The Rate of Bromate Decomposition in the Human Stomach

Final Report (Phase I)

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Summary of Key Phase I Findings

The overall purpose of the research program is to develop a quantitative understanding of the fate of bromate (and other reactive substances)in the stomach under most relevant typical human physiological conditions and exposure conditions, to aid in the estimation of the systemic uptake of bromate from drinking water. These data can be used to refine the current quantitative risk extrapolation models for bromate.

Phase I studied reactions of bromate with hydrogen chloride, hydrogen sulfide, and organothiols (RSH) under acidic pH conditions as could be found in the stomach. The analytical technique utilized was Ion Chromatography which was shown to be capable of quantifying bromate in acid solutions in the range of 1 ~~u~~µg/L. Initial laboratory studies were in HCl solution at gastric juice concentrations wherein the chloride concentrations would be more than 10^6 times the bromate challenge levels of interest, as interferences between chloride and bromate made it more difficult studies in the 10 ug/L range. Subsequently,major experimental modifications resulted in a procedure that allowed studies of bromate reactions at 200 ug/L in the HCl medium with excellent accuracy and precision. It was concluded that 200 ug/L of bromate was a sufficiently low concentration to challenge the hypothesis that bromate could be reactive at low concentrations under gastric conditions, and it was therefore used as the screening level for the subsequent experimental studies in Phase I. (ICP/Mass spectrometric techniques are now available that would be able to detect and quantify levels of bromate (and bromide) in the presence of very high chloride and such an instrument has been purchased and it will be used in subsequent studies allowing measurement at ppb levels.)

Phase I demonstrated that chloride can react with bromate by acid sensitive processes under conditions similar to the human stomach and that chloride is oxidized and bromate is chemically reduced.

The net chemical reaction in the absence of additional bromide is essentially as follows:

$$BrO_3^- + 5\,Cl^- + 6\,H^+ = BrCl + 2Cl_2 + 3\,H_2O$$

This reaction is indicated to be in equilibrium that would be shifted far to the left and the slow step is likely the formation of the halogen species which should react instantaneously as formed in the presence of oxidizable material.

The experimental rate law was determined to be:

$$-d[BrO_3^-]/dt = k\,[BrO_3^-]\,[H^+]^2\,[Cl^-]^{1.5}$$

This representation indicates that it is a complex probably multistep reaction that is highly sensitive to the hydrogen ion concentration, since the rate of decomposition is proportional to the square of the hydrogen ion concentration. Since there is approximately 10^6 molar excess of chloride versus bromate and the reaction is greatly affected by hydrogen ion, it is likely that the reaction may involve the transient formation of ~~a~~ of protonated $HBrO_3$ perhaps in an activated complex that includes hydrogen ion, bromate, and chloride . This is indicated because the rate of reaction usually drops rapidly below approximately 0.1 moles/liter of hydrogen ion.

pH	H^+	Cl^-	BrO_3^- % Reduction	$t_{1/2}$ minutes
0.8	0.17	0.17	12	153
1	0.10	0.17	5	454

Above conditions: Initial bromate concentration was 200 ug/L, at 37 degrees C. This was a static simulation that did not take into consideration that gastric juice would be continually secreted into the stomach during the contact time period, and that protein amine groups probably buffer the acidity in actual gastric juice.

Under biologically relevant conditions the rate of **this** reaction is slow given that the likely retention time halflife in the stomach of water that was ingested in the absence of food might be in the range of 15-30 minutes so only a small amount of bromate would react in that time frame by this simulation.

However, actual gastric juice is in a chemically reduced state and in addition to HCl and water it contains numerous proteins and potentially other reducing agents such as hydrogen sulfide and ascorbic acid. We were able to analyze with some difficulty a sample of human gastric juice for sulfide and demonstrated that it was present at a concentration of ~8.5×10^{-5} molar (between 34 and 340 ug/L) in that single sample. We have found no indication that this quantitative analysis of gastric juice had been conducted prior to this study. When sulfide was added to the simulated gastric juice under similar conditions we demonstrated a significantly increased rate of bromate reduction, as below, except that these bromate reductions occurred in only 15 minutes rather than 30 minutes of contact at 37 degrees C.

pH	H^+	Cl^-	% Reduction BrO_3^-		$t_{1/2}$ minutes	
			H_2S @ 10^{-4}M	10^{-5}M	H_2S @ 10^{-4} M	10^{-5} M
0.8	0.17	0.17	97	57	2	14
1	0.10	0.17	93	21	15	43

Thus, because it is a much stronger reducing agent than chloride, but still acid sensitive, the hydrogen sulfide could be reacting directly with bromic acid or possibly with the bromine chloride or chlorine that are generated as follows. We suspect the former mechanism.

$$3\ BrO_3^- + H_2S \quad (+ HCl/H_2O) \rightarrow 3\ Br^- + 2\ SO_4^{-2} + H_2O$$

$$BrCl + Cl_2 + 2\ H_2S \rightarrow 2\ S + 3\ HCl + HBr$$

Combining the reactions of bromate with HCl and H_2S the rate law can be rewritten in more specific mechanistic terms and expanded to:

$$-d[BrO_3^-]/dt = k_1[BrO_3^-][H^+]^2[Cl^-] + k_2[BrO_3^-][H^+]^2[Cl^-]^2 + k_3[BrO_3^-][H_2S][H^+]^n$$

We also simulated gastric juice supplemented with cysteine, an amino acid and/or glutathione (glutamylcysteineglycine) as protein surrogates, both of which contain organic thiol (SH), as well as amine groups that would buffer the acidity, and we found increased rates of conversion of bromate relative to HCl alone, but less than the hydrogen sulfide effect.

Thus, we have demonstrated that bromate and chloride react slowly under strongly acid conditions in simulated gastric juice. We also measured sulfide in actual gastric juice and found that small amounts of sulfide reacted rapidly with bromate in simulated gastric juice at physiologically relevant rates under acidic conditions. We also demonstrated that organic thiol amino acid type compounds will also reduce bromate under acidic conditions although slower than sulfide.

Introduction

It is known that under acidic conditions, the hydrochloric acid in gastric juice will react with chloride ion and bromide ion (Schulek, Burger, and Laszlovszky, 1960):

$$2\ BrO_3^- + 10\ Cl^- + 12\ H^+ = Br_2 + 5\ Cl_2 + 6\ H_2O \qquad [1]$$

In the absence of significant additional bromide ion, the more likely reaction is:

$$BrO_3^- + 5\ Cl^- + 6\ H^+ = BrCl + 2\ Cl_2 + 3\ H_2O \qquad [2]$$

It should be noted that these reactions are reversible, however, the products Cl_2, Br_2, and BrCl are reactive oxidizing and halogenating agents.

It is also known that in gastric juice there are inorganic ionic species such as Cl^- and Br^- and organic sulfhydryls such as cysteine and glutathione, which will also react with bromate ion (Schulek, Burger, and Laszlovszky, 1960, Tanaka et al., 1984).

Given the known (published) bromate ion chemistry and the composition of gastric juice, bromate ion should be reduced in gastric juice (Jones, Baeckstrom, 1934, Schulek, Burger, and Laszlovszky, 1960). The actual rate of reduction will be a function of the pH, reactive species and retention time in the stomach-. As a result there should be less bromate ion reaching the target cell than was originally ingested. This reduction of bromate ion would cause the risk to the target cell and ultimately the MCL to be overestimated.

In this report, the ion chromatography (IC) system, methods used to measure bromate ion in high chloride solutions, as well as our preliminary results from our bromate ion reductions in synthetic and real gastric juices, blood, and blood serum are described.

Materials and Methods

Reagents

All solutions and standards were prepared using reagent grade chemicals dissolved in deionized, distilled water (DDW). All solutions were freshly made daily or refrigerated at 4°C for a maximum of 21 days.

Ion Chromatography Reagents

The 9.0 mM carbonate eluent used in IC analyte separation was prepared by dissolving 1.91 g of sodium carbonate in 2.00 L of DDW. The stock ammonium molybdate solution (2.0 mM) was prepared by dissolving 0.247 g of ammonium molybdate in 100.00 mL of DDW. The post column reagent (PCR) was prepared by dissolving 8.62 g of potassium iodide (0.26 M) in about 500 mL of DDW, adding 43.0 µL of the stock ammonium molybdate solution (43 µM) and diluting to 1.00 L. The sulfuric acid solutions used to regenerate the suppressors were made by diluting 28.0 and 8.5 mL of concentrated H_2SO_4 (0.50 N and 0.15 N, respectively) each in 2.00 L of DDW. Both the PCR and carbonate eluent were degassed with nitrogen prior to use.

