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Development of an electrochemical sensor for the determination of the total antioxidant capacity in berries based on boron doped diamond

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Abstract

Many antioxidants can be electrochemically oxidized using graphite-based electrodes; nevertheless problems arise due to the strong adsorption of redox species at the sensing area. We have demonstrated that boron doped diamond (BDD) electrodes do not show this property, which can be exploited for the design of a new amperometric sensor for the quantification of antioxidants as “total antioxidant capacity” (AOC). As reference substances hydroquinone (HQ) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were studied in more detail. The supporting electrolyte was a phosphate buffer solution (PBS, 0.1 mol/L, pH 7.0). The limits of detection (LOD) were 1.5 mg/L and 2.5 mg/L for HQ and Trolox, respectively. The repeatability was 3 % RSD for concentration of 200 mg/L HQ. The method could be applied for the determination of AOC in different berry samples, such as strawberry, blueberry, grape and bramble. A comparison with a standard photometric assay showed good correlation between both methods. The BDD sensor features good reproducibility without fatiguing over at least two months of operation.

Keywords

Boron doped diamond electrode; total antioxidant capacity; amperometric sensor; berry extracts.
Introduction

The term “antioxidant” identifies a huge number of substances, in majority of cases phenolic or polyphenolic substances that inhibit the oxidation of molecules. They are strongly reducing agents that neutralize reactive oxygen species (ROS). These ROS are oxidizing radicals or molecules that are formed during the respiratory chain and cause damage on cell own material. Antioxidants neutralize these species and therefore they are of enormous scientific interest.

The free scavenger effect of antioxidants has been in use for many years to prolong the stability of lipid foods especially of edible oils. Reports go back to 1947 where butylated hydroxyanisole was used as the first antioxidant for the prevention of the deterioration of fat-containing food [1-2]. Nowadays antioxidants are also in the medicinal focus because of their tissue protecting effects by neutralization of ROS. Therapeutic progress has been made with many diseases that show a high degeneration rate, such are diabetes, coronary heart failure or Alzheimer disease [3-5]. Ever since in 2003 Sinclair et al. found out that resveratrol, an antioxidant occurring in grape, extends the lifespan of yeast cells and causes a decrease of typical aging effects, antioxidants became also interesting for cosmetic branches and for anti-aging research [6-7].

Antioxidants are mostly secondary plant metabolites. Especially berries, such as grape, red or black currant, bramble or raspberries, are known to form high amounts of flavonoids. Their synthesis is a response of the plant to stress attacks like strong light exposure or fungal or microbial infection. In the latter case, the plant uses them as phytoalexins for tissue protection. Unfortunately, isolated antioxidants are highly sensitive to light, temperature and oxygen. Although natural antioxidants are an interesting tool to prevent lipid products, e.g. oily food or cosmetics, to become rancid, their stabilization is still a problem to be solved.

Responding to a growing interest some analytical methods, mostly photometric assays, were developed. Standardized techniques are the ORAC-assay (oxygen radical absorbance capacity) involving hydrogen transfer reactions, the Folin-Ciocalteu-assay and the TEAC-assay (Trolox equivalent antioxidant capacity) that base on electron transfer reactions [8]. Although these methods have been well approved they are rather complex, time-consuming and use high priced chemicals or test kits.

Most antioxidants show electrochemical activity and could be determined using voltammetric or amperometric methods. Unfortunately, during the electrochemical process reduced and oxidized species adsorb very strongly on the surface of many electrode types, such as graphite or carbon paste electrodes (CPE).

Diamond electrodes offer an alternative approach. Ever since in 1987 Pleskov et al. [9] commenced studies on the electrochemistry of boron doped diamond (BDD) their development and fundamental research gained rising tendency. In contrast to graphite electrodes the C-sp³ hybridization and tetrahedral bonding of boron doped diamond electrodes (BDDE) leads to properties like chemical inertness, hardness, thermal conductivity and low fatigue [10]. Their main field of application is the treatment of wastewater [11]. The potential window of BDDEs is much wider (-0.75 to +2.35 V) than of graphite electrodes (-0.5 to +1 V) because neither oxygen nor hydrogen evolution interfere in the analysis. Because commercial BDDEs are usually synthesized by chemical vapor deposition (CVD) they contain non-diamond and metallic impurities [10]. Also, surface oxygen can cause disturbances and it needs to be removed. For surface cleaning and pretreatment of BDDEs a few suggestions, like acid washing or anodic polarization, have been offered [12,13].
In this work the pretreatment of boron doped diamond electrodes by polarization in Na$_2$SO$_4$ at +1 V, and the use of this electrode for the electrochemical determination of antioxidants in ethanolic berry extract, was investigated.

**Experimental**

**Chemicals and materials**

The investigated target analytes were phenolic and polyphenolic ingredients of different berries (grape, bramble, blueberry, strawberry) that are known to show antioxidative potential [14]. Therefore ferulic acid, vanillic acid, syringic acid, coumaric acid, sinapic acid, caffeic acid and p-hydroxybenzoic acid, all purchased from Sigma-Aldrich GmbH (Vienna, Austria), were selected. Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] and hydroquinone (HQ) were chosen as reference substances and were purchased, like all other standards, from Sigma-Aldrich GmbH (Vienna, Austria). To prepare the standard solutions the appropriate amount of substance was dissolved in deionized water. The aqueous solutions were prepared freshly before use, kept cool and protected from light. Phosphate buffer (Na$_2$HPO$_4$/NaH$_2$PO$_4$ 0.1 mol/L, pH 7) was selected as electrolyte medium.

**Apparatus**

All electrochemical experiments were performed using potentiostat (Metrohm AUTOLAB® PGstat302N) in a three electrode arrangement controlled by the corresponding software (NOVA 1.6). The BDDEs purchased from Fraunhofer (Michigan, USA), plated on titanium substrate with gold as a contact, a saturated calomel electrode (SCE) and a platinum wire were used as working, reference and auxiliary electrode respectively. All potential values in this work are given against SCE.

Cyclic voltammetry and hydrodynamic amperometry were chosen as techniques to obtain the electrochemical data. In the case of cyclic voltammetry the explored potential window was between -1 V and +1.2 V. The other operating parameters were: scan rate: 50.0 mV/s, step potential: 2.0 mV and time interval: 0.050 s. For hydrodynamic amperometry measurements the characteristic potential of the oxidation current peak was selected.

**Measuring procedure**

The BDDE was conditioned at +1 V for 1 h in 0.1 mol/L Na$_2$SO$_4$ solution prior to the start and also between the measurements to remove impurities and for the baseline correction.

All experiments were carried out in 20 mL phosphate buffer (0.1 M, pH 7) after degassing with argon. Aliquots of the standard solutions were added to the cell and cyclic voltammograms and hydrodynamic amperograms, respectively, were recorded by applying the aforementioned parameters.

**Photometric analysis**

The photometric Trolox Equivalent Antioxidant Capacity assay (TEAC-Assay) was selected as a reference method. According to the report of Li et al. [15] the redox indicator 2,2´-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) forms a radical in contact with potassium persulfate. The ABTS radical cation is of green color which decreases in intensity upon addition of substances with antioxidative potential. The solution was diluted using phosphate buffer saline (pH = 7) until it showed an absorption of 0.700 ± 0.050 at 734 nm. To 3 mL of that solution 100 µL of the sample were added and the absorption decrease was measured. For the calibration Trolox and HQ
solutions of 0.1, 0.2, 0.3 and 0.4 mol/L were used and the AOC of the berry samples was calculated as Trolox- or hydroquinone-equivalent (TE, HQE).

**Preparation of the samples**

The berry marc (donated from Grünewald Fruchtsaft GmbH) were dried at 50 °C. For the recovery of the phenolic compounds the milled raw material was extracted with a mixture of absolute ethanol and water (75:25 v/v) at a 8:1 solvent-to-sample ratio (volume to mass of oven-dried marc). The extraction was performed using sonication at a temperature of 50 °C for 15 min. After centrifugation (7000 rpm, 20 min) the extract was then filtered through a glass fiber filter (type AP2029325, Millipore) and stored at 4 °C.

