Electrochemical Investigation of Na-Salt of 2-Methyl-3-(4-nitrophenyl)acrylate on Glassy Carbon Electrode

Afzal Shah, Victor C. Diculescu, Niaz Muhammad, Rumana Qureshi, Saqib Ali, Ana Maria Oliveira-Brett

Abstract
The electrochemical behavior of Na-salt of 2-methyl-3-(4-nitrophenyl)acrylate (NPA) and its reduction product was studied by cyclic (CV), differential pulse (DPV) and square wave voltammetry (SWV) using a glassy carbon electrode (GCE). The results revealed that NPA is irreversibly reduced leading to the formation of a reduction product (PNPA). For pH < 9.0 the peak potential was linearly dependent on pH. For pH > 9.0 the peak potential was pH-independent and the value of pK_a ≈ 9.0 was determined. The adsorbed PNPA exhibited reversible redox reaction. The reduction of PNPA was pH dependent. To ensure that the electrochemical behavior of NPA is due to the reducible moiety, NO_2, closely related compounds to NPA were also studied, and a redox mechanism was proposed for NPA.

Keywords: Na-salt of 2-methyl-3-(4-nitrophenyl)acrylate, Reduction, Voltammetry, Adsorption

DOI: 10.1002/elan.200900368

1. Introduction

Nitroarenes (NA) are environmental contaminants found notably in automobile exhaust particulates, airborne particles and soot formed after wood combustion [1]. They have been reported as important intermediates in the industrial manufacture of dyes, pesticides and plastics, and declared as significant environmental hazards [2]. Besides, NA have been found to have a broad spectrum of mutagenicity, genotoxicity, and carcinogenicity [3]. A survey of literature reveals that the reduction of the nitro group to yield nitro radical anions, N-hydroxynitrosoarenes, is a crucial metabolic step for the genotoxic and cytotoxic properties of such compounds [4]. In this sense, the effect of pH and electrolytes that characterize the ease of reduction are very important to define the type of biological properties of the different nitrocompounds [5]. Consequently, the electrochemical reduction of NA is of prime importance and has been the subject of numerous investigations [6–8].

The electroreduction of NA produce a variety of intermediates, which play a key role in organic synthesis [9]. The formation of these diverse reaction products is due to the differences in the chemical follow-up reactions, nature of the electrode, composition of the medium and the groups attached to the aromatic ring. The nitro group is purposely incorporated in organic molecules of therapeutic use as the biological properties of such compounds have been claimed to depend strongly on the ease of nitroreductive processes in the cell [10, 11]. Therefore, it is considered worthwhile to study the redox characteristics of such species to anticipate cytotoxic effects in biological systems.
obtained here will throw light in understanding the metabolic fate or in vivo redox processes of these compounds.

2. Experimental

2.1. Materials and Reagents

2-Methyl-3-(4-nitrophenyl) acrylic acid, 2-methyl-3-(3-methylphenyl) acrylic acid (I), 3-(4-bromophenyl)-2-methylacrylic acid (II), 3-(4-bromophenyl)-2-ethylacrylic acid (III), 3-(4-chlorophenyl)-2-methylacrylic acid (IV), 2-(4-ethoxybenzylidene)butanoic acid (V), (E)-(2-fluorobenzylidene)butanoic acid (VI), 2-(4-isopropylbenzylidene)propanoic acid (VII) were prepared by the literature reported methods [16 – 23] and converted to sodium salts in order to improve their water solubility. Analytical grade reagents were used for the synthesis of all compounds. 4-Nitrophenylacetic acid was purchased from Fluka and converted to its sodium salt with the same objective as discussed above. Stock solutions of 2.0 mM of each compound were prepared using analytical grade reagents and purified water in deionized water and were stored at 5°C for 3 days.

All supporting electrolyte solutions (Table 1) were prepared by the literature reported methods [16 – 23] and converted to sodium salts in order to improve their water solubility. Analytical grade reagents were used for the synthesis of all compounds. 4-Nitrophenylacetic acid was purchased from Fluka and converted to its sodium salt with the same objective as discussed above. Stock solutions of 2.0 mM of each compound were prepared in deionized water and were stored at 5°C for 3 days.

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).

Table 1. Supporting electrolytes, 0.1 M ionic strength.

