed with 100 mg of carboxy methylcellulose (CMC) and then the mixture was diluted with deionized water to obtain a pretreated sodium montmorillonite colloid (PSMC). Aliquots of PSMC (5 µL) were deposited onto glassy carbon electrodes (GCE, 3 mm dia.) and allowed to dry under ambient conditions to form a montmorillonite layer on the electrode surface. The resulting clay-modified electrode which was used as the working electrode, showed no peak in cyclic voltammetry. The montmorillonite modified electrode was kept in the electrolyte solution for 5 days and thereafter, a CV was taken. There was only 4.2% decrease in the background current. After 5 days, the montmorillonite deposits partially peeled off from the glassy carbon electrode surface. The continuous potential cycling of the adsorbed montmorillonite for 48 h resulted in only 5% decrease in the current. Distilled water was used to carefully remove and clean the coating, followed by surface treatment to clean the glassy carbon electrode after each experiment. Nitric acid (6 *M*) solution was used to clean the cell.

Results and discussion

The voltammetric behavior of amlodipine calcium channel blocker drug was examined in the *p*H range 1.0–13.0 by recording their cyclic voltammograms. The effect of the *p*H on the peak current and potential is presented in Fig. 1(a & b). The dependence of *p*H on peak current results in a maximum at *p*H 13.0. Since the best shape and sensitivity of the peak current was obtained at *p*H 13.0 for the drug, this *p*H value is considered most suitable for the stripping voltammetric analysis. Figure 2 shows the

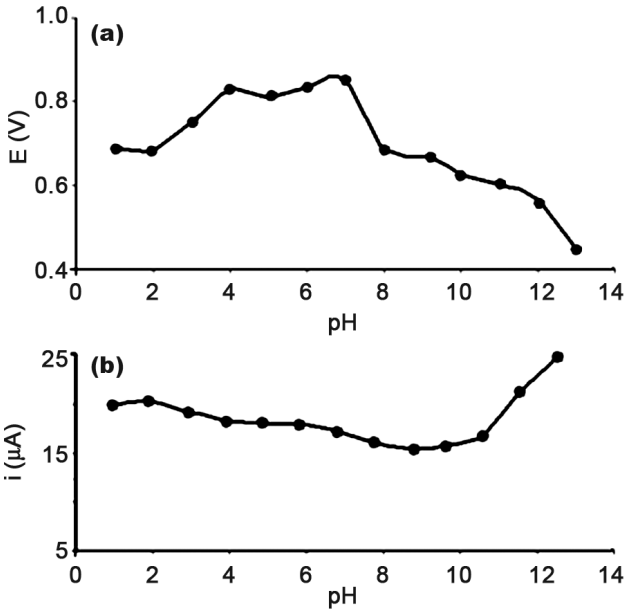

Fig. 1 – Effect of *p*H on (a) potential and (b) peak current, in the voltammetric behavior of amlodipine.

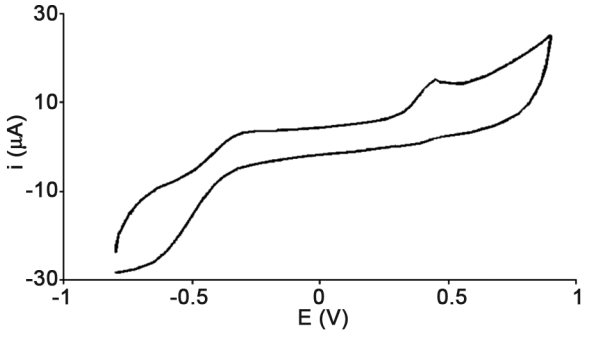

Fig. 2 – Cyclic voltammogram of amlodipine on NaMM/GCE at *p*H 13.0.

representative cyclic voltammogram of 100 μg/mL of the drug at *p*H 13.0. In acid medium, one well defined peak around 0.5 V was observed, while another one peak with poor characteristics was also observed at more negative potential. These two peaks may be due to oxidation of the secondary NH group and reduction of chlorine/nitro groups respectively. The negative shift of the peak potential towards more negative values with increasing *p*H indicates that the protonation step precedes the electron transfer. The nature of the oxidation process was studied by following the effect of the scan rate on the peak current. Both ip versus $\nu^{1/2}$ and log ip versus log ν plots were linear, indicating a diffusion-controlled process (Fig. 3(a & b)). Peak potential of both peaks shifts towards more negative values with the increase of the *p*H. The irreversibility of oxidation of the drug at NaMM/GCE was ascertained from the larger difference in anodic and cathodic peak potentials at all *p*Hs in the range of 1.0–13.0.

