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Electrochemical determination of calcium channel blocker drugs using multiwall carbon nanotube-modified glassy carbon electrode

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Abstract

Background: Calcium channel blockers belonging to the dihydropyridine family have been used as a potent arterial vasodilator in the management of angina and cardiovascular diseases. In the design of glassy carbon electrode coatings for the study of the same, multiwall carbon nanotubes (MWCNT) are favored. They are known for the reduction and oxidation of electroactive species towards cathodic and anodic direction with the simultaneous enhancement of the peak current.

Results: The MWCNT-modified glassy carbon electrode exhibited a sharp anodic peak potential at around at 0.5 V for amlodipine (AMLD) andnimodipine (NIMD), and 0.4 V for felodipine (FELD) in the cyclic voltammograms. The antihypertensive drugs were determined using a simple two-step procedure developed which comprised a preconcentration step followed by the differential pulse stripping voltammetric quantification. Concentration calibrations were linear within the range from 0.01 to 0.3 μg/mL for AMLD and FELD, and 0.025 to 0.3 μg/mL for NIMD.

Conclusion: The lower limit detection (LOD) was found to be very low on modified electrode. The LOD is 0.005 μg/mL for AMLD and FELD, and 0.01 μg/mL for NIMD. The prepared electrode showed an excellent electrocatalytic activity towards the oxidation of antihypertensive drugs leading to a remarked improvement in sensitivity. An electrochemical sensor featuring the MWCNT-modified electrode was applied successfully as the result for the calcium channel blocker determination in pharmaceutical samples.

Keywords: Amlodipine, Felodipine, Nimodipine, Electrochemical, Multiwall carbon nanotubes

Background

Calcium channel blocker belonging to the dihydropyridine family has commonly been used as a potent arterial vasodilator in the management of angina and cardiovascular diseases [1]. Electrochemistry plays an important role to study the formation of radical and its reactivity in one-pot systems [2,3]. There are several reports in literature concerning the development of stable carbon nanotube (CNT)-based electrodes for environmental samples, electrochemical sensors, electrocatalysis, and electrochemical estimation of drugs and compounds of biological interest [4,5]. However, simple but effective method for the development of homogeneously and stably assembled CNT-based electrode is particularly desired for electroanalytical determinations. Multiwall carbon nanotube (MWCNT)-based electrodes were prepared generally by casting MWCNT suspension on conventional electrode surface [6,7]. The resulting electrodes have been successfully utilized in the sensitive detection of various biological molecules such as uric acid [4], folic acid [6], and cytochrome c [7]. Generally, MWCNT-based electrodes enhance the detection sensitivity and improve reversibility as they can promote electron transfer [8].

Amlodipine besylate is a dihydropyridine derivative with calcium antagonist activity [9]. The main metabolic pathway is oxidation of dihydropyridine ring to the pyridine analog [10]. Amlodipine (AMLD) has side effects such as peripheral edema (often), dizziness, palpitations,

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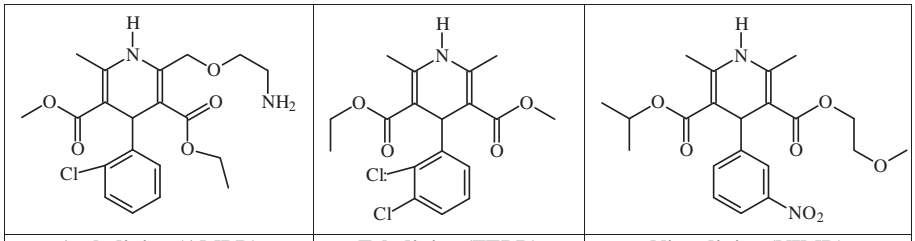

Amlodipine (AMLD)	Felodipine (FELD)	Nimodipine (NIMD)
CAS NO. 88150-42-9	CAS No.72509-76-3	CAS: 66805-59-4

Scheme 1 Calcium channel blocker drugs.

muscle-, stomach- or headache, dyspepsia, nausea (very often), blood disorders, development of breasts in men (gynecomastia), impotence, depression, insomnia, tachycardia, and gingival enlargement (sometimes). The amlodipine besylate in tablets and biological fluids was determined using chromatographic techniques such as gas chromatography (GC) [11], liquid chromatography (LC) [12], and high performance thin layer chromatography [13]. Chromatographic methods offer a high degree of selectivity but need sample clean-up and relatively heavy instrumentation. Few spectrophotometric methods have been reported for amlodipine besylate determination [14]. A differential pulse voltammetric method has been described for the determination of amlodipine besylate in tablets at pH 5.5 [15]. The electrochemical behavior at the dropping mercury and glassy carbon electrodes (GCE) of some calcium antagonist drugs has been reported [16].

