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Comparison of Methodologies of Using Colorimetry to Detect Low Concentrations of Bromide as a Hydrologic Tracer

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Abstract: The objective of this research was to compare colorimetric methods for measuring low concentrations of bromide in natural waters. The bromide ion has long been used as a tracer to study hydrodynamic processes and properties. Its conservative nature makes it ideal as a tracer. Bromide is almost entirely immune to loss from a solution via adsorption on rocks and sediments, or reaction with other solutes present in the water. Using three methodologies to use colorimetry in the detection of bromide, I analyzed samples taken from the Gila River, Jemez Creek, and the Rio Grande after injections of sodium bromide were released in a tracer experiment. Concentrations of bromide samples made up breakthrough curves to analyze stream biogeochemistry, and I compared my analyses with results of bromide concentrations derived from ion chromatography, the "gold standard" of anion analysis.

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Introduction

The objective of this research was to compare variations of the phenol red colorimetric methods for measuring low concentrations of bromide in natural waters – The APHA Method drawn from "Standard Methods for the Examination of Water and Wastewater (21st edition), David Jones' (1993) adaptation of the standard method, and Barak and Lepore's (2009) method for recasting Jones in the microscale, correcting concentrations reported too low in previous literature, and eliminating sodium thiosulfate. The reagents added to bromide samples and standards (in this case, containing amount of sodium bromide) are Chloramine-t (Tosylchloramide or N-chloro tosylamide, sodium salt) and Phenol Red (phenolsulfonphthalein) in all methodologies, and, in the Standard Method as well as Jones' 1989 method, Sodium Thiosulphate is added. Sodium Thiosulphate is intended to dechlorinate samples of bromide – the presence of chloride can cause the excess Chloramine-t to bleach oxidatively the bromophenol blue produced with phenol red, bromide, and chloramine-t. Using it in a study intended to assess its effect on color maintenance over time yielded essentially no difference between its inclusion and omission, as will be discussed. I analyzed samples taken from the Gila River, Jemez Creek, and the Rio Grande after injections of sodium bromide were released in a tracer experiment. Concentrations of bromide samples made up breakthrough curves to analyze the stream biogeochemistry, and I compared my analyses with results of bromide analyses done by ion chromatography. Use of a colorimetric method - measuring the amount of light absorbed by a sample against the concentration of an analyte in that sample- is often a quick and cost-effective means of analysis.

A literature review of bromide ion detection in natural waters – utilizing ion chromatography, non-phenol red colorimetric methodologies, potentiometric methodologies, and others not used in this work, is included in the section entitled "Methods for bromide ion detection and history of colorimetric method."

The Barak and Lepore method (2009) was the most effective means by which to use concentrations of samples containing bromide derived from colorimetry to predict concentrations of those same samples derived from ion chromatography. It was the strongest when coupled with an ion chromatography-informed "correction" or the curve, or applying a response factor to all concentrations in a sample set by comparing a sample of river water with a known concentration to the concentration derived from a calibration curve, and obtaining the ratio between those two values to determine an offset for the series.

Because of the small sample sizes collected in the lab work leading to these analyses, this research aimed at scaling down the methods in the literature, since only 1 to 3 mL of sample might be available at any given time. While the colorimetric method led to the production of good linearity in the calibration curves produced with standards of known concentration, concentrations from ion chromatography (IC) analysis and spectrophotometry for the same sample often did not yield reliably similar results, perhaps due to the extremely small concentrations present in the samples, the broadness of the wavelength at which the samples were analyzed (590 nm), the extremely high background of chloride in sampled waters, the difficulty of measuring extremely tiny aliquots of reagent by volume, and the presence of other organic matter and constituents found in natural waters which may affect colorimetric methods of analysis.