<table>
<thead>
<tr>
<th>pH</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>HCl + KCl</td>
</tr>
<tr>
<td>2.0</td>
<td>HCl + KCl</td>
</tr>
<tr>
<td>3.4</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>4.5</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>5.4</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>6.1</td>
<td>HAcO + NaAcO</td>
</tr>
<tr>
<td>7.0</td>
<td>NaH₂PO₄ + Na₂HPO₄</td>
</tr>
<tr>
<td>8.0</td>
<td>NaH₂PO₄ + Na₂HPO₄</td>
</tr>
<tr>
<td>9.4</td>
<td>NaHCO₃ + NaOH</td>
</tr>
<tr>
<td>10.2</td>
<td>NaHCO₃ + NaOH</td>
</tr>
<tr>
<td>12.0</td>
<td>KCl + NaOH</td>
</tr>
<tr>
<td>12.8</td>
<td>KCl + NaOH</td>
</tr>
</tbody>
</table>

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). 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).

2.2. Voltammetric Parameters and Electrochemical Cells

Voltammetric experiments were carried out using a μAuto-lab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a glassy carbon (GCE) (d = 1.5 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 and then placed in the desired buffer electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.3. 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 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.

3. Results and Discussion

3.1. Cyclic Voltammetry

The redox behavior of NPA was first studied by CV at 100 mV s⁻¹, in N₂ saturated solution of 1.0 mM NPA in pH 7.0. 0.1 M phosphate buffer. The CV’s were initially started at +0.00 V and recorded between potential limits of +1.40 V and −1.00 V (not shown). On the first positive-going scan of the 1st voltammogram, an oxidation peak 2a was observed at Epa = 0.59 V (Fig. 1A). After changing the scan direction, on the positive-going scan of the same voltammogram, an oxidation peak 2b was observed at Epa = +0.00 V. This peak corresponds to the oxidation of the NPA reduction product (P_NPA) formed at the GCE surface. A subsequent CV in the negative direction, recorded in the same solution and without cleaning the GCE surface,
showed a new cathodic peak 2c, at $E_{pc}^2 = -0.05$ V, which confirmed the reversibility of PNA oxidation.

CVs were also recorded in N2 saturated solutions of 1.0 mM NPA but in electrolytes with different pH values, always using a clean GCE surface (Fig. 1B). It was observed that NPA redox mechanism is pH-dependent since by increasing the pH value all peaks shifted to more negative potentials. However, for pH > 8 the oxidation peak 2a occurred with a very small current and the increase in the potential difference between peak 2a and its cathodic correspondent 2c obtained on a subsequent scan (not shown) showed a change in the NPA redox mechanism in alkaline electrolytes.

CVs were obtained for different scan rates in a N2 saturated solution of 1.0 mM NPA in pH 7.0 0.1 M phosphate buffer (Fig. 2). Between measurements, the electrode surface was always polished in order to assure a clean surface and to avoid possible problems from the adsorption of redox products onto the GCE surface. It was observed that, on increasing the scan rate, the peak 1c potential is slightly displaced to more negative values. The difference between peak potential $E_{pc}$ and the potential at peak half height $E_{1/2}$ was ca. 45 mV. Since for a diffusion-controlled irreversible system $|E_{pc} - E_{1/2}| = 47.7/(\alpha c n')$ where $\alpha c$ is the cathodic charge transfer coefficient and $n'$ the number of electrons in the rate-determining step [24], it can be calculated that $\alpha c n' = 1.09$.

Also, increasing the scan rate, the current of peak 1c increases linearly with square root of $v$ (not shown), consistent with the diffusion-limited reduction of a solution species (recording of voltammograms was to $v < 500$ mV s$^{-1}$) (Fig. 2). However, for this scan rate interval, the peak current in amperes for a diffusion-controlled irreversible system is given by $I_{pc}(A) = 2.99 \times 10^{-5} n (\alpha c n')^{-1/2} A [O] D_{Oz} v^{-1/2}$ where $n$ is the number of electrons transferred during the reduction. For NPA, $n = 2$ for pH 7.0, $A$ is the electrode area in cm$^2$, $D_O$ is the diffusion coefficient in cm$^2$ s$^{-1}$, [O] is the concentration in mol cm$^{-3}$ and $v$ is in V s$^{-1}$ [24]. By plotting $I_{pc}$ vs. $v^{-1/2}$, the value of $D_{NPA}$ is obtained. For this calculation, the GCE electroactive area of 0.012 cm$^2$ was determined as described elsewhere [25]. From the measured slope of $6.0 \times 10^{-5}$ A/(V s$^{1/2}$) the diffusion coefficient of NPA in pH 7.0 0.1 M phosphate buffer is $D_{NPA} = 6.4 \times 10^{-5}$ cm$^2$ s$^{-1}$.

