TECHNICAL NOTE

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Determining Sodium Azide Concentration in Blood by Ion Chromatography*

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ABSTRACT: We describe a simple method for measuring sodium azide concentrations in aliquots of blood and other tissues. Aliquots are acidified, converting azide to volatile hydrazoic acid (HN_3) which is then trapped in sodium hydroxide. We analyze the resulting aliquots by ion chromatography, using a sodium tetraborate eluent and suppressed conductivity detection. The method is sensitive to at least 100 ng/mL.

KEYWORDS: forensic science, azide determination in blood, ion chromatography of azide, hydrazoic acid, air bags

Not too many years ago, a few kilograms of sodium azide per year sufficed to meet the world demand. Then it was used primarily in microgram quantities as a bactericide in laboratory reagents. Now, however, with the advent of the automobile "air" bag, hundreds of metric tons are required. Sodium azide is detonated to provide the nitrogen which inflates the bags. One metric ton will outfit approximately 15,000 new cars with one air bag each. As multiple bags are being installed in newer models, even more sodium azide will need to be manufactured annually. Furthermore, it is a rapidly acting, highly toxic compound with a poorly understood mechanism of action (1). Indeed, its i.p. LD_{50} in mice is 0.57 mmol/kg (2), only five times greater than that for the notoriously deadly cyanide.

While all too little is known about azide's toxic mechanism or how to treat azide poisoning, the increased presence of this health hazard in our environment has at least spawned a number of analytical methods. A well-known spectrophotometric determination exploits the reaction of azide with ceric ammonium nitrate (3,4). After diffusion from an acidified solution, hydrazoic acid (HN_3) is trapped in a solution of ceric ammonium nitrate with which it participates in a redox reaction. This reaction results in a decrease in the absorbance of ceric ammonium nitrate at 390 nm which is proportional to the concentration of azide. In our earlier work on azide toxicity, we measured azide levels in mouse blood by a modification of this method (5). Unfortunately it is not sufficiently sensitive to detect concentrations in the range of 1 μg/mL or less. Decolorization of standard solutions of bilirubin has also been used to determine azide (6).

Another widely-used method involves reacting the azide-containing sample to form the 3,5-dinitrobenzoyl derivative and then determining its concentration by HPLC (7). This method was developed by an automobile manufacturer to be used in conjunction with the testing of air bags. It was designed to analyze primarily aqueous solutions of azide, and has a detection limit of the order of 10 ng/mL. There have been a number of modifications of this method aimed at determining azide in the presence of proteins (8,9) all of which have drawbacks. Of special interest was the measurement of the azide content of various tissue samples (blood, liver, kidney, etc.) from a suicide victim, but the concentrations, as anticipated, were in the range of several hundred μg/mL (10). A highly sensitive HPLC method (to 10 ng/mL) for measuring aqueous samples directly has been reported recently (11).

We describe a simple, relatively quick and inexpensive method for determining azide concentration in blood, and other tissues as well, using ion chromatography. The detection limit is about 30 ng/mL.

Materials and Methods

Sigma Chemical Co. supplied sodium azide while Fisher Scientific supplied sodium tetraborate (ACS certified) and sodium hydroxide. Routine hematological samples obtained from our institution's associated hospital were pooled for whole blood experiments or pooled, washed, and resuspended in Krebs-Ringer solution for red-blood cell studies. We used plasma separated in the latter procedure in one series of experiments. Deionized water at 18 MΩ-cm resistivity was used in all dilutions to eliminate or minimize overlapping peaks in the chromatography.

Our procedure for determining azide in blood consists of two parts: extracting azide from the blood sample and then analyzing the extract by means of ion chromatography. The extraction procedure is a modification of the method of Bruce et al. (12) for the determination of cyanide, except that the volatility of hydrazoic acid is exploited rather than that of hydrocyanic acid. The azide in the sample is acidified to HN_3, a gas, which is then blown over and trapped in dilute NaOH. The aeration apparatus consists of three tubes: a gas scrubber containing NaOH; an aeration tube containing trichloroacetic acid and a bubbler, into which the blood

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sample is injected; and a collection tube containing NaOH. The apparatus is connected to the house vacuum, drawing air through the scrubber and into the bubbler and finally carrying HN_3 over to the collector.