Bromate Ion Standards

The BrO_3^- standards were prepared by dissolving 0.131 g of potassium bromate in 100 mL of DDW to a final concentration of 1000.0 mg/L BrO_3^-. A 1.00 mL aliquot was removed and diluted to 100.00 mL in DDW to a final concentration of 10.0 mg/L. Bromate ion standards of 0, 2, 5, 10, 15, 50, 100, 150, 200 µg/L were prepared by adding exactly 0, 5, 12.5, 25, 37.5, 125, 250, 375, and 500 µL, respectively, of 10.0 mg/L BrO_3^-

stock solution and diluting to 25.00 mL in the appropriate matrix. Bromate ion standards of exactly 0, 2, 5, 10, 15, 50, 100, 150, 200 μg/L were also prepared by adding exactly 0, 20, 50, 100, 150, 500, 1000, 1500, and 2000 μL, respectively, of 10.0 mg/L BrO_3^- stock solution and diluting to 100.00 mL in the appropriate matrix.

Chloride Ion Standards

The Cl^- standards were prepared by making a 10,000.0 mg/L stock solution that was prepared by adding 1.648 g of NaCl to 100 mL of DDW. A second stock solution of 10.0 mg/L was prepared by diluting 100.0 μL of 10,000.0 mg/L stock solution in exactly 100 mL of DDW. The Cl^- standards of 1, 100, 250, 500, 750, and 1000 mg/L were prepared from the 10,000.0 mg/L stock solution by adding exactly 0.1, 1, 2.5, 5, 7.5, and 10 mL, respectively, and diluting in 100.00 mL of DDW. The Cl^- standards of exactly 0.005 and 0.05 mg/L were prepared from the 10.0 mg/L stock solution by adding 0.05 and 0.5 mL, respectively, and diluting in 100.00 mL with DDW.

Other Standards

The SO_4^{2-}, NO_3^-, and ClO_4^- standards were prepared by making a 10,000.0 mg/L stock solutions that were prepared by adding 1.371 g $NaNO_3$, 1.479 g Na_2SO_4, and 1.60 mL of 6.293 M $NaClO_4$ to exactly 100 mL of DDW. A second stock solution of 10.0 mg/L was prepared by diluting 100.0 μL of 10,000.0 mg/L stock solution in 100.00 mL of DDW. The SO_4^{2-}, NO_3^-, and ClO_4^- standards of exactly 1, 100,1000 mg/L were prepared by adding exactly 0.1, 1, and 10 mL, respectively, of 10,000.0 mg/L stock solution and diluting in 100.00 mL of DDW. The SO_4^{2-}, NO_3^-, and ClO_4^- standards of exactly 0.05 mg/L were prepared by adding exactly 0.5 mL of 10.0 mg/L stock solution and diluting in 100.00 mL with DDW.

HCl Solutions

The HCl solutions of 0.17 M and 0.10 M were prepared by adding 10.45 and 6.15 mL, respectively, of 4.066 M HCl and diluting to 250.00 mL with DDW. For the experiments that required a total, constant 0.17 M Cl^- concentration, 1.022 g of NaCl was added to the HCl solution and diluted to 250.00 mL with DDW.

Reducing Agents

In subsequent experiments, other potential reducing agents such as H_2S, ascorbic acid, glutathione, and cysteine were also added to the 0.17 M HCl or 0.10 M HCl/total 0.17 M Cl^- solutions. Concentrations of approximately 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} H_2S were made by preparing a saturated (~ 0.1 M) stock solution of H_2S that was prepared by adding approximately 1.20 g Na_2S, 3.10 mL of 4.066 M HCl, and diluting to 25.00

mL with DDW. It was assumed that due to the volatility of H_2S, significant amounts would be lost initially. In order to compensate for the loss, the amount of H_2S was doubled before dilutions were made. The 10^{-4} M H_2S was prepared by diluting exactly 0.5 mL of stock H_2S in 250.00 mL of the appropriate HCl solution. The 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M H_2S solutions were prepared by diluting 200.0 μL of stock H_2S in 100.00 mL DDW, and then aliquots of exactly 50, 5, 0.5, and 0.05 mL, respectively, were diluted to 250.00 mL in the appropriate HCl solution.

Solutions of 2.0 mg/L glutathione and/or cysteine were prepared by making a stock solution of 20.0 mg/L glutathione and/or cysteine, which was prepared by adding 0.005 g of glutathione and/or cysteine to 250.00 mL of DDW. A 2 mg/L solution was prepared by diluting 25.00 mL of stock solution in 250.00 mL of the appropriate HCl solution.

A solution of 5.0×10^{-5} M ascorbic acid was prepared by making a stock solution of 5.0×10^{-3} M ascorbic acid, which was prepared by adding 0.220 g of ascorbic acid to 250.00 mL of DDW. The 5.0×10^{-5} M solution was prepared by diluting 2.50 mL of stock solution in 250.00 mL of the appropriate HCl solution.

Real Gastric Juice

The real gastric juice, which was received from Bernard Bouscarel, required centrifugation in order to remove larger particulates before it could be run in the IC. The sample was centrifuged at 6000 RPM for 5 minutes, which left a layer of clear solution on the top and a layer of larger particulate matter on the bottom. The clear sample solution was transferred and refrigerated for later use.

A sample of gastric juice was spiked with HCl to a final concentration of 0.05 M (pH 1.30). The pH of the real gastric juice was 1.70 and 42.0 μL of 4.066 M HCl was added to give a final pH of 1.31.

Whole Blood and Blood Serum

The whole blood that was received, required clean-up before it could be run in the IC. Bromate ion was added to the whole blood and was mixed in a ratio of 1:2:2 for whole blood, ethanol and water, respectively, and centrifuged at 20,000 RPM for 10 minutes. This centrifugation gave a lighter red solution and a solid at the bottom. The lighter red solution was poured into an IC vial and was measured. Approximately 20 mL of water was added to the solid from the centrifugation and shaken, which was then poured into an IC vial and measured.

Two separate runs were done, because it was possible that the BrO_3^- could be in the solid or lighter red solution, or it could be in both.

The blood serum that was received was already "cleaned up" at Hoxworth Blood Center in Cincinnati, Ohio. There was no further preparation of the blood serum before it was measured in the IC.

Hydrogen Sulfide Determination

A stock sulfide solution was prepared by dissolving approximately 10.0 grams of Na_2S in 25.00 mL of deaerated DDW and allowed to stand overnight. A sulfide anti-oxidant buffer (SAOB) was prepared by adding 110.0 mL of concentrated NaOH, 67.00 g of disodium EDTA, and 35.00 g of ascorbic acid and diluted to 1.00 L with deaerated DDW. A stock sulfide/SAOB solution was prepared by adding 5.00 mL of stock sulfide solution, 250.00 mL SAOB, and diluted to 500.00 mL with deaerated DDW. An iodine solution to standardize the sulfide/SAOB stock solution was prepared by adding 1.000 g KI, 10.00 mL of 7.946×10^{-3} M KIO_3, and 1.00 mL of concentrated H_2SO_4 and diluting to 100.00 mL with deaerated DDW.

Instrumentation

A Dionex ion chromatograph model # ICS-2500 instrument was used for the measurement of bromate ion. The system is modified specifically for the detection of bromate ion; therefore only a post column reagent (PCR) is employed in the system. A conductivity detector is not present in the system, as shown in Figure 2 (Wagner et al., 2002).