Nimodipine (NIMB), a dihydropyridine calcium channel antagonist, has potent vasodilatory effect on cerebral vessels and increases cerebral blood flow. Nimodipine, in high doses (2 to 10 mg/kg), has been shown to improve memory retention and/or memory recall process in aged rats. The Food and Drug Administration has classified the side effects into groups based on dose levels given every 4 h. For the high dosage group (90 mg), less than 1% of the group experienced adverse conditions including itching, gastrointestinal hemorrhage, thrombocytopenia, neurological deterioration, vomiting, diaphoresis, congestive heart failure, hyponatremia, decreasing platelet count, disseminated intravascular coagulation, and deep vein thrombosis. The electrochemical reduction of 4-nitrophenyl-1,4-dihydropyridine compounds such as nifedipine, nitrendipine, nimodipine, nicardipine, and furnidipine in protic media [17] follows the general pattern of nitroaromatic compounds involving a single four-electron step producing the hydroxylamine derivative. However, the electrochemical reduction of nitrendipine, nimodipine, and nifedipine in mixed aqueous-DMF media resulted in the generation of the one-electron reduction product, the nitro radical anion [18].

Cyclic voltammetry was also performed on insulin, valsartan, and felodipine (FELD) separately with the potential window applied from −0.5 V to +1.00 V vs. Ag/AgCl. Infusion of valsartan and felodipine has been reported to lower the striatum glucose level significantly [19]. Stereospecific determination of nimodipine was reported using an ES-OVM column with detection at 230 nm, resulting in a high specific separation of enantiomers with a limit of

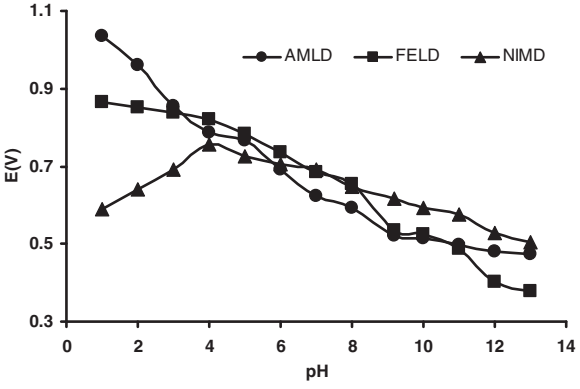

Figure 1 Plot of peak potential vs. pH.

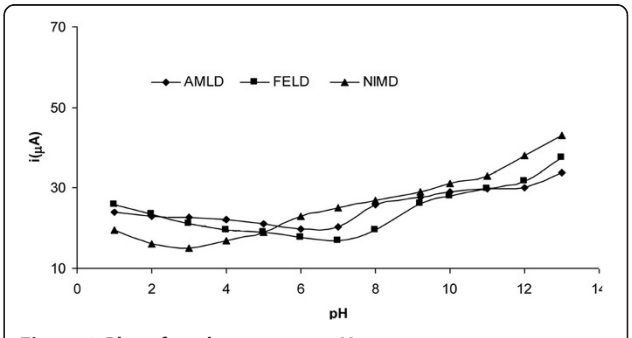

Figure 2 Plot of peak current vs. pH.

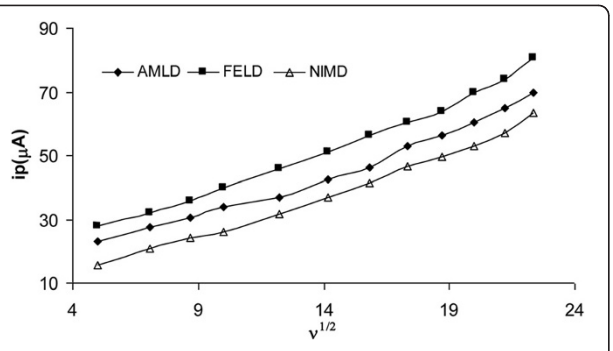

Figure 3 Plot of peak current vs. square root of scan rate.

