Electrochemical Sensor Based On Carbon Printed Electrode

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Abstract: An amperometric method for the determination of nitrite ions has been developed using disposable, Carbon printed electrodes in presence of potassium ferrocyanide (II) as a mediator. The used sensors have a gold working electrodes with an area of about 7 mm². Calibration curves exhibit a linear relationship between the electrode response and the nitrite concentration up to 2.50 μM. calibration curves exhibit a linear relationship between the electrode response and the nitrite concentration up to 2.50 μM. with correlation coefficients equal to or better than 0.983. The lowest nitrite concentration measured (LOQ) was 100 μM. The relative standard deviations were equal to or better than 6.73 % (n=5). The electrode response was examined with respect to different parameters. The sensors were evaluated on samples of unspiked and spiked tap water and agriculture drain water.

Keywords: screen-printed electrodes, Amperometry, Nitrite, Potassium hexacyanoferrate, Electroanalytical

1. Introduction

Nitrite and nitrate are ubiquitous within environmental, food, industrial and physiological systems. Nitrite is an important and common pollutant found in the environment, indicative of organic pollution in the atmosphere and natural water [1,2]. The importance of improved analytical methods for the detection of nitrite in water, food, and agricultural products has been widely recognized because of the evidence that their presence can lead to carcinogenic compounds in human bodies’ [3]. The acceptable daily intake recommended by the World Health Organization is <8 mg nitrite for an adult of about 60 kg [4]. Furthermore, measurement of nitrite ion has recently been examined as an indication for in vivo nitric oxide production [5,6]. Thus, sensitive, selective and convenient method are needed to measure nitrite ions at low levels in the complex matrices found in food, water and biological fluids. The suggested electrodes were used for the determination of Iodide [7] and sulphite [8]. The determination of nitrite has already been performed by various techniques [9--11]

2. Experimental

2.1 Chemicals and Reagents

The potassium ferrocyanide (II) and sodium nitrite were obtained from (BDH). Other chemicals used in this study were of analytical grade and all solutions were prepared in second distilled water. Stock nitrite solution was prepared to 100 mM and the solutions of lower concentration for the voltammetric and amperometric experiments were freshly prepared prior to each run by adding appropriate amounts of the standardized stock solution to 20 ml of electrolyte solution. The phosphate buffer solutions with different pH values were done by mixing the two appropriate solutions of disodium hydrogen phosphate, sodium dihydrogen phosphate and phosphoric acid until the desired pH, read on a pH meter, was obtained.

2.2 Preparation of the Screen-Printed Electrodes

The used sensors were fabricated at the GesellschaftfürBiotechnologischeForschungmbH (GBF) Braunschweig, Germany were fabricated according to procedures given in Schmidt et al.[12]. All measurements were done using a planar screen-printed platinum or gold as a reference electrode and a platinum wire as a counter electrode. Potentials with respect to the screen-printed Pt electrode are shifted compared to a Ag/AgCl reference electrode by about -200 mV depending on the concentration of the oxidized and reduced compounds, i.e., a potential of –500 mV vsPt corresponds to –300 mV vs Ag/AgCl [13].

2.3 Instrumentation

A computer-aided electrochemistry system was used in the voltammetric and amperometric studies. The system consists of a potentiostat Model 263A (EG&G PARC, Princeton Applied Research Corporation, USA) and Electroanalytical software Model 270/250 version 4.0 (PARC).

2.4 Procedures

All experiments were carried out at room temperature. Voltammetric and amperometric measurements were performed in a 50 ml cell containing appropriate concentration of a mediator in 20 ml of 0.1 M sodium phosphate buffer pH 3.0. Standard solutions of 100 mM nitrite were added. The potentials of the working electrodes were increased in steps of 100 mV; the resulting steady-state current responses were measured for each plateau and plotted against applied potential. Screen-printed electrodes were rinsed by redistilled water, dried by plotting paper after each run and stored at room temperature without special humidity adjustment.

Initial studies were performed using cyclic voltammetry to establish whether the chosen mediator [The potassium ferrocyanide (II) ] shows any redox behavior at the used screen-printed electrodes. Such behavior was a prerequisite for the development of an amperometric NO₂ sensor. Cyclic voltammetric experiments were carried out using 0.1 M sodium phosphate buffer, pH 3.0, and scan rate of 50 mV/s for a solution containing nitrite alone, mediator alone and mediator plus NO₂. The first cycle showed that an enhancement of the cathodic peak of the mediator which corresponds to the chemically oxidized portion by nitrite ion
3. Results and Discussion

3.1 Cyclic Voltammetric Measurements

The recorded cyclic voltammograms for 10 mM nitrite at different screen printed electrodes showed that the direct oxidation of nitrite at the used electrodes occurred at higher potential, more than +700 mV and the peak is insignificant. Thus, determination of nitrite by direct oxidation is not useful. To modify the method the addition of a mediator is suggested. Figure 1 shows the comparison of cyclic voltammograms of 5 mM K₄[Fe(CN)₆] mediator in the absence (a) and presence (b) of 5 mM of nitrite ion. From the figure it can be seen that, the K₄[Fe(CN)₆] mediator shows a small cathodic peak and a big anodic peak in the absence of NO₂⁻. Upon the addition of nitrite, an enhancement of the cathodic peak current was observed. Indicating the chemical oxidation of the reduced form of the mediator by nitrite ions. Fig (2): showing the suggested various reactions occurring during the operation of the proposed system.