### 3.2. Differential Pulse Voltammetry

The electrochemical reduction of NPA was studied over a wide pH range between 2 and 13 using DPV. The DPVs (Fig. 3A) were all recorded in N2 saturated solutions of 0.1 mM NPA in different electrolytes with 0.1 M ionic strength (Table 1). For $1.2 \leq pH \leq 9.0$, peaks 1c occurred and its potential decreased with increasing pH of the supporting electrolyte. The dependence was always linear as shown in Figure 3B, the slope of the line, $-60$ mV per pH unit showing that the reduction of NPA occurred with the transfer of the same number of electrons and protons. In addition, in these electrolytes, the width at half height of the NPA reduction peak 1c was $W_{1/2} \approx 60$ mV, close to the
theoretical value (45 mV) corresponding to an electrochemical reaction involving the transfer of two electrons [24]. Thus it can be concluded that the reduction of NPA occurs with the transfer of two electrons and two protons.

A different situation has been observed for pH > 9.0, where peak 1c does not depend on pH of the supporting electrolyte. The change in the slope of the line can be attributed to chemical protonation/deprotonation processes of NPA in electrolytes obeying these pH conditions. That means in these conditions, the reduction of NPA involves only the transfer of two electrons.

On the other hand, the graph for the variation of peak 1c current with the pH of the supporting electrolyte (Fig. 3B) have shown maximum values in strong acid solution whereas for pH > 5.0 a constant value being reached.

Successive DP voltammograms were recorded in a N$_2$ saturated solution of 100 µM NPA in pH 7.0 0.1 M phosphate buffer (Fig. 4). In these conditions, on the first DP voltammogram peak 1 was observed at $E_{pc}^1 = -0.48$ V. On the second DP scan, a new cathodic peak 2c occurred at $E_{pc}^2 = -0.01$ V. This peak corresponds to the reduction of P$_{NPA}$ formed at the GCE surface during the first DP scan. The current of peaks 1c decreased with the number of scans due to the decrease of the available electrode surface area owing to adsorption of P$_{NPA}$ whereas the current of peak 2c increased with the number of scans. The adsorption of NPA reduction product at the GCE surface was confirmed when, at the end of several DP scans recorded in the solution of NPA, the electrode was washed with a jet of deionized water and then transferred to the supporting electrolyte. The DP voltammogram obtained in these conditions (not shown) shows only peak 2c. Moreover, consecutively recorded DP voltammograms in buffer showed only a continuous, but slow decrease of peak 2c current due to P$_{NPA}$ consumption.

Consecutive DP voltammograms recorded in the same solution of NPA showed a new peak 2c corresponding to P$_{NPA}$ adsorbed at the GCE surface and the electrochemical reduction of this compound was also studied for different pH values. Thus, two consecutive DP voltammograms were recorded in a N$_2$ saturated solution of 0.1 mM NPA in different electrolytes and the second DP voltammograms were plotted vs. pH (Fig. 5A).

It was observed that peak 2c is displaced to more negative values with increasing pH, following a linear relationship with slope $-60$ mV per pH unit (Fig. 5B). This means that the reduction of P$_{NPA}$ involves the same number of electrons and protons. Nevertheless, in all electrolytes, the width at half height of peak 2c was about 50 mV, which suggests that the reduction P$_{NPA}$ occurred with the transfer of 2 electrons, hence also 2 protons.

The variation of peak 2c current with the pH of the supporting electrolyte has been also plotted showing a maximum cathodic current at about pH 4.0. For alkaline solution (most obvious for pH > 9.0) the current decreased tending to 0, in agreement with the change in the redox mechanism of NPA observed also during the CV studies (Fig. 1B).