Bromide analysis in the study of hydrodynamic processes

The bromide ion has long been used as a tracer to study hydrodynamic processes and properties. It is considered a conservative tracer because it participates in few reactions in the natural environment, making it ideal as a tracer– and, to capture the full breakthrough curve including micromolar quantities at the beginning and end of a tracer experiment (the "tails"), it is essential to detect bromide at the micromolar level. Bromide is almost entirely immune to loss from a solution via adsorption on rocks and sediments, or reaction with other solutes present in the water (Jones 1992). In the Rio Grande and other waters analyzed by this lab, background concentrations of bromide tend to be lower, making analysis of newly heightened (peak) concentrations easier. Dispersion coefficients, flow velocity, and stream

discharge can also be determined through the integration of a breakthrough curve derived from a series of samples taken at a given point, downstream of an injection site, over regular intervals until the solute has dissipated and the curve has decreased again to its baseline.

Detection of bromide at low concentrations is crucial for the assessment of nutrient uptake in recent research by the Gonzalez-Pinzon lab, wherein conservative (or non-reactive) tracers and reactive tracers are injected into flowing waterbodies simultaneously. The transport of these solutes downstream is discerned by grab sampling as well as, in the case of sodium nitrate, semi-continuous Eulerian sampling with sensors such as the SUNA – a sensor which measures levels of nitrate in natural waters.

The advection and dispersion of solutes through a waterway is displayed on a breakthrough curve, which shows, over time and in a constant location, the change in concentration of the given tracer reagent. These breakthrough curves can give insight into the stream dynamics of the waterway, the hyporheic zone/main channel exchange of water and analytes, and can convey crucial information about the biogeochemistry of a particular reach. Use of a reactive and conservative tracer side-by-side will give two breakthrough curves which can be viewed on the same chart: the difference between the integration of area under the curves reveals information about the nutrient uptake of the reach (nitrate will be taken up for primary production use in plants, while a conservative tracer like bromide will not undergo reaction or uptake on its journey downstream). Figure 1 displays this relationship.

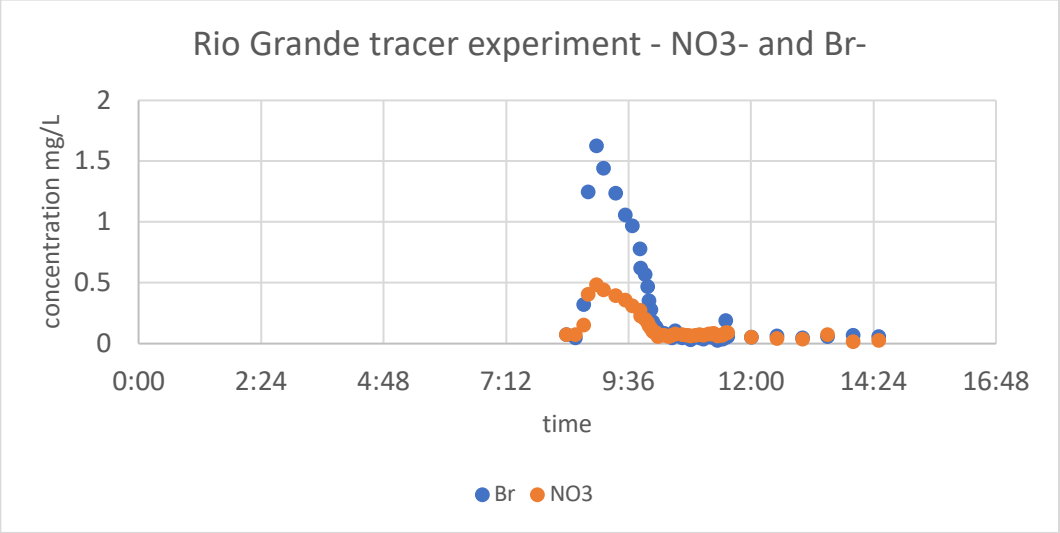

Figure 1: A conservative tracer breakthrough curve (blue) and reactive tracer curve (orange) poised in a chart can display nutrient uptake data in a given stream reach

