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Pitfalls in the Toxicological Analysis of an Isobutyl Nitrite-Adulterated Coffee Drink

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A forensic investigation was carried out on one poisoning case, where cyanide was first detected in an evidence sample of a canned coffee drink. A more complete study revealed that it had been adulterated with isobutyl nitrite (IBN) and not cyanide. We examined the detectivity of IBN and related compounds by headspace gas chromatography and capillary electrophoresis. IBN decomposes to isobutyl alcohol (^{i}BuOH) and nitrite in aqueous solution, and under higher temperature and more acidic conditions, the rate of this reaction becomes more rapid. IBN was also produced by the esterification of ^{i}BuOH with nitrite below pH 5. Cyanide was produced in a coffee solution by the addition of nitrite below pH 6. An IBN-spiked canned coffee drink solution was stored at 4 °C and periodically analyzed for IBN, ^{i}BuOH, nitrite, nitrate, and cyanide. Since the IBN level decreased rapidly, ^{i}BuOH was produced in an almost 90% molar yield. Nitrite production reached a maximum of 40% molar recovery on the first day and then gradually disappeared. The nitrate level reached a plateau of ∼60% molar recovery. Cyanide was also detected, and its level at the 14th day was ∼0.26% molar recovery. These findings suggest that, in a coffee drink solution, IBN undergoes hydrolysis to produce ^{i}BuOH and nitric acid, which is oxidized to nitrate and also produces cyanide through the nonspecific oxidation of organic compounds under acidic conditions.

During the second half of 1998, a widespread series of poisoning cases occurred in Japan.[1,2] The first case, in late July, involved the Wakayama curry poisoning case,[3] where 4 people died and more than 60 became seriously ill after eating arsenic-laced curry served at a community festival in the Sonobe district of Wakayama. In August, 10 employees of a wood processing firm in Niigata were sickened after drinking beverages made with sodium azide-laced water from the office electric pot. These poisoning cases can be characterized in terms of three important aspects. First, new and unusual poisons such as azide and arsenic were used for the crimes. Not only toxic substances but common articles used in daily life, such as commercial detergents were used to adulterate food and drink. Second, copycat crimes involving food tampering cases also occurred rather quickly. Third, there were several mistaken cases of detection in the early stages after the cases were documented. In the Wakayama case, a false positive reaction for the presence of cyanide had been reported within a week.[3] Forensic science laboratories (FSL) of the prefectural police H.Q. and also, when requested, National Research Institute of Police Science (NRIPS) have been involved in the forensic investigation of such poisoning cases, having analyzed victim's samples and evidence samples taken from the crime scenes. A number of attempted cases have been reported, where alien substance-tampered drinks and food were found in public places, such as vending machines, and were reported to the police. In such cases, no specific information on symptoms of casualties were available. Therefore, it is difficult to identify substances adulterated in food and drink samples, because a wide range of substances must be considered as candidates for detection.

Toxic substances can be converted to different compounds through metabolism in the human body and chemical reactions in the environment. The substances detected by the forensic analysis may not necessarily coincide with those used to originally adulterate the food and drink. The following issues need to be considered: the alteration of toxic substances in the body (phase I changes), in samples during storage (phase II changes), and also during analysis (phase III changes).[4] Among toxic substances, cyanide is an important compound and is a highly relevant candidate for testing. One fatal cyanide poisoning case was reported in the late summer of 1998 in Nagano, where a resident died after drinking cyanide-laced canned oolong tea.[1,5] In the autumn of 1998, we experienced one peculiar poisoning case, where, initially, we detected cyanide in the evidence, a canned coffee drink, and finally concluded that isobutyl nitrite (IBN) had been the original adulterant. Alkyl nitrite esters are volatile organic liquids and show vasodilatation effects.[6] The physiological effect is derived from its biological breakdown product, nitric oxide.[7−9]

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Alkyl nitrite esters have been abused, particularly among male homosexuals,[10] and high doses result in toxic[11,12] and fatal[13−15] effects, such as methemoglobinemia.[13] Immunosuppressive[10] and blood cell destructive[16] effects have been also reported. These esters are unstable in blood and water and readily undergo hydrolysis to nitrite and alcohol.[6,17,18] In this report, we introduce briefly a case of IBN poisoning, report the experimental results concerning the behavior of IBN in a canned coffee drink solution, and report important information for toxicologists concerning the decomposition of toxic substances and related formation on other toxic substances with regard to artifacts.

