Indirect voltammetric determination of trace hydroxylamine using magnetic microspheres

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A new indirect voltammetric method for the determination of hydroxylamine is described. It is based on the reduction of an electroactive derivative of hydroxylamine on the surface of a magnetic electrode. The electroactive derivative produced by hydroxylamine reacted with magnetic polymer microspheres containing carbonyl groups on the surface. The experimental conditions are discussed. It was found that the peak potential ($E_p$) of the derivative was $-0.46$ V (vs. Ag/AgCl) under optimum conditions. Hydroxylamine could be determined in the range of 5–2000 μg l$^{-1}$ with the detection limit of 2 μg l$^{-1}$ and relative standard deviation for the determination of 100 μg l$^{-1}$ hydroxylamine was 2.35%. Satisfactory results were obtained for the determination of hydroxylamine in aqueous medium.

1. Introduction

The quantitative determination of hydroxylamine is very important both in studies of biological processes and for industrial purposes. It has been confirmed that hydroxylamine is produced during the reduction of nitrates by E. coli and Torula yeast. It has also been detected in bacterial media and in the tissues of a number of organisms. Ammonia is reported to have been produced in vivo from hydroxylamine by various microorganisms. Hydroxylamine is known as a kind of reducing reagent that is routinely used in industrial and pharmaceutical processes. Hydroxylamine also is a well-known mutagen, moderately toxic and harmful to microorganisms that could interfere with biological sewage plant performance. Many methods have been proposed for the determination of hydroxylamine. In recent years, an ion chromatographic method has been described for the determination of hydroxylamine. The detection limit of this procedure is 15 μg l$^{-1}$. The method is defined to be linear for concentration ranges that are equivalent to 0.5–20 mg l$^{-1}$. Another report described a flow injection biamperometric method. Hydroxylamine can be determined in the range of $6 \times 10^{-7}$–$4 \times 10^{-5}$ mol l$^{-1}$ with the detection limit of $1 \times 10^{-7}$ mol l$^{-1}$. The composite magnetic polymer microspheres usually contain two parts: one is the magnetic core which is always an inorganic magnetic material, such as Fe$_3$O$_4$, δFe$_2$O$_3$, etc.; the other is the polymer shell around the magnetic core. The microspheres can move directly in magnetic field and be separated from the medium. The polymer shell, always containing some active groups, can covalently bond organic molecules, biomolecules and cells. Magnetic microspheres have been applied to fluororesiimmunoassay, flow injection analysis, chemiluminescence enzymatic immunoassay, environmental analysis and DNA carriers. In this paper, a new voltammetric method for the determination of hydroxylamine is reported. The reaction path can be expressed as follows: The microspheres containing carbonyl groups on the surface can react with hydroxylamine to produce oxime compounds which are concentrated on a magnetic electrode. Oxime-groups can be reduced at a silver-based mercury film magnetic electrode.

The current of the reduction peak was measured at $-0.46$ V (vs. Ag/AgCl) by differential pulse voltammetry (DPV). This determination is a highly sensitive and selective procedure.

2. Experimental

2.1 Reagents

All chemicals were of the highest purity. Tripoly distilled water was used throughout. A hydroxylamine stock standard solution (1.00 g l$^{-1}$) was prepared by dissolving 2.106 g of hydroxylamine hydrochloride (Beijing Chemical Company, China) in distilled water and diluting to 1000 ml. Hydroxylamine working standard solutions were prepared daily from the stock standard solution by appropriate dilution with water.

2.2 Apparatus

All electrochemical experiments were carried out using a BAS100w electrochemical analyzer. The working electrode used in DPV was a silver-based mercury film magnetic electrode with an area of 0.10 cm$^2$. The structure of the silver-based magnetic electrode is shown in Fig. 1, it was obtained from the magnetic material factory of Lanzhou. Inner sleeve was made of diamagnetic copper. The mercury film was electroplated on the polished electrode surface for 10 min with a constant current of 5 mA in a saturated Hg(NO$_3$)$_2$ solution. Then the electrode was washed with distilled water. A large area platinum plate was used as the counter electrode. All potentials measured and reported in this paper were vs. an Ag/AgCl (saturated KCl) electrode.