Methods for bromide ion detection and the colorimetric method theory

Ion Chromatography. Ion chromatography allows for the analysis of ions present in samples of water by utilizing the process of separating ionic and polar molecules based on the charge they carry. In particular, it is an effective, yet extremely costly, way to determine concentrations of anions, or negatively charged molecules. For the Gonzalez-Pinzon lab, this method had been needed to measure non-conservative tracers such as nitrate, but ion chromatography is decreasingly needed with the introduction of high quality and increased-accuracy sensors such as the SUNA and S-CAN. IC samples have much finer resolution than any other method, being less affected by background constituents present in samples. This research found that, due to the difficulty of approximating water sample concentrations derived from the IC using spectrophotometric analysis, utilizing IC analysis for a few reference points (plotting IC concentrations against absorbances of the same samples) will still produce a close comparison – and can decrease the number of samples run through the IC, often at about $10 per sample, from the 60-100 range

to three or four, running the rest in the spectrophotometer which itself is a fraction of the cost to operate. The instrument is often priced at $20,000 or more.

Flow injection analysis. In flow injection analysis, the phenol red method is adapted to a flow-injection system. A sample of water is mixed with the same reagents as used in colorimetry before flowing toward a detector which measures absorbance at the same wavelength of 590 nm (Standard Methods 2015).

Colorimetry. Colorimetry is the process of plotting light absorbance at a certain wavelength of a given sample of analyte against the concentration of a substance in that sample. Either the analyte must exhibit light absorbance in the UV-visible spectrum or, most commonly, solution chemistry is used to develop a colored compound whose intensity is proportional to the concentration of the analyte. Ideally there should be a linear relationship between absorbance and the concentration of the analyte, which is referred to as Beer's Law. However, non-linear relationships are common and simply require recognition that the calibration curve is not linear. Concentration and absorbance should have a linear relationship to each other, allowing for the creation of calibration curves with which to determine the concentration of unknown samples. A spectrophotometer is an instrument which measures the amount of light absorbed, or the intensity of color at a given wavelength. The intensity of color can be given a numerical value by comparing the attenuation of light passing it through a sample. Quantitative measurements of light absorbed are transmittance and absorbance (Cheng 2003).

Because several other methods of bromide detection exist – including potentiometric as well as non-phenol red colorimetric methods, the following section outlines a literature review describing published methods of bromide detection at low concentrations, and areas for potential future research.

Literature Review of Colorimetric Methods

Besides the phenol red method, and falling into the method of colorimetry, there are several published articles detailing various approaches to colorimetric determinations of bromide in water, based on the needed detection amount, field or lab basis, and more considerations. This review presents a list of detection methods for bromide in water.

Margaret Neal, Colin Neal, Heather Wickham, Sarah Harman. Determination of bromide, chloride, fluoride, nitrate, and sulphate by ion chromatography: comparisons of methodologies for rainfall, cloud water, and river waters at the Plynlimon catchments of Mid-Wales. Center for Ecology and Hydrology. Hydrol Earth Syst. Sci., 11(1), 294-300, 2007.
Colorimetric methods of detecting bromide and the other anions listed, utilizing the phenol red method. Compares results of colorimetry with ion chromatography and inductively coupled plasma optical emission spectroscopy. The authors found that colorimetric determinations of bromide were higher than IC determinations – due to, the authors posit, the colorimetric method determining "bromide plus organo-bromide compounds."

S. Katzenelbogen, T. Czarski. Experimental Biology and Medicine. Improved Colorimetric Method for Determination of Bromide Concentration in Blood and Cerebrospinal Fluid, First Published October 1, 1934 Research Article
Employs the phenol red method with oxone as oxidizing agent.

Grace Chiu and Randy D. Eubanks. Microchimica Acta. .July 1989, Volume 98, Issue 4–6, pp 145–148| "Spectrophotometric determination of bromide."
This spectrophotometric approach is based on the conversion of bromide to tribromide. Absorption is at 267 nm, which may give more sensitivity than 590 nm. Detection limit for this method is 0.4 ug in an excess of chloride and sulfate.