EXPERIMENTAL PROCEDURES

Reagent. IBN was purchased from Tokyo Chemical Industry (Tokyo, Japan). All other chemicals used were of analytical grade. All aqueous solutions were prepared with deionized, distilled water.

Headspace Incubation of Isobutyl Nitrite in Various Types of Solutions. In a screw cap septum vial (7 mL, GL Science, Tokyo, Japan), 0.05 mL of acetone solution containing IBN or isobutyl alcohol (^{i}BuOH) and 0.95 mL of an aqueous solution which contained various additives (buffer or coffee solution) were added and the vial was then sealed with a Tuf-Bond disk. For the IBN and ^{i}BuOH assay, the mixture was allowed to stand at 30 °C for an appropriate time interval, and 0.5 mL of the gas phase in the headspace was injected into a GC using a glass syringe. For the cyanide assay, the mixture was allowed to stand at 50 °C for 30 min, and 0.5 mL of the gas phase was injected into a GC.

Storage Experiment for Isobutyl Nitrite in Water and a Coffee Solution. Twenty milliliters of water or a commercial canned coffee drink (Gorgia, Japan Coca Cola Co., Tokyo, Japan) and 140 mg of IBN were added to a glass-stoppered conical beaker and stored at 4 °C. Two hundred microliters of the mixed solution was periodically sampled into an HS vial containing 0.5 mL of 1 M phosphate buffer (pH 7.0), 0.25 mL of water, and 50 μL of acetone and incubated at 30 °C for 10 min, and 0.5 mL of the gas phase was injected into GC for IBN and ^{i}BuOH analysis. For the cyanide analysis, 0.2 mL of the storage solution was sampled into an HS vial containing 0.5 mL of 1 M phosphate buffer (pH 6.0) and 0.3 mL of water and incubated at 50 °C for 30 min, and 0.5 mL of the gas phase was injected into GC. For the nitrite and nitrate assay, an aliquot of the storage sample was diluted 100-fold with water, filtered through a 0.45-μm cellulose acetate membrane, and applied to capillary electrophoresis (CE).

Headspace Gas Chromatographic Determination of Isobutyl Nitrite and the Isobutyl Alcohol. IBN and ^{i}BuOH concentrations were determined by the headspace gas chromatography (HS-GC) method. A total of 500 μL of the above-mentioned gas phase was injected into the following GC. The GC instrument used was a model HP 6890 gas chromatograph (Yokogawa Analytical Systems, Tokyo, Japan) equipped with a flame ionization detector and split injector. A capillary column HP-WAX (30 m × 0.25 mm i.d., 0.25-μm thickness, Yokogawa Analytical Systems) was used as the stationary phase. The column head pressure of carrier gas (helium) flow was adjusted to 7.67 psi, and the splitter ratio was adjusted to 30. The oven temperature was controlled by a temperature program (initially at 40 °C (3-min hold), then to 115 °C at 15 °C/min). The injection port and detector were maintained at 200 and 220 °C, respectively.

The presence of IBN and ^{i}BuOH were confirmed by GC/mass spectrometry (MS), which consisted of an HP 5890 series II gas chromatograph combined with an HP 5989B quadrupole mass spectrometer (Yokogawa Analytical Systems). The stationary phase was DB-WAX (30 m × 0.25 mm i.d., 0.25-μm thickness, J&W Scientific, Folson, CA). The carrier gas (helium) flow rate was 0.67 mL/min. The injection port, transfer line, and ion source were maintained at 200, 280, and 200 °C, respectively. The splitter ratio was adjusted to 47. Electron impact ionization (EI, ionization energy 70 eV, ionization current 60 μA) was used as the ionization mode. The oven temperature was controlled by a temperature program (initially at 40 °C (3-min hold), then to 200 °C at 10 °C/min (3-min hold)). The acquisition mass range was 33−150, and the sampling was 0.8 scan/s. Acquisition was started 4 min after sample injection.