2.3 Preparation of magnetic microspheres

A series of microspheres were synthesized by dispersion copolymerization. Fe$_3$O$_4$ powder (offered by Lanzhou Institute of
of Magnetic Material) with a particle size of 10–80 nm was dispersed ultrasonically in a polyethylene glycol and water solution. The mixture was placed in a 250 ml round-bottomed, four-necked flask with an incubating dispersion medium of H$_2$O/C$_2$H$_5$OH. Styrene and 1-pentene-4-one were copolymerized using potassium persulfate as the initiator. The reaction mixture was stirred at 400 rpm for 6 h, while the reaction temperature was kept at 70 °C and N$_2$ gas was passed continuously through the flask. Then the magnetic microspheres were separated by a magnetic field of 4200 G and washed with portions of 30% ethanol until the liquid washed out was colorless. The suspension of magnetic microspheres (2 g l$^{-1}$) was prepared by appropriate dilution with ethanol and water. These magnetic microspheres had carbonyl groups on the surface. The TEM photograph of magnetic microspheres is shown in Fig. 2.

### 2.4 Procedure

The supporting electrolyte was 0.1 mol l$^{-1}$ HOAc–NaOAc (pH = 4.5). A 25 ml calibrated flask containing a suitable amount of hydroxylamine, buffer solution and 5.00 ml of magnetic microspheres solution was heated in a boiling water-bath for 20 min and diluted to volume. This solution was placed in a H-shape cell, and then a stream of nitrogen was passed through for 5 min in order to remove oxygen. An initial potential of −0.25 V (vs. Ag/AgCl), a final potential of −0.65 V, a scan rate of 5 mV s$^{-1}$, a pulse amplitude of 90 mV, a sample width of 15 ms, a pulse width of 50 ms, a pulse period of 1200 ms, a quiet time of 60 s were found to be the optimum conditions. The current of the reduction peak (as shown in Fig. 3) was measured at −0.46 V (vs. Ag/AgCl) by using DPV on a silver-based mercury film magnetic electrode. The results were obtained by plotting the related peak current against the concentration of freshly prepared standard solutions.

### 3. Results and discussion

#### 3.1 Supporting electrolyte and pH

At relative lower pH, Fe$_3$O$_4$ nanoparticles coated in the magnetic microspheres will be partly corrupted by H$^+$. In the experiment, the magnetic microspheres were found to have poor stability at pH value lower than 2.5. Hence buffers of pH 3.0–8.0 were selected to study. DPV curves were observed in the following electrolytes: Na$_2$HPO$_4$–KH$_2$PO$_4$, TRIS–HCl and HOAc–NaOAc, but the background current was lower only in HOAc–NaOAc buffer. As shown in Table 1, the peak height increased with the increasing pH from 3.0 to 4.0, then it was stable at pH 4.0–5.0, and decreased on increasing pH from 5.0 to 8.0. On the basis of these results, a buffer solution of 0.1 mol l$^{-1}$ HOAc–NaOAc (pH = 4.5) was chosen in our experiments.

#### 3.2 Effect of heating time

The reaction of hydroxylamine and the magnetic microspheres is slow at room temperature. The peak heights were used to

### Table 1

<table>
<thead>
<tr>
<th>pH</th>
<th>3.0</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak current/nA</td>
<td>661</td>
<td>831</td>
<td>834</td>
<td>832</td>
<td>804</td>
<td>762</td>
<td>529</td>
</tr>
</tbody>
</table>

*a Conditions: scan rate = 5 mV s$^{-1}$, pulse amplitude = 90mV, sample width = 15 ms, pulse width = 50 ms, pulse period = 1200 ms.*
adjust the extent of derivative reaction between hydroxylamine and the magnetic microspheres. It was found that the peak current of the formed derivative increased with the heating time up to 20 min and then reached a maximum, constant value (Fig. 4).

### 3.3 Influence of the scan rate on the peak current

The change of the peak current with the scan rate was also investigated. With increasing sweep rate \((v < 10 \text{ mV s}^{-1})\), the peak current was found to be increased. Larger peak current values can be obtained at higher scan rates, but the reduction peak broadens. On the basis of these experimental results obtained, a scan rate of 5 mV s\(^{-1}\) was chosen for all further measurements.

