Electrochemical Oxidation at a Glassy Carbon Electrode of the Anti-Arrhythmia Drug Disopyramide

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Abstract: A voltammetric study of the oxidation of disopyramide has been carried out using a glassy carbon electrode. The electrochemical oxidation of disopyramide was investigated by cyclic, differential pulse, and square wave voltammetry. The oxidation of disopyramide is an irreversible, diffusion-controlled process. The diffusion coefficient of disopyramide was calculated in pH 7.0 phosphate buffer to be $D_{\text{disopyramide}} = 3.8 \times 10^{-6}$ cm$^2$ s$^{-1}$. The oxidation of disopyramide is also pH dependent and for electrolytes with pH between 4 and 7 occurs with the transfer of one electron and one proton. In alkaline electrolytes, two consecutive charge transfer reactions are observed: both oxidation reactions involve the transfer of two electrons but only the first also involves the transfer of two protons. Two procedures for the analytical determination of disopyramide in pH 7.0 phosphate buffer were developed and compared and a detection limit LOD = 1.27 μM was obtained.

Keywords: Disopyramide, voltammetry, oxidation, anti-arrhythmic drug

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INTRODUCTION

Disopyramide, alpha-(2-(diisopropylamino)ethyl)-alphaphenyl-2-pyridine-acetamide, Scheme 1, is a drug known to modify cardiac electrophysiology, with an anti-arrhythmic effect and was first used experimentally in 1962 (Mokler and Van Arman 1962). In further studies seeking for oral drugs with no side effects for the ventricular fibrillation and “sudden death”, disopyramide has gained more and more importance in the therapy of this disease (Vismara et al. 1974). Nowadays, according to the Vanghan Williams Classification, which is widely used in clinical trials, disopyramide is classified as a 1A anti-arrhythmic drug (Singh et al. 1970).

Although the mechanism of action of disopyramide is not completely elucidated, it appears that it acts similarly to other drugs belonging to the 1A anti-arrhythmic class, e.g., quinidine, by blocking the Na\(^+\) channels and prolonging the action potential duration by also blocking one or more K\(^+\) channels (Campbell 1983; Sanchez-Chapula 1999). Microelectrode studies have shown that disopyramide depresses the maximum rate of repolarization, increases conduction time, and prolongs the terminal phase of cardiac repolarization (Kus and Sasyniuk 1975). In isolated cardiac myocytes, disopyramide blocks the sodium current in a use-dependent manner, probably by binding to the activated state of the channel (Zilberter et al. 1994; Sanchez-Chapula 1999, and the references there in). Disopyramide also blocks cardiac potassium currents, including the inward rectifier (Coraboeuf et al. 1988) and the ATP-sensitive K\(^+\) current (De Lorenzi et al. 1995).

Disopyramide has shown an effective prophylaxis and treatment of ventricular and supraventricular arrhythmias (Mayer at al. 1991), and has also been shown to reduce gradient and improve symptoms in patients who suffer obstructive hypertrophic cardiomyopathy (Sherrid et al. 1988).

However, besides these benefits, several studies and medical observation have shown complications during the administration of disopyramide. These effects are mostly mediated by its action on the cardiovascular system.
showing causing heart failure and hypotension. Adverse effects were also observed on the respiratory and nervous systems as well as second-generation effects on pregnancy and lactation (Aronson 2006). In order to avoid these side effects disopyramide could be administered in combination with other drugs. As a result, there is an ongoing need for the determination of disopyramide pharmacokinetics so that issues as drug-to-drug or drug-biomolecular complex interaction could be evaluated. Detection and quantitation of disopyramide in biological fluids is very important. Therefore, to support pharmacokinetic studies with sufficient speed, suitable analytical procedures are required. The few reported methods in the literature for the determination of disopyramide are capillary electrophoresis (Kuroda et al. 2003; Fang et al. 2005), gas chromatography (Quaglio et al. 1995), HPLC (Witek et al. 1994; Bortocan et al. 2000), spectrophotometry (Abdellatef 2007), and a disopyramide-sensitive membrane electrode (Katsu et al. 1997).