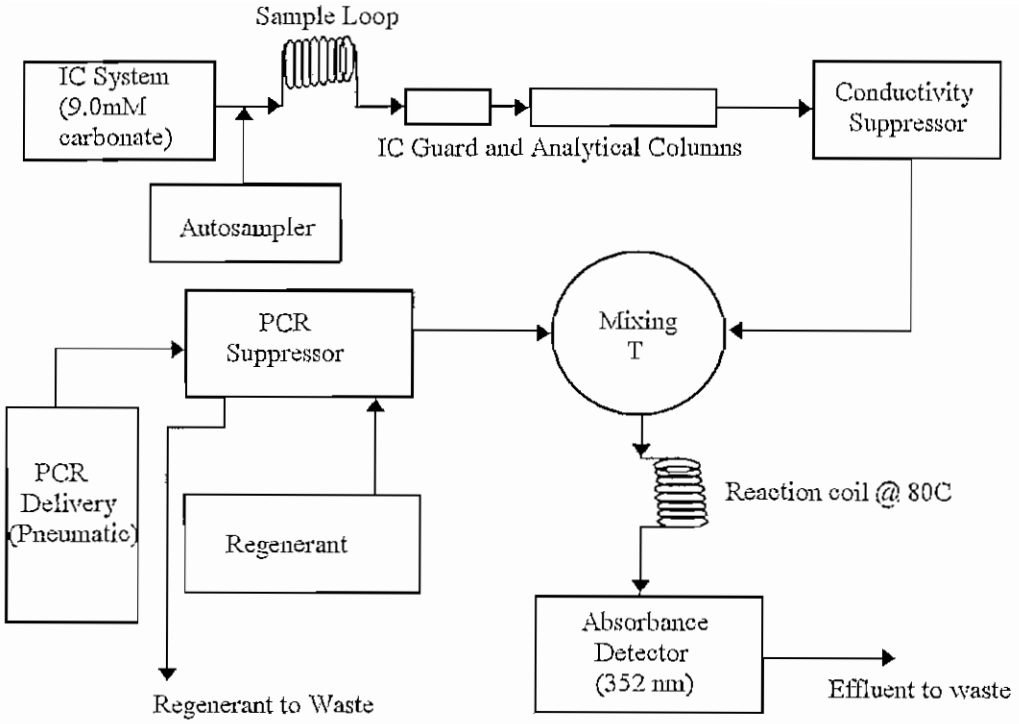


Figure 2. Schematic of an IC-PCR system

A sample is introduced into the carbonate eluent flow stream by the autosampler (AS40) and 225 µL sample loop at a flow rate of 1.3 mL/min. Larger particles in the sample are removed on the guard column (AG9-HC) and separation of the sample into various components occurs in the analytical column (AS9-HC). The sample is acidified by one of the suppressors, ASRS-ULTRA (Wagner et al., 2002).

The post column reagent is delivered into the system pneumatically and is also acidified by another suppressor (ASRS-ULTRA). Both the acidified PCR and sample converge at the mixing T and mix thoroughly in the reaction coil, which is heated to 80°C. The mixed sample and PCR form a yellow color that is detected by a UV-Visible detector at a wavelength of 352 nanometers (Wagner et al., 2002). The experimental parameters for the experiment were determined by using EPA Method 326.0 Revision 1.0. Data acquisition and analysis of the sample was accomplished using The Dionex Chromeleon Chromatography Management System Version 6.5.

Procedures

IC System Initial Capabilities

Initial accuracy and precision of our IC system were determined for our system. Seven replicate samples of pristine water were fortified with 2.0 µg/L BrO_3^- and measured. The precision (percent relative standard deviation) of the seven samples was determined using the equation:

$$\% \text{ RSD} = [\text{sqrt}\{[(x_1-x_{avg})^2 + (x_2- x_{avg})^2 + (x_3- x_{avg})^2 + \ldots + (x_7- x_{avg})^2]/n-1\}] \text{ X } 100 \qquad [3]$$

where x corresponds to the BrO_3^- measured in each sample, n is the number of samples, and x_{avg} is the average value of the samples. The accuracy of the seven samples was determined using the equation:

$$\text{Accuracy} = [\sum (i = 1\ldots7)\ x_{avg} - x_i / x_i\ ] \text{ X } 100 \qquad [4]$$

where $\sum$ is the summation of the errors of each sample, x_{avg} is the average value of the seven samples, and x_i is the actual amount of BrO_3^- in each sample. The average percent recovery of a 2.0 µg/L BrO_3^- sample in pristine water was determined by measuring seven replicate samples. The equation used to determine the percent recovery is:

$$\% \text{ Recovered} = [(A - B)/ C] * 100 \qquad [5]$$

where A is the measured concentration in the fortified sample, B is the measured in the unfortified sample, and C is the fortification concentration (Wagner et al., 2002).

The Method Detection Limit (MDL) was determined by measuring seven replicate samples of pristine water that were fortified with 2.0 µg/L BrO_3^- on three separate days. The MDL was determined using the equation:

$$\text{MDL} = \text{St}(n - 1,\ 1 - \text{alpha} = 0.99) \qquad [6]$$

where St(n – 1, 1 – alpha = 0.99) is the student's t value for the 99% confidence level with n – 1 degrees of freedom, n is the number of replicates, and S is the standard deviation of replicates (Wagner et al., 2002). The minimum reporting level (MRL) was determined by the equation:

$$MRL = MDL * 3 \quad [7]$$

where MDL is the method detection limit (Wagner et al., 2002).

A calibration curve of BrO_3^- standards in pristine water was developed. The BrO_3^- standards run were 0, 0.5, 0.7, 0.9, 1.0, 1.1, 1.3, 1.5, 2.0, 5.0, 10.0, 15.0, 50.0, and 100.0 µg/L which were prepared directly by pipetting appropriate amounts of the stock solution into a 100 mL volumetric flask and diluting to exactly 100 mL with DDW.

Chloride Interference

The effect of Cl^- (found in gastric juice) on the BrO_3^- peak shape was measured by comparing the BrO_3^- peak shape in pristine water with water fortified with varying amounts of Cl^-. Bromate ion standards from 0 – 100 µg/L in pristine water were fortified with Cl^- standards from 0 – 1000 mg/L and measured on the IC. It was found that the BrO_3^- peak shape was affected by Cl^-.

Reduction of the Cl^- interference was measured by diluting the sample prior to measurement. Dilution of the sample by factors of 2, 5, and 10 resulted in improvement of the BrO_3^- peak shape. Similar results were noted with other inorganic anions such as SO_4^{2-}, NO_3^-, and ClO_4^-.

Methods Used to Measure Bromate Ion in HCl

Two complementary methods were developed for measuring bromate ion in presence of HCl. The development of both methods requires that a calibration curve be constructed for each matrix or set of operating conditions. Before BrO_3^- reduction can be measured in a specific matrix, a BrO_3^- calibration curve must be developed in the same matrix. Each calibration standard was made immediately before it was to run in the IC, because bromate ion reduction was also occurring simultaneously. After a calibration curve was established, BrO_3^- reduction could be measured quantitatively. Bromate ion reduction was run in the same matrix, except the matrix was at 37°C, as compared to room temperature for the calibration standards. Reduction of bromate ion was measured at time 0, 15, 30, 60, 90, 120, 180, and 240 minutes or until a 50 % reduction of BrO_3^- had

occurred. The same techniques were used for the comparison method, except that before the standards and sample were run in the IC, the standards and sample were diluted in DDW by a factor of five. A comparison of the error in BrO_3^- measurement of less than 4 % ensured us that both methods were working properly.

Method Used to Measure Bromate Ion Reduction

Both methods used to measure BrO_3^- in HCl worked well. Therefore, BrO_3^- reduction was measured using the method that did not require dilution. Before BrO_3^- reduction reactions were to take place, the pH of the matrix was measured after it had been prepared to confirm that the pH was in the specified range. Calibration standards of exactly 0, 2, 5, 10, 15, 50, 100, 150, and 200 μg/L BrO_3^- were prepared at room temperature and measured immediately in the IC. The pH of the 200 ug/L BrO_3^- standard was again tested to confirm that the pH was in the specified range. After the calibration standards were measured, three check standards of 2 (or 5), 15, and 200 μg/L BrO_3^- were run to confirm that the IC was performing properly.

A 25.00 mL sample of matrix was brought to 37°C in a water bath in order to mimic human body temperature. An aliquot of matrix was removed and 500.0 μL of 10.0 mg/L BrO_3^- stock solution was added to the matrix and filled to 25.00 mL. The sample was shaken and a timer was started for the experiment. The change in temperature was less than 1°C. A sample was immediately run on the IC. The sample was placed back in the water bath and reduction was measured at time: 0, 15, 30, 60, 90, 120, 180, and 240 minutes or until a 50 % reduction of BrO_3^- had occurred. In between the measurements starting after 30 minutes, a check standard was run and a difference of less than 3% between the two measurements confirmed that the IC was running properly. Integration of the standards and samples was done and data was plotted in Microsoft Excel.

This procedure was used in all synthetic gastric juice matrices including: HCl, HCl/total 0.17 M Cl^-, HCl/glutathione, HCl/total 0.17 M Cl^-/glutathione, HCl/cysteine, HCl/total 0.17 M Cl^-/cysteine, HCl/glutathione/cysteine, HCl/total 0.17 M Cl^-/glutathione/cysteine, HCl/H_2S, HCl/total 0.17 M Cl^-/H_2S, HCl/glutathione/cysteine/H_2S, and HCl/total 0.17 M Cl^-/glutathione/cysteine/H_2S. This procedure was also used in measuring BrO_3^- reduction in blood, blood serum, real gastric juice, and real gastric juice spiked with HCl.

