CHAPTER 4

ELECTROCATALYTIC OXIDATION OF AMLODIPINEBESYLATE WITH PHENYLHYDRAZINE AS A MEDIATOR AT CARBON PASTE ELECTRODE
Chapter 4—Electrocatalytic oxidation of amlodipinebesylate with phenylhydrazine

4.1. Introduction

The anodic oxidation of amlodipinebesylate has been studied at a carbon paste electrodes by electrocatalytic effect of phenyl hydrazine homogeneous mediator, using cyclic voltammetry and linear sweep voltammetry as diagnostic technique. The cyclic voltammetry study showed that, the catalytic current of the system depends on the concentration of amlodipinebesylate magnitude of the peak current for phenyl hydrazine increased sharply in the presence of phenylhydrazine, and proportional to amlodipinebesylate concentration.

4.2. Chemistry and Biological Relevance of Amlodipinebesylate

Amlodipinebesylate 2[(2-aminoethoxy) methyl]-4-(2-chloro-phenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl,5-methylester besylate Fig.1 is a dihydro pyridine derivative with calcium antagonist activity. It is used mainly as an antihypertensive and anti anginal agent.

![Structure of Amlodipinebesylate](image)

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and 2 nondihydropyridine binding sites. The contractile
processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extra cellular calcium ions into these cells through specific ion channels.

Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Negative inotropic effects can be detected in vitro but such effects have not been seen in intact animals at therapeutic doses. Serum calcium concentration is not affected by amlodipine. Within the physiologic pH range, amlodipine is an ionized compound (pKa=8.6), and its kinetic interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociation with the receptor binding site, resulting in a gradual onset of effect.

Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. After oral administration of therapeutic doses of NORVASC, absorption produces peak plasma concentrations between 6 and 12 hours. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of NORVASC is not altered by the presence of food.

Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine. Ex vivo studies have shown that approximately 93% of the circulating drug is bound to plasma proteins in hypertensive patients. Elimination from the plasma is biphasic with a terminal elimination half-life of about 30–50 hours. Steady-state plasma levels of amlodipine are reached after 7 to 8 days of consecutive daily dosing.

Amlodipine (Besylate) is a calcium channel blocker. It lowers the blood pressure, relaxes heart muscles and dilates the heart blood vessels to prevent spasm. It has been
known since the late 1800s that calcium influx was necessary for the contraction of muscle. It inhibits the calcium influx across the cell membrane.

4.3. Review of Electrochemistry of Amlodipinebesylate

The literature revealed that all the reported methods for the assay of amlodipinebesylate in tablets and biological fluids rely on the use of chromatographic techniques such as GC, LC, and HPTLC. Few spectrophotometric methods have been reported for amlodipinebesylate determination. The drug and its formulation are not official in USP or BP pharmacopoeia. The assay procedure listed. Chromatographic methods offer a high degree of selectivity but need sample clean up and relatively heavy instruments.

The hydrazines constitute an important class of xenobiotic agents occurring in natural organisms, industrial settings, and medical therapeutics. Agents with a hydrazine functionality can be metabolized to radical intermediates which have toxic effects, such as carcinogenesis and haemolysis. Phenyl hydrazine is one of the most widely distributed organic pollutants, which may irritate the eyes, the skin and the trachea, and may produce a rapid haemolysis, resulting in kidney impairment, liver impairment and total anaemia. It is produced and used in the manufacture of rocket propellant, dyes, pesticides and pharmaceuticals, and discharged into the environment through various waste streams. Furthermore, it is also ingested in considerable quantities by the human population in edible mushrooms and tobacco. Here tofore, previously reported analytical methods for phenylhydrazine in water include titration, spectrophotometry. Detection of phenyl hydrazine based on lectin-glycoenzyme multilayer-film modified biosensor. Electrocatalytic oxidation of hydrazine with pyrogallol red as a mediator on glassy carbon electrode. Identifying quinine-like species on the surface of graphite carbon and multi-walled carbon nanotubes using reactions with 2,4-dinitrophenylhydrazine to provide a voltammetric fingerprint. The voltammetric
 behavior of amlodipinebesylate in tablets or biological fluids using differential-pulse or square-wave adsorptive stripping voltammetry at glassy carbon electrode. The polarographic and voltammetric behaviors at the dropping mercury and glassy carbon electrodes (GCE) of some calcium antagonist drugs have been reported. A literature survey revealed that no attempt has been made to study the voltammetric behavior of amlodipinebesylate with phenylhydrazine as a mediator at a carbon paste electrode using cyclic voltammetry. The diffusion nature of the drug at the carbon paste electrode surface from the bases for the electro analytical determination of amlodipinebesylate with phenyl hydrazine mediator.