Initially, the K₄[Fe(CN)₆] mediator undergoes chemical oxidation by nitrite which reduced to NO. The oxidized form of the mediator is reduced at the screen-printed electrodes leading to an increase in the cathodic current which constitutes the analytical signal, according to the following equations:

\[
\text{[Fe(CN)₆]^{4-} + NO₂^- + 2H^+ \rightarrow [Fe(CN)₆]^{3-} + NO + H₂O}
\]

Thus, nitrite can be determined by following the increase in the cathodic peak in the presence of K₄[Fe(CN)₆] mediator.

3.2 Amperometric Measurements

To establish the optimum operating potential for amperometric determination of nitrite in presence of K₄[Fe(CN)₆], hydrodynamic voltammograms were constructed for 5 mM K₄[Fe(CN)₆] and 2 mM NO₂⁻ using Au/Au, Au/Pt, C/Pt and Pt/Pt screen-printed electrodes. The response clearly increased with increasing the negative potential reaching a plateau at −400 mV. Thus, this potential was chosen for all further experiments. Different buffers were examined for the determination of nitrite in presence of K₄[Fe(CN)₆]. The buffers include: sodium borate, sodium phosphate and Britton-Robinson buffer. The electrode response was investigated with respect to buffer kind., buffer concentration and buffer pH. The best results were obtained with a 0.10 M sodium phosphate buffer and pH = 3.0. The effect of the pH value of the used buffer on the response of NO₂⁻ was also investigated, the amperometric responses were measured over a wide range of NO₂⁻ concentrations (ranged from 100 μM to 2.5 mM). The response time was fast; steady-state current signals were obtained in less than (90%) < 15s. The amperometric responses of the mediator alone and after the addition of different concentration of nitrite ranged from 0.5 mM to 2.5 mM were recorded.

The collected results from the electrode response on addition of different concentration of the aimed analyte showed a linear relationship between the electrode response in μA and nitrite concentration in mM. Calibration curves exhibit a linear relationship between the electrodes response and the nitrite concentration up to 2.5 mM (Figure 3) and the lowest nitrite concentration measured was 100 μM. The correlation coefficients of the linear regressions for all calibration curves were equal to or better than 0.983. The repeatability of five successive measurements for 1mM NO₂⁻ in term of relative standard deviations was also calculated. The results in this table indicate that, C/Pt sensor showed the highest sensitivity for nitrite of 4.42 μA/mM followed by Pt/Pt with sensitivity of 4.08 μA/mM, Au/Pt with sensitivity of 3.72 μA/mM and Au/Au electrode with the lowest sensitivity of 3.55 μA/mM. (Table 1)

3.3 Influence of Interferences

The interference resulting from some anions (SO₄²⁻, S₂O₃²⁻, SO₃²⁻, S²⁻, NO₃⁻) and electroactive substances (e.g. ascorbic acid) was examined in presence of 1 mM nitrite and 1 mM K₄[Fe(CN)₆]. The results indicated that SO₄²⁻, SO₃²⁻, S₂O₃²⁻, S²⁻ and NO₃⁻ did not have a significant effect on the nitrite response obtained with different sensors up to amounts similar to that of nitrite. Ascorbic acid enhancement the nitrite response and the effect increase with increasing the concentration of these species. The interference from ascorbic acid can be compensated by the use of a standard addition method for the quantification of nitrite in real samples.

3.4 Analytical Application

The amperometric response of the studied electrodes for the determination of nitrite in presence of potassium ferrocyanide (II) as mediator were evaluated by monitoring samples of tap and agriculture drain waters before and after the addition of known NO₂⁻ concentration. The pH of water samples was adjusted to 3.0 by addition of phosphate buffer. Table (2) reported the results of determination carried out on the water samples, the standard deviations shown in the table have been calculated on five replicate determinations. The percentage recoveries in the range of 98 to 103 % and the RSD of 2 to 8 % obtained for the various concentration of nitrite in the solutions are reasonable. These results indicate that the used screen-printed electrodes should give reliable results for the determination of nitrite at the levels investigated. The advantage of the used sensors is, therefore, that it provides a rapid means of detecting elevated levels of NO₂⁻ arising as a result of pollution. In addition, as amperometric sensors, in general, have the potential to be portable, we envisage that the used electrodes may be applied in the field. This approach would eliminate any need to collect and stabilize water samples.
3.5 Operational and Storage Stability

Sensors fabricated in batches of several hundred by screen-printing technology have to be preserved for several weeks until they are used for real applications. Thus, the storage stability of any type of sensors has a great of importance. The influence of storage period and storage conditions on the signals of the used sensors with the nitrite ion in presence of potassium ferrocyanide (II) was investigated. For a period of three months, ten subsequent measurements of 1 mM NO$_2^-$ in presence of 1 mM potassium ferrocyanide (II) as mediator in 0.1 M sodium phosphate buffer, pH 3.0, were carried out by the used sensors. After each measurement the sensor was rinsed by bidistilled water, dried and stored at room temperature (25°C) until the next measurement. A gradual decrease of the response current was observed and the current of all sensors dropped to about 85 % after three month of operation.