Cyanide Assay by Headspace Gas Chromatography. Cyanide concentrations were determined by the HS-GC method, as described previously.[19,20] A total of 500 μL of the above-mentioned gas phase was injected into an HP 5890A gas chromatograph (Yokogawa Analytical Systems), which was equipped with a nitrogen−phosphorus detector and a GS-Q column (30 m × 0.53 mm i.d., J&W Scientific). The carrier gas (helium) flow rate was 4.7 mL/min. The injection port, detector, and column oven were maintained at 200, 250, and 140 °C, respectively. The splitter ratio was adjusted to 3.5. The quantification limit was roughly 0.05 nmol (S/N = 3.5) of cyanide in the HS vial.

Capillary Electrophoretic Determination of Nitrite and Nitrate Anions. Nitrite and nitrate anions were determined using a Quanta 4000E CE system (Waters, Milford, MA). The capillary column used was a fused-silica column (75 μm i.d. × 60 cm), and the electrophoresis buffer was 5 mM sodium chromate (pH 8.0) containing 0.5 mM CIA-Pak anion-BT (Waters). The voltage was set at 20 kV with a negative power supply. The detection was by indirect ultraviolet absorption at 254 nm. The column temperature was maintained at 25 °C. Samples were applied hydrodynamically for 30 s. Nitrite and nitrate migrated at 3.5 and 3.6 min and were well separated from chloride (3.3 min) and sulfate (3.4 min).

RESULTS AND DISCUSSION

Report of the Poisoning Case. In the autumn of 1998 at a local motor hotel, one of a young couple drank one portion of a canned coffee drink taken from a refrigerator. She immediately noticed a strange taste and spit most of the liquid from her mouth.

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The local police began an investigation of the case. Diagnosis of the injured casualty showed no unusual symptoms. The remaining canned coffee drink, which was left at the crime scene, was transferred to the FSL, and a preliminary copper−guaiacum paper test[21] indicated a positive cyanide reaction, and its presence was subsequently confirmed by HS-GC. Any organic-extractable, toxic substances and drugs were not detected by GC/MS from either the diethyl ether or the chloroform layer, which was obtained by extraction of the sample solution under acidic (tartaric acid) or ammonia-alkaline conditions, respectively. A portion of the coffee drink sample was sent to NRIPS for further forensic investigation. The pH of the sample was 4.8 and that of the same brand of the commercial canned coffee drink (control sample) was 5.9. 3.2 μg/mL cyanide as determined in the evidence coffee drink sample, using the HS-GC method.[20] CE analysis for a search of toxic anions indicated that about 200 ppm nitrate and 310 ppm nitrite were present, and these values surpassed those of the control sample (nitrate 9.5 ppm; nitrite not detected). However, CE analysis for alkaline and alkaline earth metal cations[22] indicated that there were essentially no differences in the levels for the evidence coffee drink (K^+ 850 ppm, Ca^{2+} 230 ppm, Na^+ 300 ppm, Mg^{2+} 68 ppm) and the control coffee drink (840, 240, 300, and 70 ppm, respectively). We deduced that the nitrite found was not adulterated as a salt form into the evidence canned coffee drink. Finally, ~3000 ppm isobutyl alcohol and ~6 ppm IBN were detected by HS-GC when the evidence sample was directly incubated at 50 °C for 30 min and the resultant HS gas was injected to GC. We concluded that the cyanide was derived from artifacts and was not part of the original adulterant mixture.