Fred. Zitomer, J. L. Lambert. Spectrophotometric Determination of Bromide Ion in Water. Analytical Chemistry 35(11). DOI: 10.1021/ac60204a057
The method of detection in this paper is based on the inhibition of bromide to chlorination of ammonia, forming trichloramine. Bromide is measured as a function of ammonia lost, "because of oxidation by hypobromite to nitrogen."

Parameter and Code. Bromide, colorimetric, fluorescein, automated-segmented flow
Bromide, dissolved, I-2129-85.
Chloramine-t is added first after buffering, instead of phenol red. Bromide is converted to hypobromous acid, which then forms a pink "eosin" after reacting with fluorescin.

https://www.epa.gov/sites/production/files/2015-12/documents/9211.pdf
Environmental Protection Agency: METHOD 921. POTENTIOMETRIC DETERMINATION OF BROMIDE IN AQUEOUS SAMPLES WITH ION-SELECTIVE ELECTRODE.
With a detection limit of 0.2 mg/l, bromide is determined potentiometrically using a "bromide ionselective electrode (ISE) in conjunction with a double-junction reference electrode and a pH meter with an expanded millivolt scale or an ISE meter capable of being calibrated directly in terms of bromide concentration."

Field determination of bromide in water. Hajna F. Dobolyi. Analytical Chemistry 1984 56 (14), 2961-2963. DOI: 10.1021/ac00278a076
Bromide is oxidized by peroxymonosulfate (oxone) to bromine, then reacting with phenol red. Ammonium sulfate and sodium thiosulfate are added to mask chloride interference and stop fading.

Beer-Lambert Law. The Beer-Lambert Law (a.k.a. Beer's law) describes the linear relationship between absorbance and concentration of an absorbing species. The law is described by the following equation

$$A = a(\lambda) * b * c$$

where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, **b** is the path length, and **c** is the analyte concentration. (Afrasiabi 2003)

The foundation upon which the methods utilized in this study are based is the conversion of phenol red (PR, phenolsulfonphthalein) to bromophenol blue (3,3′,5,5′-tetrabromophenolsulfonephthalein), developed originally in 1935 (Barak et. al 2009). Initially, hypobromite was created by oxidizing bromide by the addition of sodium hypochlorite. Sodium hypochlorite subsequently replaced by chloramine-t as the oxidizing agent.

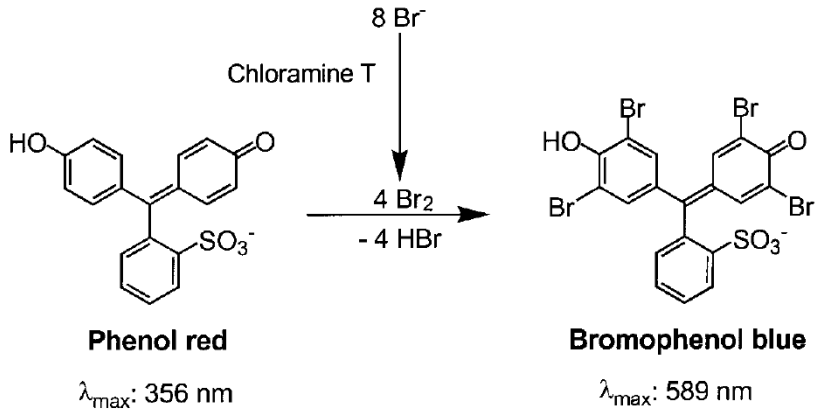

Figure 2: Reaction whereby phenol red is converted to bromophenol blue (Biomonitoring methods, Vol. 10)

The reaction equation follows (see figure 2 for more detail):

