Inhibition of Nitrosamine Formation by Inorganic and Organic Salts

MIEKO IITSUKA, TETSUTA KATO, and KIYOMI KIKUGAWA*

Tokyo College of Pharmacy, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan

(Received January 24, 1986)

Formation of N-nitrosamines from nitrite and secondary amines in acidic solutions was affected by inorganic and organic salts such as chloride, succinate, citrate and tartrate. Formation of N-nitrosodimethylamine at pH 5 was inhibited more effectively than at pH 3. The dissociation constant of nitrous acid–nitrite in the solution was decreased by addition of these salts. The salts influenced the nitrous acid–nitrite equilibrium to inhibit N-nitrosation of secondary amines.

Keywords—N-nitrosamine; inorganic salt; organic salt; nitrous acid; nitrite

Nitrite reacts with secondary amines to produce carcinogenic N-nitrosamines in acidic solutions. Kinetic analysis showed that nitrous acid in the nitrous acid–nitrite equilibrium is transformed to dinitrogen trioxide, an active nitrosating species. We demonstrated that addition of organic solvents to the reaction systems increased the nitrous acid concentration, accelerating the formation of N-nitrosamines. We will show in this communication that addition of inorganic and organic salts to the reaction systems decreased the nitrous acid concentration and thus inhibited the formation of N-nitrosamines.

Materials and Methods

Materials—N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) standards for gas chromatography were obtained from Wako Pure Chemical Industries, Ltd. Sodium nitrite, dimethyl (or diethyl) amine, sodium chloride, succinic acid, citric acid and tartaric acid were special grade reagents.

Analytical Methods—A Yanaco G80 gas chromatograph, equipped with a glass column (3 mm i.d. × 3 m) packed with polyethylene glycol 6000 (25%) on 80–100 mesh Chromosorb W AW, was used to determine the N-nitrosamines. The chromatograph was operated isothermally at 125 °C (column temperature) and at 140 °C (injector and detector temperature) with a nitrogen carrier gas flow of 25 ml/min. The amount of the N-nitrosamine was determined by comparing the peak area of the sample with that of standard NDMA or NDEA in chloroform. Ultraviolet (UV) absorption spectra were measured with a Hitachi 557 dual-wavelength double-beam spectrophotometer.

Formation of the N-Nitrosamines—A 5.0 ml aliquot of 4 mM dimethyl(or diethyl)amine hydrochloride was added to 80 ml of solution containing 5–20 g of sodium chloride, succinic acid, citric acid or tartaric acid, and the pH value of the mixture was adjusted to 3 or 5 by addition of 10% HCl or 10% NaOH solution. A 5.0 ml aliquot of 1 M sodium nitrite was added to the mixture, and then the pH value was readjusted. The mixture was made up to 100 ml with distilled water and incubated at 37 °C in a stopped flask. The pH value of the reaction mixture was readjusted at 1 h intervals by addition of 10% HCl or 10% NaOH solution. After 4 h, a 10 ml portion of the reaction mixture was withdrawn and supplemented with 2 g of sodium chloride and 1 g of sulfamic acid. The N-nitrosamine in the mixture was extracted with 40 ml of chloroform and the extract was subjected to gas chromatographic analysis.

Measurement of Apparent pKₐ value of Nitrite—A solution (80 ml) containing 5–20 g of sodium chloride, succinic acid, citric acid or tartaric acid was adjusted to the required pH value by addition of 10% HCl or 10% NaOH solution. To this solution, a 10 ml aliquot of 0.1 M sodium nitrite was added, and the mixture was made up to 100 ml with distilled water. The UV absorption spectrum and pH value of the mixture were measured as soon as possible. Apparent pKₐ values were determined by plotting absorbance at the absorption maximum of nitrous acid (371 nm) versus the pH value of the mixture.
Results and Discussion

The effects of inorganic and organic salts on the formation of \( N \)-nitrosamines from nitrite and secondary amines in pH-controlled aqueous solutions were investigated. The salts were chloride, succinate, citrate and tartrate. The pH-specific ratios of the formation of NDMA and NDEA in aqueous solutions containing these salts to that in the control system without the salts are given in Table I. Formation of NDMA and NDEA was effectively inhibited by sodium chloride. The inhibitory effect of sodium chloride on NDMA formation was greater

<table>
<thead>
<tr>
<th>Salts</th>
<th>Concentration (m)</th>
<th>Formation ratio of ( N )-nitrosamines</th>
<th>NDMA</th>
<th>NDEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without salts)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>1.7</td>
<td>1.0(^{(a)}) 1.0(^{(a)})</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>0.65 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>0.4</td>
<td>0.37 0.10</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.04 0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>0.5</td>
<td>0.94 0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.84 0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartrate</td>
<td>0.3</td>
<td>0.92 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.69 0.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A mixture of 50 mM sodium nitrite and 200 mM dimethyl(dimethyl)amine was incubated at 37°C for 4 h in the presence and absence of the salts.  
- \( a \) The amounts of NDMA formed in the control system at pH 3 and 5 were 13.9—14.7 and 0.7—1.0 mM, respectively.  
- \( b \) The amount of NDMA formed in the control system at pH 3 was 2.65—3.35 mM.

![Image](image_url)

**Fig. 1.** Plots of Absorbance at 371 nm versus pH Value of Aqueous Solutions of Nitrite in the Presence and Absence of A, Sodium Chloride; B, Sodium Succinate; C, Sodium Citrate; D, Sodium Tartrate

The molar concentrations of salts are indicated.
at pH 5 than at pH 3. Formation of NDMA at pH 5 was significantly inhibited by succinate, citrate and tartrate, but that at pH 3 was not. These results are consistent with the earlier observations\textsuperscript{5,6} that nitrosation of proline and sarcosine was inhibited by these salts preferentially at higher pH values.

Nitrous acid–nitrite equilibrium in the presence and absence of the salts was investigated spectrophotometrically. Plots of absorbance at the absorption maximum of nitrous acid\textsuperscript{6} versus pH value show that the dissociation constant ($pK_a$ 3.30) of the nitrous acid–nitrite equilibrium was decreased by addition of inorganic and organic salts (Fig. 1). Thus, the conversion of nitrite to nitrous acid at pH values around the $pK_a$ was suppressed by addition of these salts. All the $\Delta pK_a$ values in the presence of the salts are negative (Table II). The inhibitory effect of the salts on the formation of NDMA may be attributed to the decrease of the dissociation constant (Table II). While it has been shown that chloride forms nitrosyl chloride and thus accelerates $N$-nitrosation of a secondary amine at pH 0.5,\textsuperscript{7} it inhibited the $N$-nitrosation at pH values around the $pK_a$ value of nitrous acid in the present experiments. We have shown that addition of organic solvents to an aqueous solution of nitrite increases the concentration of nitrous acid and accelerates the formation of $N$-nitrosamines (Table II).\textsuperscript{3}

Increase and decrease of nitrous acid concentration by addition of both organic solvents and salts can thus influence the formation of $N$-nitrosamines.

**References**