4.4. Experimental

Reagents and chemicals

Amlodipinebesylate was obtained from [Aldrich] Company. The stock solution of amlodipinebesylate [25mM] was prepared in methanol. The diluted solutions were prepared daily by accurate dilution with deionized water just before use and should be protected from light. Tetra sodium pyro phosphate buffer [0.1M, pH 9], 2N sulphuric acid and 0.1N sodium hydroxide were used to get the required pH. Phenyl hydrazine was prepared by using double distilled water. All the chemicals used were of analar grade.

Procedure

To prepare a blank solution, 25ml of buffer solution [Tetra sodium pyrophosphate, sulphuric acid, pH 9.0] 25ml of phenylhydrazine [0.5mM] solution were transferred in to a 25ml volumetric flask. Then the solution was transferred in to the electrochemical cell [with three electrodes, carbon paste as a working, saturated calomel as a reference electrode and a platinum wire as an auxillary electrode]. The initial and final potentials were adjusted to +0 to +1000 mV vs. SCE, respectively, with a scan rate of 100mVs⁻¹.
4.5. Results and Discussion.

Electrocatalytic oxidation of amlodipinebesylate.

Our experiments showed that phenyl hydrazine acts as a suitable intermediate for electron transfer in the oxidation of amlodipinebesylate at the surface of the carbon paste electrode. The amlodipinebesylate peak current increases sharply in presence of phenyl hydrazine and the peak potential of phenyl hydrazine is in lower potential than that of a amlodipinebesylate. Therefore, phenyl hydrazine was considered as a suitable homogenous electro catalyst for amlodipinebesylate by electrochemical determination. The proposed cyclic voltammetric method for the determination of amlodipinebesylate is based on the following sequence of reactions.

\[
\text{Phenylhydrazine}^{\text{aq}} \rightarrow \text{(Phenylhydrazine) ox}^{\text{aq}}
\]

\[
\text{(Phenylhydrazine) ox}^{\text{aq}} + \text{Amlodipinebesylate}^{\text{aq}} \rightarrow \text{Phenylhydrazine}^{\text{aq}} + \text{(Amlodipinebesylate) ox}^{\text{aq}}
\]

As it can be seen in Diagram 1, in the first step phenyl hydrazine can be oxidized at the surface of the carbon paste electrode, then in the presence of amlodipinebesylate, the oxidized mediator can oxidize amlodipinebesylate and converts to its initial form, while the phenyl hydrazine itself can be oxidized further so the peak current of amlodipinebesylate increases in the presence of phenyl hydrazine.

![Diagram 1](attachment:diagram.png)

The cyclic voltammetric responses of a carbon paste electrode in tetra sodium pyrophosphate buffer [pH 9.0] without and with amlodipinebesylate are shown in Fig.
4.1(a) and 4.1(b), respectively. A small anodic current by the oxidation of amlodipinebesylate is observed but no cathodic peak found, indicating an irreversible homogeneous transfer in the system. In the presence of phenyl hydrazine with the same amlodipinebesylate concentration, a large anodic peak is observed without a cathodic counterpart Fig. 4.1(c), that the current observed is associated with amlodipinebesylate is demonstrated by comparing the current in the curve 4.1(c) with that in the curve 4.1(b), which shows the cyclic voltammetric behaviour of an electrode in 0.5mM phenyl hydrazine solution in a amlodipinebesylate free solution. (Tetra sodium pyrophosphate buffer pH 9.0). It is apparent that the anodic current associated with phenyl hydrazine is significantly less than that obtained in solution containing amlodipinebesylate. The anodic peak potential for oxidation of amlodipinebesylate in the presence of phenyl hydrazine is near +622mV vs. SCE. [Fig. 4.1(c)], while amlodipinebesylate starts to oxidize at about +590mV vs. SCE [Fig. 4.1(b)] in the absence of mediator under identical conditions, so a decrease in over potential 30mV is observed. In the presence of amlodipinebesylate the oxidized phenyl hydrazine reacts with amlodipinebesylate presented in the solution and converts into an initial form. This reaction is an EC\(^1\) and because of relatively fast charge transfer between amlodipinebesylate and oxidized form of phenyl hydrazine, the peak current depends on the diffusion of amlodipinebesylate to diffuse layer of phenylhydrazine and cause the phenylhydrazine peak current to increase, therefore, \( \Delta i_p \) depends on amlodipinebesylate concentration. The anodic oxidation of amlodipinebesylate in aqueous solution has been studied extensively on glassy carbon electrode. In aqueous solution amlodipinebesylate is oxidized mainly through a two electron process with the final product \(^{24}\).
In order to get information on the rate-determining step, a Tafel plot was
developed for the blank containing phenyl hydrazine only (Fig. 4.2) derived from data of
the following part of the current voltage curve at a scan rate of 50mVs⁻¹. A slope of 6.25
mV decade⁻¹ is obtained indicating that the process follow four electron transfer in rate
determining step and charge transfer coefficient of α = 0.2.