PR + 4Br- + 4H+ +4CT (o) → BPB + 4CT (r)
One setback this method has encountered through the history of the phenol red/chloramine-t technique is the interference of chloride ions. Oher interferences include iodide, ammonium (NH4+), very high concentration of bicarbonate ions and oxidizing agents such as free chlorine present as hypochlorite (HOCl) or ozone (O3). When the presence of chloride leads to production of chlorine monoxide from chloramine-t's reaction with phenol red, chlorophenol blue can be produced, which has absorbance similar to bromophenol blue, and can result in high absorbance (thus indicating high concentrations) readings in blanks – samples of water to which reagents are added, with no bromide present. The standard methodology (Standard Methods 2015) - upon which APHA (2015) is based - as well as Jones (1993), prescribe the addition of sodium thiosulphate after 20 minutes of chloramine-t and phenol red reaction, to "quench" (Barak et al 2009) the side reaction, consuming the oxidizing ion which leads to the formation of chlorophenol blue and thus results in high blanks.

Jones (1993) writes: "The initial colour development is very rapid but the chromophore is then bleached by a slower secondary reaction with hypochlorite generated by decomposition of the excess of CT present. It is clear that under these conditions the timing of the addition of the quenching reagent, thiosulphate, is extremely critical."

However, as Barak & Lepore (2009) state: "Jones (1993) demonstrated that addition of 0.4 Mmol/L NH4+ (as acetate) to the phenol red method reacted with excess free chlorine to produce chloramine (NH3Cl), thereby avoiding the formation of chlorophenol blue that otherwise led to high blanks." Thus, the addition of ammonia to the samples subjected to phenol red/chloramine-t reagents appeared to suppress chlorination and yield stable absorbance levels.

More attention will be paid to the apparent effect of sodium thiosulphate in the analysis which follows.

Methods

These methods intended to compare only the different variations of the phenol red method instead of a comparison of different colorimetric methods, since there are several non-phenol red methods as well, outlined in the appendix. The methods I used were as follows, and will be described in detail:

1. Modified versions of Jones' (1992) method, increasing concentrations where the paper seemed to report reagent concentrations much too small to yield meaningful results

2. Barak and Lepore's (2009) method of recasting Jones for use in the microscale, using microwell plate readers and taking advantage of newly developed multi-channel pipettes and microwell assay plates
3. A scaled-up version of Barak and Lepore (2009), analyzing samples in cuvettes for use in the UV Vis spectrophotometer available in the laboratory in which this research was centralized
4. The APHA 4500 Method based on the APHA Standard Methods for the Examination of Water and Wastewater 20th Edition (2015).

Different volumes and concentrations of samples, phenol red, chloramine-t, sodium thiosulphate, and ammonium were used in combination in the various methodologies of bromide detection via colorimetry. Because of the challenge presented by the small sample sizes collected in tracer analysis performed by the Gonzalez-Pinzon lab, with only 1 – 3 mL of sample available for analysis at times, some of the volumes prescribed by the methodologies had to be scaled down, both for the water samples being analyzed and the volumes of reagent added to them. Grab sampling protocol for this project require - due to the degradation of reactive tracers necessary to measure, such as nitrate – that samples are filtered immediately upon extracting them from the river, and since the waters of the Rio Grande are highly turbid, filtering is a slow process and can contribute to the small size of water samples collected.

A note on tracer injection x-axes: Each grab sample from the field contains water collected and filtered at a certain point in time, showing the rise and fall over time of concentration as a breakthrough curve is formed. The samples I used from Gila River tracer injection experiments were contained in bottles labeled with either a number designating the order in which the sample was collected (for example, Gila5_1, Gila5_2, Gila5_3), or labeled with the time of collection. In some cases I was unable to locate records associating the bottles of the former labeling style with the time of collection, in which case the x-axis is labeled 1, 2, 3, 4… etc.

Due to the uncertainties which can arise in scaling of already-small volumes, samples were analyzed in triplicate and the importance of using different methodologies became extremely apparent. Chlorine has a high likelihood of interfering with colorimetric results; therefore, I wanted to accompany the methodologies which eliminate sodium thiosulphate with those that include it.