**Results and Discussion**

**Cyclic voltammetry**

Cyclic voltammetry (CV) was basically used to determine the oxidation potential of the standard berry antioxidants with CPEs and BDDEs (Table 1). The quoted potentials correspond to the maximum of the oxidation current registered in the anodic scan. Typical CVs of hydroquinone and Trolox are shown in Fig. 1. As can be seen the electrochemical behavior of both substances on both electrodes is rather similar with the exception of carbon paste electrode where the currents are significantly higher. This behavior is due to the adsorption of the analyte at CPE and therefore due to the strong π electron interaction of the aromatic ring with the graphite structure. Both substances were used as reference compounds, by converting the response of other antioxidants to Trolox- (TE) and hydroquinone-equalities (HQE), respectively.

![Figure 1](image_url)

**Figure 1.** Cyclic voltammograms of 30 mg/L (a) Trolox and (b) HQ measured in PBS (0.1 mol/L, pH 7) on *• BDDE and *• CPE.

The investigated antioxidants (Table 1) produce typical peak currents in CV at BDDEs in the range of 4 to 8 mA mol⁻¹ with the exception of HQ which is about four times higher; probably because HQ ideally interacts with the graphite surface finally yielding benzoquinone, whereas...
other oxidants are reduced to the corresponding R-O· radical with ensuing follow-up effects. Again it is evident that carbon paste electrode favors adsorption of the oxidation product which exerts a positive effect for the first cycle in cyclic voltammetry but it is deteriorating the signals in further scans and in amperometry (see below) by blocking the active surface area.

Since the sensors based on boron-doped diamond should be used for alcoholic extracts of berries, the influence of ethanol on the signal was investigated. Concentrations from 0.5 to 20 % ethanol were added to the supporting electrolyte; no influence on peak position or peak height was observed.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula</th>
<th>CPE</th>
<th>BDDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$E_{pa}$ / V</td>
<td>$i_{pa}$ / mA mol$^{-1}$</td>
</tr>
<tr>
<td>Syringic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.95</td>
<td>23.3</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.70</td>
<td>15.2</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.48</td>
<td>10.2</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.88</td>
<td>78.6</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.50</td>
<td>19.2</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.90</td>
<td>37.5</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.90</td>
<td>148.5</td>
</tr>
<tr>
<td>Trolox</td>
<td><img src="image" alt="Formula" /></td>
<td>0.50</td>
<td>14.2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td><img src="image" alt="Formula" /></td>
<td>0.61</td>
<td>56.4</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td><img src="image" alt="Formula" /></td>
<td>0.50</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Table 1. Oxidation potentials, $E_{pa}$, and peak currents, $i_{pa}$, from cyclic voltammetry of typical antioxidants found in berries. Concentration: 30, respectively 90 mg/L, supporting electrolyte: PBS (0.1 mol/L, pH 7); potential window: -1.5 to +1.5 V, scan rate: 50 mV/s.
Hydrodynamic amperometry

Basic studies

Hydrodynamic amperometry was applied to the antioxidants listed in Table 1 applying a working potential which corresponds to the maximum of the oxidation current in CV. After attaining a stable baseline the analytes were added to a stirred solution.

Figure 2 shows a comparison of the electrochemical behavior of HQ between BDDE (a) and CPE (b) electrodes under identical conditions. As can be seen a strong adsorption of the quinone on the CPE leads to a fast decrease of the signal due to blocking of the active electrode surface area with a very small current steps after ensuing additions. BDDE does not show any obvious adsorption therefore, after addition of the electrochemically active analyte, the signal remains constant. Repetitive additions lead to clear step-like increases of current. For this reason BDDE seems to be an ideal electrode material for the determination of phenolic antioxidants due to the absent tendency for adsorption.

![Figure 2. Hydrodynamic amperograms of subsequent additions of 30 mg/L HQ in phosphate buffer (0.1 M, pH 7) using (a) BDDE and (b) CPE. Working potential: +1V.](image)

Validation of analytical parameters

The analytical method based on hydrodynamic amperometry was evaluated with hydroquinone and Trolox as standards. Under optimized conditions the linearity range of the BDD-sensor was estimated to be between 10 and 400 mg/L for Trolox and 10 to 300 mg/L for HQ (Fig. 3). The calibration curves can be described by the following regression equations:

\[ i_{\text{HYDROQUINONE}} / \mu A = 43.04 \times y / (\text{mg/L}) + 0.387 \]

\[ i_{\text{TROLOX}} / \mu A = 22.09 \times y / (\text{mg/L}) + 0.609, \]

both with a mean correlation coefficient of \( R = 0.999 \). For the concentrations above 400 mg/L (Trolox) and 300 mg/L (HQ) the calibration graph levels off but still remains linear to concentrations above 1 g/L, in fact with a very small sensitivity. The limit of detection (LOD) and the limit of quantification (LOQ estimated as the three-fold of LOD) were determined as 1.5 mg/L and 4.5 mg/L for HQ, and 2.5 mg/L and 7.5 mg/L for Trolox according to the International Conference on Harmonisation, ICH, Quality Guidelines for Analytical Evaluation [Q2(R1)] guidelines for visual evaluation [16].
The precision experiments (100, 200, 375, 625, 750 and 875 mg/L; n = 4) showed a relative standard deviation (RSD) of 3-8 % and a RSD of the slope of 3 %. The intercept of the calibration curves ranged from around 0.4 (HQ) to 0.6 µA (Trolox) with a RSD of about 5 % in different calibrations (n = 4).

**Figure 3.** Calibration curve of the BDD-sensor 10-1200 mg/L concentration range of *HQ and *Trolox.

**Application of the sensor in samples**

After it was demonstrated that the BDDEs showed only insignificant adsorption effects to the redox products of the investigated antioxidants they were applied to chosen samples. Thus, ethanolic extracts were prepared using red grape, bramble, blueberry and strawberry. The extracts were injected directly to the analytical cell without further dissolution or preparation. 20 mL of phosphate buffer was transferred into the electrochemical cell and after the baseline stabilization, 100 µL of the extract was injected. The extracts were analyzed using hydrodynamic amperometry at +1 V and the AOC was estimated as Trolox equivalent (TE) or hydroquinone equivalent (HQE) using the corresponding calibration curves. The capacities of the berry samples were determined in the range of 8 and 23 mM TE/HQE (Table 2) and are in a good agreement with the results of the photometric reference method.

**Table 2.** Comparison of the electrochemical and photometric total antioxidant capacity measurements of berry extracts.

<table>
<thead>
<tr>
<th></th>
<th>Trolox equivalent, mmol/L</th>
<th>Hydroquinone equivalent, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TEAC Assay</td>
<td>Electrochemical BDD sensor</td>
</tr>
<tr>
<td>Grape</td>
<td>23.4 ± 1.2</td>
<td>23.9 ± 1.2</td>
</tr>
<tr>
<td>Bramble</td>
<td>12.4 ± 0.6</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>Blueberry</td>
<td>18.5 ± 0.9</td>
<td>18.1 ± 0.9</td>
</tr>
<tr>
<td>Strawberry</td>
<td>8.9 ± 0.4</td>
<td>8.9 ± 0.4</td>
</tr>
</tbody>
</table>

Figure 4 shows a comparison between electrochemical signals determined using the BDD sensor and the decrease of absorption (ΔA) measured using the TEAC-Assay. It can be seen clearly that there is a directly proportional relation between the absorption signal decrease and...
amperometric currents. The method employing electrochemical sensors seems well applicable to detect the antioxidant capacity of ethanolic berry extracts.

Figure 4. Comparison of electrochemical data recorded in current intensity and photometric results charted as decrease of absorption.

Our studies show that the evaluated Trolox and hydroquinone equivalents from amperometric measurements with BDDEs are correlating well with the optical reference method (Table 2). This was also verified with individual antioxidants (Table 1) which produce the same antioxidant equivalent capacities with both procedures, amperometric and optical.