Influence of variables

The influences of chemical variables such as pH, phenyl hydrazine concentration,
and instrumental variable such as scan rate on the sensitive were studied.

Effect of pH

The voltammetric behavior of the system was characterized at various pH values.
The results showed that pH effects of the electrochemical behaviour of phenyl hydrazine
and also amlodipinebesylate activity (Fig. 4.3). It can be for the phenyl hydrazine
solution when the pH was varied from 4.0 to 8.0, the phenyl hydrazine peak current
increased and from 8.0 to 10.0, the peak current decreased, in the presence of
amlodipinebesylate when the pH was increased from 6.0 to 8.0 the peak current increased
and the peak potential shifted towards more negative values. Since the pKa of
amlodipinebesylate is 8.6, below this pH value amlodipinebesylate will be present in its
protonated form. Fig. 4.4 shows the effect of pH on the electrocatalytic oxidation of amlodipinebesylate at carbon paste electrode in the presence of phenyl hydrazine. The sensitivity increased with increasing pH from 4.0 to 8.0 and then decreased for higher pH values. However the better sensitivity and shape of the voltammogram of the peak at [pH =9.0] suggested it as optimal pH value.

**Effect of phenylhydrazine concentration**

The influence of phenylhydrazine concentration on the peak currents was studied for the range of 0.25-2.0 mM phenylhydrazine concentration in the solutions containing different concentrations at [pH=9.0] (Fig. 4.5a,b). The results showed that by increasing phenylhydrazine concentration up to 0.5mM the peak current increased, where as higher concentration of phenylhydrazine causes a slight decrease on the magnitude of peak current due to the formation of phenylhydrazine aggregations. After 2.0mM the peak was disappeared therefore 0.5mM phenylhydrazine concentrations were selected as the optimal mediator concentration.

**Effect of amlodipinebesylate concentration**

The cyclic voltammogram showed successive enhancement of peak current on increasing amlodipinebesylate concentration. The plot of peak current (obtained by measuring the peak height) verses the respective concentration of amlodipinebesylate was found to be linear in the range 0.5 to1.0 mM. The variation of peak current ($i_p$) with amlodipinebesylate concentration in the linear concentration range Fig. 4.6a,b.

**Effect of scan rate**

The dependence of peak current ($i_p$) as well as peak current function ($i_p/ACv^{-1/2}$) on the scan rate (v) were studied in the range 50-350 mVs$^{-1}$ a linear relationship was
observed between log/ip and log v Fig. 4.7a was linear with a correlation coefficient of 0.997 and this behavior was consistent with the EC nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step $^{29,30}$. The plot of $i_p/v^{1/2}$ vs. log v indicated an increase in peak current with an increase in sweep rate Fig. 4.7b confirming that the electrode process at the electrode surface has some adsorption. Also the plot of peak potential ($E_p$) vs. logarithm of scan rate Fig. 4.7c was linear.

**Effect of surfactants**

To study the effects of surfactants, the experiments were carried out using cationic surfactant cetyl trimethyl ammonium bromide (CTAB) and anionic surfactant sodium dodecyl sulphate (SDS). Initially, cyclic voltammograms were recorded for a solution containing 0.5mM amlodipinebesylate and 0.5mM phenyl hydrazine in supporting electrolyte [pH=9.0]. Keeping the concentration of amlodipinebesylate and phenylhydrazine constant, the concentration of surfactant was varied from 0.5 to 2.5 x 10^{-5} M and the cyclic voltammograms were recorded at a scan rate 100mVs^{-1} as shown in Fig. 4.8a,b. In both the surfactants after 0.5 x 10^{-5} M there was a slight decrease in the peak current and peak potential $^{31}$.

