Cyanide Concentration Changes in Environmental Water Samples as a Function of Sample Preservation, and Holding Time

Introduction

Most cyanide analysis sampling protocols specify preservation of samples at a pH of 12 or higher. Sample preservation is intended to minimize changes in analyte concentration to allow sample storage for up to 14 days prior to analysis. The source of a water sample, sample pretreatment, and the presence of possible matrix interferences must also be considered in determining the appropriate sampling and preservation procedure to avoid inadvertent formation or destruction of cyanide species.

Matrix interferences are known to cause either negative or positive analytical biases depending upon the composition of a sample being tested. The species of cyanide present in a sample is directly affected by the pH, and presence of; oxidizers, sulfur or sulfur compounds, and transition metals. The U.S. EPA acknowledges the problem matrix interferences pose in cyanide analysis in the “Pumpkin Guide”(1) stating; “Next to oil and grease, cyanide is the pollutant for which the most matrix interferences have been reported.” Table 1 provides a summary of cyanide analysis interferences associated with sampling, preservation, and storage.

Table 1. Summary of Cyanide Analysis Interferences Associated with Sampling, Preservation, and Storage

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description of Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual chlorine, peroxide, or other oxidizers</td>
<td>React with cyanide in solution rapidly decreasing the cyanide concentration. Oxidizers can co-exist with cyanide.</td>
</tr>
<tr>
<td>Chloramines</td>
<td>React with sample at pH&gt;10 increasing the cyanide concentration.</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Reacts with cyanide to form thiocyanate decreasing the cyanide concentration. Reaction is especially rapid if metal sulfides, such as lead sulfide, are present. The reaction is fairly slow without metal sulfides.</td>
</tr>
<tr>
<td>Native Sulfur (colloidal sulfur)</td>
<td>Reacts with cyanide to form thiocyanate decreasing the cyanide concentration. Reaction is very fast with colloidal sulfur.</td>
</tr>
<tr>
<td>Sulfite</td>
<td>Reacts with strong cyanide complexes at pH&gt;10 decreasing the cyanide concentration. The reaction is almost immediate at pH&gt;12.</td>
</tr>
</tbody>
</table>
A number of compounds cited in Table 1 that can cause generation or loss of cyanide analysis during sample preparation and storage are introduced in drinking and waste water treatment processes. Chlorine, chloramine, peroxide and UV light are used for disinfection and to oxidize contaminants. Sulfur dioxide, sodium thiosulfate (Na₂S₂O₃), calcium thiosulfate (Ca₂S₂O₃), sodium sulfite (Na₂SO₃), and ascorbic acid are reducing agents used to dechlorinate water.(2,3)

Research supported by the Water Environment Research Foundation (WERF) showed that chlorination of wastewater effluent resulted in cyanide formation.(4) The implications of this finding were stated in the executive summary of a WERF report on Cyanide Formation and Fate in Complex Effluents and its Relation to Water Quality Criteria.(5) “Numerous wastewater treatment plants (WWTPs) across the United States have detected cyanide in chlorinated effluents at levels exceeding those in influent waters. In some cases, these levels exceed National Pollution Discharge Elimination System (NPDES) discharge limits. Cyanide can be formed during wastewater chlorination. In addition, false positives are possible, caused by artifacts of sample handling and analytical techniques.”

Compounding this problem is the fact that the formation of cyanide after disinfection is accelerated by USEPA requirements for sample preservation at pH 12.

The U.S. EPA is aware of problems related to sampling and preservation practices and has recently taken steps to address these problems. ASTM D 7365-09a Standard Practice for Sampling, Preservation, and Mitigating Interferences in Water Samples for Analysis of Cyanide(6) was included in the U.S. EPA Method Update Rule (MUR) of September 23, 2010(7) applicable to 40 CFR 136 Clean Water Act methods for testing of wastewater samples.

This application note presents data demonstrating decreases and increases in total and available cyanide concentration in samples as a function of the metals present, treatment chemicals added, and pH.

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</tr>
</thead>
<tbody>
<tr>
<td>Light (&lt;350nm)</td>
<td>Reacts with strong metal-cyanide complexes releasing free cyanide.</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Reacts with cyanide decreasing the cyanide concentration. Sample holding time when ascorbic acid is added is less than 48 hours. In some samples, ascorbic acid can react with ammonia or other nitrogen sources and increase the cyanide concentration.</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Reacts with cyanide decreasing the concentration. In some samples, formaldehyde reacts with ammonia or other nitrogen sources and increases the cyanide concentrations.</td>
</tr>
</tbody>
</table>
**Instrumentation**

Instrumentation used for this study was an OI Analytical CNSolution™ 3100 Cyanide Analyzer (Figure 1).