Degradation of Isobutyl Nitrite in Aqueous Solution. After completing the forensic investigation, we attempted to establish an analytical method for the determination of IBN and related compounds. Alkyl nitrites have been analyzed by derivatization methods,[6] GC with direct injection,[17] HS-GC,[18,23] solid-phase microextraction GC,[24] or infrared spectrometry.[25] In this paper, IBN was determined by HS-GC. Many volatile substances exert phase III alteration,[4] which takes place during analysis, especially HS equilibrium. The HS conditions have been improved to suppress such a change. IBN and ^{i}BuOH were well separated on the HB-WAX capillary column (retention time: 3.4, 7.4 min, respectively) and confirmed by EI-MS of their peaks for IBN (base peak, m/z 43 [$(CH_3)_2CH^+$]; 57 [$(CH_3)_2CHCH_2^+$]; 73 [$(CH_3)_2CHCH_2O^+$]) and ^{i}BuOH (base peak, m/z 43 [$(CH_3)_2CH_2^+$]; 74 [M^+]). IBN easily undergoes hydrolysis, especially in acidic solution.[17] As shown in Figure 1, in water solution, IBN rapidly decomposed in an exponential manner, independent of temperature. During the incubation of IBN in aqueous solution, the pH of the solution became acidic. As a result, the decomposition proceeded autocatalytically. However, for a long incubation, IBN decomposition was suppressed due to the reverse reaction of esterification from nitrite and ^{i}BuOH. IBN rapidly decomposed at 40 and 50 °C even under neutral pH conditions. For the case of a 30 °C incubation using neutral pH buffer, IBN degradation was not so rapid. Because the boiling point of IBN is 67 °C, the sensitivity of the HS-GC method at 30 °C would be anticipated to be rather high. Therefore, we adopted the incubation temperature at 30 °C for 10 min in a neutral, buffered solution.

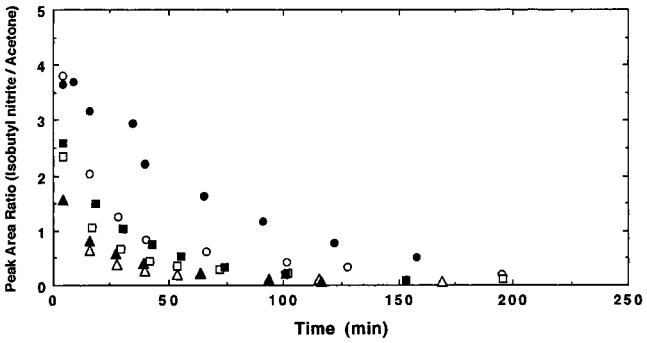

Figure 1. Degradation of isobutyl nitrite in aqueous solution. In an HS vial, 0.05 mL of acetone solution containing 5 mg of IBN, 0.45 mL of water, and 0.5 mL of 0 (open) or 1 M (closed) phosphate buffer (pH 7.0) were added, and the vial was sealed and incubated at 30 (circle), 40 (triangle), and 50 °C (square). After an appropriate interval, 0.5 mL of the gas phase was injected into the GC. The peak areas corresponding to IBN and acetone on gas chromatogram were integrated, and the ratio of peak areas of IBN to acetone was plotted against the incubation time.

We also investigated the pH dependence of IBN degradation. IBN decomposes as a function of time, and a finite amount of time is required to attain HS equilibrium. Thus, from a theoretical point of view, it is impossible to accurately determine IBN levels by the HS-GC method. The ionic strength of the phosphate buffer solution in the HS liquid phase was increased with increasing pH. Although the HS behavior of IBN and acetone in relation to the salting-out effect was not the same, we used a value obtained by dividing the peak areas of IBN to acetone as a representative IBN level, to suppress the error of HS injection volume and to offset differences in ionic strength. As shown in Figure 2A, IBN decomposed rapidly under acidic conditions, and the decomposition was suppressed by neutral pH conditions.

Production of Isobutyl Nitrite from Isobutyl Alcohol and Nitrite. Although IBN decomposes to produce ^{i}BuOH and nitrite, the reverse reaction should also be considered. We investigated the pH dependence of IBN formation. As shown in Figure 2B, at pH values below 4, IBN was produced in significant amounts from a mixture of nitrite and ^{i}BuOH. With long-term incubation, the newly produced IBN again decomposed. At a lower pH, IBN production and the subsequent IBN breakdown proceeded faster and more profoundly. A total of 64.4 mM IBN was produced from 100 mM nitrite and 68 mM ^{i}BuOH at pH 2.0. IBN production was dose dependent with respect to nitrite and ^{i}BuOH levels at pH 2.0 and pH 4.0. With a high dose of nitrite, IBN production tended to be saturated from the level of 25 mM nitrite. In contrast, IBN production was nearly proportional to the level of ^{i}BuOH up to 130 mM. Because the pK_a of nitrous acid is 3.37, the chemical form required to esterify ^{i}BuOH might be the protonated form of nitrite.