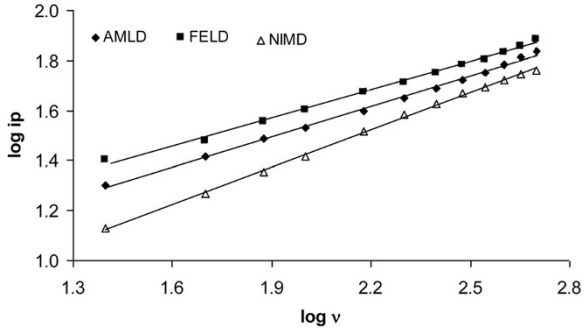

Figure 4 Plot of log peak current vs. log scan rate.

quantitation for each enantiomer of about 1 ng/ml from 1-ml plasma [20]. The stability of the retention volumes of the enantiomers of nimodipine by LC has been checked to guarantee the reproducibility of enantiomer collection to allow automated LC analysis [21]. Introduction of felodipine as an enantioselective determination method for felodipine in human plasma has been described involving off-line detection using capillary GC on a DB-1 column with ECD following chiral separation on a Chiralcel OJ column (Chiral Technologies, Illkirch, France) monitored at 240 nm [22]. Quantitation limits of 0.1 ng/ml were demonstrated for a sample size of 0.5-ml biological fluid, while felodipine [23] and nisoldipine [24] were finally determined by GC-MS in PEI mode [25].

Until now, no publications concerning the electroanalytical determination of antihypertensive drugs in pharmaceutical formulations are available in the literatures. Therefore, the aim of the present investigation is to investigate the electrochemical behavior of antihypertensive drugs on MWCNT-modified GCE and to develop sensitive stripping voltammetric methods for their determination.

Methods

Electrochemical workstation (760C model, CH Instruments, TX, USA) was employed mainly for carrying out electroanalytical studies. The three calcium channel blocker drugs of amlodipine, felodipine, and nimodipine (Scheme 1) were received from CIPLA Ltd, Mumbai, India and used as such.

The stock solutions were made up in methanol/double distilled purified water (TKA-LAB, Thermo Electron LED GmbH, Germany) (80:20). For studies in aqueous methanol media, Britton-Robinson buffers, 4.0, 7.0, 9.2, and 0.1 moldm^{-3} KOH, and 0.1 mol dm^{-3} H_2SO_4 were used as the media for the analysis. Multiwall CNT produced by arc method was purchased from Sigma-Aldrich (MO, USA) and sodium dodecyl sulfate (SDS) from Merck (Merck & Co., Inc, NJ, USA).

Purging of nitrogen was done for analyte solution placed in the electrochemical cell of 15-ml capacity for 25 min under stirring, and then voltammograms were recorded while blanketing nitrogen gas. To get reproducible results, great care was taken in the electrode pretreatment. The GCE was pretreated in two ways as described earlier [26].

Preparation of MWCNT-modified GCE

One milligram MWCNT was dispersed in 1 mL of 0.1 M sodium dodecyl sulfate using an ultrasonicator to give black suspensions [27]. Cast films were prepared by placing 5 μL of the MWCNT/surfactant suspensions on GCE and then evaporating them in an oven at 50°C.

Results and discussion

In the experiments carried out for the effect of pH, pH 13.0 was chosen as the best for further electrochemical studies. In the case of experiments on the influence of modifier, the *i*p and the *E*p values are presented in Figures 1 and 2. As it is observed, the MWCNT-modified electrode gives higher peak intensity values for all the pharmaceuticals. The modifier also influences the *E*p values, although the shifts caused in the peaks of the analytes are not significantly relevant.