Due to their high sensitivity, voltammetric methods have been successfully used for the detection and determination of various biological compounds (Hart 1990; Diculescu et al. 2006a and 2006b). Besides the analytical goals, investigations of the redox behavior of different compounds by means of electrochemical techniques have the potential for providing valuable insights into the redox reactions of these molecules.

Therefore, the present study is concerned with the investigation of the electron transfer properties of disopyramide using a glassy carbon electrode with cyclic, differential pulse, and square-wave voltammetry. The investigation of the electrochemical oxidation mechanisms of disopyramide is important since this could result in a better understanding of the data already known (Taguchi et al. 2003) and increases the overall knowledge of disopyramide’s physiological mechanisms of action.

EXPERIMENTAL

Materials and Reagents

Disopyramide from Aventis, was used without further purification. A stock solution of 600 μM disopyramide was prepared in deionized water and was stored at −4°C.

All supporting electrolyte solutions, Table 1, were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤0.1 μS cm⁻¹).

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pipettes (Rainin Instrument Co. Inc., Woburn). The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1°C).
Voltammetric Parameters and Electrochemical Cells

Voltammetric experiments were carried out using a μElectroanalytical Instrument running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a glassy carbon (GCE) \((d = 1.5 \text{ mm})\) working electrode, a Pt wire counter electrode, and a Ag/AgCl (3 M KCl) as reference, in a 0.5 ml one-compartment electrochemical cell. The experimental conditions for differential pulse voltammetry (DPV) were pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s\(^{-1}\). For square wave voltammetry (SWV) the experimental conditions were frequency 25 Hz and potential increment 2 mV, corresponding to an effective scan rate of 50 mV s\(^{-1}\).

The GCE was polished using diamond spray (particle size 1 μm) before each experiment. After polishing, the electrode was rinsed thoroughly with Milli-Q water for 30 s; then it was sonicated for 1 min in an ultrasound bath and again rinsed with water. After this mechanical treatment, the GCE was placed in pH 7.0 0.2 M phosphate buffer electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

Acquisition and Presentation of Voltammetric Data

All the voltammograms presented were background-subtracted and baseline-corrected using the moving average with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak height is in some cases

<table>
<thead>
<tr>
<th>pH</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>HCl + KCl</td>
</tr>
<tr>
<td>3.4</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>4.3</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>5.1</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>6.1</td>
<td>NaH(_2)PO(_4) + Na(_2)HPO(_4)</td>
</tr>
<tr>
<td>7</td>
<td>NaH(_2)PO(_4) + Na(_2)HPO(_4)</td>
</tr>
<tr>
<td>8.1</td>
<td>NaH(_2)PO(_4) + Na(_2)HPO(_4)</td>
</tr>
<tr>
<td>8.6</td>
<td>NaH(_2)PO(_4) + Na(_2)HPO(_4)</td>
</tr>
<tr>
<td>10</td>
<td>NH(_3) + NH(_4)Cl</td>
</tr>
<tr>
<td>12</td>
<td>NaOH + KCl</td>
</tr>
<tr>
<td>12.5</td>
<td>NaOH + KCl</td>
</tr>
</tbody>
</table>
reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all graphs were determined from the original untreated voltammograms after subtraction of the baseline.

RESULTS AND DISCUSSION

Cyclic Voltammetry

The oxidation behavior of disopyramide was first studied by cyclic voltammetry (CV) at 50 mV s\(^{-1}\), in 250 \(\mu\)M disopyramide in different pH electrolyte solutions Fig. 1A. The oxidation is irreversible and follows different oxidation pathways according to the pH of the electrolyte solution.

In alkaline media, in pH 10.0 0.2 M ammonia buffer, two well-separated consecutive oxidation peaks \(P_1, E_{pa}^1 = +0.56 \text{ V} \), and \(P_2, E_{pa}^2 = +0.75 \text{ V} \), occurred on the anodic scan.

In pH 7.0 0.2 M phosphate buffer a small shoulder \(P_1, E_{pa}^1 \sim +0.75 \text{ V} \), could be identified close to the occurrence of peak \(P_2, E_{pa}^2 = +0.81 \text{ V} \), showing that the oxidation of disopyramide in neutral electrolytes also occurs in a two-step irreversible mechanism. Recording successive scans in the same solution, only a small decrease of both peaks was observed. No other oxidation peak was observed showing that the oxidation product of disopyramide is electrochemically inactive.