There are several parameters in this method which can be varied to achieve optimum results in a particular situation: volume of acid in aeration tube, volume of sample, volume of NaOH in collector, flow rate of bubbling air, and time of aeration. After considerable experimentation, we chose to inject one mL of sample into 5 mL of 20% (w/v) trichloroacetic acid (which did not change the pH) and collect in 1 mL 0.1 mol/L NaOH. The flow meter was set at 0.4 L/min and the aeration proceeded for 30 min, a practical upper limit. Numerous other variations will probably work equally well.

We ran aliquots of the samples thus extracted isocratically through a Dionex™ DX-100 ion chromatograph, an integrated single-channel instrument, using a 12.7 mmol/L sodium tetraborate eluent and suppressed conductivity detection. The Dionex IonPac® AS4A-SC anion exchange column, 4 mm in diameter and 250 mm long, was preceded by its associated guard column, the AG4A-SC. The AS4A-SC column, and comparable ones from other manufacturers, are widely employed in analyses following EPA Method 300. Highly-conductive eluents, such as are commonly used in ion chromatography, provide good separation of the analytes of interest but at the price of high background conductivity at the detector. To suppress this background, water is electrolyzed to hydronium and hydroxide ions which, by passing through or being excluded by appropriate exchange membranes, serve to convert the eluent to its poorly-conductive weak acid form while enhancing the conductivity of the analytes. In order to optimize the sensitivity, we found it necessary to use the external water mode with the suppressor rather than the built-in eluent recycling which had insufficient capacity. Our method is a modification of that described by Annable and Sly (8). We acquired the data and analyzed it with the MacIntegrator hardware and software package (Rainin Instrument Co., Inc.) connected to a MacIntosh computer.

Results

We routinely work at the 1 μg/mL level and, with care, can quantitate at 100 ng/mL. Figure 1 is a sample chromatogram of azide at 100 ng/mL, in which the azide peak is clear and quantifiable. This is the limit of detection of the method at present, although peaks corresponding to 30 ng/mL can be observed but not easily quantified. Figure 2 shows a calibration curve for azide concentrations ranging from 0.1 μg/mL (100 ng/mL) to 10 μg/mL. As indicated, the samples were azide-doped, pooled whole blood, and doped washed red blood cells resuspended in Krebs-Ringer solution to whole blood concentrations of heme (7–8 mmol/L). The two sets of data were subjected to a paired t-test and found not to differ significantly ($p < 0.05$); thus the single line.

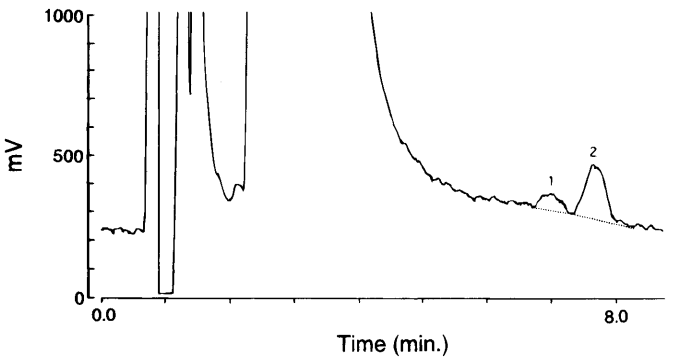

FIG. 1—*Chromatogram of sodium azide at 100 ng/mL from spiked whole blood sample. Peak 1: azide, area = 1.16 × 10⁶ μV-sec; Peak 2: nitrate impurity in 18 MΩ-cm water. eluent: 12.7 mmol/L sodium tetraborate, flow Rate: 2.0 mL/min, injection volume: 25 μL, and detector: Suppressed conductivity.*