In the investigation of cyclic voltammetric behavior of drugs, the peak potentials correlated well with log scan rate and resulted in straight lines. The fractional '*α*n' value calculated from the slope confirmed irreversible electron transfer. The peak current showed an increasing trend with sweep rate and straight lines with good correlation coefficients, indicating adsorption. The plots, i_p vs. $v^{1/2}$, were curve lines (Figure 3). The log peak current vs. log scan rate showed straight line (Figure 4), and its slope value is above 0.5. Thus, it is further confirmed that adsorption of the substrate on the pores and surface of the electrode and the overall reaction was adsorption-controlled.

In the investigation of differential pulse stripping voltammetric behavior of drugs, maximum peak current was observed for AMLD, FELD, and NIMD at 30, 20, and 10 s respectively. The decreased current above the

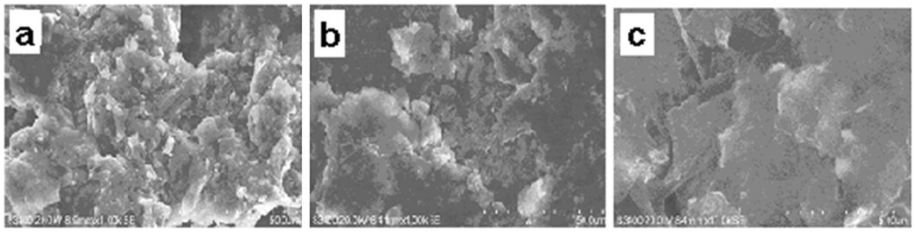

Figure 5 SEM photographs. (**a**) AMLD, (**b**) FELD, and (**c**) NIMD on MWCNT-modified GCE.

maximum current signal condition might be due to the saturation of the electrode surface and blocking of the products formed on the surface. The accumulation of the drugs on the modified electrode surface was ascertained by carrying out SEM analysis.

SEM was employed to study the surface morphology of the three accumulated drugs on MWCNT-coated glassy carbon electrode. The stem-like structure of the coating confirmed the presence of MWCNTs on GCE. The average tube size of the material is 50 nm as reported earlier by us [27,28]. The drug AMLD was adsorbed on MWCNT electrode during the accumulation and exhibited granular sponge-like structure (Figure 5a). FELD exhibited broken pitch and sponge-like structure (Figure 5b), and NIMD exhibited tide sponge-like structure (Figure 5c). Different surface morphology confirmed the accumulation of drugs on the MWCNT/GCE.

The initial scan potential was also an important parameter in controlling both peak potential and peak height in the stripping voltammogram. The initial potential was varied between −0.4 to 0.2 V, and an initial scan potential at −0.3 V for AMLD, −0.1 V for FELD, and 0 V for NIMD led to higher peak current response. Pulse height was varied between 0.025 and 0.25 V. This variation had shown a decrease in peak current with increase in applied pulse height after 0.05 V. Hence, pulse height of 0.05 V was chosen due to increased current response for all drugs. The effect of pulse period demonstrated that the stripping peak current increased up to 50 ms and then decreased with an increase in pulse width from 75 to 125 ms for three drugs. The peak current decreased with an increase in pulse width from 25 to 100 ms, and a pulse width of 50 ms was selected. Thus, the maximum peak current conditions were arrived at, and the results are presented in Table 1. These conditions were used to study the effect of concentration.

Analytical characteristics

Typical differential pulse stripping voltammograms for AMLD, FELD, and NIMD obtained under the maximum peak current experimental conditions were presented in Figure 6. As the concentration of the drugs increased, the stripping peak current increased. Calibration plots were made and presented in Figure 7. The limits of concentration were 0.01 to 0.3 μg/mL for AMLD and FELD, and 0.025 to 0.3 μg/mL for NIMD. The LOD is 0.005 μg/mL for AMLD and FELD, and 0.01 μg/mL for NIMD. The precision of the method was ascertained by measuring the peak current of the drugs' response in five standard samples. Ten replicates were analyzed, and standard deviations were calculated. The relative standard deviation was 2.5% for a concentration 50 μg/mL of AMLD, 2.7% for the same concentration of FELD, and 3.1% for 50 μg/mL of NIMD. The low value of standard deviation indicated good reproducibility and feasibility of this method for the determination of drugs.