![CNSolution™ 3100 Cyanide Analyzer](image1)

**Experimental**

**Oxidants**

To illustrate the affect of oxidants, three synthetic wastewater matrices containing 20-mg/L thiocyanate (SCN-) and 10 mg/L Nitrate Nitrogen (NO$_3$-N) were treated with hypochlorite (ClO^-), hydrogen peroxide (H$_2$O$_2$), and ascorbic acid (commonly used as a dechlorination agent during sampling) at 0.5 mg/L each. An identical matrix without oxidizer was also prepared. Each solution was adjusted to pH 12 with Sodium Hydroxide (NaOH) and analyzed for available cyanide (without ligand addition) using an OI Analytical CNSolution™ 3100. Samples were stored at room temperature, in the dark. The results of this study are shown in Figure 2.

![Generation of cyanide at pH 12 with various oxidants/reductants Sample Matrix - 20 ppm SCN, 10 ppm NO$_3$-N, 0.01 M NaOH](image2)

The control matrix contained only trace cyanide (0.2–0.3 ppb) while the addition of oxidant generated cyanide almost immediately. Cyanide formation continued throughout the entire two days of the test. The amount of
cyanide generation is greater with hypochlorite than with peroxide. Even the control sample, the synthetic matrix adjusted to pH12, generated trace cyanide during the 48-hour test. The ascorbic acid containing solution, generated cyanide immediately after NaOH addition, then decreased with cyanide over time. Figure 3, illustrates the rapid loss of cyanide in samples containing ascorbic acid and adjusted to pH 12.

![Graph showing loss of cyanide with ascorbic acid at pH12](image)

**Figure 3.** Loss of cyanide with ascorbic acid at pH12

**Sulfite**

Reducing agents, such as ascorbic acid and sodium thiosulfate, are often recommended to dechlorinate samples during sample collection, and then sample pH is adjusted to pH 12 with NaOH for storage up to 14 days prior to analysis. A negative bias by distillation of samples containing thiosulfate has been demonstrated(8) previously. Many municipalities use sodium sulfite, or sulfur dioxide, to dechlorinate wastewater streams prior to discharge. Preservation of sulfite containing samples at pH 12 with NaOH results in rapid loss of cyanide concentration. According to the data in Figure 4, cyanide samples that have been treated with sulfites should not be preserved to pH 12 prior to analysis.

![Graph showing cyanide loss in samples containing sulfite](image)

**Figure 4.** Cyanide loss in samples containing sulfite
Ozonation
In an effort to minimize the generation of chlorination by-products many water treatment plants have turned to UV irradiation and ozone. While these techniques have lowered halogenated organics emissions, they may produce cyanide in samples preserved for analysis. It is known that formaldehyde is generated as a result of ozonation and UV disinfection\(^9\). Formaldehyde can react with ammonia, and other organics generating cyanide (Figure 5).

![Figure 5. Cyanide generation from formaldehyde + chlorine + ammonia at pH 12](image)

**Holding Times**
While data suggests that low level cyanide test samples should not be adjusted to pH 12 prior to analysis, holding time studies should be conducted on each matrix to confirm that cyanide will not be lost if samples are not pH adjusted. For example (see Figure 6), an ASTM D 6888 cyanide holding time study of non-preserved samples lost cyanide within a few days for some samples, while cyanide concentrations seemed to remain stable for other matrices.

![Figure 6. Holding Time Study on “As Received” Samples, (no NaOH added)](image)
These same samples obtained quantitative recovery on portions that were adjusted to pH 12 prior to analysis (Figure 7). The surface water sample decreased in available cyanide (Figure 6), yet obtained the correct result when analyzed for total cyanide (indicating that cyanide is slowly being “bound” to the ferrous iron present in the sample)\(^{(10)}\).

![Figure 7. Comparison of Holding Time on Cyanide Recovery with and without NaOH Added](image)

Cyanide samples suspected of containing insoluble cyanides, such as Prussian blue, must be adjusted to pH 12–13 and held at room temperature for four hours prior to analysis or results will be low\(^{(11)}\).

**Summary and Conclusions**

ASTM D 7365-09a Standard Practice for Sampling, Preservation and Mitigating Interferences in Water Samples for Analysis of Cyanide was included in the U.S. EPA Method Update Rule (MUR) of September 23, 2010\(^{(7)}\) applicable to 40 CFR 136 Clean Water Act methods for testing of wastewater samples. This standard describes new procedures for preservation of samples and warns that pH adjustment could result in the generation of cyanide in concentrations high enough to exceed NPDES permits.

Formation of cyanide in sodium hydroxide preserved synthetic matrices containing contaminants that are likely to be present in natural waters was confirmed. Data presented also demonstrates cyanide loss as a result of pH adjustment. While this data suggests cyanide samples should not be preserved at an elevated pH, other data demonstrated that without pH adjustment cyanide results could be too low. When in doubt, samples should be processed immediately, or within minutes, after collection.

**References**