Maybe, in complex samples, substances which can be oxidized at an operational potential of 1.0 V could be present and do not contribute to the antioxidant capacity. In fact, we could not, so far, observe such a behavior with ethanolic extracts of berries.

Conclusions

The investigations show that boron doped diamond used as working electrode in electrochemical analytics is an attractive tool because of its many advantages. Due to its low feature for the adsorption of redox products it can be used for quantification of antioxidants even in complex material. The designed sensor is inexpensive because it can be used for repetitive measurements, easy to prepare, and with high reproducibility.

Acknowledgements: The financial support of the Österreichische Forschungsförderungsgesellschaft (FFG) for project “Antiflavo” (No.827334) is highly acknowledged.

References
A comparison of pitting susceptibility of Q235 and HRB335 carbon steels used for reinforced concrete

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Abstract

The phase structure and the pitting susceptibility of two carbon steels, Q235 and HRB335, used for reinforced concrete, are investigated by phase observation, polarization curve measurements, electrochemical impedance spectroscopy, and Mott-Schottky analysis. It is found that Q235 is ferrite and HRB335 is pearlite. Q235 is more susceptible to chloride ions leading to pitting than HRB335. The polarization curves show that the breakdown potential of the passive film in saturated Ca(OH)₂ solution containing 0.4 M NaCl is 0 V for Q235 and 0.34 V for HRB335. The Mott-Schottky analyses show that passive films formed on Q235 and HRB335 in saturated Ca(OH)₂ solution containing chloride ions behave like an n-type semiconductor. The passive film formed on Q235 has a higher donor density, which explains why Q235 is more susceptible to pitting than HRB335.

Keywords

Pitting susceptibility; Carbon steel; Phase structure; Polarization curve; Electrochemical impedance spectroscopy; Mott-Schottky.

Introduction

Reinforced concrete is widely used as building material because the corrosion resistance of the embedded carbon steel for the reinforcement plays a significant role in the life of reinforced concrete. In a high quality concrete, the embedded carbon steel prevents corrosion by forming a passive film on a steel surface, which slows down the access of oxygen, moisture, and various aggressive species to the interface between steel and concrete [1]. Among all the aggressive species, chloride ions exhibit the strongest attack on passive film. Chloride ions may be introduced to the concrete from raw material, such as water and sand, or they can penetrate from the outside in highway viaducts where de-icing salts are used as well as in marine structures [2]. In practice,
the corrosive attack due to chloride penetration usually leads to pitting corrosion, which causes catastrophe because its initiation and propagation is difficult to predict.

The pitting susceptibility of carbon steel is related to the microstructure and composition of the steel and inhibitors used for the formation of passive films [3-12]. Various carbon steels usually are combined for use because of the need for strength and tenacity. For example, two kinds of carbon steels, Q235 and HRB335, are usually used together in reinforced concrete structures to improve the strength of concrete. It is necessary to understand the pitting susceptibility of various kinds of carbon steel for their successful application in reinforced concrete. The aim of this work is to understand the difference in pitting susceptibility between Q235 and HRB335.

**Experimental**

**Electrodes and solutions**

The composition of Q235 and HRB335 is given in Table 1. The steel specimens (ф 0.8 cm × 0.5 cm) were embedded in epoxy resin, with a test area of 0.5 cm². Prior to each measurement, the working surface of specimens was polished with different SiC₂ abrasive papers and Al₂O₃ powder of 0.05 μm, then degreased with ethanol and rinsed with de-ionized water successively.

The saturated Ca(OH)₂ solution was prepared as simulated concrete pore solution (SPS) with de-ionized water. NaCl was added to form the solution containing chloride ions. All the chemicals used were of analytical grade.

| Table 1. Composition of Q235 and HRB335, wt. % |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Samples         | C     | Mn    | P     | S     | Si    | Cr    | Ni    | Al    | As    |
| Q235            | 0.15  | 0.326 | 0.039 | 0.03  | 0.115 | 0.024 | 0.032 | 0.127 | 0.028 |
| HRB335          | 0.26  | 0.698 | 0.046 | 0.05  | 0.324 | 0.033 | 0.039 | 0.016 | 0.026 |

**Phase-structure observation**

Optical microscope (ECLIPSE 50iPOL, Nikon Corporation) was used to observe the microstructure of Q235 and HRB335. Before the observation, the specimens were etched with 0.2 % Nital for 10s, degreased with ethanol and rinsed with de-ionized water.

**Electrochemical measurement**

The electrochemical measurements were carried out with PGSTAT-30 (Autolab, Eco Chemise B. V. Company). A classical three electrodes electrochemical cell was used. A platinum sheet with a geometric area of 1 cm² was used as the counter electrode and a saturated calomel electrode (SCE) was used as the reference electrode. All the potentials in this paper are versus the SCE.

The electrolyte was deaerated with nitrogen for 30 min before each measurement, and all the electrochemical measurements were carried out at ambient temperature without stirring. The potential scanning rate used in the polarization curve measurements was 0.5 mV s⁻¹. The impedance measurements were carried out in frequency range from 100 kHz to 0.01 Hz. In the Mott-Schottky measurement, the frequency used was 1 kHz and the potential step was 5 mV. Prior to each measurement, the working electrode was firstly kept at -1.15 V for 30 min, then at 0 V for 60 min to form a passive film, and finally it was stabilized at open circuit potential (OCP) for 30 min.
Results and Discussion

Phase structure

Fig. 1 presents the phase structure of Q235 (A) and HRB335 (B). Both, Q235 and HRB335 have two phases, pearlite and ferrite [11,13]. However, the proportion of ferrite and pearlite is different in HRB335 and Q235. Ferrite phase prevails in Q235 while in HRB335 prevails mainly pearlite. The composition of two carbon steel is similar except for the contents of Mn and Si (Table 1), which might account for the difference in microstructure between two carbon steels. From the different microstructure of the two carbon steels, it can be expected that they exhibit different pitting susceptibility [3,9].

![Figure 1. Phase structure of Q235 (A) and HRB335 (B)](image)

Polarization curve

In order to understand the formation process of passive film and to determine the passive potential range, a potential scan ranging from -0.6 to 0.65 V is performed. Fig. 2 presents the formation of passive film on Q235 and HRB335 obtained in the simulated concrete pore solution (SPS). It can be seen from Fig. 2 that both electrodes, Q235 and HRB335, exhibit a similar formation process of passive film. A cathodic process takes place when the potential is scanned from point a (-0.6V) to b (-0.41 V) for Q235, and from point a (-0.6 V) to b' (-0.45 V) for HRB335. This cathodic process should be ascribed to the hydrogen evolution reaction, because the experiments were carried out under nitrogen atmosphere and there were no other reducible species in the solution. When potential is scanned from point b (-0.41 V) and b' (-0.45 V) toward positive potential, the current in both cases (Q235 and HRB335 electrodes) increases till the potential approaches point c (-0.18 V). From point c (-0.18 V) to point d (0.65 V) the electrodes reach passive region. Both electrodes, Q235 and HRB335, have similar passive current density of about 4 μA cm⁻². The sharp increase of the current density at point d (0.65 V) is ascribed to the O₂ evolution, which depends on pH of the solution [13]. The current plateau of the anodic polarization curve indicates the formation of passive film within the potential domain from -0.18 to 0.65 V for both carbon steel electrodes.

Fig. 3 shows the polarization curves of Q235 and HRB335 in the SPS containing 0.4 M chloride ions. Before the measurement, the two carbon steel electrodes were passivated in SPS, in absence of chloride ions, at the potential of 0 V for 60 min. In SPS containing 0.4 M chloride ions, the breakdown of passive films on both electrodes, Q235 and HRB335, occurs at the potential, which is indicated by a drastic increase of current. However, the breakdown potential values of passive
films were different, i.e. about 0 V for Q235 and 0.34 V for HRB335. Therefore, the passive film formed on Q235 is more susceptible to chloride ions than the one formed on HRB335 electrode.