Table 1 Optimum experimental conditions in DPSV

Variable	Range studied			Optimum value		
	AMLD	FELD	NIMD	AMLD	FELD	NIMD
pH	1 to13	1 to 13	1 to 13	13.0	13.0	13.0
Accumulation potential (V)	0 to 0.6	0 to 0.6	0 to 0.6	0.3	0.4	0.3
Accumulation time (s)	15 to 90	15 to 90	15 to 90	15	30	30
Initial scan potential (V)	−0.2 to 0.3	−0.2 to 0.3	−0.2 to 0.3	0.1	0.1	0
Pulse height (PH; mV)	25 to 150	25 to 150	25 to 150	50	75	50
Pulse width (PW; ms)	25 to 150	25 to 150	25 to 150	50	50	50
Scan increment (SI; mV)	2 to 20	2 to 20	2 to 20	4	6	6
Stirring rate (rpm)	50 to 250	50 to 250	50 to 250	250	250	250
Rest period (s)	2 to 10	2 to 10	2 to 10	5	5	5

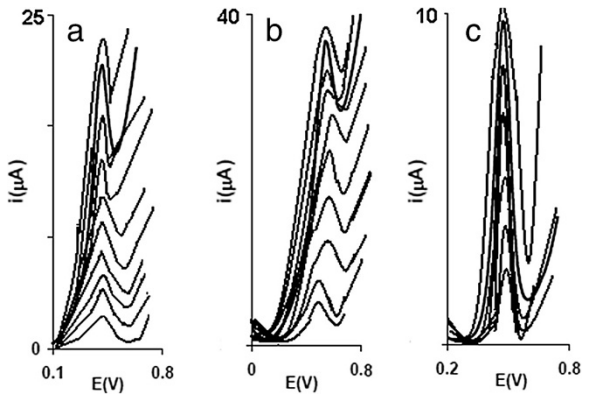

Figure 6 Differential pulse stripping voltammetry. DPSV of (**a**) AMLD (**b**) FELD and (**c**) NIMD under optimum conditions on MWCNT-modified GCE.

In the square wave voltammetric determination of AMLD on SWCNT/EPPGE and MWCNT/EPPGE for 1.0 × 10^{-9} mol L^{-1} and 5.0 × 10^{-9} mol L^{-1} [29], respectively, 0.025 μg mL^{-1} by spectrofluorimetric method was reported [30]. Another compound of FELD was also reported: 0.3 to 1.5 μg cm^{-1} by AAS [31] and range studies of 1.0 to 40.0 μg/mL by spectrophotometric method [32].

Selectivity of electrode is very important. Among the electrode system, the modified MWCNT is very sensitivity for all three drug compounds. Range studies are also very low compared to those reported in the literature since MWCNT was selected for the determination of the drugs.

The reproducibility of electrode was also an important parameter for electrochemical determination technique. The MWCNT modified system is highly stable up to 100 cycles of experimental conditions. After the 100 cycles, the reproducibility decreased slowly. The number of experiments continued from101to 200 cycles, and the 10% reproducibility decreased.

Pharmaceutical sample analysis

In order to evaluate the applicability of the proposed method, three commercial samples in combination or in pure form containing anyone of AMLD, FELD, and NIMD were selected. The pharmaceutical samples were collected from medical shops at Karaikudi, Tamilnadu, India. Various tablets having AMLD, FELD, and NIMD were examined for the estimation of content of drugs. The tablets were dissolved in methanol, and then the filtrate was further evaporated to get the drug in pure form. The residue was dissolved in known quantity of methanol and transferred into a 250-ml calibrated flask and made up to the mark. A 10-ml portion of this solution was transferred into a 50-ml calibrated flask, and 0.1 mM NaOH containing 50% aqueous methanol was used to dilute the contents of the flask to the required volume. The standard addition method was used. An aliquot of 0.05 ml of the 0.1 μg/mL standard stock solution was added to the solution prepared as described above. Differential pulse stripping voltammetric studies under the maximum current signal experimental conditions were carried out and the trace amount of drugs in the sample were determined. A relative standard deviation of 2.9% was obtained for 0.1 μg/mL AMLD for ten identical measurements. The relative standard deviation of 2.9% was obtained for 0.1 μg/mL of FELD and NIMD for ten identical measurements. Thus, the suitability of this method for the determination of AMLD, FELD, and

