Notes & Tips

Spectrophotometric determination of hydroxylamine and nitrite in mixture in water and biological samples after micelle-mediated extraction

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Hydroxylamine (HA)¹ and nitrite recycle through the hydrosphere as a result of microbial processes [1–3]. These compounds are important intermediates in the biological nitrogen cycle and are present in soils and surface waters [4,5]. The important role of nitrite in producing N-nitrosamines, some of which have been shown to be carcinogens in the human body, is well established. Nitrite is also a versatile chemical agent that has found numerous applications ranging from dye manufacture to food preservation [6]. HA is often used as a raw material for the synthesis of pharmaceutical intermediates and final drug substances. Modest levels of HA can be toxic to humans, animals, and even plants [7]. The need for selective and sensitive methods for determination of these compounds is well documented.

Several methods have been reported for the spectrophotometric determination of HA and nitrite [8–14]. To the best of our knowledge, there is no report on the determination of HA and nitrite in mixtures.

In this work, we present a spectrophotometric determination method for resolving nitrite and HA mixtures after cloud point extraction (CPE). The method is based on the combination of two well-known reactions: oxidation of HA to nitrite and nitrite determination with N,N-dimethylaniline and p-nitroaniline followed by micelle-mediated extraction of the produced azo dye. The nonionic surfactant Triton X-114 was chosen as the extraction agent.

A PerkinElmer Lambda 45 UV/Vis spectrometer was used for recording absorbance spectra. Absorption measurements at fixed wavelength were performed using a Shimadzu UV-mini-1240V spectrophotometer with 1-cm quartz cells (0.5 ml). A Metrohm pH meter (model 713) with a combined glass electrode was used for pH measurements. A water bath with good temperature control and a centrifuge with 10-ml calibrated centrifuge tubes (Superior, Germany) were used to accelerate the phase separation process. Triply distilled water was used, and analytical reagent-grade chemicals were purchased from Merck.

Three runs were needed for each sample. In the first run, an aliquot of the solution containing 1.1–32.6 nmol of nitrite ion, 2.1–45.5 nmol of HA, 1 ml of 5.71 £ 10⁻³ mol L⁻¹ iodate solution, and 1 ml of 0.1 mol L⁻¹ sulfuric acid solution was added and allowed to stand for 5 min at room temperature. Then 1 ml of 2% Triton X-114 solution and 0.2 ml of 2.5% (m/v) p-nitroaniline solution were transferred into a 10-ml tube and allowed to stand for 1 min. Next 0.2 ml of 10% (v/v) N,N-dimethylaniline solution was added and made up to the mark with water. Subsequently, the sample was shaken and left to stand in a thermostatted water bath for 10 min at 60 °C before centrifugation. Separation of two phases was achieved by centrifugation for 10 min at 2600 g. The mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily decanted by simply inverting the tube. The micellar extract of this procedure was diluted with 0.3 ml of ethanol and transferred into a 0.5-ml quartz cell to measure its absorbance at 494 nm against a blank solution. The absorbance of the solution (A₁) was proportional to the total concentrations of HA and nitrite.

The second run was performed in a similar fashion to the first run except that iodate solution was not added. The absorbance of the solution at 494 nm (A₂) was proportional to the nitrite concentration.

The third run was performed in a similar fashion to the second run except that the standard solution of nitrite was...
selected instead of the HA–nitrite mixture. The absorbance of the solution at 494 nm ($A_j$) was proportional to the nitrite concentration.

Iodate ion oxidizes HA in acidic media to produce nitrite ions. In the presence of nitrite ions, the composite diazotization coupling reaction of p-nitroaniline (as a diazotizable aromatic amine) and $N,N$-dimethylaniline (as a coupling agent) proceeds in acidic media to produce an azo product. The produced azo compound is insoluble in water. However, it was observed that the azo compound becomes soluble in water on the addition of the neutral surfactant Triton X-114. The absorbance intensity of the solution at 494 nm, the $\lambda_{\text{max}}$ of the product, is proportional to the nitrite concentration. Therefore, the system is suitable for spectrophotometric determination of nitrite and HA.

To take full advantage of the procedure, the reagent concentrations and reaction conditions must be optimized. The effects of sulfuric acid concentration, iodate concentration, $N,N$-dimethylaniline concentration, p-nitroaniline concentration, and Triton X-114 concentration were investigated. The results showed that 0.01 mol L$^{-1}$ sulfuric acid, 0.57 mmol L$^{-1}$ iodate, 0.2% (v/v) $N,N$-dimethylaniline, 0.05% (m/v) p-nitroaniline, and 0.2% (v/v) Triton X-114 were optimal.

The effect of time on the reaction of HA with iodate and both reaction steps of azo dye formation (diazotization and coupling steps), as well as on the CPE procedure, was investigated. The results showed that the reaction of HA was completed in 5 min and that the diazotization and coupling reactions were completed in 1 and 10 min, respectively. A 10-min centrifugation at 2600g was found to be adequate for successful CPE.

The effect of equilibration temperature and incubation time was investigated. It is desirable to employ the shortest incubation time and the lowest possible equilibration temperature that are compatible with completion of the reaction and efficient separation of the phases. As mentioned above, the coupling reaction was completed in 10 min. Therefore, the incubation time was kept at 10 min, which is sufficient for the completion of the coupling reaction and for the CPE procedure. It was also observed that a temperature of 60°C is sufficient for nearly complete recovery of the analyte.

Because the surfactant-rich phase was very viscous, ethanol was added to the surfactant-rich phase after CPE to facilitate its transfer into a spectrophotometric cell. The amount of ethanol added (0.3 ml) was chosen to have an appropriate amount of sample for transferring and measuring the sample absorbance.

