Destruction of Cyanogen Bromide and Inorganic Cyanides¹,²

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Cyanogen bromide in water and seven organic solvents and sodium cyanide in water may safely and efficiently (>99.7%) be destroyed using sodium hydroxide (1 M) solution and commercially available sodium or calcium hypochlorite. Details are given of an analytical procedure which can be used to check the final reaction mixture for the presence of residual cyanogen bromide or cyanide.

KEY WORDS: cyanogen bromide; cyanides; disposal; protein structure; affinity chromatography.

Many experiments in biomedical research laboratories involve the use of highly toxic reagents yet comparatively little research has been done on their safe disposal. Recently, in a program sponsored by the Division of Safety, National Institutes of Health, we have investigated the destruction of N-nitrosamines (1), N-nitrosamides (2), hydrazines (3), and dimethyl sulfate (4). Destruction methods for these and other compounds have been described in a series of monographs published by the International Agency for Research on Cancer (5-11). We now wish to report the results of a study on the safe disposal of cyanogen bromide (BrCN), a reagent used in laboratories in the analysis of protein structure (12-15), for the activation of agarose beads in affinity chromatography (16-18), and for the detection of pyridine compounds (19).

Cyanogen bromide is highly toxic (92 ppm for 10 min has caused a fatality (20)) with its toxic effects being those of hydrogen cyanide (21). At low concentrations in the air (10 ppm) it is very irritating to the eyes, nose, and respiratory tract (20, 21). We are not aware of any systematic, validated study on the degradation of cyanogen bromide. One report (15) states that BrCN in excess base can be destroyed by adding an equimolar amount of hypochlorite for 24 h. The use of cyanogen bromide in organic solvents has been advocated (16, 17) but no consideration appears to have been given to whether these solvents might interfere with disposal techniques.

Many articles have been published on the detection of cyanide, but these are generally concerned with the determination of cyanide in waste water (22, 23) or in dilute solutions containing low concentrations of agents that might interfere with analysis (24, 25). The detection of cyanogen bromide or cyanide in waste solutions that may contain high concentrations of other species appears to have received little attention, although Lonsdale-Eccles et al. (15) recommended the addition of ferrous ions to the final reaction mixture. They stated that a Prussian blue color indicated the presence of cyanide but they gave no indication of sensitivity or indeed any experimental details.

We have investigated the destruction of cyanogen bromide in a variety of solvents, using sodium hydroxide solution and sodium hypochlorite.
hypochlorite or calcium hypochlorite. By modifying the method of Nagashima (25) we have developed a method for detecting cyanogen bromide or cyanide ions in the final reaction mixture. A similar method has been recommended by Kohn and Wilchek (26) for monitoring the activation of Sepharose.

PROCEDURES

Cyanogen bromide and sodium cyanide are highly toxic. They should be handled using gloves in a properly functioning chemical fume hood. The addition of hypochlorites to these solutions is an exothermic process. For large-scale reactions, hypochlorites should be added over a period of time, cooling in an ice bath if desired. Sodium and calcium hypochlorite are corrosive and may cause burns; sodium hydroxide, potassium hydroxide, and hydrochloric acid solutions are corrosive and chloramine-T and 4-methylpyridine are irritants. All should be handled with due care.

Materials. Sodium hydroxide, calcium hypochlorite, sodium cyanide, and hydrochloric acid were obtained from Fisher Scientific Company, Fairlawn, New Jersey. Sodium hypochlorite was commercially available Clorox bleach and was used as supplied. All other reagents were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Distilled water was used throughout. Sodium ascorbate solution (10 g/liter) and chloramine-T solution (100 g/liter) were prepared fresh daily. A standard solution of sodium cyanide (about 100 mg/liter) was prepared fresh each week. Buffer solution: potassium dihydrogen phosphate (13.6 g), disodium hydrogen phosphate (0.28 g), and potassium bromide (3.0 g) were dissolved in water and the resulting solution made up to 1 liter. 4-Methylpyridine/barbituric acid reagent: barbituric acid (3.0 g) was stirred in a small amount of water (±10 ml) and 4-methylpyridine (15 ml) and concentrated hydrochloric acid (3 ml) were added while stirring. After cooling, the solution was diluted to 50 ml with water.

Destruction of bulk quantities of cyanogen bromide. Cyanogen bromide was dissolved in water to give a solution not exceeding about 60 g BrCN/liter. An equal volume of 1 M NaOH was added to this mixture and the basified solution was mixed with the same volume of NaOCl (i.e., BrCN solution: NaOH: NaOCl, 1:1:2). Alternatively, to each liter of basified solution, 60 g of Ca(OCl)₂ was added. After the mixture was stirred for 3 h it was tested for completeness of destruction and discarded.