Measurement of Hydrogen Sulfide in Gastric Juice

Approximately 0.10 M sodium thiosulfate was standardized to determine its concentration. An iodine solution of 0.500 g KI, 500.0 μL concentrated H_2SO_4, and 5.00 mL of 7.946×10^{-3} M KIO_3 were titrated. The following equations were used to determine the $S_2O_3^{2-}$ concentration:

$$6H^+ + IO_3^- + 5I^- \rightarrow 3I_2 + 3H_2O \quad [8]$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-} \quad [9]$$

The first equation was used to determine the number of moles IO_3^- used in the reaction, as well as the number of moles of I_2 formed in the reaction. The moles of I_2 formed in the first reaction reacted with the $S_2O_3^{2-}$ in a 1:2 molar ratio of I_2 to $S_2O_3^-$. The sulfide/SAOB stock solution was standardized appropriate to the equation:

$$I_2 + S^{2-} \rightarrow S\ (s) + 2I^- \quad [10]$$

Standards of 1.126×10^{-1} to 1.126×10^{-7} were prepared by dilution from the stock sulfide/SAOB solution. A calibration curve was constructed by measuring the Standard Potential with a sulfide ion selective electrode (Orion Research Incorporated). A 1.00 mL gastric juice sample was mixed with 25.0 mL of SAOB and diluted to 50.00 mL with deaerated DDW and was measured using the ion selective electrode. The concentration of S^{2-} was found to be 7.72×10^{-5} M. A method of standard additions using the 1.126×10^{-1} standard was also used. Nine additions for a total of 590.0 μL of 1.126×10^{-1} standard were added to the 50.00 mL of gastric juice, SAOB, DDW sample and a Microsoft Excel plot of log of standard added vs. mV gave a y-intercept that was substituted into the equation from the plot of calibration standards to give the concentration of S^{2-}. The concentration of S^{2-} was found to be 9.15×10^{-5} M. The average of the two methods is approximately 8.4×10^{-5} M (± 10 %).

Results and Discussion

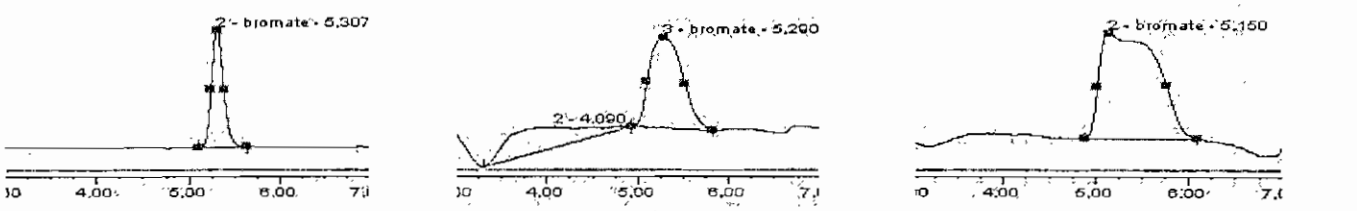
Initial Experiments

Initial experiments were designed to determine the method detection limit (MDL), minimum reporting level (MRL), average percent recovery, accuracy, and precision. These initial values were determined by using

US EPA Method 326.0. The accuracy and relative standard deviation (precision) of our system are 4.5 and 6.8 %, respectively, which is well within the accuracy and precision of 15 and 20 % required by the US EPA. The average percent recovery of our system is 103.2 %, which again is within the US EPA range of 75 – 125 %. The MDL was determined to be 0.32 µg/L, with the MRL being approximately 1.0 µg/L. The results from these experiments show that the IC's detection limit, accuracy, and precision are well within the EPA requirements (Wagner et al., 2002).

Bromate Ion Measurement in High Chloride

The literature indicates that gastric juice contains approximately 170 mM chloride ion, which corresponds to approximately 6 g/L (Hollander, 1934). This data was used to develop experiments to measure the effect of varying chloride ion concentration on the shape of the bromate ion peak. Initial measurements showed that solutions with greater than 100 mg/L chloride ion caused a broadening of the bromate ion peak (see Figure 3).



10 µg/L BrO_3^- with 0 mg/L Cl^- 10 µg/L BrO_3^- with 500 mg/L Cl^- 10 µg/L BrO_3^-- with 1000 mg/L Cl^-

Figure 3. Effect of bromate ion peak broadening due to chloride ion

The broad bromate ion peaks caused by chloride ion makes it difficult to measure the bromate ion peak accurately and precisely as compared to pristine water.

Resolution of the broad bromate ion peak was needed before it was possible to measure bromate ion in synthetic gastric juice. Many different techniques were evaluated in order to improve the shape of the bromate ion peak. These included varying the concentration of eluent, diluting the sample prior to measurement, and substituting other inorganic ions for Cl^-. It was found that the best way to measure the reduction of bromate ion in synthetic gastric juice was to develop a calibration curve specific to the operating conditions. This approach did not resolve peak broadening, but both accurate and precise bromate ion measurements with an error of less than 4 % could be made.

Methods Used to Measure Bromate Ion in HCl

The two complementary methods developed for measuring bromate ion in presence of HCl use a calibration curve that is developed for each matrix or operating condition. A comparison of the undiluted method and the method that requires a dilution by a factor of five resulted in an average error of 1.3 percent. This value tells us that both sets of data are statistically indistinguishable, as shown in Table 1.

Table 1
Data and Calculations from Bromate Ion Measurement Using Diluted and Non-diluted Methods

Time (min)		Non-diluted (µg/L)		Diluted (µg/L)		% Difference
0		203		202		0.5
15		188		191		1.6
30		176		177		0.6
60		155		157		1.3
100		134		131		2.3
Avg.						1.3

Bromate Ion Reduction in Varying H^+ with Constant Cl^-

The major component of gastric juice is hydrochloric acid (Sobala, 1991). The initial bromate ion reductions were at H^+ concentrations ranging from 0.01 to 0.17 M. This corresponds to a pH range of 2 to 0.8, which are common pH levels in stomach contents of normal humans. The Cl^- concentration was kept constant at 0.17 M. In order to minimize IC error associated with the measurement of BrO_3^- after it has been reduced beyond 75 %, an initial concentration of 200 µg/L of BrO_3^- was chosen, instead of the normal human consumption of 10 – 15 µg/L, for all reduction reactions. All bromate ion reductions were carried out at 37 degrees Celsius, in order to mimic the temperature of gastric juice in the human stomach. The results from the bromate ion reductions are shown in Tables 2 and 3.

Table 2
Bromate Ion Reduction – Varying H^+ Concentration at Constant 0.17 M Cl^-

0.17 M H^+				0.135 M H^+				0.10 M H^+		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	192			0	205	
30	172	12		30	177	8		30	194	5

60	151	23		60	160	17		60	184	10
90	130	34		90	146	24		90	176	14
120	110	44		120	135	30		120	168	18
180	87	56		192	112	42		180	155	24
240	67	66		257	91	53		240	142	31
								1389	25	88

Table 3
Bromate Ion Reduction – Varying H^+ Concentration at Constant 0.17 M Cl^- (continued)

0.075 M H^+				0.05M H^+				0.01 M H^+		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	188			0	196			0	206	
30	188	0		30	198	0		30	209	0
60	183	3		60	195	1		60	208	0
90	178	5		90	192	2		90	207	0
120	173	8		120	189	4		120	209	0
180	161	14		180	184	6		180	207	0
240	159	15		240	178	9		240	207	0
2128	35	81		1279	119	39		1224	207	0
				2822	68	65		2659	203	1
								4214	200	3
								27640	136	34
								43238	102	50

The data presented in Table 2 and 3 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 4.

Table 4
Time Required for 50% Reduction (Half-life) of Bromate Ion at Various H^+ Concentrations and Constant 0.17 M Cl^-

	0.17 M H^+	0.135 M H^+	0.10 M H^+	0.075 M H^+	0.05 M H^+	0.01 M H^+
$t_{1/2}$	153 min	238 min	454 min	14.9 hrs	30.6 hrs	30.2 days

The results show that as the H^+ concentration decreases from 0.17 M (pH 0.8) to 0.01 M (pH 2) that the rate of BrO_3^- reduction increases and the half-life increases from 153 minutes to ~30 days.

The results from these experiments are shown graphically in Figure 4. The overall order with respect to hydrogen ion for bromate ion reduction is 2.0 ± 0.05 at 37°C. This means that changes in acidity in the stomach markedly affect the decomposition of bromate ion.