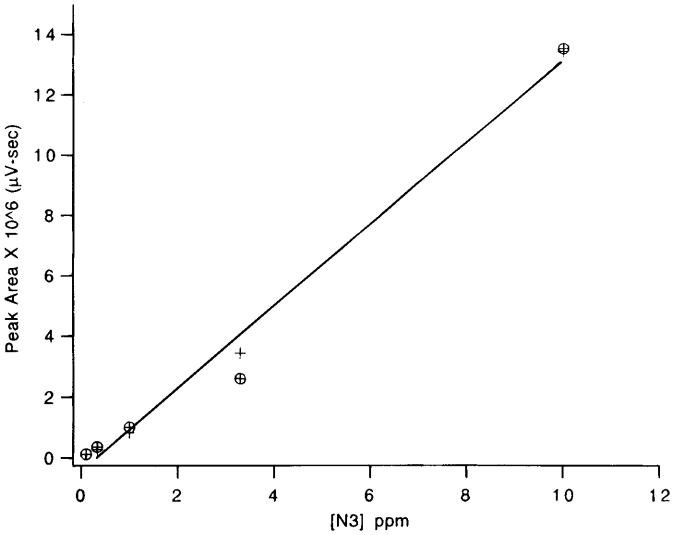

FIG. 2—*Calibration for sodium azide from spiked whole blood (+) and washed, resuspended red cell (○) samples. The equation for the line is: $A = 1.366\ [N_3] - 0.4413$, with $R^2 = 0.994$. $[N_3]$ expressed in ppm or μg/mL.*

As reported by Annable and Sly (8), azide is not resolved from bromide and nitrate if one uses a standard carbonate-bicarbonate eluent in anion-exchange chromatography, but it is cleanly separated from these interfering anions if a tetraborate eluent of appropriate concentration is used, a finding which we have verified. Other potential interferences, such as fluoride, formate, and nitrite, elute earlier, while doubly-charged anions, such as phosphate, oxalate, and sulfate, elute later (13). Because of their hydrophobicity, anions like iodide and cyanide are not retained by the Dionex AS4A-SC column or comparable ones from other manufacturers.

We tested for azide recovery by injecting standard samples of azide dissolved in blood into the aeration apparatus (two at each concentration), collecting the blown-over HN_3 in NaOH, and then running these in the chromatograph. These were compared with standards dissolved in NaOH and injected directly into the instrument. Table 1 shows the percent recovery. While the recovery may appear somewhat low, it is consistent and yields a linear calibration. We do recommend, however, that a standard in the appropriate concentration regime be run with each batch of unknowns.

To test for reproducibility, we ran three aliquots of the same azide-doped whole blood sample through the aeration apparatus and then injected each resulting aliquot in the chromatograph. We

TABLE 1—*Percent recovery.*

Concentration (μg/mL)	Recovery (%)
0.1	84
0.33	83
1.0	84
10.0	85

TABLE 2—*Disappearance of azide in whole blood with time.*

Time (h)	Peak Area* (T = 20°C)	Peak Area* (T = 0°C)
0	9.570	8.696
4	9.000	8.528
8	8.540	8.194
22	8.190	
24		7.696
144		5.760
192		5.511

*Units of 10^6 μV-sec.

obtained eleven replicate values with a mean of 6.62 μg/mL, a standard deviation of 0.118 (1.8%), and a standard error of 0.035 (0.5%).

One important difficulty with any method of determining azide concentrations in blood is that it is metabolized by red blood cells, both in the intact animal and *in vitro.* We found it imperative to process doped blood immediately upon addition of the azide or to quick-freeze it and store it at −70°C. Samples taken from animals or humans require similar treatment. We studied the decrease in azide concentration in whole blood with time; data appear in Table 2. These data were fitted by a computer program (Cricket Graph) to a declining exponential curve. At room temperature (20°C), we obtained the equation $A = 9.305 \times 10^{-0.00279t}$; at 0°C, $A = 8.439 \times 10^{-0.001038t}$. The room temperature half-life was 108 hours ($4^1/_2$ days). When the blood was kept at 0°C, the half-life was extended to 290 hours, about 12 days. Even when spiked blood samples were shipped to us with appropriate handling (overnight in dry ice), we observed some biotransformation by detecting nitric oxide hemoglobin in the specimens (14).

Discussion

Numerous physicochemical methods have been applied to the determination of azide in various matrices, but only reversed phase liquid or ion-exchange chromatography offer interference-free approaches to its quantification in solutions containing proteins (8). The modification of the method of Annable and Sly (8), which we used here, was employed in conjunction with an isolation/purification procedure that separates azide not only from proteins, but from nonvolatile organic and inorganic constituents of biological tissues as well (12). This cleanup method was originally devised for cyanide, but we encountered no evidence for azide conversion to cyanide in shed blood (10). Instead, azide was clearly converted to nitric oxide in blood (14).

We are the first to report the half-lives for azide in blood at various temperatures. We have also applied the method to preliminary studies analyzing homogenized organ tissues, as well as blood, from poisoned mice at various times after injection, and the results are encouraging.

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