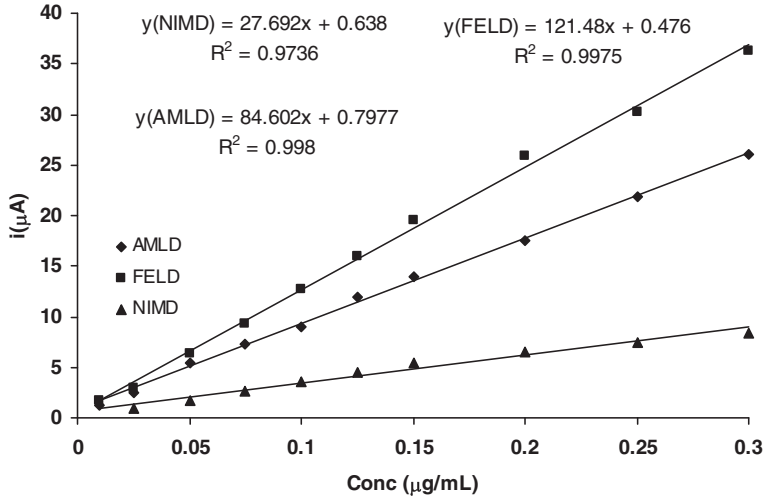

Figure 7 Calibration plot of peak current vs. concentration.

Table 2 Amount of drugs in tablets determined by DPSV in tablets

Brand name	Company name	Tablets (mg)	Experimental value(mg)	% RSD value
Amlodipine				
Amace	Systopic	5	4.80	2.1
Amlodac	ZydusMedica	10	9.96	2.2
Amlopres	Cipla	10	9.85	2.1
Myodura	WOCKHARDT	10	9.90	2.2
Lama	Stadmed	10	9.88	2.5
Card	Jagsonpal	10	9.95	2.3
Calchek	Ipca	10	9.94	2.7
Felodipine				
Felogard	Cipla	10	9.93	2.1
Plendil	Astra Zeneca	10	9.90	2.4
Nimodipine				
Nimodip	USV	30	29.27	2.5

NIMD in real sample was verified. The results are presented in the Table 2.

Experimental

Effect of pH

Britton-Robinson buffer/acid/alkaline solution was selected as the support electrolyte to find the optimal pH values for every analyte. The range of pH investigated was from 1.0 to 13.0. Values outside of this interval did not give either oxidation or reduction waves. Moreover, in some cases, signals were very close to the discharging current of the background, making the quantification very difficult. The pH affects both peak potential (*E*p) and peak intensity (*i*p) values. With respect to the first parameter, *E*p values for the three pharmaceuticals decrease (or increase in absolute value) with pH, being more positive (Figure 1). Figure 2 shows the dependence of *i*p with respect to pH for all the three drugs. From the curves, the optimal pH values for every one of them were deduced. This study was focused in order to find particular zones of potential for every compound that allowed the sequential determination of the three pharmaceuticals in a unique biological sample.

Influence of modifier

*i*p and the *E*p values using unmodified GCE and MWCNT-modified carbon electrode were studied. The modifier is expected to give to higher *i*p value and the longest distance from the discharging current of the background.

Cyclic voltammetric behavior of drugs

Cyclic voltammetric behavior of amlodipine, felodipine, and nimodipine on MWCNT-modified GCE was carried

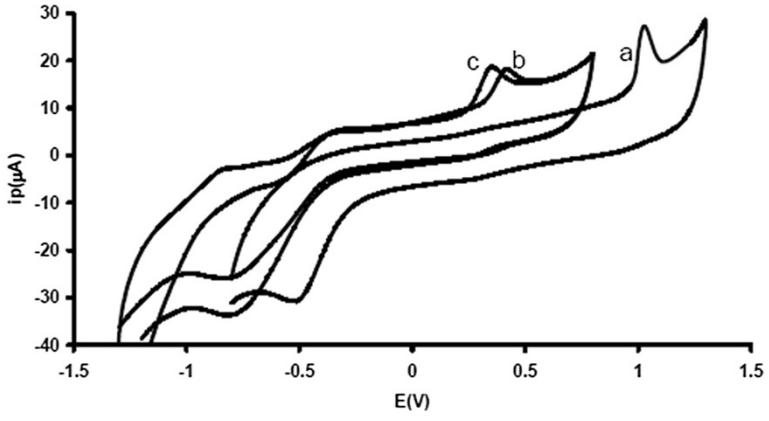

Figure 8 Cyclic voltammogram. (**a**) 305 μg/mL amlodipine, (**b**) 190 μg/mL felodipine, and (**c**) 210 μg/mL nimodipine on GCE at pH 13.0. Scan rate is 100 mV/s.