![Polarization curves of Q235 and HRB335 in SPS in absence of chloride ions (scan rate: 0.5 mV s\(^{-1}\)).](image)

**Figure 2.** Polarization curves of Q235 and HRB335 in SPS in absence of chloride ions (scan rate: 0.5 mV s\(^{-1}\)).

![Polarization curves of Q235 and HRB335 in SPS containing 0.4 M chloride ions (scan rate: 0.5 mV s\(^{-1}\)).](image)

**Figure 3.** Polarization curves of Q235 and HRB335 in SPS containing 0.4 M chloride ions (scan rate: 0.5 mV s\(^{-1}\)).

**Electrochemical impedance**

Fig. 4 presents the Nyquist plots of passivated Q235 and HRB335 in the SPS containing 0 and 0.4 M chloride ions at open circuit potential. It can be seen from Fig. 4 that two electrodes have similar behavior which does not involve any diffusion process, although there is a significant difference in polarization impedance for the electrodes in the solution with and without chloride ions.

A passivated electrode can be modeled by the equivalent circuit of Fig. 5. In Fig. 5, \(R_s\) represents the solution resistance; \(R_f\) and \(Q\) are the resistance and the space charge layer capacitance of the passive film. The element \(Q\) is usually represented by the constant phase element (CPE) in which \(n\) is in the range between 0.5 and 1 due to the surface heterogeneity and surface roughness of the passivated electrodes [14]. The impedance of a CPE is given by

\[
Z_{\text{CPE}} = \left[\gamma^0 (jw)^n\right]^{-1}
\]  

(1)
The capacitance element $Q$ (CPE) is pure capacitance when $n = 1$ and pure resistance when $n = 0$. The results obtained from fitting with Fig. 5 are shown by solid lines in Fig. 4 and by the data in Table 2. It can be seen from Table 2 that, in the solution without chloride ions, the film resistance ($R_f$) of Q235 is smaller than the one of HRB335, indicating that the passivated Q235 tends to react more easily than the passivated HRB335. When chloride ions are added in the solution, the resistance of the film decreases for both carbon steel electrodes.

**Figure 4. Nyquist plots of passivated Q235 and HRB335 electrodes in SPS: (a) without chloride ions; (b) containing 0.4 M chloride ions**

**Figure 5. Equivalent circuit for a passivated electrode.**

**Mott-Schottky analysis**

Passive films of most metals behave as semiconductor [15-18]. In the high frequency domain, the Mott-Schottly approach is a good tool for characterizing the semiconducting properties of a passive film [19,20]. When the frequency used for the impedance measurement is high enough, potential dependence of the capacitance of space-charge layer ($C_{sc}$) is expressed by Mott-Schottky relationship [5]:

For n-type semiconductor

$$\frac{1}{C_{sc}^2} = \frac{2}{e\varepsilon_r \varepsilon_0 N_D} \left( E - \phi_{fb} - \frac{kT}{e} \right)$$

(2)

For p-type semiconductor

$$\frac{1}{C_{sc}^2} = -\frac{2}{e\varepsilon_r \varepsilon_0 N_A} \left( E - \phi_{fb} - \frac{kT}{e} \right)$$

(3)

where $e$ is electron charge ($1.6\times10^{-19}$ C), $\varepsilon_r$ is dielectric constant, taken as 15.6 [17]. $\varepsilon_0$ is the vacuum permittivity ($8.85\times10^{-14}$ F cm$^{-1}$), $N_D$ is donor density, $N_A$ is acceptor density, $E$ is the applied potential, $\phi_{fb}$ is flat-band potential, $k$ is Boltzmann constant ($1.38 \times 10^{-23}$ J K$^{-1}$) and $T$ is absolute temperature. $N_D$ and $N_A$ can be determined from the slope of the linear relationship between $C_{sc}^{-2}$ and $E$, while $\phi_{fb}$ is obtained from the extrapolation to $C_{sc}^{-2} = 0$. 
Fig. 6 shows Mott-Schottky plots of passivated Q235 and HRB335 electrodes in SPS containing 0.4 M chloride ions. It can be seen from Fig. 6 that, at the potentials lower than 0.35V, $C_{sc}^{-2}$ increases with increasing the potential. There is a positive linear relationship between $C_{sc}^{-2}$ and $E$ in the potential range from 0 to 0.15 V for both electrodes, indicating that both passive films are n-type semiconductors [4]. The donor densities values calculated from the slope of the linear relationship between $C_{sc}^{-2}$ and $E$ in Fig. 6 are shown in Table 2. It is found that the donor densities values are very high, i.e. in the order of $10^{26}$ m$^{-3}$. This order is characteristic of heavily doped and disordered passive films, which was also found by Cheng and Luo ($10^{26}$ m$^{-3} \leq N_D \leq 10^{27}$ m$^{-3}$) [21]. A donor density of a passive film determines its pitting susceptibility. The larger the donor density is, the more susceptible to pitting is. The donor density of passive film formed on Q235 is $4.42 \times 10^{26}$ m$^{-3}$, which is higher than the one of HRB335 ($2.29 \times 10^{26}$ m$^{-3}$), confirming that Q235 is more susceptible to pitting than HRB335.

**Table 2. Fitting results of the experimental data in Fig. 4 and Fig. 6**

<table>
<thead>
<tr>
<th>Samples</th>
<th>$C_{NaCl}$/ M</th>
<th>$R_t$ / k$\Omega$ cm$^2$</th>
<th>$\gamma^0$ /$\mu$S cm$^{-2}$ s$^{-1}$</th>
<th>n</th>
<th>$N_D$ /$10^{26}$ m$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q235</td>
<td>0</td>
<td>285.3</td>
<td>10.95</td>
<td>0.9607</td>
<td>-</td>
</tr>
<tr>
<td>Q235</td>
<td>0.4</td>
<td>6.0</td>
<td>18.42</td>
<td>0.9236</td>
<td>4.42</td>
</tr>
<tr>
<td>HRB335</td>
<td>0</td>
<td>402</td>
<td>10.81</td>
<td>0.9610</td>
<td>-</td>
</tr>
<tr>
<td>HRB335</td>
<td>0.4</td>
<td>22.5</td>
<td>12.55</td>
<td>0.9512</td>
<td>2.29</td>
</tr>
</tbody>
</table>

**Conclusions**

Based on the results from phase observation, potentiodynamic polarization, electrochemical impedance measurements and Mott-Schottky analysis of passive films of two carbon steel electrodes, it can be concluded that Q235 is more susceptible to pitting than HRB335. Q235 has a phase structure of ferrite, which tends to form a less stable passive film and thus is more susceptible to chloride ions than HRB335, whose phase structure is pearlite.

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Abstract
The redox behaviour of two potential anticancer anthraquinones, 9,10-anthraquinone and 2-chloromethyl-9,10-anthraquinone was investigated in a wide pH range. Cyclic voltammetry based assay was developed for the assessment of the effect of medium, substituents, potential scan rate and number of scans on the voltammetric response of anthraquinones. The electrode reaction mechanism was suggested on the basis of cyclic and differential pulse voltammetric results. The effect of DNA on anthraquinones was also probed at physiological pH which could lead to further investigation of possible citotoxic activity in vitro. The results revealed that anthraquinones interact with DNA more strongly than the clinically used anticancer drug, epirubicin.

Keywords
Anthraquinones; redox mechanism; voltammetry; binding constant.