Production of Cyanide in Nitrite-Spiked Coffee Solution. Cyanide is easily produced through various types of reactions, especially the oxidation of nitrogen-containing compounds.[26] In this experiment, even without coffee, a minute amount of cyanide

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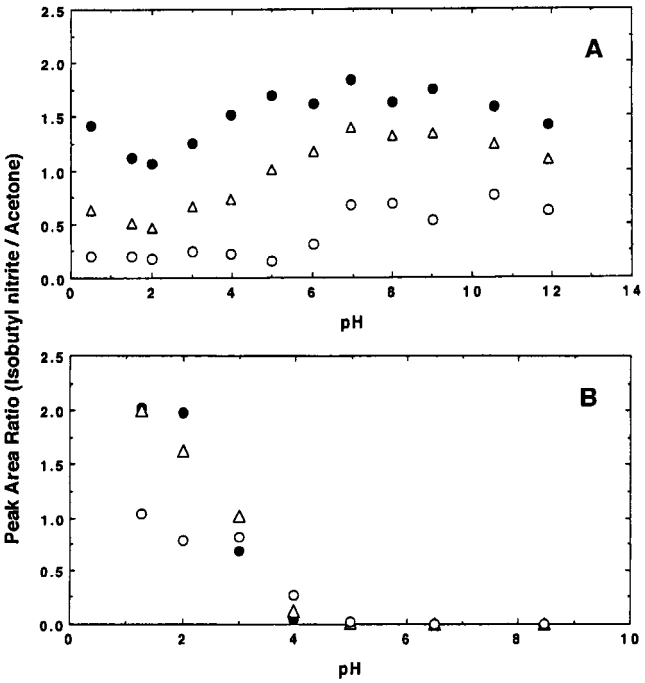

Figure 2. pH dependence of isobutyl nitrite degradation and production. In (A), into an HS vial, 0.05 mL of acetone solution containing 5 mg of IBN, 0.45 mL of water, and 0.5 mL of 1 M phosphate buffer were added and the vial was sealed and incubated at 30 °C. After 10 (closed circle), 37 (open triangle), and 140 min (open circle), 0.5 mL of the gas phase was injected into the GC. In (B), into an HS vial, 0.05 mL of acetone solution containing 5 mg of ^{i}BuOH, 0.05 mL of 1 M sodium nitrite solution, 0.4 mL of water, and 0.5 mL of 1 M phosphate buffer were added, and the vial was sealed and incubated at 30 °C. After 10 (closed circle), 37 (open triangle), and 140 min (open circle), 0.5 mL of the gas phase was injected into the GC. The peak areas of IBN and acetone on gas chromatogram were integrated, and the ratio of peak areas of IBN to acetone was plotted against pH of buffer.

was produced in the nitrite solution at low-pH conditions, but its level was insignificant. This issue was not pursued further. At pH 2.0, ~4.1 μM cyanide was produced from 100 mM nitrite. As shown in Figure 3, cyanide production in a coffee solution that contained nitrite was pH dependent. Under lower pH conditions, more cyanide was produced. The addition of ascorbic acid, which suppresses cyanide production from thiocyanate,[26] particularly, but not completely, suppressed cyanide production. Cyanide production was dose dependent with respect to both coffee volume and nitrite level. As shown in Figure 4, with a high dose above 2% coffee and 2 mM nitrite, cyanide production tended to be saturated. About 76 μM (2.0 μg/mL) cyanide was produced in a solution containing 20% coffee and 100 mM nitrite at pH 2.0.