Jones' 1993 paper, "Difficulties with The Chloramine-T-Phenol Red Method for Bromide Determination," prescribes the use of initial concentrations of Chloramine-t at 38.6 μM and Phenol red at 26 μM. An initial run of standards with those concentrations (Figure 3) proved inconclusive and did not yield a linear relationship between concentration of Br- and absorbance at wavelength 590.

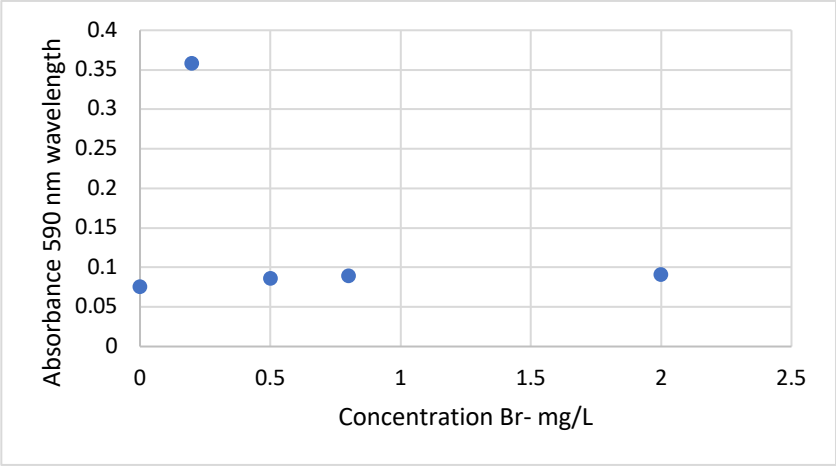

Figure 3: calibration curve showing relationship between known bromide concentration standards and the associated absorbance at 590 nm wavelength. This run was performed with Phenol Red and Chloramine-t initial concentrations of 26 µM and 38.6µM, respectively, and using Jones (1993) methods, which misreported Phenol Red concentrations as too low.

In researching Barak and Lepore's (2009) analysis of Jones' work, and their "recasting" of the final Chloramine-t (CT) and Phenol Red (PR) concentrations, I back-calculated new initial concentrations for CT and PR (figure 4). In Jones (1993), the final concentrations are indicated to be 0.16 mM for CT, 0.053 mM for PR, 0.4 mM for Ammonium, and 18.169 mM for Sodium Thiosulphate. In this research new initial concentrations were derived for each by using the formula:

$$[C]_0 = [C]_{final} \times (\frac{volume_{final}}{aliquot})$$

CUVETTE (JONES) - Sample size 10 mL				
Final concentrations	Original conc (mM or g/l)	Aliquot (mL)	Final volume (mL)	Final solution conc (mM)
Chloramine-t	3.7216	0.5	11.63	0.16
phenol red	1.23278	0.5	11.63	0.053
Ammonium	9.304	0.5	11.63	0.4
Thiosulphate	49.8 g/l	0.13	11.63	18.169

Figure 4: new concentrations for Jones' (1993) method of colorimetrically detecting bromide, based on recasting and correction to reach Barak and Lepore (2009) final concentrations

As well, this research scaled down Jones' method, which calls for 10 mL of sample, to require only 2 mL of sample, taking the ratio of each reagent to correspond to the new sample size.

APHA 4500 is based on the "Standard Methods for the Examination of Water and Wastewater 20th Edition." David Jones also based his research on the Standard Method. In 1989 and 1993, Jones developed his method by making a change to the "Standard Method" in use at the time: decreasing Chloramine-t concentrations from 386 µM to 38.6 µM (Jones 1989). Both Jones and APHA 4500 use the same initial molarity of phenol red and utilize sodium thiosulphate (ST) as a means of de-chlorinating

samples, whereas the Barak and Lepore methods eliminate sodium thiosulfate, relying on the addition of NH4+ as acetate to remove free chlorine.