Introduction
Quinones are known to have a variety of biochemical and physiological functions. Literature survey reveals that these compounds are endowed with antibacterial, antifungal, anti-inflammatory, wound healing, analgesic, antipyretic, antimicrobial, antibiotic and antitumor activities [1,2]. Some derivatives of quinones are common constituents of biologically important molecules such as vitamins K [3]. Others, like mitoxantrone and pixantrone, have been reported to have appreciable antineoplastic activity [4]. It has been reported that the biological action of quinones is due to their electron transfer rates and redox potentials [5]. Consequently, the knowledge of the redox properties of quinones derivatives seems conceivable for a better understanding of their biological action.
Anthraquinones (AQs), an important class of tricyclic compounds, have drawn the utmost attention in medical field as anticancer drugs [6]. The main role of AQs in biological electron transport [7] and industrial processes as redox catalysts [8] have led to the extensive investigations of their electrochemical behavior [9,10]. Quinone-hydroquinone redox complexes are widely used in voltammetric studies due to their robust electrochemistry. The electron transfer process of most of the quinones has been reported to occur through two one-electron steps via a mechanism involving semiquinone free radical [11]. Thus, the study of electron transfer reactions is expected to provide an useful information about molecular structure [12]. Literature survey reveals that in spite of the broad range activities of quinones the electrochemical redox mechanism and DNA binding studies of biologically important AQs, 9,10-anthraquinone (AQ) and 2-chloromethyl-9,10-anthraquinone (CM), is an unexplored matter at physiological pH. To bridge this gap and to offer an explanation for the role of the selected AQs (Scheme 1) in exercising anticancer effect their detailed electrochemistry has been carried out in a wide pH range. Due to the existing resemblance between electrochemical and biological reactions it can be assumed that the redox mechanisms taking place at the electrode and in the body share similar principles [13]. By monitoring the variation in the electrochemical signal of the DNA binding drug it is possible to propose the mechanism of the interaction and the nature of the complex formed. Since AQs participate in several electron transfer processes in the cellular milieu, the present study is expected to provide valuable insights into molecular mechanisms of their action and designing specific DNA-targeted drugs [14].

**Scheme 1. Structures of the chemicals used.**

**Experimental**

Anthraquinones were purchased from Sigma and used without further purification. 0.5 mM working solutions of AQs were prepared in 50% ethanol and 50% buffer. All supporting electrolyte solutions (Table 1) were prepared using analytical grade reagents. Double stranded DNA was extracted from chicken blood by Falcon method [15,16]. The concentration of the DNA stock solution (2.0 mM) was determined from UV absorbance at 260 nm using a molar extinction coefficient ($\varepsilon$) of 6600 M$^{-1}$ cm$^{-1}$ [17,18]. A ratio of absorbance at 260 and 280 nm of ($A_{260}/A_{280}$) > 1.8 indicated protein free DNA [19]. Doubly distilled water was used throughout.

Voltammetric experiments were performed using µAutolab running with GPES 4.9 software, Eco-Chemie, The Netherlands. A glassy carbon (GC) ($A = 0.07$ cm$^2$) was used as a working electrode, a Pt wire served as a counter electrode and a saturated Ag/AgCl electrode was employed as the reference electrode. Before each experiment the surface of GCE was polished with alumina powder followed by thorough rinsing with distilled water. For reproducible experimental results the clean GC electrode was placed in the supporting electrolyte solution and
various cyclic voltammograms were recorded until the achievement of a steady state baseline voltammogram. All the voltammetric experiments were conducted in a high purity argon atmosphere at room temperature (25±1°C). The pH measurements were carried out with a Crison micro pH 2001 pH-meter with an Ingold combined glass electrode.

Table 1. Supporting electrolytes of 0.1 M ionic strength.

<table>
<thead>
<tr>
<th>pH</th>
<th>Composition</th>
<th>pH</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>HCl + KCl</td>
<td>7.0</td>
<td>NaH₂PO₄ + Na₂HPO₄</td>
</tr>
<tr>
<td>3.0</td>
<td>HAcO + NaAcO</td>
<td>8.0</td>
<td>NaH₂PO₄ + Na₂HPO₄</td>
</tr>
<tr>
<td>4.0</td>
<td>HAcO + NaAcO</td>
<td>9.0</td>
<td>NH₃ + NH₄Cl</td>
</tr>
<tr>
<td>5.0</td>
<td>HAcO + NaAcO</td>
<td>10.0</td>
<td>NH₃ + NH₄Cl</td>
</tr>
<tr>
<td>6.0</td>
<td>NaH₂PO₄ + Na₂HPO₄</td>
<td>12.0</td>
<td>NaOH + KCl</td>
</tr>
</tbody>
</table>

Results and Discussion

The cyclic voltammograms of 0.5 mM AQ (Fig. 1) obtained in 0.1 M acetate based supporting electrolyte (pH 3.0) registered a prominent cathodic peak 1c (more concentration sensitive) at -0.41 V followed by a small reduction peak 2c at -0.60 V. A single anodic peak 1a corresponding to oxidation of the reduction product of AQ was recorded in the reverse scan. The $E_{p1c}-E_{p1c/2}$ of 60 mV (peak 1c) indicated reversible one electron transfer process as reported by the previous investigators [20]. However, the unequal anodic and cathodic peak currents pointed to the quasireversible nature of the overall electrochemical process. The decrease in the cathodic peak current of AQ, as evident from 2nd, 3rd and 4th voltammograms obtained under the same conditions, indicated the adsorption of the reduction product at the electrode surface.

The appearance of two cathodic peaks in the differential pulse voltammogram -DVP (shown in Fig. 2) confirmed the CV result of two step reduction of AQ. In non-aqueous media quinones undergo two reversible1e processes, but the results of our experiments indicate the appearance
of one quasi reversible and another irreversible peak in a 50:50 mixture of ethanol and water. The width at the half peak height \( W_{1/2} \) of 91 and 93 mV (close to the theoretical value of 90.4 mV) indicated the involvement of one electron in each reduction step.

![Figure 2. First scan DPV of AQ obtained in pH 3.0 at 5 mV s\(^{-1}\); pulse amplitude = 50 mV and pulse period = 200 ms](image)

For the assessment of the effect of pH, CVs of AQ (Fig. 3) were obtained in acidic, neutral and alkaline media. In contrast to acidic conditions, the reduction of AQ at pH 7.0 was evidenced by a sharp single cathodic peak. The appearance of two broad anodic peaks in the reverse scan showed two step oxidation of the AQ reduction product. The cathodic peak width \( E_{pc} - E_{pc/2} \) of 34 mV (close to the theoretical value of 30 mV) indicated the transfer of two-electrons as reported by Bard et al. [20]. The equal anodic and cathodic currents at pH 9.0, with peak potential difference of 75 mV can be attributed to the almost reversible nature of the overall redox process under these conditions [21]. The variation in voltammetric response indicated the strong dependency of the AQ redox mechanism on the pH of the medium. With the increase in pH, the potential of \( 1_e \) displaces to more negative values. This indicated the involvement of protons in the electropositive reduction of AQ. Two peaks were also noticed in the CV of CM at pH 4.5 (Fig. 4) but at positions different from AQ indicating the modulation of the electronophore by the substituent attached at the aromatic ring. The reduction potential of CM was also found to shift cathodically with the increase in pH indicating the involvement of protons during electron transfer processes. On the basis of results obtained from CV and DPV the mechanism shown in Scheme 2 was suggested.

![Figure 3. 1st scan CVs of 0.5 mM AQ obtained in pH (a) 4.0, (b) 7.0 and (c) 9.0 at 200 mV s\(^{-1}\).](image)
In order to rapidly screen the selected compounds for their anticancer activities, a relatively simple cyclic voltammetric based assay was used without performing other elaborate assays. Although easy to implement, this assay cannot be applied to electroinactive compounds in the potential window of the working electrode. In addition to it, the method is limited to compounds exerting their anticancer effect through direct interaction with DNA. In spite of these obvious limitations the assay can differentiate the binding strength and modes of action of DNA-binding compounds. As the redox behaviour of quinones is sensitive to their covalent or non covalent binding to other molecules, therefore, cyclic voltammetry was used for their DNA binding studies. For CV titrations both the concentration and volume of anthraquinones were kept constant while varying the concentration of DNA in solution. The CVs of anthraquinones in the presence of DNA have been presented at physiological pH 4.5 (pH of lysosomes of the cell) as no report is available in literature on their DNA-binding behavior under such conditions.