Change in Isobutyl Nitrite-Spiked Water or a Standard Coffee Solution. To investigate the behavior of IBN in aqueous and coffee drink solutions during storage at 4 °C, the HS condition was optimized to determine IBN and related compounds. Regarding IBN decomposition and the production for IBN and ^{i}BuOH analysis, we adopted an HS incubation using pH 7.0 buffer at 30 °C for 10 min. Under this condition, a calibration curve was linear with respect to IBN concentration ranging from 125 ppb to 5000 ppm in HS vial. The within-day repeatability (1250 ppm, $n = 5$) was 2.8% (RSD), and the detection limit was 62 ppb (S/N = 3). A calibration curve was linear with respect to ^{i}BuOH concentration ranging from 2.5 to 10 000 ppm in HS vial. The within-day repeatability (1250 ppm, $n = 5$) was 0.98% (RSD), and the detection limit was 1.9 ppm (S/N = 3). We adopted the HS incubation using

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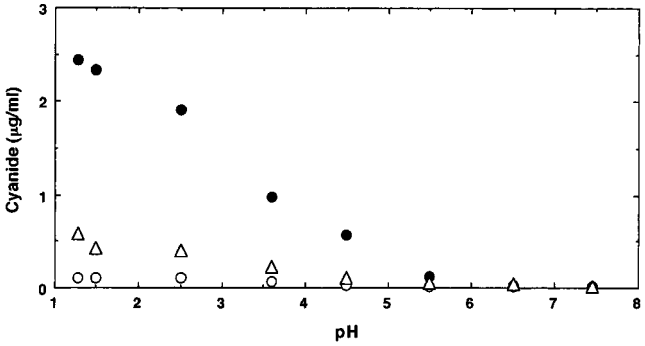


Figure 3. pH dependence of cyanide production in coffee solution. In an HS vial, 0.05 mL of 1 M sodium nitrite and 0.5 mL of 1 M phosphate buffer with (closed circle) or without (open circle) 0.2 mL of canned coffee solution or with 0.2 mL of canned coffee solution and ascorbic acid (open triangle) were added (liquid-phase final 1.0 mL), and the vial was sealed and incubated at 50 °C. After 30 min, 0.5 mL of the gas phase was injected into the GC. The cyanide levels were plotted against the pH values of the buffer solutions.

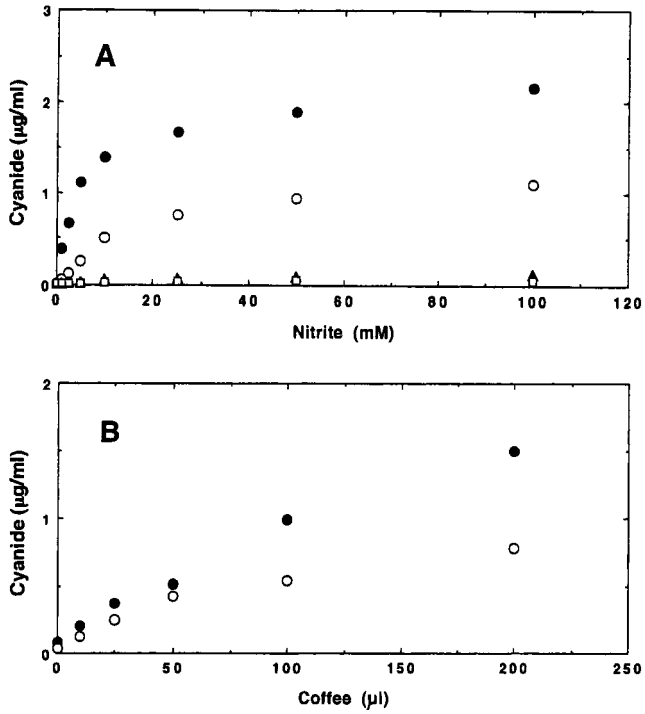


Figure 4. Dose dependence of cyanide production in coffee solution. In a HS vial, 0.05 mL of acetone, 0.5 mL of 1 M phosphate buffer (pH 2.0, closed circle; pH 4.0, open circle), 0.2 mL of canned coffee solution, and 0.25 mL of aqueous solution containing various concentration of nitrite were added (A), and the vial was sealed and incubated at 50 °C. Instead, in an HS vial, 0.5 mL of 1 M phosphate buffer (pH 2.0, closed circle; pH 4.0, open circle), 0.05 mL of 1 M sodium nitrite solution, and various volumes of canned coffee solution (liquid-phase final 1.0 mL) were added (B), and the vial was sealed and incubated at 50 °C. After 30 min, 0.5 mL of the gas phase was injected into the GC. The peak areas of cyanide were plotted against coffee volume (A) or nitrite concentration (B) in the HS vial.