Cyclic voltammetric results reveal good electroactivity of the substrate on the electrode at *p*H 13.0. Differential pulse mode was employed for stripping voltammetric studies and good performance was seen in the determination of the drug on clay modified glassy carbon electrode. Experiments were carried to find out the best accumulation potential at the chosen *p*H 13.0 with solution containing 0.1 μg/mL of the drug. The accumulation potential (E_{acc}) was varied from −05 to 0.6 V at deposition time of 15 s. The maximum peak current was observed for an accumulation potential at -0.1 V for amlodipine (Fig. 4). This may be due to electrostatic attraction between the protonated substrate and negatively charged working electrode. Deposition time was varied from 15 s to 90 s and the differential pulse voltammogram was recorded. The maximum current response was observed at 15 s for amlodipine. The initial scan potential (ip) is also an important parameter like accumulation potential. The initial scan potential was varied between −0.5 and 0.3V for drug and the maximum stripping peak current was observed at −0.2 amlodipine.

The accumulation of the antihypertensive drug, amlodipine, on the modified electrode surface under the optimum accumulation conditions was identify from the changes in the electrode surface before and after accumulation. SEM was employed to study the surface morphology of the accumulated drug on NaMM coated glassy carbon electrode. The SEM image of NaMM shows small uniform granular surface as previously reported[15-18]. The drug amlodipine was adsorbed on NaMM electrode during accumulation and exhibited large flake-like structure (Fig. 5). Due to the better accumulation, stripping leads to good results and hence stripping parameters was optimized.

The factors affecting the stripping step, i.e., primary oxidation process, were varied and optimum conditions were arrived at. The influences of pulse height, pulse width and potential scan increment were studied by varying their values and the maximum peak current conditions were obtained. The range of study and optimized conditions are presented in

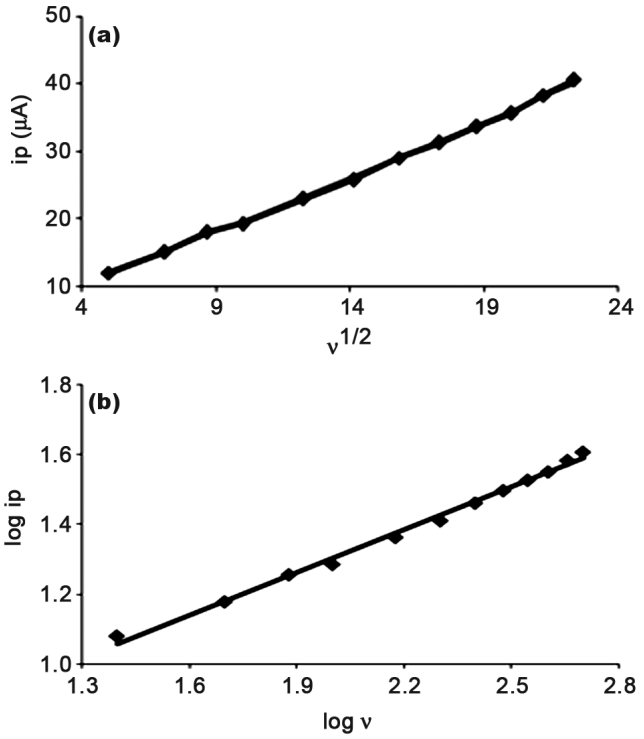

Fig. 3 – (a) Plot of peak current versus scan rate, and, (b) plot of log peak current versus log scan rate.

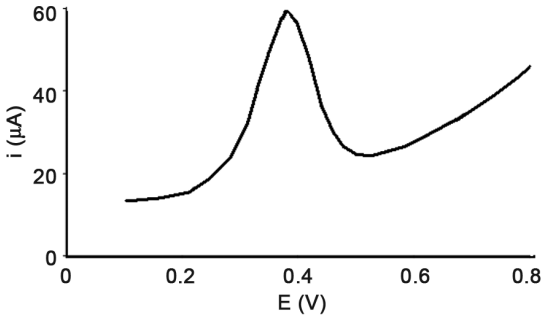

Fig. 4 – Plot of peak current versus accumulation potential.