3.3. Square Wave Voltammetry

The advantages of square wave voltammetry (SWV) are greater speed of analysis, lower consumption of the electro-
active species in relation with DPV, and reduced problems with poisoning of the electrode surface [24]. Thus, SWVs recorded in N\textsubscript{2} saturated solution of 0.1 mM NPA in pH 4.5 0.1 M acetate buffer showed peak 1\textsubscript{c} on the 1\textsuperscript{st} scan (Fig. 6A). By recording successive SW voltammograms in the same solution without cleaning the GCE surface, peak 2\textsubscript{c} occurred at a lower potential and its current increased with the number of scans due to the formation of P\textsubscript{NPA} at the electrode surface. On the other hand, the values of \( W^{1/2} \) for both peaks 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 the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment. Thus, the reversibility of peak 2\textsubscript{c} is confirmed by plotting the forward and backward components of the total current where the oxidation and the reduction currents are equal (Fig 6B). Moreover, the identical value of the potentials of peak 2\textsubscript{c} and 2\textsubscript{a} on the forward and backward current components is an indication of the adsorption of NPA reduction products on the GCE surface [26].

3.4. Redox Mechanism

In order to elucidate the redox mechanism of NPA, the electrochemical behavior of 1.0 mM Na-salt of 4-nitrophenylethanoate, NPE and several compounds (mentioned in the experimental section) with closely related structures to NPA but with different substitutes at the aromatic ring was also studied and compared. The CV’s of 1.0 mM NPE (Scheme 1B) were recorded in N\textsubscript{2} saturated solutions of several electrolytes with different pH values. A similar behavior between NPA and NPE was observed in all electrolytes although the peak potentials for the reduction of NPE and its reduction product occurred at more negative values (Fig. 7). On the other hand, the substitution of chloro, bromo, fluoro, methyl, isopropyl and ethoxy in the aromatic ring gave no redox peaks in the CV experiments carried out in N\textsubscript{2} saturated solution. This ensured that the electrochemical signals of NPA and NPE are due to the redox behavior of NO\textsubscript{2}.

The results presented above indicate that NPA is electrochemically reduced to compound (2) (Scheme 2) by a mechanism involving the conversion of NO\textsubscript{2} group to a N,N-
dihydroxylamine by a 2 electrons and 2 protons process (peak 1c in Fig. 6B). The formation of such a reduction product is in agreement with the reduction of NO₂ group of metronidazole and related compounds [27]. Its stabilization by electron-withdrawing groups and destabilization by electron-donating groups at the aromatic ring has been reported [10]. The presence of electron-donating group at the aromatic ring is expected to make the N,N-dihydroxylamine unstable which may be dehydrated to a Na-salt of 2-methyl-3-(4-nitrosophenyl)acrylate (3).

The reduction peak 2c observed in the SWV (Fig. 6B) is attributed to the reduction of nitroso group of compound (3) to hydroxylamine of compound (4) in a reversible reaction that involves the transfer of 2 electrons and 2 protons (Scheme 2).

The plot of $E_{pc}$ vs. pH for peak 1c showed that the reduction process of NPA is pH dependent in acidic and neutral media and pH independent for values higher than 9.0. This implies that in alkaline solutions with pH > 9.0 electrons transfer is not accompanied by protons in the rate determining step.

Similarly, $E_{pc}$ vs. pH for peak 2c showed that the reduction process is pH dependent for electrolytes with pH ≤ 12.

4. Conclusions

The electrochemical behavior of Na-salt of 2-methyl-3-(4-nitrophenyl)acrylate (NPA) and its reduction product was studied by cyclic voltammetry, square wave voltammetry and differential pulse voltammetry in pH 1.2 – 12.8 using a glassy carbon electrode. The results presented demonstrated that NPA is irreversibly reduced leading to the formation of a reduction product that is strongly adsorbed at the electrode surface. The results revealed that the reduction peaks of NPA and its product were pH dependent. The NPA reduction process was an electrochemically-coupled reaction, where charge transfer is followed by a chemical reaction while the reduction of the product was electrochemical. The comparison of NPA with NPE revealed that the reduction potential of the reducing moiety, NO₂ can be modulated by changing the electronic properties of the substituents attached to the aromatic ring. The results suggest that the electrochemical techniques can be successfully employed for the determination of reaction mechanism. Moreover, the physical parameters like $pK_b$ and the diffusion coefficient can be conveniently determined from voltammetric results.

5. Acknowledgements

Financial support from Higher Education Commission (HEC), Islamabad, Pakistan, (A. Shah), Fundação para a Ciência e Tecnologia (FCT), Post-Doctoral Grants SFRH/BDP/36110/2007 (V. C. Diculescu.), Project PTDC/QUI/65255/2006, POCI (cofinanced by the European Commun-
ity Fund FEDER), and CEMUC-R (Research Unit 285), is gratefully acknowledged.

6. References