From measurements made under the optimal conditions described above, the calibration graphs were obtained. Table 1 summarizes the analytical characteristics of the optimized method, including regression equation, linear range, and limits of detection (LODs) for nitrite and HA. The concentration of the nitrite in the mixture could be determined from the absorbance obtained in the second run ($A_j$) using Eq. (2). As Table 1 shows, the calibration graph obtained for the nitrite in the presence of iodate was not the same as that obtained in the absence of iodate. Therefore, the HA concentration could not be obtained by a simple subtraction. For the determination of HA concentration in the mixture, the nitrite concentration obtained from Eq. (2) was substituted into Eq. (3) and the corresponding absorbance ($A_i$) was calculated. The calculated absorbance was subtracted from the absorbance obtained in the first run ($A_i$), and then the HA concentration was calculated from Eq. (1).

Because the amount of HA and nitrite in 10 ml of the sample solution was measured after preconcentration by CPE in a final volume of 0.5 ml (0.2 ml surfactant-rich phase + 0.3 ml ethanol), the solution was concentrated by a factor of 20 and the phase volume ratio, defined as the initial volume to surfactant-rich phase volume, was 50.

The improvement factor, defined as the ratio of the slope of the calibration graph for the CPE method to the slope of the calibration graph in micellar media without preconcentration, was 9.4 for nitrite and 7.7 for HA. The relative standard deviation (RSDs) for six replicate measurements of a mixture containing 0.65 μmol L$^{-1}$ nitrite and 2.7 μmol L$^{-1}$ HA were 2.8 and 2.4% for nitrite and HA, respectively.

To study the selectivity of the proposed methods, the effect of various chemical species on the determination of a mixture of 1.5 μmol L$^{-1}$ HA and 1.1 μmol L$^{-1}$ nitrite was tested under the optimal conditions. The tolerance limit was defined as the concentration of added ion causing less than ±3% relative error. The following ions did not interfere even when their concentration was a 1000-fold excess over HA and nitrite: SO$_4^{2-}$, Co$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, CH$_3$COO$^{-}$, K$^+$, Na$^+$, Cl$^-$, Cd$^{2+}$, Ca$^{2+}$, NH$_3$, Cu$^{2+}$, Sb$^{3+}$, and NO$_3^-$.

The ions SCN$^-$ and SO$_4^{2-}$ interfered at 150 μmol L$^{-1}$, hydrazine interfered at 15.6 μmol L$^{-1}$, and phenylhydrazine interfered at 5 μmol L$^{-1}$. The interfering effect of Fe$^{3+}$ up to 150 μmol L$^{-1}$ was completely removed by the addition of 1.0 × 10$^{-5}$ mol L$^{-1}$ tartrate.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics for the calibration graphs for the determination of HA and nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td>Equation number</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>(1)</td>
</tr>
<tr>
<td>Nitrite in the presence of iodate</td>
<td>(2)</td>
</tr>
<tr>
<td>Nitrite in the absence of iodate</td>
<td>(3)</td>
</tr>
</tbody>
</table>

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$^a$ $C$ is the concentration of analyte (in μmol L$^{-1}$).

$^b$ Squared correlation coefficient.

$^c$ Defined as $C_L = 3S_D/m$, where $C_L$, $S_D$, and $m$ are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively.
Various synthetic mixtures of HA–nitrite were analyzed by the proposed method. The results for the analysis of mixtures with the ratio 20:1–1:15 for HA–nitrite showed that the relative errors of measurements were ≤5%. The maximum relative errors for HA and nitrite were −5.0 and −4.3%, respectively.

As observed, the method is applicable to the determination of mixtures having different concentration ratios of HA and nitrite.

The proposed method was applied successfully to the determination of nitrite and HA in well water and urine samples. The results are shown in Table 2. The recoveries are close to 100% and indicate that the proposed method was effective for the determination of nitrite in the samples.

A comparison of the current method with a previous report for determination of nitrite by a CPE procedure [10] shows that the current method provides a wider calibration range, whereas the figures of merit of the two methods are not significantly different. In the previously reported work [10], diphenylamine was used as coupling agent. Diphenylamine is oxidized by iodate in acidic media to produce diphenylbenzidine violet. The produced diphenylbenzidine violet interferes with the determination of the produced azo dye due to the overlap of their absorption spectra. Therefore, it is concluded that the current procedure, unlike the previously reported method, can be used to determine HA.

References


Table 2
Determination of HA and nitrite in mixture in samples by the proposed method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitrite (μmol L⁻¹)</th>
<th>Recovery (%)</th>
<th>HA (μmol L⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
<td></td>
<td>Added</td>
</tr>
<tr>
<td>Well water (Hamadan, Iran)</td>
<td>0.00</td>
<td>0.84 ± 0.02</td>
<td>—</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>1.51 ± 0.02</td>
<td>103</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>1.74</td>
<td>2.60 ± 0.03</td>
<td>101</td>
<td>2.42</td>
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<tr>
<td></td>
<td>2.61</td>
<td>3.42 ± 0.04</td>
<td>99</td>
<td>4.85</td>
</tr>
<tr>
<td>Urine</td>
<td>0.00</td>
<td>1.78 ± 0.05</td>
<td>—</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>1.99 ± 0.03</td>
<td>95</td>
<td>1.21</td>
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<tr>
<td></td>
<td>0.44</td>
<td>2.24 ± 0.08</td>
<td>104</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Note: Values are averages of three determinations.