### 3.4 Interferences

In order to investigate the selectivity of the reaction, several cations, anions and organic compounds were tested in a standard solution of hydroxylamine (100 \(\mu g \text{ l}^{-1}\)). No interference from \(K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Pb^{2+}, Zn^{2+}, Cl^-, F^-, SO_4^{2-}, CO_3^{2-}\) and \(PO_4^{3-}\) at concentrations up to 0.5 \(g \text{ l}^{-1}\) was observed. No interference was also observed with methanol and ethanol at concentrations of 50 \(g \text{ l}^{-1}\), with nitrate and ammonia at concentrations up to 0.2 \(g \text{ l}^{-1}\). Selectivity towards other \(N\)-alkylhydroxylamines is sufficient due to the higher reactivity of hydroxylamine and the different electrochemical potentials. There was no peak current in the range of scan potential for \(N\)-methylhydroxylamine and \(N,N\)-dimethylhydroxylamine. Hydrazine interfered only at concentrations higher than 5 \(mg \text{ l}^{-1}\).

### 3.5 Comparative measurement

Comparative measurements were carried out in the optimum conditions. The results of the tests were shown in Table 2. There was no peak current for hydroxylamine (100 \(\mu g \text{ l}^{-1}\)) without the derivative reagent. When formaldehyde was used as the derivative reagent, the peak current \((E_P = -0.43 \text{ V})\) was small. Based on these results, the magnetic microspheres have a clear effect in sensitivity of voltammetric determination.

### 3.6 Applications to samples

The proposed method was applied to the determination hydroxylamine in samples of industrial waste water (Lin Fen pharmaceutical factory) and simulated sample. The simulated sample by mixing of sodium nitrate, ammonia chloride, \(N\)-methylhydroxylamine, \(N,N\)-dimethylhydroxylamine and hydroxylamine hydrochloride with the ratio 5:5:5:5:1 was employed for voltammetric determination of hydroxylamine. The chromatographic method\(^d\) was used as comparing method. The results for two samples are given in Table 3 and the results of recovery test are given in Table 4.

### 3.7 Calibration, precision and detection limit

The DPV peak was found to increase linearly with the concentration in the range of 5–2000 \(\mu g \text{ l}^{-1}\) of hydroxylamine. The linear regression equation is \(I_p (\text{nA}) = 9.6 + 8.24C (\mu g \text{ l}^{-1})\) (correlation coefficient = 0.995). Five successive determinations at three concentrations of hydroxylamine of 20, 200 and 2000 \(\mu g \text{ l}^{-1}\), respectively gave relative standard deviations (RSDs) of 3.31, 1.89 and 1.43\%. The detection limit of 2 \(\mu g \text{ l}^{-1}\) could be estimated based on a 3 \(\sigma\) concept.

### 4. Conclusion

This procedure described could be shown to be one of the most sensitive voltammetric determination methods for hydroxylamine. Interference encountered is very low. The procedure can be used as a selective method for the determination of hydroxylamine in aqueous medium.

#### Table 3 Determination of hydroxylamine in real sample and simulated sample \((n = 5)^e\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method</th>
<th>Chromatographic method(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found/ (\mu g \text{ l}^{-1})</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Industrial water</td>
<td>68</td>
<td>3.05</td>
</tr>
<tr>
<td>Simulated sample</td>
<td>142</td>
<td>2.03</td>
</tr>
</tbody>
</table>

\(^e\) Conditions: 0.1 mol \(l^{-1}\) HOAc–NaOAc + 0.40 g \(l^{-1}\) magnetic microspheres + (a) 100 \(\mu g \text{ l}^{-1}\); (b) 300 \(\mu g \text{ l}^{-1}\) hydroxylamine. Pulse amplitude = 90 mV, scan rate = 5 mV \(s^{-1}\).

#### Table 4 Recovery results for hydroxylamine in aqueous solutions \((n = 5)^e\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added/ (\mu g \text{ l}^{-1})</th>
<th>Found/ (\mu g \text{ l}^{-1})</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial water</td>
<td>50</td>
<td>49.6</td>
<td>99.2</td>
<td>3.12</td>
</tr>
<tr>
<td>Simulated sample</td>
<td>200</td>
<td>198</td>
<td>99.0</td>
<td>1.87</td>
</tr>
</tbody>
</table>

\(^e\) Conditions: scan rate = 5 mV \(s^{-1}\), pulse amplitude = 90 mV, sample width = 15 ms, pulse width = 50 ms, pulse period = 1200 ms.
Acknowledgement

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References