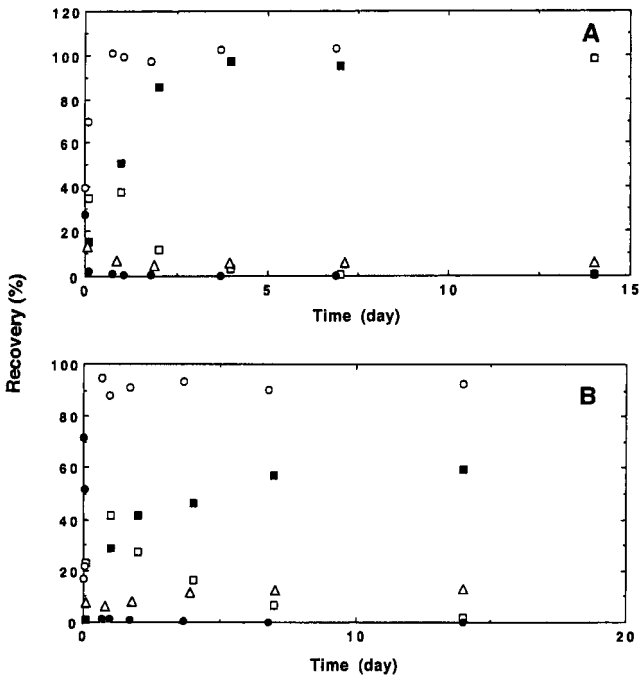


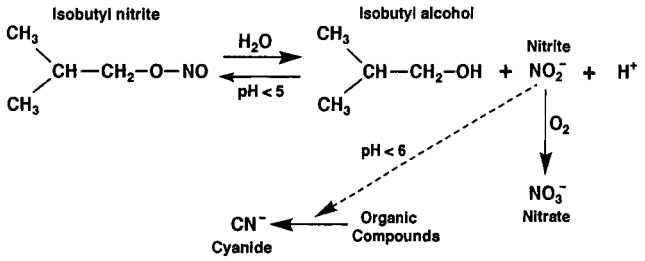
Figure 5. Change of concentrations of IBN and related compounds in aqueous solution. IBN (7 mg/mL) was added to a water solution (A) or a control coffee solution (B) and stored at 4 °C in a sealed container. Periodically, an aliquot was analyzed for IBN (closed circle) and ^{i}BuOH (open circle) by HS-GC, nitrite (open square) and nitrate (closed square) by CE, and cyanide (open triangle) by HS-GC, respectively. The concentration levels represent the molar recovery percentage corresponding to the added IBN except for cyanide, which was multiplied by 100 (A) or 50 (B).

a phosphate buffer at pH 6.0 at 50 °C for 30 min for cyanide analysis.

IBN was added to water (7 mg/mL) and stored at 4 °C. Periodically an aliquot was sampled and analyzed. Figure 5A shows the change in the concentrations of IBN and related compounds. IBN disappeared rapidly, and at 2.5 h, only ~2.2% of the originally added IBN could be detected. Instead, ^{i}BuOH was produced, and its level reached a plateau which was molar equivalent to the added IBN. Nitrite ion was produced just after the addition, and its level reached a maximum at 1 day (~40% molar recovery) and then decreased gradually. Nitrate ion was produced just after IBN addition, and its level reached a plateau which was molar equivalent to the added IBN. Furthermore, cyanide was detected, and its level remained nearly constant throughout the experiment. At the seventh day, levels of IBN, ^{i}BuOH, nitrite, nitrate, and cyanide were 0.05, 103, 0.6, 95, and 0.06% of the added IBN as molar conversion. The pH of the solution was 1.2 at 14th day.