Destruction of cyanogen bromide in solution. When necessary the solution was diluted with the same solvent so that the BrCN concentration did not exceed 60 g/liter for water or acetonitrile, 30 g/liter for DMSO, DMF, 2-methoxyethanol, or 0.1 M hydrochloric acid, 25 g/liter for ethanol, or 19 g/liter for N-methyl-2-pyrrolidinone. To these solutions an equal volume of 1 M NaOH and two vol of NaOCl (i.e., BrCN solution: NaOH: NaOCl, 1:1:2) were added. Alternatively, an equal vol of 1 M NaOH was added to the BrCN solution and 60 g of Ca(OCl)₂ was added per liter of the basified solution. After it was stirred for 3 h the solution was tested for completeness of destruction and discarded.

Destruction of cyanogen bromide in 70% formic acid. When necessary the solution was diluted so that the concentration of BrCN did not exceed 60 g/liter. This solution was basified by the slow addition of 2 vol of 10 M KOH. Caution: This reaction is highly exothermic. After it is cooled, using an ice bath if desired, the solution may be degraded as described above by adding 1 M NaOH and NaOCl or Ca(OCl)₂.

Destruction of sodium cyanide. Bulk quantities were dissolved in water to give a concentration of 25 g/liter. Aqueous solutions were diluted when necessary so that the concentration did not exceed 25 g/liter. The solutions were degraded by adding 1 M NaOH and NaOCl or Ca(OCl)₂ as described above.

Analysis for residual cyanogen bromide or
Two portions of the reaction mixture (1 ml each) were centrifuged, if necessary, to remove suspended solids and each was added to 4 ml of the buffer. If an orange or yellow color appeared, sodium ascorbate solution was added dropwise until the reaction mixtures were colorless. (No more than 2.0 ml sodium ascorbate solution should be added.) One of the duplicate solutions was spiked with 200 µl of sodium cyanide solution. Chloramine-T solution (1 ml) was added to each solution and the mixtures were shaken and allowed to stand for 1 to 2 min. One milliliter of the 4-methylpyridine/barbituric acid reagent was added to each solution and the mixtures were shaken and allowed to stand for 5 min. A blue color indicated the presence of cyanogen bromide or cyanide. (For confirmation of complete destruction the spiked solution should be blue and the unspiked solution should be colorless.) If necessary the solutions were centrifuged to remove suspended solids and the absorbance of the supernatants was measured. Appropriate blanks should always be run. Either distilled water or a reaction mixture using water instead of the cyanogen bromide or sodium cyanide solution should be analyzed as above and this will provide the spectrometer blank.

The absorbance was determined at 605 nm using a Gilford 240 uv/vis spectrophotometer and disposable 10-mm plastic cuvettes (Müller Ratiolab, Dreieich, W. Germany).

**RESULTS AND DISCUSSION**

We found that both methods degraded cyanogen bromide or sodium cyanide solutions efficiently (Table 1). However, solvents other than water interfered with the destruction process, which limited the amount of cyanogen bromide that could be degraded.

When solutions of cyanogen bromide in water (which would not be expected to interfere with the destruction process) were treated, relatively large amounts of cyanogen bromide, about 60 g/liter, could be present. At similar concentrations acetonitrile did not appear to interfere with the destruction process. How-

<table>
<thead>
<tr>
<th>Amount of BrCN present (mg)</th>
<th>Solvent</th>
<th>Residual BrCN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NaOCl method</td>
</tr>
<tr>
<td>250</td>
<td>H_2O</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>236</td>
<td>CH_3CN</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>124</td>
<td>DMSO</td>
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<tr>
<td>125</td>
<td>DMF</td>
<td>&lt;0.12</td>
</tr>
<tr>
<td>125</td>
<td>CH_3OCH_2CH_2OH</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>132</td>
<td>HCl (0.1 M)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>100</td>
<td>CH_3CH_2OH</td>
<td>&lt;0.12</td>
</tr>
<tr>
<td>85</td>
<td>Formic acid (70%)</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>74</td>
<td>N-methyl-2-pyrrolidinone</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

*The amount of BrCN indicated was dissolved in 4 ml of the solvent. Four milliliters of 1 M sodium hydroxide solution was added followed by 8 ml of sodium hypochlorite solution or 0.5 g of calcium hypochlorite. After stirring for 3 h residual cyanide was determined. The values shown are the limits of detection.