In acid media, for pH < 4.3, no oxidation peak for disopyramide was observed. Increasing the electrolyte pH, in pH 4.3 0.2 M acetate buffer,
disopyramide undergoes oxidation in a single step and only the peak $P_2$, $E_{pa}^2 = +1.00$ V, occurred on the positive-going scan. The current of $P_2$ is pH dependent and increased with pH.

CVs were obtained for different scan rates in a solution of 250 μM disopyramide in pH 7 0.2 M phosphate buffer, Fig. 1B. Between measurements, the electrode surface was always polished in order to assure a clean surface and to avoid possible problems from the adsorption of disopyramide oxidation products onto the GCE surface. It was observed that, on increasing the scan rate, the peak $P_2$ potential is slightly displaced to more positive values. The difference between peak potential $E_{pa}$ and the potential at peak half height $E_{p/2}$ was ~60 mV. Since for a diffusion-controlled irreversible system $1/2(E_{pa} - E_{p/2}) = 47.7/(\alpha\alpha'n')$ where $\alpha$ is the anodic charge transfer coefficient and $n'$ the number of electrons in the rate-determining step (Brett et al. 1993), it can be calculated that $\alpha\alpha'n' = 0.8$.

Also, increasing the scan rate, the current of peak $P_2$ increases linearly with square root of $\nu$ (not shown), consistent with the diffusion-limited oxidation of a solution species. Recording of voltammograms was to $\nu < 500$ mV s$^{-1}$ since at higher scan rates, peak $P_2$ tends to merge with the background making the measurement of the peak current difficult, Fig. 1B. However, for this scan rate interval, the peak current in amperes for a diffusion-controlled irreversible system is given by $I_{pa}(A) = 2.99 \times 10^5 n (\alpha\alpha'n')^{1/2} \ A [R]_\infty D_\Omega^{1/2} \nu^{1/2} \ A$ where $n$ is the number of electrons transferred during the oxidation. For disopyramide, $n = 2$ for pH 7.0. $A$ is the electrode area in cm$^2$, $D_\Omega$ is the diffusion coefficient in cm$^2$ s$^{-1}$, $[R]_\infty$ is the concentration in mol cm$^{-3}$ and $\nu$ is in V s$^{-1}$ (Brett et al. 1993). By plotting $I_{pa}$ versus $\nu^{1/2}$, the value of $D_\Omega$ is obtained. From the measured slope of $2.98 \times 10^{-6} \ A/(V \ s^{-1})^{1/2}$ the diffusion coefficient of disopyramide in pH 7.0 0.2 M phosphate buffer is $D_{disopyramide} = 3.8 \times 10^{-6} \ cm^2 \ s^{-1}$. For this calculation, the GCE electroactive area of 0.012 cm$^2$ was determined from a plot of $I_{pc}$ versus $\nu^{1/2}$ using a solution of 1 mM hexacyanoferrates (II) and the value of the diffusion coefficient of hexacyanoferrate (II) in phosphate buffer of $D_O = 7.35 \times 10^{-6} \ cm^2 \ s^{-1}$ (http://www.hbcpnetbase.com/).

**Differential Pulse Voltammetry**

The electrochemical oxidation of disopyramide was studied over a wide pH range between 1.2 and 12.5 using DPV. The DPVs were all recorded in a solution of 60 μM disopyramide in different electrolytes with 0.2 M ionic strength. For pH < 4.3, no oxidation peak was observed even for higher concentrations, showing that disopyramide is not oxidizable in these conditions. For pH > 4.3, one or two oxidation peaks were observed depending on the electrolyte pH, Fig. 2A.

For 4.3 < pH < 7.0, one main anodic peak $P_2$ occurred and the oxidation potential was displaced to less positive values with increasing pH, Fig. 2A. The
dependence is linear and follows the relationship $E_{pa} (V) = 1.064 - 0.059 \text{pH}$, Fig. 2B. The slope of the line, 59 mV per pH unit, showed that the oxidation of disopyramide involves the same number of electrons and protons. In addition, in these electrolytes, the width at half height of the disopyramide oxidation peak $P_2$ was $W_{1/2} \sim 100$ mV, close to the theoretical value of 90 mV corresponding to an electrochemical reaction involving the transfer of one electron (Brett et al. 1993). Thus it can be concluded that the oxidation of disopyramide in acid media occurs with the transfer of one electron and one proton.