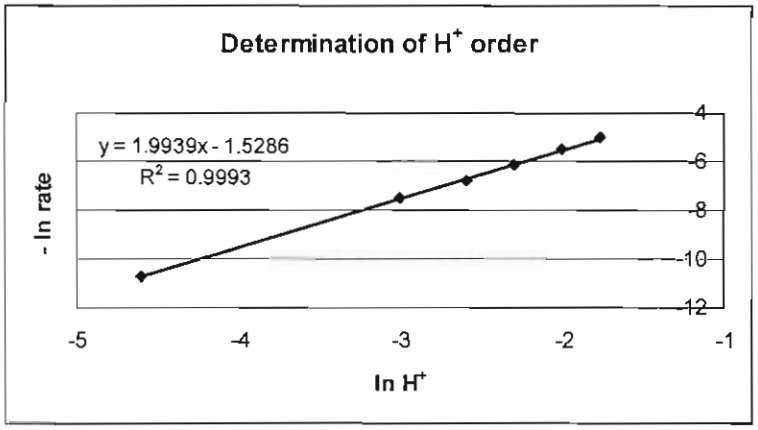


Figure 4. Plot of ln rate versus ln H^+ to determine the order of H^+ for the bromate ion reduction rate law

Bromate Ion Reduction in Varying Cl^- with Constant H^+

Additional experiments were used to determine the effect of Cl^- concentration on bromate ion reduction. The experiments were carried out by varying the Cl^- concentration at constant H^+. The results from the bromate ion reductions are shown in Tables 5, 6, 7, and 8.

Table 5
Bromate Ion Reduction in 0.135 M H^+ as a Function of Cl^-

0.135 M H^+/0.17 M Cl^-				0.135 M H^+/0.135 M Cl^-		
Time (min)	**BrO_3^- (ug/L)**	**% Reduction**		**Time (min)**	**BrO_3^- (ug/L)**	**% Reduction**
0	192			0	190	
30	177	8		30	178	6
60	160	17		60	166	13
90	146	24		90	155	18
120	135	30		120	145	24
192	112	42		180	125	34
257	91	53		256	108	43
				610	54	72

Table 6
Bromate Ion Reduction in 0.10 M H^+ as a Fnnction of Cl^- (continued)

0.10 M H^+/0.17 M Cl^-				0.10 M H^+/0.10 M Cl^-		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	205			0	200	
30	194	5		30	197	2
60	184	10		60	192	4
90	176	14		90	190	5
120	168	18		120	187	7
180	155	24		180	181	10
240	142	31		240	172	14
1389	25	88				

Table 7
Bromate Ion Reduction in 0.075 M H^+ as a Function of Cl^- (continued)

0.075 M H^+/0.17 M Cl^-				0.075 M H^+/0.075 M Cl^-		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	188			0	193	
30	188	0		30	190	2
60	183	3		60	191	1
90	178	5		90	189	2
120	173	8		120	187	3
180	161	14		180	178	8
240	159	15		240	172	11
				1654	128	34
				3110	88	54

Table 8
Bromate Ion Reduction in 0.05 M H^+ as a Function of Cl^- (continued)

0.05 M H^+/0.17 M Cl^-				0.05 M H^+/0.05 M Cl^-		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	204	
30	198	0		30	203	0
60	195	1		60	203	0
90	192	2		90	203	0
120	189	4		120	202	1
180	184	6		180	201	1
240	178	9		240	201	1
1279	119	39		1225	192	6

2822	68	65				

The data presented in Tables 5, 6, 7, and 8 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 9.

Table 9
Time Required for 50% Reduction (Half-life) of Bromate Ion at Various H^+ and Cl^-

	0.135 M H^+/0.17 M Cl^-	0.135 M H^+/0.135 M Cl^-	0.10 M H^+/0.17 M Cl^-	0.10 M H^+/0.10 M Cl^-	0.075 M H^+/0.17 M Cl^-	0.075 M H^+/0.075 M Cl^-	0.05 M H^+/0.17 M Cl^-	0.05 M H^+/0.05 M Cl^-
$t_{1/2}$	238 min	323 min	454 min	19.7 hrs	14.8 hrs	45.6 hrs	30.6 hrs	229 hrs

The table shows that at 0.05 M H^+/0.17M Cl^- the half-life is approximately 31 hours, but at 0.05 M H^+/0.05 M Cl^- the half-life is approximately 10 days. The results from the BrO_3^- reductions show that increasing Cl^- does increase the rate of bromate ion reduction, but the effect is not as significant as compared to changes in the concentration of H^+.

The results from these experiments show that the overall order with respect to chloride ion for bromate ion reduction is 1.5 ± 0.05 at 37°C.

Rate Law

The preceding data has been used in the development of a preliminary rate law for the reduction of bromate ion in an acidic medium:

$$-d[BrO_3^-]/dt = k[BrO_3^-]\,[H^+]^2\,[Cl^-]^{1.5} \qquad [11]$$

This rate law can be rewritten in more specific mechanistic terms as:

$$-d[BrO_3^-]/dt = k_1[BrO_3^-]\,[H^+]^2\,[Cl^-] + k_2[BrO_3^-]\,[H^+]^2\,[Cl^-]^2 \qquad [12]$$

The determination of the experimental rate law is important because the rate law can be used to estimate (within $\pm$ 3 – 4 %) the rate of reaction of bromate ion at different H^+ or Cl^- concentrations in the stomach. The

experimental rate law can also be used to help reduce the number of experiments needed when other possible reducing agents such as amino acids and sulfide ion are added. The rate can be estimated by using the rate law to predict the estimated rate for the new experiment under the exact H^+ and Cl^- conditions used. The difference in rate with and without the addition (e.g. hydrogen sulfide) gives the specific effect of the new species on the bromate ion reduction. The experimental rate law can be expanded as other components of real gastric juice are added to the HCl solution.

Bromate Ion Reduction in Varying Glutathione with Constant H^+ and Cl^-

Gastric juice contains different types of sulfur containing compounds such as proteins containing thiols and hydrogen sulfide. Thiols and other sulfur-containing species will react with bromate ion (Shanthi and Balasubramanian, 1996). A concentration of glutathione in gastric juice in the literature could not be found, so an initial concentration of 2.0 mg/L was used. The results from the bromate ion reductions are shown in Tables 10, 11, and 12.

Table 10
Bromate Ion Reduction in 0.17 M H^+ and 0.17 M Cl^- with Glutathione

0.17 M H^+				0.17 M H^+-2mg/L glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	199	
30	172	12		36	168	16
60	151	23		61	145	27
90	130	34		90	124	38
120	110	44		120	106	47
180	87	56		180	76	62
240	67	66		240	56	72

Table 11
Bromate Ion Reduction in 0.10 M H^+ and 0.17 M Cl^- with Glutathione

0.10 M H^+				0.10 M H^+-2mg/L glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	205			0	201	
30	194	5		30	190	5
60	184	10		60	180	10
90	176	14		90	171	15
120	168	18		120	163	19
180	155	24		180	147	27
240	142	31		240	132	34
1389	25	88		1292	18	91

Table 12
Bromate Ion Reduction in 0.05 M H^+ and 0.17 M Cl^- with Glutathione

0.05 M H^+				0.05 M H^+-2mg/L glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	199	
30	198	0		30	200	0
60	195	1		60	202	0
120	189	4		120	196	2
180	184	6		180	184	8
240	178	9		240	173	13
1279	119	39		1459	86	57
2822	68	65				

The data presented in Tables 10, 11, and 12 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 13.

Table 13
Time Required for 50% Reduction (Half-life) of Bromate Ion as a Function of H^+ with Constant 0.17 M Cl^- and 2.0 mg/L Glutathione

			H^+ - only		H^+ and 2mg/L glutathione
$t_{1/2}$	0.17 M H^+		153 min		132 min
$t_{1/2}$	0.10 M H^+		454 min		370 min

$t_{1/2}$	0.05 M H^+		30.6 hrs		20.1 hrs

The half-life table shows that at 0.17 M H^+ the half-life only decreases by 21 minutes, but at 0.05 M H^+ the half-life decreases by approximately 600 minutes. This shows that at a lower H^+ that glutathione has more of an effect on BrO_3^- reduction. The results from the BrO_3^- reductions show that the addition of glutathione does increase the rate of bromate ion reduction, but the effect is not significantly different from H^+ alone. Once a literature search reveals the concentration of glutathione in gastric juice, further tests at that concentration should be run.

Bromate Ion Reduction in Varying Cysteine with Constant H^+ and Cl^-

The sulfur containing compound, cysteine, can also be found in gastric juice, but its concentration could not be found in a literature search. Again, an initial concentration of 2.0 mg/L was used for initial experiments. The results from the bromate ion reductions are shown in Tables 14, 15, and 16.