IBN was added to the control coffee drink (7 mg/mL) and stored at 4 °C. Periodically an aliquot was sampled and analyzed. Figure 5B shows the change in the concentrations of IBN and related compounds in the coffee solution. The alterations of the levels of IBN, ^{i}BuOH, nitrite, nitrate, and cyanide were similar to those in water (Figure 5A). However, a notable difference can be observed. IBN was readily detected, showing 1.3% remaining even after the first day. The plateau level for the produced nitrate ion reached about a 60% molar recovery. The produced cyanide level was higher than in water. At the seventh day, the levels of IBN, ^{i}BuOH, nitrite, nitrate, and cyanide were 0.14, 90, 6.8, 57, and 0.25% of the added IBN as a molar conversion. The pH of the solution was pH 2.6 at 14th day.

Scheme 1. Isobutyl Nitrite Degradation in a Coffee Solution



Commercial canned coffee drink consists of a complex matrix, including milk proteins, carbohydrates, lipids, minerals, caffeine, and polyphenols. When IBN is added to the coffee drink solution, a hydrolysis product of IBN, nitrite ion, is produced, and the solution becomes acidic. Nitrite ion is, under acidic conditions, strongly reactive. Although a considerable portion of the nitrite is oxidized to nitrate by atmospheric oxygen, some part exerts oxidative or reductive interaction with organic compounds in the coffee solution. One of the nonspecific oxidative reactions is the production of cyanide. The protonated form of nitrite, HNO_2, may react with organic compounds as a part of this overall reaction. The proposed molecular mechamism is shown in Scheme 1.

The cyanide (3.2 ppm) found in our forensic investigation of the poisoning case was not sufficiently high to show lethal effects for humans with one bottle or can. However, we cannot simply conclude that such a low level of cyanide is not related to a lethal dose, for it is probable that in the usual cyanide-tampered cases the original level of cyanide added was higher than that of the measured level because of the unstability of cyanide in such a beverage sample. In the case presented in this paper, the cyanide was, in fact, derived from artifacts and was not part of the original adulterant mixture.

In our forensic investigation, we found IBN by HS-GC where the HS conditions were fixed at 50 °C and pH of the solution was 4.8. Therefore, judging from the pH dependence (Figure 2B), the obtained IBN level may be derived from the IBN left in the sample but not an artifact of esterification from ^{i}BuOH and nitrite. If the sample solution is more acidic, IBN from the reverse reaction can be readily detected from the remaining nitrite. Under the conditions that IBN was adulterated into alcohol drinks, ethyl nitrite is found from hydrolyzed nitrite and ethanol, showing a false detection; i.e., ethyl nitrite was produced as an artifact.

Cyanide-Producing Component in Reaction with Nitrite under Acidic Conditions. Cyanide production for a reaction mixture of nitrite and various types of organic compounds was examined. Previously we reported that nitrite produced considerable amounts of cyanide via a reaction with thiocyanate.[25] Glycine (0.2 M), sucrose (0.2 M), citric acid (0.2 M), and caffeine (0.02%) did not produce cyanide when reacted with nitrite (50 mM). Bovine serum albumin (2%) produced 4.9 and 1.7 μM cyanide in 10% phosphoric acid and 0.6 M acetate buffer at pH 5.0. Starch (2%) produced 5.8 and 3.8 μM cyanide in a 10% phosphoric acid and a 0.6 M acetate buffer at pH 5.0. Under the same conditions,

a canned coffee drink (0.2 mL) produced 130 and 25 μM cyanide, respectively. The other brands of commercial canned coffee drinks (three types of UCC coffee, The Blend Rich Type, Special Blend, Black Nonsugar, UCC Ueshima Coffee Co., Kobe, Japan) also produced similar levels of cyanide. Ingredients contained in canned coffee drink, i.e., proteins, carbohydrates, and caffeine, gave rise to less cyanide in a reaction with nitrite than a real coffee solution. Substances that are extracted from coffee beans, such as polyphenols, may produce cyanide in reaction with nitrous acid. At present, the search for compounds in coffee extracts that react with nitrous acid to produce cyanide is now under investigation.

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