![Graph](image)

**Figure 4.** 1st scan CV of 0.5 mM CM obtained in pH 4.5 at 200 mV s⁻¹.

Typical CV behaviour of 0.5 mM AQ at pH 4.5 and \( v = 200 \text{ mV s}^{-1} \) in the absence and presence of 40, 100 and 120 nM DNA has been shown in Fig. 5. With the gradual addition of DNA, peak 1c got diminished and peak 2c increased in height. The positive peak potential shift accompanied with the decrease in current of 1c is attributed to the disappearance of reactant, AQ and its 1e⁻, 1H⁺ reduction product (HAQ) due to their intercalation into the stacked base pair domain of DNA. The increase in current of peak 2c can be related to the greater current carrying ability of negatively charged AQ⁻ in the presence of DNA having anionic phosphate backbone.

The CV of 0.5 mM CM in the presence of 40 nM DNA (shown in Fig. 6) showed quite different behavior from AQ. The cathodic peak potential was shifted (80.3 mV) anodically while the anodic peak potential underwent a drift of 30.2 mV in the cathodic direction accompanied with the decrease in peak currents.
ANTHRAQUINONES AND THEIR DNA BINDING

Figure 5. CVs of 0.5 mM AQ in the (a) absence and presence of (b) 40, (c) 100 and (d) 120 nM DNA at pH 4.5; v = 200 mV s\(^{-1}\).

Figure 6. CVs of 0.5 mM CM in the (a) absence and presence of (b) 40 nM DNA at pH 4.5; v = 200 mV s\(^{-1}\).

In general the positive shift in peak potential is suggested for intercalation of the drug into the stacked base pair pockets of DNA [22], while negative shift is related to electrostatic interaction of the drug with the anionic phosphate backbone of DNA [23]. So, the obvious positive peak potential shift in the cathodic peak of CM can be attributed to the intercalation of CM into the base pair pockets of DNA. The shift in the anodic direction further indicates that CM is easier to reduce in the presence of DNA because its reduced form is more strongly bound to DNA than its oxidized form (neutral form). The displacement of the anodic peak in the negative going direction can be linked with the electrostatic interaction of the reduction product of CM with anionic oxygen of DNA. The interaction is expected to alter the DNA replication machinery that may lead to the death of cancerous cells. For such a system, where both forms of the drug interact with DNA, Scheme 3 can be applied [24].
Based upon the process discussed in Scheme 3, the following equation is obtained [25]

\[ E_b^0 - E_f^0 = 0.059 \log \left( \frac{K_{ox}}{K_{red}} \right) \]  

(1)

where \( E_f^0 \) and \( E_b^0 \) are the formal potentials of the free and DNA-bound forms of CM respectively.

For a shift of 60 mV caused by the addition of 40 nM DNA into 0.5 mM CM a ratio of \( K_{ox}/K_{red} \) was calculated as 0.096, which indicates 10.40 times stronger interaction of the reduced form of the drug with DNA than its oxidized form. The substantial diminution in peak current can be attributed to the formation of slowly diffusing CM-DNA supramolecular complex due to which the concentration of the free drug (mainly responsible for the transfer of current) is lowered.

The dependence of the current function \( I_p \), on the scan rate, \( \nu \), is an important diagnostic criterion for establishing the type of mechanism. The peak currents of these compounds were found to vary linearly with the square root of the scan rate (\( \nu^{1/2} \)) as expected for diffusion-limited electrochemical process. The diffusion coefficient was determined by the following form of Randles-Sevcik equation

\[ I_{pc} = -2.99 \times 10^5 n (\alpha_c n)^{1/2} A C_c D_o^{1/2} \nu^{1/2} \]  

(2)

where \( n \) is the number of electrons transferred during the reduction, \( \alpha_c \) is the cathodic charge transfer coefficient, \( A \) the electrode area in cm\(^2\), \( D_o \) the diffusion coefficient in cm\(^2\) s\(^{-1}\), \( C_c \) the concentration in mol cm\(^{-3}\) and \( \nu \) the scan rate in V s\(^{-1}\) [26]. Taking \( n = 1 \) (as determined from \( W_{1/2} \) of DPV) and \( \alpha_c = 0.73 \) (evaluated from \( E_p - E_{p/2} = 47.7/\alpha n \)), the values of \( D \) were obtained from the plots of \( I_{pc} \) vs. \( \nu^{1/2} \) at pH 4.5. The electroactive area (0.071 cm\(^2\)) of the working electrode was determined as described in literature [27]. From the measured slopes of 1.24 \( \times \) 10\(^{-5}\) and 2.17 \( \times \) 10\(^{-5}\) the diffusion coefficients of AQ and CM were determined as 2.49 \( \times \) 10\(^{-6}\) and 7.58 \( \times \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\). The general idea about diffusivity is that the molecule having smaller molecular mass should have greater diffusion coefficient but the results of our experiments showing the opposite trend disproved the common concept. The high \( D \) value of CM may be due to the presence of electron-withdrawing chlorine substituent group that will lower the electronic density at the electrophore thus increasing its affinity for reduction at the electrode surface. The lower diffusion coefficients of the DNA bound AQ (9.28 \( \times \) 10\(^{-7}\)) and CM (1.42 \( \times \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\)) as compared to their free forms is suggestive of slowly diffusing supramolecular drug-DNA adduct formation.

Heterogeneous electron transfer rate constant (\( k_{sh}^0 \)) was evaluated by using Nicholson equation [28] which is based on correlation between \( \Delta E_p \) and \( k_{sh}^0 \) through a dimensionless parameter \( \psi \) by following equation,

\[ \psi = \frac{\gamma k_{sh}^0}{(\pi a D_c)^{1/2}} \]  

(3)
where $\gamma = D_d/D_R$, $a = nFv/RT$ and $v$ is the scan rate. $\psi$ for different values of $\Delta E_p$ can be obtained from the table reported in literature [20]. From the knowledge of $\psi$, $k_m^a$ values of AQ and CM were calculated as $1.02 \times 10^{-3}$ and $8.51 \times 10^{-4}$ cm$^{-1}$ s$^{-1}$ using equation (3). The $k_m^a$ values also characterized the reduction processes to be quasi-reversible in nature.

Based upon the decrease in peak current of AQ and CM by the addition of different concentration of DNA, ranging from 20 to 120 nM, the binding constant was calculated from the intercept of the plot of log (1/[DNA]) versus log ($I/(I_o-I)$) [29]

$$\log (1/[DNA]) = \log K + \log (I/(I_o-I))$$

(4)

where $K$ is the binding constant, $I_o$ and $I$ are the peak currents of the drug in the absence and presence of DNA respectively.

The linear fitting of the amperometric data yielded the binding constant of CM and AQ as $2.14 \times 10^6$ and $1.50 \times 10^6$ M$^{-1}$ indicating their high affinity with DNA. The higher binding constant values than the $K = 4.1 \times 10^5$ M$^{-1}$ of the classical intercalator, proflavin and $K = 3.4 \times 10^4$ M$^{-1}$ of clinically used intercalating anticancer drug, epirubicin [30-32] suggested their preferred anticancer drug candidature. However, the clinical use of these drugs demands more research work on their side effects, reactions with Fe(III) in the blood and production of radicals.

Conclusions

The present study shows that anthraquinones can be reduced at the glassy carbon electrode in a pH dependent diffusion controlled manner involving the same number of electrons and protons. The two cathodic peaks, noticed in the CV of CM at positions different from AQ, indicated the modulation of the electrophore by the substituent attached at the aromatic ring. The potential anticancer AQ and CM at physiological pH were found to interact with DNA by intercalative mode as evidenced by the decrease in current intensity and positive peak potential shift. The diffusion coefficients of free anthraquinones were found greater than their DNA-bound forms as expected. The binding constant varied in the sequence: $K_{CM} > K_{AQ}$ with values greater than the clinically used anticancer drugs. The investigation of the electrochemical behaviour of AQ and CM has the potential of providing valuable insights into biological redox reactions of this class of molecules, resulting in a better understanding of the data described for biological systems and increasing the overall knowledge of anthraquinones physiological mechanism of action.