The storage stability of the used sensors was tested during storage at dry state in room temperature (25°C). The measurements were carried out every month with 1.00 mM NO$_2^-$ in presence of 1.00 mM potassium ferrocyanide (II) as a mediator in 0.1 M sodium phosphate buffer, pH 3.0. From the obtained results it was found that, the used screen-printed electrodes have its initial response after storage in dry state at room temperature up to eight months.

Table 1: Characteristic parameters for determination of NO$_2^-$ obtained by amperometric measurements in presence of K$_4$[Fe(CN)$_6$] as a mediator for the different used electrodes.

<table>
<thead>
<tr>
<th></th>
<th>C/Pt</th>
<th>Au/Pt</th>
<th>Au/Au</th>
<th>Pt/Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (μM – mM)</td>
<td>100 – 2.50</td>
<td>100 – 2.50</td>
<td>100 – 2.50</td>
<td>100 – 2.50</td>
</tr>
<tr>
<td>Linear correlation coefficient</td>
<td>0.990</td>
<td>0.988</td>
<td>0.983</td>
<td>0.985</td>
</tr>
<tr>
<td>Sensitivity (μA/mM)</td>
<td>4.42</td>
<td>3.72</td>
<td>3.55</td>
<td>4.08</td>
</tr>
<tr>
<td>Detection limit (μM)</td>
<td>100</td>
<td>130</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Relative standard deviation</td>
<td>5.80 %</td>
<td>6.73 %</td>
<td>6.37 %</td>
<td>4.94 %</td>
</tr>
</tbody>
</table>

Table 2: Recovery of nitrite ion concentrations in spiked potable water and environmental water samples. Water samples were obtained from local sources. Standard of deviations were estimated for n=5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added mM</th>
<th>Found, mM</th>
<th>Recovery (%)</th>
<th>Found, mM</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>0.50</td>
<td>0.49 ± 0.02</td>
<td>98 ± 5</td>
<td>0.49 ± 0.04</td>
<td>98 ± 7</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00 ± 0.04</td>
<td>100 ± 4</td>
<td>1.01 ± 0.06</td>
<td>101 ± 5</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.98 ± 0.08</td>
<td>99 ± 4</td>
<td>2.00 ± 0.06</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>Agriculture Drain Water</td>
<td>0.50</td>
<td>0.51 ± 0.02</td>
<td>101 ± 4</td>
<td>0.50 ± 0.03</td>
<td>100 ± 6</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.99 ± 0.05</td>
<td>99 ± 5</td>
<td>1.01 ± 0.06</td>
<td>101 ± 5</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.99 ± 0.04</td>
<td>99 ± 2</td>
<td>2.00 ± 0.08</td>
<td>100 ± 4</td>
</tr>
</tbody>
</table>

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<th>Sample</th>
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<th>Found, mM</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>0.50</td>
<td>0.50 ± 0.04</td>
<td>99 ± 8</td>
<td>0.50 ± 0.03</td>
<td>100 ± 7</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.04 ± 0.05</td>
<td>103 ± 5</td>
<td>0.99 ± 0.04</td>
<td>99 ± 4</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.99 ± 0.06</td>
<td>99 ± 3</td>
<td>2.02 ± 0.06</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>Agriculture Drain Water</td>
<td>0.50</td>
<td>0.51 ± 0.04</td>
<td>101 ± 7</td>
<td>0.49 ± 0.04</td>
<td>98 ± 7</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00 ± 0.06</td>
<td>100 ± 6</td>
<td>1.01 ± 0.06</td>
<td>101 ± 5</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.01 ± 0.05</td>
<td>100 ± 3</td>
<td>1.99 ± 0.05</td>
<td>99 ± 2</td>
</tr>
</tbody>
</table>
Fig. 1: Cyclic voltammograms of 20 ml 0.1 M sodium phosphate buffer, pH 3.0, containing 5.00 mM of K₄[Fe(CN)₆] mediator in absence (a) and presence (b) of 5.00 mM NO₂ at the screen-printed electrodes Au/Au (A), Au/Pt (B), C/Pt (C) and Pt/Pt (D). The scan rate was 50 mV/s.

Figure 2: Scheme showing the various reactions occurring during the operation of the proposed system.

Figure 3: Calibration plots of nitrite obtained by amperometric measurements in presence of K₄[Fe(CN)₆] as mediator for the four different electrodes.

References