Table 14
Bromate Ion Reduction in 0.17 M H^+ and 0.17 M Cl^- with 2.0 mg/L Cysteine

0.17 M H^+				0.17 M H^+-2mg/L cysteine		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	201	
30	172	12		30	146	27
60	151	23		60	108	46
90	130	34		90	79	61
120	110	44		120	59	71
180	87	56		180	35	83
240	67	66		240	21	90

Table 15
Bromate Ion Reduction in 0.10 M H^+ and 0.17 M Cl^- with Cysteine

0.10 M H^+				0.10 M H^+-2mg/L cysteine		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	205			0	200	
30	194	5		30	172	14
60	184	10		60	149	26
90	176	14		90	129	36
120	168	18		120	114	43
180	155	24		180	90	55
240	142	31		240	71	65
1389	25	88				

Table 16
Bromate Ion Reduction in 0.05 M H^+ and 0.17 M Cl^- with Cysteine

0.05 M H^+				0.05 M H^+-2mg/L cysteine		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	195	
30	198	0		30	179	8
60	195	1		60	169	13
90	192	2		90	155	21
120	189	4		120	143	27
180	184	6		180	117	40
240	178	9		240	102	48
1279	119	39				
2822	68	65				

The data presented in Tables 14, 15, and 16 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 17.

Table 17
Time Required for 50% Reduction (Half-life) of Bromate Ion as a Function of H^+ with Constant 0.17 M Cl^- and Cysteine

			H^+ - only		H^+ and 2mg/L cysteine
$t_{1/2}$	0.17 M H^+		153 min		69 min
$t_{1/2}$	0.10 M H^+		454 min		155 min

$t_{1/2}$	0.05 M H^+		30.6 hrs		251 min

The results from the BrO_3^- reductions show that the addition of cysteine increases the rate of bromate ion reduction significantly, as compared to H^+. Cysteine has a greater effect on BrO_3^- reduction as the H^+ concentration decreases. This can be seen in the half-life table, which shows that at 0.17 M H^+, the addition of cysteine only decreases the half-life by 84 minutes or approximately 55 percent. However, at 0.05 M H^+ the half-life decreases by approximately 1600 minutes or approximately 86 percent.

Bromate Ion Reduction in Varying Cysteine and Glutathione with Constant H^+ and Cl^-

Both cysteine and glutathione were added to the appropriate HCl mixture in order to determine the effect of both amino acids on BrO_3^- reduction. Cysteine and glutathione were both added at a concentration of 2 mg/L. The results from the bromate ion reductions are shown in Tables 18, 19, 20, and 21.

Table 18
Bromate Ion Reduction in 0.17 M H^+ and 0.17 M Cl^- with Glutathione and Cysteine

0.17 M H^+				0.17 M H^+-2mg/L cysteine and glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	194	
30	172	12		30	153	21
60	151	23		60	121	38
90	130	34		90	94	52
120	110	44		120	75	61
180	87	56		180	47	76

Table 19
Bromate Ion Reduction in 0.10 M H^+ and 0.17 M Cl^- with

Glutathione and Cysteine

0.10 M H^+				0.10 M H^+-2mg/L cysteine and glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	205			0	201	
30	194	5		30	177	12
60	184	10		60	155	23
90	176	14		90	135	33
120	168	18		120	118	41
180	155	24		180	90	55
240	142	31		240	69	66
1389	25	88				

Table 20
Bromate Ion Reduction in 0.05 M H^+ and 0.17 M Cl^- with Glutathione and Cysteine

0.05 M H^+				0.05 M H^+-2mg/L cysteine and glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	193	
30	198	0		30	184	5
60	195	1		60	171	11
90	192	2		90	158	18
120	189	4		120	146	24
180	184	6		180	124	36
240	178	9		240	109	44
1279	119	39				
2822	68	65				

Table 21
Bromate Ion Reduction in 0.01 M H^+ and 0.17 M Cl^- with Glutathione and Cysteine

0.01 M H^+				0.01 M H^+-2mg/L cysteine and glutathione		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	206			0	200	
30	209	0		30	191	5
60	208	0		60	182	9
105	207	0		90	174	13
135	209	0		120	168	16
180	207	0		180	157	22
240	207	0		240	140	30
1224	207	0		1265	40	80
2659	203	1				

4214	200	3				
27640	136	34				
43238	102	50				

The data presented in Tables 18, 19, 20, and 21 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 22.

Table 22
Time Required for 50% Reduction (Half-life) of Bromate Ion as a Function of H^+ with Constant Cl^- and Glutathione and/or Cysteine

			H^+ - only	H^+ and 2mg/L cysteine		H^+ and 2mg/L cysteine and glutathione
$t_{1/2}$	0.17 M H^+		153 min	69 min		88 min
$t_{1/2}$	0.10 M H^+		454 min	155 min		155 min
$t_{1/2}$	0.05 M H^+		30.6 hrs	251 min		283 min
$t_{1/2}$	0.01 M H^+		30.2 days			517 min

The results from the BrO_3^- reductions show that the addition of cysteine and glutathione increases the rate of bromate ion reduction significantly, but the increase is not as significant as compared to an addition of only cysteine. This can be noted in Table 22, at 0.17 M H^+ the half-life with only the addition of cysteine occurs in 69 minutes, while half-life with the addition of glutathione and cysteine requires 83 minutes. This difference in time required for a 50 percent reduction is 14 minutes. This effect is even greater at 0.05 M H^+, as the difference in time required for a 50 percent reduction is 32 minutes. Again, the addition of cysteine and glutathione has the greatest effect on BrO_3^- reduction as the H^+ concentration decreases. This can be seen in the half-life table, which shows that at 0.17 M H^+, the addition of cysteine and glutathione only decreases the half-life by 65 minutes or approximately 42 percent. However, at 0.05 M H^+ the half-life decreases by approximately 1550 minutes or approximately 85 percent. The addition of glutathione and cysteine has a very significant effect on BrO_3^- reduction at 0.01 M H^+. The half-life in 0.01 M H^+ is approximately 30 days, while the addition of glutathione and cysteine reduce the half-life to approximately 9 hours.

Bromate Ion Reduction in Ascorbic Acid with Constant H^+ and Cl^-

Ascorbic acid, another component of gastric juice, has a concentration range of 13 – 86 μM in gastric juice. A concentration of 50.0 μM ascorbic acid was added to the HCl solution. The results from the bromate ion reductions are shown in Table 23.

Table 23
Bromate Ion Reduction in
0.17 M HCl-5 X 10^{-5} M Ascorbic Acid

0.17 M H^+, 0.17 M Cl^-				0.17 M H^+, 0.17 M Cl^-, Asc		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	191	
30	172	12		15	183	4
60	151	23		30	170	11
90	130	34		60	140	27
120	110	44		90	119	38
180	87	56		120	100	48
				150	85	55

The data presented in Table 23 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 24.

Table 24
Time Required for 50% Reduction (Half-life) of Bromate Ion at Constant 0.17 M Cl^- and H^+ with 5 X 10^{-5} M Ascorbic Acid

	0.17 M HCl		0.17 M HCl and 5 X 10^{-5} M Ascorbic Acid
$t_{1/2}$	153 min		131 min

The data from BrO_3^- reductions show ascorbic acid does increase the reduction rate as compared to H^+. The half-life is decreased from 153 minutes in HCl to 131 minutes in HCl with the addition of ascorbic acid,

which corresponds to an approximately 14 percent increase in reduction rate. Additional experiments will be needed to determine if varying the ascorbic acid concentration has an effect on rate of reduction of bromate ion.

Bromate Ion Reduction in Varying H_2S with Constant H^+ and Cl^-

The concentration of hydrogen sulfide, a sulfur containing component of gastric juice, was varied in solutions of 0.17 M H^+/Cl^- and 0.10 M H^+/0.17 M Cl^-. The results from the bromate ion reductions are shown in Tables 25, 26, 27, and 28.