For pH $> 7.0$, the oxidation potential of peak $P_2$ does not depend on the electrolyte pH, Fig. 2B, indicating a mechanism above pH 7 involving only one electron and no proton corresponding to the oxidation of the deprotonated disopyramide in alkaline solutions (Oliveira et al. 2007). Nevertheless, for this pH values, the width at half height of the disopyramide oxidation peak $P_2$ decreased to a value of about $W_{1/2} \sim 50$ mV corresponding to an electrochemical reaction involving the transfer of two electrons (Brett et al. 1993).

On the other hand, for pH $> 7.0$ a new peak $P_1$ appears, Fig. 2A. The oxidation potential of peak $P_1$ is also pH dependent for $7.0 < \text{pH} < 12.5$ and its value decrease with increasing pH. The dependence is linear and follows the relationship $E_{pa} (V) = 1.094 - 0.059 \text{pH}$, Fig. 2B. The slope of the line, 59 mV per pH unit, showed that the oxidation of disopyramide involves the same number of electrons and protons. Taking into consideration that the width at half height of the disopyramide oxidation peak $P_1$ is $W_{1/2} \sim 50$ mV, it can be concluded that this oxidation reaction involves the transfer of two electrons and two protons.

Although in a solution of 60 μM disopyramide in pH 7.0 0.2 M phosphate buffer only the oxidation peak $P_1$ was observed with an apparent $W_{1/2} \sim 100$ mV, this is due to the high concentration used. As
will be shown below, for low concentration, e.g., \( C_{\text{disopyramide}} \leq 14 \, \mu M \), both peak \( P_1 \) and \( P_2 \) can be easily observed, with a width at half height \( W_{1/2} \approx 50 \, \text{mV} \) that corresponds to the transfer of two electrons. For this reason, the calculation of disopyramide diffusion coefficient in pH 7.0 was based on the transfer of two electrons. Nevertheless, the pH study was effectively carried out using a concentration of 60 \( \mu M \) disopyramide because for lower concentrations no peak could have been observed in electrolytes with pH < 5.1.

### Square Wave Voltammetry

The advantages of square wave voltammetry (SWV) are greater speed of analysis, lower consumption of the electroactive species in relation with DPV, and reduced problems with poisoning of the electrode surface (Brett et al. 1993). SWVs recorded in 60 \( \mu M \) disopyramide solutions showed similar features to DPV, i.e., the oxidation peak \( P_2 \) in 4.3 < pH < 7.0 and the appearance of peak \( P_1 \) for supporting electrolytes with pH > 7.0, Fig. 3A. Also, for 4.3 < pH < 7.0 the potential of peak \( P_2 \) is pH dependent, the slope of the line being 59 mV per pH unit. For pH > 7.0, the potential of peak \( P_2 \) does not depend on the pH whereas the potential of peak \( P_1 \) decreased with increasing pH also following a slope of 59 mV per pH unit. On the other hand, the values of \( W_{1/2} \) confirmed the results obtained with DPV.

A greater advantage of SWV is the possibility to see during only one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both positive and negative-going pulses, peaks corresponding to

![Figure 3](image-url)

**Figure 3.** SWV of 60 \( \mu M \) disopyramide: (A) different pH supporting electrolytes and (B) pH 7.0 0.2 M phosphate buffer; \( f = 25 \, \text{Hz, } \Delta E_0 = 2 \, \text{mV, } v_{\text{eff}} = 50 \, \text{mV s}^{-1} \), pulse amplitude 50 mV; \( I_t \) – total current, \( I_f \) – forward current, \( I_b \) – backward current.
the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment. Thus, the irreversibility of both peaks P1 and P2 is confirmed by plotting the forward and backward components of the total current obtained in a solution of 60 μM disopyramide in pH 7.0 0.2 M phosphate buffer, Fig. 3B. Whereas the forward component showed both peaks at the same potential and with the same current as the total current obtained, the backward component showed no cathodic peak.

Even though the exact oxidation mechanism was not determined, some conclusions concerning the electroactive centers under the working conditions, could be reached. Taking into consideration all the electrochemical studies performed, it could be considered that the anodic reaction is the oxidation of nitrogen in the pyridine moiety in the disopyramide molecule.