**4.6. Conclusion**

The new method approach is cyclic voltammetry for the determination of amlodipinebesylate, is reproducible, selective and sensitive. The importance of the technique is its ability to determination of amlodipinebesylate with phenylhydrazine as electrocatalyst, while for the determination it does not need to prepare as for modified electrode.
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Fig. 4.1. Cyclic voltammograms of a solution containing reactants at a carbon paste electrode with a scan rate of 100 mV s⁻¹ at pH 9.0, the buffer, (a) solid line in the absence of amlodipinebesylate, (b) line in the presence of 0.5 mM amlodipinebesylate and (c) in the presence of 0.5 mM amlodipinebesylate and Phenylhydrazine.

Fig. 4.2. A Tafel plot at pH 9.0, 0.1M KCl and 0.5mM Phenylhydrazine.
Fig. 4.3a. Effect of pH on the peak potential, 0.5mM amlodipinebesylate, in 0.1M pyro buffer, scan rate 100mVs⁻¹.

Fig. 4.3b. Effect of pH on the peak current, 0.5mM amlodipinebesylate, in 0.1M pyrophosphate buffer, scan rate 100mVs⁻¹.
Fig. 4.4. Influence of pH on the electro-oxidation of different amlodipinebesylate concentrations (0.6, 0.7, 0.8 and 0.9 mM) at a carbon paste electrode. Conditions: 0.5 mM Phenylhydrazine, 0.1 M pyrophosphate buffer, scan rate 100 mV s⁻¹, potential range of 0 to +1000 mV vs SCE, pH values are (■) 4.0, (•) 5.0, (▲) 6.0, (▼) 7.0, (♦) 8.0, (+) 9.0 and (x) 10.0
Fig. 4.5a. Effect of Phenylhydrazine concentration on the peak current conditions amlodipinebesylate concentrations (■) 0.6, (○) 0.7, (▲) 0.8, (▼) 0.9 and (△) 1.0mM, pH of 9.0, 0.1M pyrophosphate buffer, scan rate 100mVs⁻¹.

Fig. 4.5b. Effect of Phenylhydrazine concentration on the peak current conditions for amlodipinebesylate, a = 0.0, b = 0.25, c = 0.75, d = 0.5mM, pH of 9.0, 0.1M pyrophosphate buffer, scan rate 100mVs⁻¹.
Fig. 4.6a. Effect of amlodipinebesylate concentration, pH of 9.0, 0.1M pyro phosphate buffer, scan rate 100mVs⁻¹.

Fig. 4.6b. Effect of amlodipinebesylate concentration, a = 0.6, b = 0.7, c = 0.8, d = 0.9 and e = 1.0mM, pH of 9.0, 0.1M pyro phosphate buffer, scan rate 100mVs⁻¹.
Fig. 4.7a. Variation of the logarithm of peak current with the logarithm of the sweep rate for 0.5 mM amlodipine besylate and 0.5mM Phenylhydrazine, pH of 9.0, 0.1M pyrophosphate buffer.

Fig. 4.7b. Dependence of $i_p/v^{1/2}$ on logv for peak $i_a$ of 0.5 mM amlodipinebesylate and 0.5mM Phenylhydrazine, pH of 9.0, 0.1M pyro phosphate buffer.

Fig. 4.7c. Plot of $E_p$ vs logarithm of sweep rate of 0.5 mM amlodipinebesylate and 0.5mM Phenylhydrazine, pH of 9.0, 0.1M pyrophosphate buffer.
Fig. 4.8a. Effect of CTAB on the peak current, a =0ml, b =1ml and c =0.5ml CTAB, pH of 9.0, 0.1M pyrophosphate buffer, scan rate 100mVs\(^{-1}\).

Fig. 4.8b. Effect of SDS on the peak current, a =0ml, b =1ml and c =0.5ml SDS, pH of 9.0, 0.1M pyrophosphate buffer, scan rate 100mVs\(^{-1}\).
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4.7. References