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Ni-MoO₂ cathodes for hydrogen evolution in alkaline solutions. Effect of the conditions of their electrodeposition

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Abstract

The electrodeposition of Ni-MoO₂ composite coatings is a specific process taking place under defined hydrodynamic conditions. In this work the influence of hydrodynamics, current density, and MoO₂ concentration on the electrodeposition of Ni-MoO₂ coatings from a suspension of MoO₂ particles in a Watt’s type bath were investigated by comparison of their polarization characteristics for the hydrogen evolution reaction (HER) in 1 M NaOH at room temperature. It was shown that electrolyte and air flow rates influence the process of coating electrodeposition at low concentrations of MoO₂. With increases in MoO₂ concentration the current density became the main parameter influencing the coatings’ properties. The best coating, with the lowest over-voltage for the HER, was obtained from the suspension containing 3 g dm⁻³ of MoO₂ particles. The over-voltage of this coating was 57 mV lower for the HER at j = −0.3 A cm⁻² (the current density used in industrial applications) than that for the commercial De Nora’s Ni + RuO₂ electrode.

Keywords

Ni-MoO₂; composite coatings; electrodeposition; HER; morphology.
Introduction

One of about 90 hydrogen production routes [1] is alkaline water electrolysis [2]. It is a well-established process that is technologically very simple and produces very clean gases, but it is also energy-inefficient, slow, and expensive. One of the ways to increase the energy efficiency of electrolysers is by developing advanced electrocatalytic materials in order to reduce the electrode over-voltage. In conventional water electrolysis, the cathode is usually made of stainless steel or nickel-based materials (Raney Ni), and operates in 6–9 M KOH solution in a temperature range of 60–90 °C. The overall energy efficiency of electrolysis is related to the hydrogen evolution reaction (HER). In chlor-alkaline electrolysis, HER, as the cathodic reaction in 32 wt.% NaOH at 90 °C, is also responsible for the energy efficiency of electrolysers [3]. Hence, for industrial application, advanced electrocatalytic materials for HER are of great importance to increase energy efficiency. Since porous Ni (Raney Ni) electrodes showed extensive deactivation during electrolysis, several approaches to design and produce new electrocatalytic cathode materials have been presented in the past [4–20]. One of these approaches is based on the Brewer intermetallic bonding theory, which predicts a synergistic activation effect whenever metals of the left half of the d-series are alloyed with metals of the right half of the d-series [4–10]. Accordingly, an increase in electrocatalytic activity has been widely observed for such materials: Ti–Ni [11], Pt–Mo [12], Ni–Mo [9], Ni–Co–LaNi5 [13], Ni–RuO2 [14], and Ni–transition metals (Fe, Mo, W) [15]).

Recently, particular attention has been given to the replacement of expensive Ni + RuO2 [14] composite coating with coatings obtained by the electrodeposition of Ni with molybdenum oxide species [16–20]. It was shown that Ni–MoO3 [16,20] composite coatings, electrodeposited from a suspension containing different concentrations of MoO3 particles in a Watt’s type bath, possess good catalytic activity, but their properties during the “service life” test were not good enough for application in industrial electrolysis [18]. On the other hand, Ni–MoO2 composite coatings electrodeposited from a suspension containing 3 g dm−3 MoO2 particles in a solution of 0.2 M NiCl2 + 2 M NH4Cl were found to be a promising replacement [17,18] for commercial Ni + RuO2 coating obtained by a similar electrodeposition procedure.

Taking into account that the commercial Ni + RuO2 cathodes were electrodeposited by incorporation of RuO2 particles in an Ni matrix, in this work an attempt was made to produce Ni–MoO2 composite coatings from a suspension of MoO2 particles in a Watt’s type bath under different conditions (different concentrations of MoO2 particles, different current densities, and different hydrodynamic conditions) and to compare their polarization characteristics for HER in 1 M NaOH with that for the commercial Ni + RuO2 coating. In this way most of the possibilities of obtaining Ni–Mo–oxide coatings by electrochemical deposition would be fulfilled.

Experimental

Electrodeposition of the Ni–MoO2 composite coatings

The MoO2 powder was synthesized by a rheological phase reaction route [18]. The average size of MoO2 powder particles, determined with a Brookhaven Instruments light-scattering system equipped with a BI-200SM goniometer, a BI-9000AT correlator, a temperature controller, and a Coherent INNOVA 70C argon-ion laser, amounted to about 200 nm. Dynamic light scattering measurements were performed using 135 mW laser excitation at a wavelength of 514.5 nm and a detection angle of 90°. All samples were electrodeposited onto Ni 40 mesh from the suspension of MoO2 powder particles (1–3 g dm−3) in a Watt’s type electrolyte containing 210 g dm−3 NiSO4 × 7H2O, 60 g
dm$^{-3}$ NiCl$_2 \times 6$H$_2$O, 60 g dm$^{-3}$ Na$_2$SO$_4$, and 30 g dm$^{-3}$ H$_2$BO$_3$ at pH = 3.8 and $t$ = 50 °C in the pilot plant cell (Fig. 1a,b,c). The electrolyte was circulated with the pump, while the flow rate was measured with the flow meter (Fig. 1a). Additional mixing of the electrolyte was provided by the air flow (measured with the flow meter, Fig. 1b) through two pipes with small openings facing the bottom of the cell in order to remove eventually precipitated molybdenum oxide particles from the bottom of the cell and to force particles to float and circulate with the electrolyte (Fig. 1b,c).

The temperature of the electrolyte was kept constant by the thermocouple, heater, and control unit (as shown in Fig. 1a). The Ni 40 mesh cathode (dimensions: $5 \times 6$ cm$^2$) was connected to a Ni holder (frame, Fig. 1d) and placed between two Ni anode plates ($18 \times 22$ cm, Fig. 1b). A homemade power supply, with a ripple smaller than 1 %, was used to apply the required current/voltage [16,17].

All Ni 40 mesh substrates were first cathodically degreased in a solution of 30 g dm$^{-3}$ NaOH, 30 g dm$^{-3}$ Na$_2$CO$_3$, and 40 g dm$^{-3}$ Na$_3$PO$_4 \times 12$H$_2$O for 5 min at $j = -100$ mA cm$^{-2}$ and $t = 60$ °C. Neutralization was performed in a solution of 20 g dm$^{-3}$ H$_2$SO$_4$ for 5 min. The Ni 40 meshes were then shortly etched in a 1:3 mixture of H$_2$O:HNO$_3$ and washed with distilled water before the electrodeposition of the Ni–MoO$_2$ composite coatings.

**HER investigations**

All experiments were carried out in 1 M NaOH at room temperature in a three-compartment electrochemical cell with two Pt mesh counter electrodes placed parallel (in separate compartments) to the working electrode and a saturated calomel electrode (SCE) as the reference electrode, connected to the working electrode (in the central compartment) by means of a Luggin capillary. The solution of 1 M NaOH was made from extra pure UV water (Smart2PureUV, TKA) and p.a. chemicals.

All experiments were performed with the potentiostat Reference 600 and PHE 200 or DC 105 Software (Gamry Instruments).

**Polarization curve measurements**

Electrodes were first submitted to hydrogen evolution at a constant current density of $j = -0.3$ A cm$^{-2}$ for 800 s (step 1), followed by HER at a constant potential at which the cathodic current density for HER was higher than $-0.3$ A cm$^{-2}$ (step 2) for 800 s. After this pre-electrolysis, polarization curves were recorded by sweeping the potential at 1 mV s$^{-1}$ from the potential applied in step 2 to the potential value of $-1.10$ V and recording the current density. The potential values were corrected for the IR drop by the current interrupt technique (DC 105).

