

PRESERVATION OF WATER SAMPLES FOR INORGANIC NITROGEN ANALYSES WITH MERCURIC CHLORIDE

PATRICK L. BREZONIK and G. FRED LEE*

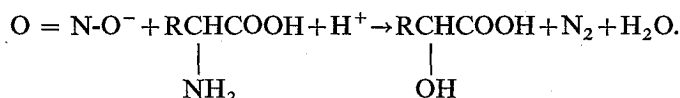
Water Chemistry Laboratory, University of Wisconsin Madison, Wisconsin 53706, U.S.A.

(Received 28 April 1966)

INTRODUCTION

SULFURIC acid has long been the accepted preservative of water samples for inorganic nitrogen determinations. STANDARD METHODS (1965) recommends the use of 1500 mg/l H_2SO_4 in water samples; this is equivalent to about 0.8 ml concentrated H_2SO_4 /l of sample. The authors have long felt this to be an unfortunate selection of preservatives. STANDARD METHODS itself cautions that acid may not preserve a nitrogen balance longer than 24 hr. Analysis of water samples within 24 hr is not always possible, however, especially for samples collected in the field. Storage of samples for a week or more is probably not uncommon, and in many cases such delays are unavoidable.

The use of sulfuric acid to preserve samples for nitrite determinations is even more unfortunate, in light of the possible occurrence of the Van Slyke reaction (VAN SLYKE, 1912). This is a reaction whereby nitrite reacts with amino groups—especially α -amino acids—and also with ammonia to produce nitrogen gas:



The reaction occurs at significant rates only in low pH solutions (BREZONIK and LEE, 1966). Thus rather than having a preserving effect, sulfuric acid would seem to have a catalyzing effect on the disappearance of nitrite from water samples. This is especially the case for natural waters having significant concentrations of ammonia or free amino acids.

Accordingly, a brief study has been conducted to determine the effect of acid preservation on the nitrite content of a natural water. Preservation by simple refrigeration and with mercuric chloride was compared to preservation with sulfuric acid. Mercuric chloride was chosen since it has been employed successfully in the past. For instance, COOPER (1933) found that 1 ml of a saturated mercuric chloride solution per l. of sample was sufficient to preserve marine water samples for ammonia analyses for several months. HELWIG (1964) reported that 5 ml of a saturated mercuric chloride solution per gallon of sample was satisfactory in preserving strongly polluted water samples with respect to 19 parameters. Among these parameters were all the forms of nitrogen, pH, conductivity, phosphate, and dissolved solids. Other preservatives studied—chloroform, thymol, formalin, and potassium cyanide—either influenced certain parameters adversely or could not preserve the water satisfactorily.

* Presently, G. Fred Lee & Associates, El Macero, CA www.gfredlee.com

EXPERIMENTAL

A sample of surface water from Lake Mendota, Wisconsin, was taken, and to a portion of it was added sufficient sodium nitrite and glycine to make a solution 10 mg/l in NO_2^- -N and glycine-N. Dilutions of this water to 1.0 mg NO_2^- -N/l and 0.1 mg NO_2^- -N/l were made with additional lake water. Each of the three waters enriched in nitrite and glycine was divided among three 300 ml bottles, and in each set of three bottles, one was preserved with 1 ml concentrated sulfuric acid, another was preserved with 1 ml of a saturated mercuric chloride solution, and the final one was left unpreserved. Another portion of the original lake water sample was enriched 10 mg/l in NO_2^- -N alone. Dilutions of 1.0 and 0.1 mg NO_2^- -N were made, and a sample of each of the three concentrations was preserved in a 300 ml bottle with 1 ml concentrated sulfuric acid. Upon addition of the preservative, all samples were immediately refrigerated at 6° C.

Nitrite analyses were performed on the samples over a period of six days. The diazotization method with sulfanilic acid and α -naphthylamine hydrochloride (STANDARD METHODS, 1965) was used for nitrite determinations, and a Beckman DU spectrophotometer was used for absorbance measurements.

A second experiment was conducted with an unenriched natural water sample. A sample of Lake Mendota surface water containing about 17 μg NO_2^- -N/l was divided among three 2.5 l. glass bottles. One of the samples was preserved with 2 ml saturated mercuric chloride solution, another with 2 ml concentrated sulfuric acid, and the final sample was left unpreserved. The volume of each sample was 2 l. The samples were again stored in a refrigerator at 6° C, and analyses for nitrite were made over a period of 11 days in the same manner as in the first experiment.