Table 25
Bromate Ion Reduction – Varying H_2S Concentration at Constant 0.17 M H^+ and Cl^-

0 M H_2S				~1X 10^{-4} M H_2S				~1X 10^{-5} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	196			0	200			0	202	
30	172	12		15	6	97		15	86	57
60	151	23		30				30	47	77
90	130	34		60				60	15	93
120	110	44		90				90	5	98
				120				120		

Table 26
Bromate Ion Reduction – Varying H_2S Concentration at Constant 0.17 M H^+ and Cl^-
(continued)

~1X 10^{-6} M H_2S				~1X 10^{-7} M H_2S				~1X 10^{-8} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	199			0	192			0	194	
15	146	27		15	168	13		15	179	8
30	107	46		30	129	33		30	165	15
60	53	73		60	64	67		60	143	26
90	27	86		90	31	84		90	121	38
120	15	92		120	15	92		120	104	46

								180	79	59

Table 27
Bromate Ion Reduction – Varying H_2S Concentration
at Constant 0.10 M H^+ and 0.17 M Cl^-

0 M H_2S				~ 1X 10^{-4} M H_2S				~ 1X 10^{-5} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	205			0	230			0	219	
30	194	5		15	17	93		15	172	21
60	184	10		30	~0	100		30	142	35
90	176	14		60				60	90	59
120	168	18		90				90	51	77
				120				120	28	87

Table 28
Bromate Ion Reduction – Varying H_2S Concentration
at Constant 0.10 M H^+ and 0.17 M Cl^-
(continued)

~ 1X 10^{-6} M H_2S				~ 1X 10^{-7} M H_2S				~ 1X 10^{-8} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	201			0	204			0	203	
15	185	8		15	194	5		15	191	6
30	168	16		30	180	12		30	186	8
60	132	34		60	143	30		60	178	12
90	95	53		90	106	48		90	167	18

120	68	66		120	77	62		120	157	23
								180	143	30
								240	130	36
								1329	23	89

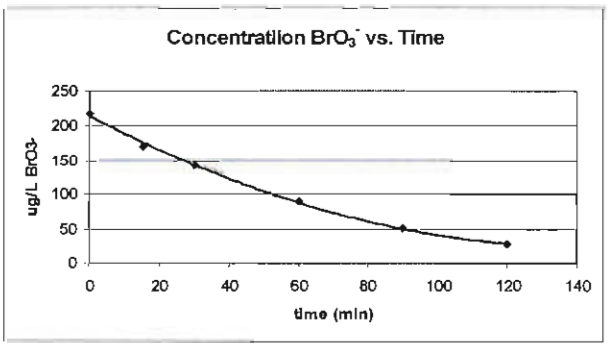
The data presented in Tables 25, 26, 27, and 28 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 29.

Table 29
Time Required for 50% Reduction (Half-life) of Bromate Ion as a Function of H^+ and H_2S with Constant Cl^-

			0 M H_2S		~ 1X 10^{-4} M H_2S		~ 1X 10^{-5} M H_2S		~ 1X 10^{-6} M H_2S		~ 1X 10^{-7} M H_2S		~ 1X 10^{-8} M H_2S
$t_{1/2}$	0.17 M H+ 0.17 M Cl-		153 min		2 min		14 min		32 min		39 min		137 min
$t_{1/2}$	0.10 M H+ 0.17 M Cl-		454 min		15 min		43 min		83 min		93 min		410 min

The results from these experiments show that hydrogen sulfide has a significant effect on bromate ion reduction. The data show that a reduction in the half-life occurs from 153 minutes in the absence of H_2S to 1.5 – 2 minutes with 10^{-4} M H_2S. This means that the combination of acid (H^+) and H_2S can effectively reduce the bromate ion in the stomach by more than 90 % in less than 10 minutes. Data from a bromate ion reduction using 0.10 M H^+, 0.17 M Cl^-, and 1 X 10^{-5} H_2S are shown in Figures 5a and 5b.

a)



b)

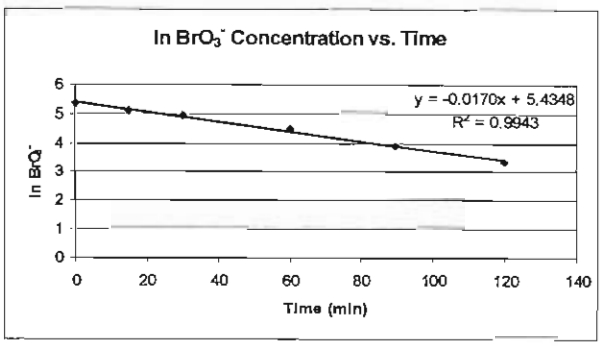


Figure 5. a) Plot of BrO_3^- versus time **b)** Plot of ln BrO_3^- versus time

The data from Figure 5a is a zero-order non-linear plot of BrO_3^- versus time, while Figure 5b is a first-order linear plot of ln BrO_3^- versus time. The linearity of the first-order ln plot tells us that the overall order with respect to hydrogen sulfide for bromate ion reduction is 1.0 ± 0.02 at 37°C.

Bromate Ion Reduction in Varying H_2S with Constant H^+, Cl^-, Glutathione, and Cysteine

A solution of glutathione, cysteine, H^+, Cl^-, and H_2S was prepared in order to determine the effect of varying H_2S as a function of H^+. The results from the bromate ion reductions are shown in Tables 29, 30, 31, and 32.

Table 29
Bromate Ion Reduction – Varying H_2S Concentration at Constant 2 mg/L Glutathione, 2 mg/L Cysteine, 0.17 M H^+, 0.17 M Cl^-

0 M H_2S				~ 1X 10^{-4} M H_2S				~ 1X 10^{-5} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	194			0	192			0	203	
30	153	21		15	11	94		15	128	37
60	121	38		30	~0	~100		30	95	53
90	94	52		60				60	50	75
120	75	61		90				90	34	83
				120				120		

Table 30
Bromate Ion Reduction – Varying H_2S Concentration at Constant 2 mg/L Glutathione, 2 mg/L Cysteine, 0.17 M H^+, 0.17 M Cl^- (continued)

~ 1X 10^{-6} M H_2S				~ 1X 10^{-7} M H_2S				~ 1X 10^{-8} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	198			0	193			0	200	
15	166	16		15	172	11		15	181	10
30	136	31		30	153	21		30	161	20
60	94	53		60	114	41		60	117	42
90	68	66		90	88	54		90	87	57
120	51	74		120	76	61		120	65	68

Table 31
Bromate Ion Reduction – Varying H_2S Concentration at Constant 2 mg/L Glutathione, 2 mg/L Cysteine, 0.10 M H^+, 0.17 M Cl^-

0 M H_2S				~ 1X 10^{-4} M H_2S				~ 1X 10^{-5} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	201			0	233			0	208	
30	177	12		15	129	45		15	146	30
60	155	23		30	66	72		30	113	46
90	135	33		60	21	91		60	78	63
120	118	41		90				90		
180	90	55		120				120		

Table 32
Bromate Ion Reduction – Varying H_2S Concentration at Constant 2 mg/L Glutathione, 2 mg/L Cysteine, 0.10 M H^+, 0.17 M Cl^- (continued)

~ 1X 10^{-6} M H_2S				~ 1X 10^{-7} M H_2S		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	208			0	206	
15	185	11		15	189	8
30	170	18		30	175	15
60	148	29		60	151	27
90	129	38		90	130	37
135	106	49		124	112	46
				180	86	58

The data presented in Tables 29, 30, 31, and 32 have been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 33.

Table 33
Time Required for 50% Reduction (Half-life) of Bromate Ion as a Function of H^+ and H_2S with Constant 0.17 M Cl^-, 2 mg/L Glutathione, 2 mg/L Cysteine

			0 M H_2S		~ 1X 10^{-4} M H_2S		~ 1X 10^{-5} M H_2S		~ 1X 10^{-6} M H_2S		~ 1X 10^{-7} M H_2S		~ 1X 10^{-8} M H_2S
$t_{1/2}$	0.17 M H+		88 min		4 min		29 min		59 min		85 min		76 min
$t_{1/2}$	0.10 M H+		155 min		17 min		39 min		134 min		142 min		IN PROCESS

The data in Table 33 shows that the addition of H_2S to the HCl, glutathione, cysteine solution does increase the rate of BrO_3^- reduction as compared to a solution without H_2S. A comparison of the half-lives in Tables 33 and 29 also shows that the rate of BrO_3^- reduction is faster in an HCl solution with only H_2S as compared to solutions of HCl, glutathione, cysteine, and H_2S. The only exception is at 10^{-8} M H_2S. At 10^{-8} M H_2S, the addition of glutathione and cysteine does increase the rate of reduction as compared to H_2S alone.

The addition of glutathione, cysteine, ascorbic acid and hydrogen sulfide allows the rate law to be expanded to:

$$-d[BrO_3^-]/dt = k_1[BrO_3^-][H^+]^2[Cl^-] + k_2[BrO_3^-][H^+]^2[Cl^-]^2 + k_3[BrO_3^-][H_2S][H^+]^n + k_4[BrO_3^-][Glu]^p[H^+]^q + k_5[BrO_3^-][Cys]^r[H^+]^s + k_6[BrO_3^-][Asc]^t[H^+]^v \quad [13]$$

Additional experiments are required to determine the order of each component in the rate law. With this completed rate law, it will be possible to predict the rate and half-life of BrO_3^- in gastric juice over a wide range of initial concentrations.

Bromate Ion Reduction in Real Gastric Juice

One sample of gastric juice were received that allowed BrO_3^- reduction to be measured in this matrix. Due to the limited gastric juice quantities, a calibration curve could not be constructed in this matrix, so a calibration curve from previous experiments was used instead. The results from two separate BrO_3^- reductions are shown in Table 34.