*See text for procedure.
ever, when the cyanogen bromide was dissolved in other organic solvents (dimethyl sulfoxide, dimethylformamide, 2-methoxyethanol) or in hydrochloric acid (0.1 M) only smaller amounts of cyanogen bromide, about 30 g/liter, could be present in the sample. If larger amounts were present, traces of cyanogen bromide or cyanide were found in the final reaction mixtures after 3 h. Longer reaction times gave little improvement. N-Methyl-2-pyrrolidinone, a solvent which has been recommended (16) for use with cyanogen bromide, interfered considerably with the destruction process and only small amounts of cyanogen bromide, about 19 g/liter, could be degraded. When ethanol was the solvent about 25 g/liter could be degraded. When cyanogen bromide was dissolved in formic acid (70%) the reaction mixture had first to be basified and this caused dilution of the sample. About 20 g/liter could be degraded.

We also investigated the destruction of sodium cyanide in aqueous solution. Since the fundamental destruction process is the oxidation of cyanide ions first to the much less toxic cyanate ions and then to nitrogen and carbon dioxide (27), the weight of sodium cyanide present in a sample was reduced to preserve approximately the same concentration of cyanide ions as when BrCN was destroyed. No problems were observed with this destruction procedure. An initial concentration of 25 g/liter was used and no trace of cyanide was found when NaOCl or Ca(OCl)₂ was used (limit of detection < 0.02% in each case).

A slightly modified analytical procedure (25) was tested and found to work well. In this procedure cyanide ions are converted to cyanogen chloride by the addition of chloramine-T. The cyanogen chloride then reacts with 4-methylpyridine in the presence of barbituric acid to give a blue color which can be measured spectrophotometrically at 605 nm. Cyanogen bromide undergoes a similar reaction (19,28), as was shown by numerous spiking experiments, and so the method is equally sensitive for cyanogen bromide or cyanide ions. The main problem encountered with the analytical procedure was the failure of the blue color to develop, even when the sample had been spiked with a known quantity of sodium cyanide or cyanogen bromide. If a large amount of oxidant was present, any material used to spike the sample would rapidly be converted to cyanate and thus the spiking would be in vain. To get around this problem, sodium ascorbate solution (10 g/liter) was added until the yellow color, produced by the oxidation of bromide (from the buffer or from cyanogen bromide) to bromine, disappeared. With the oxidizing power of the solution removed the sample may be spiked. It was found empirically, however, that if more than 2 ml of the sodium ascorbate solution were added no blue color developed. We suppose that large amounts of ascorbate prevented formation of the blue dye. We recommend that no more than 2 ml sodium ascorbate solution be used, whether the yellow color disappears or not. Each time a reaction mixture is tested for cyanide or cyanogen bromide the determination should be done in duplicate with one reaction spiked with a small amount of sodium cyanide (or cyanogen bromide) solution. In this way one can be sure that the absence of a blue color means that no cyanide or cyanogen bromide is present, not just that something has interfered with the test.

The absorbances produced by given amounts of cyanide were found to vary depending on the presence of other components. Thus the spiked sample can be used to establish the limit of detection. Our practice was to treat absorbances of less than 0.050 as not significant, being within the range of random variation. If spiking with $x \mu g$ of NaCN gives an absorbance of $y$, then the limit of detection is $0.050 \times \frac{x}{y} \mu g$.

The blue color was found to be relatively stable, but the determination should be made promptly because colorless crystals tend
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separate on prolonged standing and these will have to be removed by centrifuging (preferably) or filtering or the absorbance value will be inflated. For this reason we recommend using a water blank if large numbers of samples are to be run. The absorbance of a reaction mixture blank can be determined at the beginning of the analysis.

Although we have given directions for degrading solutions of cyanogen bromide of certain concentrations we recognize that many people will not want to dilute more concentrated solutions before beginning the disposal procedures but instead will simply add more sodium hydroxide solution and more hypochlorite. Clearly, it would be impossible for us to recommend procedures to cover every conceivable case but from the data given above it should be possible to work out satisfactory conditions. In every case the final reaction mixtures should be checked for completeness of destruction.

ACKNOWLEDGMENTS

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REFERENCES

A one-step procedure (CRBP and CRABP) from anion-exchange high-perf of Mono Q, which perm © 1985 Academic Press, Inc.

KEY WORDS: cellular r

Cellular retinol and retinc proteins (CRBP and CRAI discovered in recent years (1 to be involved in the met; function of retinoids (4–9).

We present an anion-exchai for the separation and det-proteins after incubation o fraction from rat testis with rac and retinoic acid.

MATERIALS AND MIC

Preparation of cytosol from Sprague–Dawley rats (250 g) under ether anesthesia. Testes w the “tunica albuginea” and hon vol of ice-cold 10 mM Tris–Htaining 1 mM EDTA at pH 8.0 were then centrifuged at 150.( 4°C. Glycerol was added to ti to a final concentration of 19

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2Abbreviations used: CRBP, cellu; protein; CRABP, cellular retinoic acid SIG, sucrose density gradient.