Analytical Determination

Two different procedures for the electroanalytical determination of disopyramide were evaluated. Supporting electrolyte, pH 7.0 0.2 M phosphate buffer, was preferred since higher oxidation peaks are obtained, as shown in Figs. 2A and 2B.

In the first procedure the electrode surface was polished between measurements and conditioned in order to ensure a clean GCE surface, Fig. 4A. Since neither disopyramide nor the oxidation products adsorb on the GCE surface, in the second procedure, DP voltammograms for disopyramide oxidation were consecutively recorded in solutions of different concentrations of disopyramide Fig. 4B.

![Figure 4](image)

*Figure 4.* DPVs of 1 to 40 μM disopyramide in pH 7.0 0.2 M phosphate buffer: (A) procedure 1 and (B) procedure 2. Scan rate = 5 mV s⁻¹.
The detection limit (LOD) was determined as the disopyramide concentration that caused a peak with a height three times the baseline noise level, i.e., \( \text{LOD} = \frac{3 \times \text{S.D.}}{\text{sensitivity}} \).

The quantification limit (LOQ) is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical signal/noise ratio of 10 is generally considered to be acceptable; therefore:

\[
\text{LOQ} = \frac{10 \times \text{S.D.}}{\text{sensitivity}}
\]

The data obtained from the calibration curves are presented in Table 2. Procedure 1 leads to a wider linear range and a lower LOD when compared with the results obtained using procedure 2. Nevertheless, a better linearity was observed when procedure 2 was followed, as demonstrated by the values of \( R^2 \) in Table 2.

The reproducibility of these methods was evaluated by plotting different calibration curves. It must be mentioned that each set of measurements was always done using a freshly polished GCE, a process that give rise to small modifications of the electrode surface area which can in turn cause variations in the oxidation currents. Also, another important factor is that disopyramide oxidation occurs at high positive potentials and some contribution from the decomposition of the electrolyte can influence the peak current measurements.

**CONCLUSIONS**

The present study shows that disopyramide, a class 1A anti-arrhythmic agent, undergoes oxidation at a glassy carbon electrode. The electrochemical oxidation of disopyramide was investigated by cyclic, differential pulse and square wave voltammetry over a wide pH range. In all cases, it was shown that the oxidation is an irreversible, diffusion-controlled process. Cyclic voltammetry allowed the determination of the disopyramide diffusion coefficient as \( D_{\text{disopyramide}} = 3.8 \times 10^{-6} \text{ cm}^2 \text{s}^{-1} \), in pH 7.0 0.2 M phosphate buffer. The present study also shows that the oxidation of disopyramide occurs in a complex, pH dependent mechanism. It was found that for electrolytes with \( 4.3 < \text{pH} < 7.0 \) the oxidation occurs with the transfer of one electron and one proton. On the contrary, in alkaline solutions, two charge transfer reactions are observed, both oxidation reactions involving the transfer of two electrons but only one of them.

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**Table 2.** Analytical data for disopyramide obtained by DPV using the GCE

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (nA µM(^{-1}))</th>
<th>Intercept (nA)</th>
<th>LOD (µM)</th>
<th>LOQ (µM)</th>
<th>S.D. (nA)</th>
<th>( R^2 )</th>
<th>Upper limit of linear range (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure 1</td>
<td>5.05 ± 0.69</td>
<td>22.93 ± 10.33</td>
<td>1.27</td>
<td>4.24</td>
<td>0.989</td>
<td>2.15</td>
<td>120</td>
</tr>
<tr>
<td>Procedure 2</td>
<td>1.51 ± 0.03</td>
<td>7.39 ± 2.42</td>
<td>7.45</td>
<td>24.8</td>
<td>0.997</td>
<td>3.75</td>
<td>60</td>
</tr>
</tbody>
</table>
associated with the transfer of two protons. A oxidation mechanism corresponding to the oxidation of a nitrogen atom in the pyridine moiety in the disopyramide molecule is proposed. Also, in the present study, two procedures for analytical determination were developed and compared. The lowest detection limit LOD = 1.27 μM was achieved using Procedure 1.

REFERENCES


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