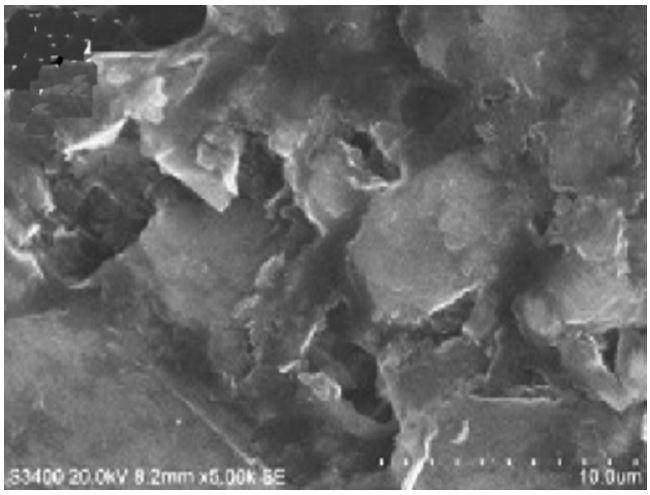

Fig. 5 – SEM image of amlodipine adsorbed on NaMM electrode.

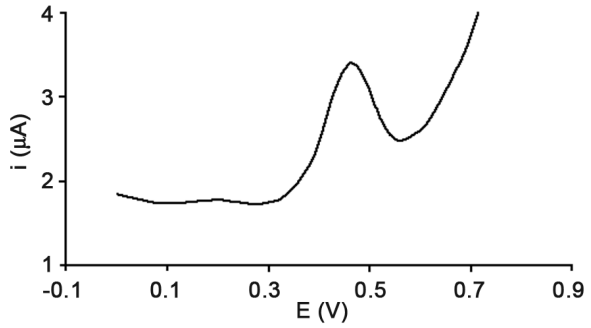

Fig. 6 – DPSV of amlodipine under optimum conditions.

Table 1. The optimum conditions that resulted in maximum peak current response were used to study the effect of analyte concentration.

Since differential pulse stripping voltammetry (DPSV) is a technique with a better peak shape and higher sensitivity, DPSV was employed for the determination of the drug. Figure 6 shows the DPSV curves of the drug, amlodipine, under optimum conditions. The experimental results showed that the peak current increased with the increase in concentration of drugs. In the range studied from 0.03–0.35 μg/mL for amlodipine, the anodic peak current is linearly proportional to the concentration of the drug. The calibration plot indicates the linear dependence of peak current with concentration under optimum experimental conditions. The limit of detection was found to be 0.01μg/mL for amlodipine. The reproducibility of the stripping signal was realized in terms of relative standard deviation for seven readings at a concentration level of 0.05 μg/mL.

Table 1 – Optimum experimental conditions in DPSV studies

Variable	Range studied	Optimum value
*p*H	1-13	13.0
Accumulation potential (V)	0–0.6	0.3
Accumulation time (s)	15–90	15
Initial scan potential (V)	-0.2 to 0.3	0.1
Pulse height (PH) (mV)	25–150	50
Pulse width (PW) (ms)	25–150	50
Scan increment (SI) mV	2–20	4
Stirring rate (rpm)	50–250	250
Rest period (s)	2–10	5

Table 2 – Estimation of amlodipine by DPSV with the proposed electrode

Sample	Amlodipine (mg)		% RSD
	Cert.	Expt.	
Amace (Systopic)	5	4.90	2.4
Amlodac (Zydus Medica)	10	9.86	2.5
Amlopres (Cipla)	10	9.75	2.3
Myodura (Wockhardt)	10	9.92	2.2
Lama (Stadmed)	10	9.85	2.8
Card (Jagsonpal)	10	9.91	2.1
Calchek (Ipca)	10	9.95	2.0

The pharmaceutical formulations of amlodipine in the form of tablets from various pharmaceutical companies were analyzed. Stripping voltammograms of the drug at *p*H 13.0 were recorded under optimum conditions. The concentration of the calcium channel blocker in commercial formulations determined by the proposed method was in good agreement with the value certified by the manufacturer (Table 2).

In the present study, the electrochemical oxidation of calcium channel blocker drug at a sodium montmorillonite modified GCE was studied in different buffer solutions and a validated voltammetric procedure described for its determination. The proposed procedure provides a sensitive and simple approach to the determination of drugs in pharmaceutical formulations. The accuracy, reproducibility, simplicity and selectivity suggest its application in quality control analysis, clinical laboratories and pharmacokinetic studies.

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