**SEM and EDS analysis of the Ni–MoO$_2$ composite coatings**

The appearance of the surface and the chemical composition of the coatings were investigated by SEM (VEGA TS 5130 MM, Tescan) equipped with an energy-dispersive x-ray spectrocope (EDS; INCAPentaFET-x3, Oxford Instruments).

**Results and Discussion**

**Electrodeposition of Ni–MoO$_2$ samples**

Samples 1–12 were deposited with a charge of 60 C cm$^{-2}$, while samples 13–21 were deposited with a charge of 120 C cm$^{-2}$. The conditions of electrodeposition are given in Table 1, while
corresponding values of potential taken from the polarization curves for HER in 1 M NaOH at $j = -0.3$ A cm$^{-2}$ are presented in the last column of Table 1.

![Figure 1](image-url)

**Figure 1.** (a) Schematic presentation of the system for electrodeposition of coatings. (b) System for additional mixing of electrolyte with air bubbling through two pipes on the bottom of the tank. (c) Position of anodes and cathode. (d) Position of the mesh cathode on the Ni frame.

Before the addition of MoO$_2$ powder particles to the electrolyte, electrodeposition of several pure Ni samples in a Watt’s type bath was performed at $j = -50$ mA cm$^{-2}$ with different electrolyte and air flow rates. In all cases good quality deposits were obtained with a high current efficiency (> 85 %). When the first amount of MoO$_2$ powder particles of 1 g dm$^{-3}$ was added to the electrolyte, electrolyte was cycled at the highest rate of 20 dm$^3$ min$^{-1}$ (with the pump, Fig. 1a) and mixed with the highest air flow of 20 dm$^3$ min$^{-1}$ (using a compressor, Fig. 1b) for several hours. Electrodeposition of Ni–MoO$_2$ composite coating started after 24 h of electrolyte aging (following experience from our previous work [17]).

The electrolyte flow rates were set at two values, 10 and 20 dm$^3$ min$^{-1}$, while the air flow rates were 1, 5, 10, and 20 dm$^3$ min$^{-1}$. Three values of the deposition current densities ($j_{dep}$) were used: $-25$ mA cm$^{-2}$, $-50$ mA cm$^{-2}$, and $-100$ mA cm$^{-2}$. 


Polarization curves for the best samples

Four samples were deposited in the suspension containing 1 g dm\(^{-3}\) MoO\(_2\). Their polarization curves for HER were similar, with the best one being that for sample 4. All samples possessed lower over-voltage for HER than Ni 40 mesh with about 50 μm of pure Ni deposited from the Watt’s type bath (Ni). The best sample (4) was deposited at the highest current density and flow rates of electrolyte and air (see Table 1). Nine samples were deposited in the suspension containing 2 g dm\(^{-3}\) MoO\(_2\). The best polarization characteristics were recorded for sample 13 and the worst ones for sample 11, while all others were placed between these two. Eight samples were deposited in the suspension containing 3 g dm\(^{-3}\) MoO\(_2\). The best polarization characteristics were recorded for sample 19, while all others were placed close to the best one. The best polarization characteristics for samples deposited from all three suspensions are presented in Fig. 2, together with polarization characteristics for the deposited Ni coating (Ni) and commercial De Nora’s Ni + RuO\(_2\) (DN) coating.

**Table 1. Conditions for electrodeposition of Ni-MoO\(_2\) coatings.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>MoO(_2) g dm(^{-3})</th>
<th>( -j_{\text{dep}} ) mA cm(^{-2})</th>
<th>Electrolyte flow rate, dm(^3) min(^{-1})</th>
<th>Air flow rate, dm(^3) min(^{-1})</th>
<th>( E_{(0.3 \text{ A cm}^{-2})} ) V</th>
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doi: 10.5599/jese.2012.0027
Figure 2. The best polarization curves recorded in 1 M NaOH at room temperature for samples deposited in the suspensions containing 1 g dm\(^{-3}\) MoO\(_2\) (sample 4), 2 g dm\(^{-3}\) MoO\(_2\) (sample 13), and 3 g dm\(^{-3}\) MoO\(_2\) (sample 19). Polarization curves for deposited Ni (Ni) and commercial De Nora’s Ni + RuO\(_2\) electrode (DN).

Morphology of electrodeposited samples

The typical morphology of the electrodeposited samples is presented in Fig. 3a. Two different types of deposits could be clearly seen: a bright, compact deposit, rich in Ni, around the position where the Ni wires crossed, and a dark deposit characterized by a large number of cracks, rich in Mo, located on the rest of the surfaces of the Ni wires. The Ni-rich and Mo-rich parts of the surfaces were analysed by EDS, as shown in Fig. 3b. The results of the EDS analysis are presented in Table 2.

Figure 3. (a) SEM of the surface of a typical deposit; (b) EDS analysis of Ni-rich and Mo-rich surfaces.
Table 2. Chemical compositions of sample surface presented in Fig. 3b.

<table>
<thead>
<tr>
<th>Spectrum No.</th>
<th>O content, at. %</th>
<th>Ni content, at. %</th>
<th>Mo content, at. %</th>
<th>Average values</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td>O content, at. %</td>
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<td>76.10</td>
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</table>

As can be seen, spectra 1, 2, and 6 correspond to the Ni-rich surface, while spectra 3, 4, and 5 correspond to the Mo-rich surface. Similar morphologies and compositions were obtained for samples electrodeposited from ammoniacal NiCl₂ solution [17–19], except that a larger part of the surface of the Ni–MoO₂ composite coatings obtained in our previous work [17–19] was richer in Ni, while a smaller part contained a high percentage of Mo.

In chlor-alkali electrolysis the efficiency of the cathodes results from the combination of a certain activity and stability at the high current densities (3–6 kA m⁻²) used in technological applications. One of the main reasons for the loss of activity and stability of cathodes during long-term operation is the so-called polarity inversion of the electrodes which takes place during the replacement of old electrodes of an electrolyser with new ones in zero-gap cells. During this operation anodes and cathodes are short-circuited, causing a reverse current flow which may damage the cathodes and negatively affect their activity for HER [3]. Manufacturers can predict how often this operation should be performed during a certain period of time and, in accordance with that, design appropriate accelerated “service-life” tests for cathodes. The service-life test of cathode materials that are promising for use in industrial chlor-alkali electrolysis is the subject of only two papers in the literature [18,21]. The procedure for testing the service life is based on a sequence of galvanostatic polarizations in the HER range and cyclic voltammetries (CVs) in a wide potential range, inducing hydrogen evolution at its negative limit and oxygen evolution at its positive limit (reproducing the conditions of polarity inversion [18,21]). As shown in our previous paper [18] the main reason for the loss of activity of Ni–MoO₂ composite coatings is the dissolution of Mo-rich parts of the coating during the anodic polarization. Although the morphology and composition analysis of Ni–MoO₂ composite coatings obtained in this work indicates the presence of large amount of deposit with Mo-rich parts, their performance during the service-life test will be the subject of our further work.

Conclusions

In this work the influence of hydrodynamics, current density, and MoO₂ concentration on the electrodeposition of Ni-MoO₂ coatings from a suspension of MoO₂ particles in a Watt’s type bath was investigated by comparison of their polarization characteristics for HER in 1 M NaOH at room temperature. It was shown that electrolyte and air flow rates influence the process of coatings electrodeposition at low concentrations of MoO₂, while at higher concentrations of MoO₂ the current density became the main parameter influencing the coatings’ properties. The best coating was obtained from the suspension containing 3 g dm⁻³ of MoO₂ particles. This coating had a 57 mV lower over-voltage for the HER at j = −0.3 A cm⁻² than a commercial De Nora’s Ni + RuO₂ electrode.
**Acknowledgements:** The authors are indebted to the Ministry of Education and Science of the Republic of Serbia (Project No. 172054) for the financial support of this work as well as to the Department for the Research and Development of De Nora Industries S.p.A. for providing them with the commercial De Nora’s cathode for hydrogen evolution in the chlor-alkali plants.

**References**


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