RESULTS AND DISCUSSION

The results of the first experiment, as shown in Figs. 1, 2 and 3, indicate that mercuric chloride is a satisfactory preservative for nitrite samples, but that sulfuric acid is completely inadequate for this purpose. In fact, the use of no preservative at all (other than refrigeration) gave much better results than sulfuric acid preservation. The samples to which no preservative was added closely paralleled the samples to which mercuric chloride was added and remained at essentially the original concentration throughout the duration of the experiment. On the other hand, dramatic losses of nitrite were seen in the samples preserved with sulfuric acid. The losses occurred most rapidly in the first 20 hr and were slower thereafter.

Although it was expected that the sulfuric acid preserved samples with added α -amino acid (glycine) would lose nitrite more rapidly than the acid preserved samples without glycine, this was not found to be the case. Apparently there was sufficient amino nitrogen in the samples so that the amino acid was not rate controlling. Alternatively, it might be considered that loss of nitrite occurs by a mechanism other than the Van Slyke reaction, but what this would be is not certain. Regardless of the mechanism, however, it is apparent that sulfuric acid does not effect a preservation of nitrite in water samples.

Another point that is apparent from Figs. 1, 2 and 3 is that the relative loss of nitrite is greater in the samples with higher original concentrations of nitrite. Thus the acid preserved samples with 10 mg NO_2^- -N/l lost nearly 40 per cent of their nitrite content

in 17 hr, whereas the acid preserved 1.0 mg NO_2^- -N samples lost only about 10 per cent of their nitrite in the same period of time. While 10 mg NO_2^- -N/l represents the approximate upper limit of nitrite in biologically treated, nitrified sewage effluent, nitrite concentrations are usually below 1 mg/l as N in the natural environment, and

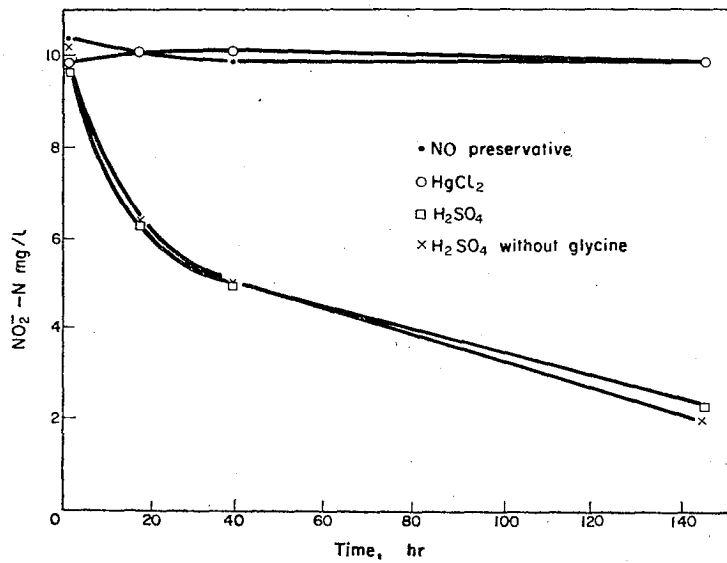


FIG. 1. Effect of preservatives on nitrite content of water enriched with 10 mg/l NO_2^- -N.

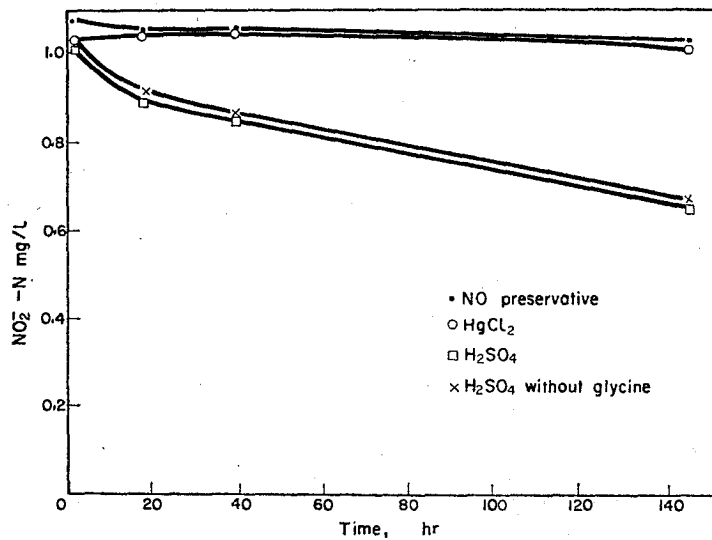


FIG. 2. Effect of preservatives on nitrite content of water enriched with 1.0 mg/l NO_2^- -N.