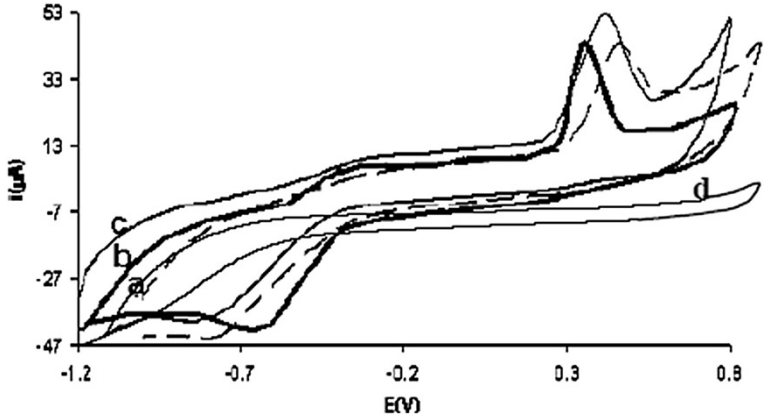

Figure 9 Cyclic voltammogram. (**a**) 305 μg/mL amlodipine, (**b**) 190 μg/mL felodipine,(**c**) 210 μg/mL nimodipine, and (**d**) absence of drugs on MWCNT/GCE at pH 13.0. Scan rate is 100 mV/s.

out in pH 13.0. Figures 8 and 9 represent the cyclic voltammograms recorded for all the three drugs on GCE and modified glassy carbon electrode. They exhibited one oxidation peak with larger current and one reduction peak with lesser current for modified GCE, but bare GCE shows lower current and high potential difference from modified electrode. The anodic peak was taken for further discussion due its analytical characteristic because of larger peak current.

Differential pulse stripping voltammetry

Differential pulse stripping voltammetry (DPSV) experiments were carried out to ascertain the best conditions for the adsorption process. Many preconcentration-stripping experiments were performed for different accumulation potentials and at an accumulation time of 15 s to evaluate the electrostatic attraction/repulsion between electrode surface and the drugs. When accumulation potential changed from −0.1 to +0.5 V, the maximum responses were obtained at 0.1 V for all three drugs. Maximum peak current was found for an accumulation potential in the positive region at 0.1 V because of the electrostatic interaction between the positive nature of electrode at this potential and the electron-rich substrate. After fixing the accumulation potential, the accumulation time was varied between 10 to 60 s.

Conclusion

MWCNT-modified GCE allowed the successful determination of AMLD, FELD, and NIMD drugs with a detection limit of 0.005 μg/mL for AMLD and FELD, and 0.01 μg/mL for NIMD. The anodic peak current varies linearly under optimized conditions in the concentration range from 0.01 to 0.3 μg/mL for AMLD and FELD, and 0.025 to 0.3 μg/mL for NIMD. The results obtained are promising and demonstrate the utility of the developed method for the determination of drugs in pharmaceutical formulations. The specificity of the voltammetric method was also investigated in the presence of substances present in drugs. Thus, the present investigation revealed that the proposed method is simple, specific, sensitive, and effective for the determination of three calcium channel blocker at MWCNT-modified glassy carbon electrode in pharmaceutical formulations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AMS carried out all the experimental components of the study. CV and PM monitored the results and conclusions drawn by AMS. All authors read and approved the final manuscript.

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AMS is an assistant professor of the Department of Chemistry, Velammal Engineering College, Chennai, India. CV is an assistant professor of the Department of Chemistry, V.O.C College, Tuticorin, India. PM is currently the director of Distance Education and professor and head of the Department of Industrial Chemistry, Alagappa University, Karaikudi, India.

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