Table 34
Bromate Ion Reduction – "Real" Gastric Juice at 37°C, pH 1.7, and ~ 8.5 X 10^{-5} M H_2S

Trial 1				Trial 2		
Time (min)	BrO_3^- (ug/L)	% Reduction		Time (min)	BrO_3^- (ug/L)	% Reduction
0	192			0	217	
15	187	3		15	215	1
30	185	4		30	196	10
60	140	27		60	163	25
120	116	40		90	147	32
180	60	69		120	127	41
				180	104	52

The data presented in Table 34 has been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 35.

Table 35
Time Required for 50% Reduction (Half-life) of Bromate Ion in "Real" Gastric Juice at 37°C, pH 1.7, and 8.5 X 10^{-5} M H_2S

	Trial 1	Trial 2	Average
$t_{1/2}$	124 min	164 min	142 min ± 18 min

The data from the experiments show that after 120 minutes, the rate of reduction is nearly the same, but after 180 minutes the reduction rate is significantly faster in the Trial 1 sample. This can also be seen in Table 35, as the half-life for Trial 2 requires 40 extra minutes. This difference could be due to the volatility of H_2S. The experiments were run on separate days and it is possible that some H_2S was lost before Trial 2 causing the rate of BrO_3^- reduction to be slower.

Measuring Hydrogen Sulfide in Real Gastric Juice

A literature search for the concentration of hydrogen sulfide in real gastric juice produced no results. Since, the effect of H_2S on BrO_3^- reduction is rather significant, measurement of the H_2S concentration in gastric juice was needed. The use of the procedure described earlier was used to measure the concentration. The sample of gastric juice that was received contained ~ 8.5 X 10^{-5} M H_2S with an error of ± 10%. The error associated with this measurement can be improved by additional experiments with more gastric juice.

Bromate Ion Reduction in Real Gastric Juice Spiked to a Final Concentration of 0.05 M H^+

A small quantity of real gastric juice was spiked with an HCl until a final concentration of 0.05 M H^+ was obtained. The results from the bromate ion reduction are shown in Table 36.

Table 36
Bromate Ion Reduction – "Real" Gastric Juice Spiked to Final Concentration of 0.05 M H^+

Trial 1		
Time (min)	BrO_3^- (ug/L)	% Reduction

0	194	
15	159	18
30	104	46
60	50	74
90	37	81
120	21	89

The addition of HCl to the real gastric juice significantly increased the rate of BrO_3^- reduction as compared to real gastric juice. The half-life for real gastric juice with the addition of HCl was decreased to 36 minutes as compared to an average of 142 minutes for real gastric juice. This significant increase shows that HCl has a significant effect on BrO_3^- reduction in gastric juice..

Bromate Ion Reduction Comparison in Real and Synthetic Gastric Juice

A synthetic gastric juice was made with 0.02 M H^+, 1 X 10^{-5} M H_2S, and 2 mg/L glutathione and cysteine. This mixture had a pH, concentration of H_2S, and quantities of glutathione and cysteine as surrogates for thiol containing proteins that may be present in gastric juice. The results from the bromate ion reduction are shown in Table 37.

Table 37
Bromate Ion Reduction Comparison of "Real" Gastric Juice (Trial 1) and 0.02 M H^+-0.17 M Cl^--2mg/L glutathione-2mg/L cysteine-1 X 10^{-5} M H_2S

Real Gastric Juice – Trial 1				0.02 M H^+, 0.17 M Cl^-, Cys, Glu, H_2S		
Time (min)	**BrO_3^- (ug/L)**	**% Redncton**		**Time (min)**	**BrO_3^- (ug/L)**	**% Reduction**
0	192			0	210	
15	187	3		15	182	13
30	185	4		30	150	29
60	140	27		60	126	40
120	116	40		90	106	50
180	60	69		120	94	55

The data presented in Table 37 has been used to calculate a 50% reduction (half-life) of the 200 ug/L bromate ion sample. The results are shown in Table 38.

Table 38
Time Required for 50% Reduction (Half-life) of Bromate Ion in Real Gastric Juice and 0.02 M H^+-0.17 M Cl^--2mg/L glutathione-2mg/L cysteine-1 X 10^{-5} M H_2S

		Real Gastric Juice – Trial 1		0.02 M H^+, 0.17 M Cl^-, Cys, Glu, H_2S
$t_{1/2}$		124 min		90 min

A comparison of the real and synthetic gastric juice half-lives shows that the reduction of BrO_3^- in real gastric juice occurs in 124 minutes, while in synthetic juice the reduction only takes 90 minutes. The reason for this significant difference in rate is most likely due to components that have not yet been added to the synthetic gastric juice.

The addition of other components, found in gastric juice, to the synthetic HCl solution will make it possible for BrO_3^- reduction experiments to mimic reduction in real gastric juice.

Bromate Ion Reduction Comparison in Blood Serum

In some preliminary experiments bromate ion reduction was measured in a matrix of human blood serum. There was only a small quantity of blood serum, so a calibration curve was not constructed. A calibration curve from previous experiments was used instead. The results showed that after 30 hours there was negligible BrO_3^- reduction in the initial solution under these conditions. An aliquot of initial solution containing BrO_3^- was added to a second solution of blood serum and measured. The results showed that there was no further BrO_3^- reduction, which eliminates the possibility that blood serum components were consumed by the initial experiment.

Further experiments are needed in order to develop a calibration curve that is representative of the matrix, which will allow for more accurate quantitation of the results.

Bromate Ion Reduction Comparison in Whole Blood

In a very preliminary experiment bromate ion reduction was also measured in a whole blood matrix. A calibration curve from previous experiments was used. The results showed that there was some BrO_3^- reduction in the whole blood, but we were unable to quantitate the results. Many problems occurred during the measurements that made it difficult to measure reduction. The biggest problem is that whole blood contains many large protein molecules, which cause rather large interferences when attempting to accurately measure the BrO_3^- peak. The second problem associated with whole blood is that the large protein molecules cause damage to the IC column.

Many techniques have been attempted to remove the large proteins, but the interferences are still making it difficult to measure BrO_3^- accurately. The damage to the IC column is still a problem. Further whole blood experiments will not be run until a method is developed to remove the large protein molecules.

Papers/Presentations Written and in Preparation

The following presentations have been completed:

- Keith, J., Cotruvo, J., Pacey, G., Gordon, G. "Methodology for Detection of Bromate Ion in Artificial Gastric Juice". Pittcon Poster Presentation (2004)
- Gordon, G., Keith, J., Cotruvo, J., Pacey, G."The Rate of Bromate Reduction in the Human Stomach". Report (2004)

The following presentations/papers are in preparation:

- Keith, J., Cotruvo, J., Bull, R., Pacey, G., Gordon, G. "Measurement of Bromate Ion Reduction in Gastric Juices Using Ion Chromatography". Pittcon Presentation (2005)
- Keith, J., Cotruvo, J., Bull, R., Pacey, G., Gordon, G. "Preliminary Data on the Fate of Bromate Ion in Simulated Gastric Juices" to be presented at International Ozone Association Meeting in Windsor, ON (Sept. 2004)
-
- Keith, J., Cotruvo, J., Bull, R., Pacey, G., Gordon, G. "Methodology for Measuring Bromate Ion in Complicated Matrices". Anal. Chem. (to be submitted, 2004)

Conclusions

The experiments reported here show that BrO_3^- is reactive with H^+ and Cl^- in the synthetic gastric juice. The experiments show that H^+ has more of an effect than Cl^- on bromate ion reduction. The data have been used to derive a preliminary rate law for synthetic gastric juice that is second order in H^+ and is 1.5 order in chloride ion. The addition of the reducing agents glutathione, cysteine, hydrogen sulfide, and ascorbic acid to an acidic medium increase BrO_3^- reduction as compared to an acidic medium alone. It was found that hydrogen sulfide has the greatest effect on BrO_3^- reduction.

Measurement of BrO_3^- in a sample of gastric juice showed that a reduction was occurring. Bromate ion was also measured in blood serum and whole blood in some preliminary tests. It was concluded that there is a negligible reduction in blood serum and that there was some reduction in whole blood. The results of the BrO_3^- reduction in whole blood and blood serum were not quantitated due to insufficient sample volumes, as well as interferences in the sample matrix.

Further experiments in the presence of hydrogen sulfide, cysteine, glutathione, and ascorbic acid will be needed to further develop the rate law. Additional quantities of gastric juice are needed to develop a calibration curve that can be used to quantitate BrO_3^- reduction. These experiments will make it possible to further understand the rate of BrO_3^- reduction in the stomach.

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