are of the order of a few parts per billion ($\mu\text{g/l}$) in many lakes. In order to determine whether the loss of nitrite by acid preservation is significant at low levels of nitrite commonly found in unpolluted lakes, the second experiment was conducted with unenriched Lake Mendota water. The results are shown in FIG. 4 and again indicate

the inadequacy of sulfuric acid as a preservative for nitrite samples. As in the first experiment, the greatest loss of nitrite occurred in the first 24 hr. However, the loss of nitrite continued after that at only a slightly reduced rate till only about 25 per cent of the original nitrite concentration remained after 171 hr. The unpreserved sample

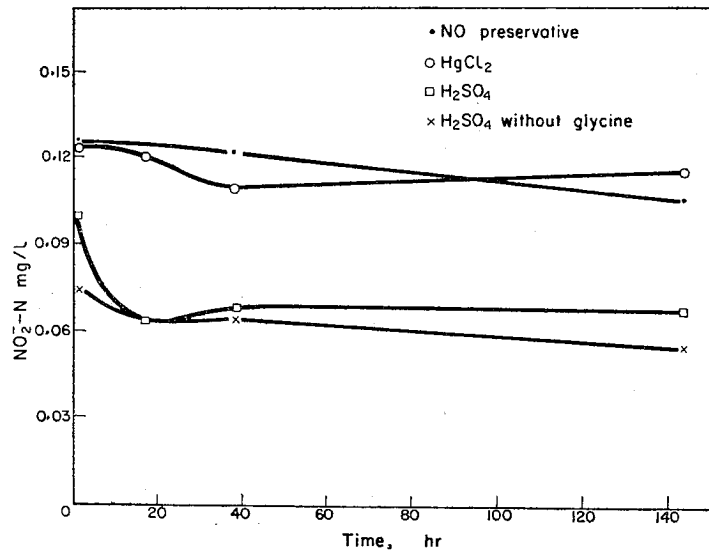


FIG. 3. Effect of preservatives on nitrite content of water enriched with 0.1 mg/l NO_2^- -N.

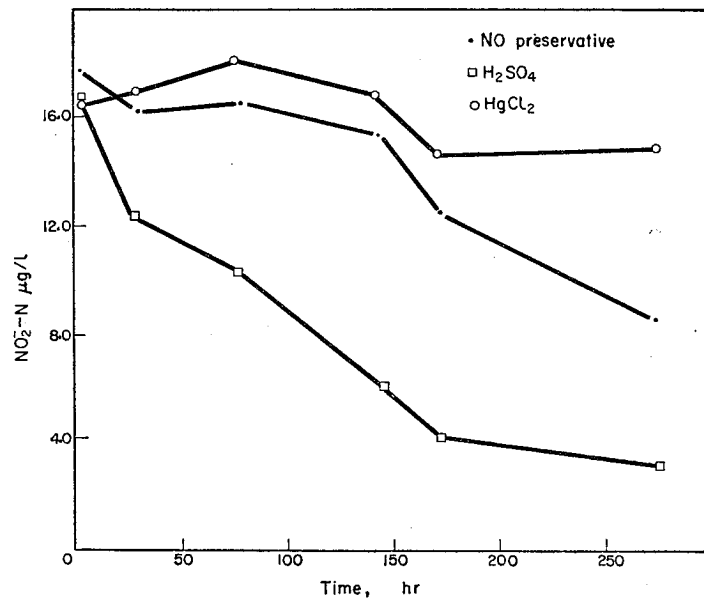


FIG. 4. Effect of preservatives on nitrite content of Lake Mendota water.

followed the mercuric chloride preserved sample fairly closely for the first 150 hr, but thereafter lost nitrite more rapidly. The mercuric chloride preserved sample fluctuated slightly through the experiment and had about 2 $\mu\text{g NO}_2^-$ -N/l less at the end of the run than it had originally.

In summary, then, sulfuric acid is not an effective preservative for nitrite in natural water samples containing μg to mg/l quantities of nitrite-N. Rather, it has a catalytic effect on the loss of nitrite. On the other hand, mercuric chloride is an effective preservative for nitrite samples, and its use in preserving water samples for nitrite analyses in limnological, oceanographic and pollution studies is highly recommended.

Acknowledgment—This investigation was supported in part by Training Grant Number 5T1-WP-22-04 and Predoctoral Fellowship Number 1-F1-WP-26, 103-01, both from the Federal Water Pollution Control Administration. The investigation was conducted in the Laboratory of Limnology. The use of the facilities in this laboratory is greatly appreciated. The support of this study by the Engineering Experiment Station and the Department of Civil Engineering is also appreciated.

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