Figure 5: reagent concentrations, volumes, and apparatus for each methodology.

	Jones (1993)	**Barak & Lepore (2009), microscale**	**Barak & Lepore (2009), macroscale**	**APHA 4500 Method**
Sample aliquot	10 mL (1.999 mL for this study)	300 µL	1.999 mL	50 mL (2.11 for this study)
Acetate buffer aliquot and final concentration	0.5 mL (0.099 for this study) 0.4 mM	11.25 uL (can combine 1:1 with phenol red for total aliquot of 22.5 µL) 0.4 mM	74.9 µL (can combine 1:1 with phenol red for total aliquot of 150 µL) 0.4 mM	2 mL (0.084 mL for this study) 0.0448 mM
Phenol red aliquot and final concentration	0.5 mL (0.099 for this study) 0.053 mM	11.25 µL (can combine 1:1 with buffer for total aliquot of 22.5 µL) 0.053 mM	74.9 µL (can combine 1:1 with buffer for total aliquot of 150 µL) 0.053 mM	2 mL (0.084 mL for this study) 0.0203 mM
Chloramine-t aliquot and final concentration	0.5 mL (0.099 for this study) 0.16 mM – 0.25 mg/mL	22.5 µL 0.160 mM	149.9 µL 0.160 mM	0.5 mL (0.021 mL for this study) 0.160 mM
Sodium thiosulphate aliquot and final concentration	0.13 mL (0.013 for this study when used) 18.169 mM	Not used	Not used	0.5 mL (0.021 mL for this study) 18.169 mM
Total volume	11.63 mL (2.3 for this study)	345 µL	2.3 mL	54.5 mL (2.3 for this study)
Apparatus	Cary UV-Vis 50 Conc Spectrophotometer	Synergy H1 Hybrid Microwell plate reader	Cary UV-Vis 50 Conc Spectrophotometer	Cary UV-Vis 50 conc Spectrophotometer
Reaction time	20 minutes	15-20 minutes	15-20 minutes	20 minutes

Procedure for each methodology

I. Preparation of acetate buffer solution. For each method, a buffer solution of 4.6 pH is required. I prepared one to use continuously with each method. A 1-liter buffer was prepared by diluting 0.5 mol L−1 NaOAc, 0.5 mol L−1 glacial acetic acid, and 12.32 mmol L−1 NH4OAc to 1000 mL.

II. Preparation of calibration standards. A 1000 ppm stock solution of NaBr was prepared by diluting about 1.35 g NaBr to 1000 mL milli-Q (ultrapure) water. 100 ppm, 10 ppm, and 1 ppm solutions were prepared by diluting, respectively, 10 mL of stock into 100 mL DI water, 1 mL of stock into 100 mL DI water, and 1 mL of 100 ppm solution into 100 mL of DI water. Standards were prepared at known concentrations of 0.01, 0.02, 0.03, 0.05, 0.08, 0.1, 0.2, 0.5, 0.6, 0.8, 1, 2, 5, 10, and 20 mg/L Br-.

III. Jones (1993). The following solutions were prepared: 0.25 mg/L Chloramine-t, 0.23 mg/mL Phenol red , and 49.8 g/L sodium thiosulfate. Into each cuvette, 1 mL of each standard (starting with 0 mg/L Br-) or sample was added. Next, 0.05 mL buffer was added to each cuvette in turn. 0.05 mL PR solution was then added, followed by 0.05 mL CT. Cuvettes were shaken to initialize reaction. After 20 minutes, 0.013 mL sodium thiosulfate was added. Each cuvette is read in triplicate on the Cary UV-Vis spectrophotometer to obtain absorbance at 590 nm wavelength. Standard concentrations are plotted against absorbance to derive a calibration curve, from which concentrations of samples can be derived by using their absorbance values.

IV. Barak and Lepore (2009), microscale. The following solutions were prepared: 0.69 g/L Chloramine-t (2.45 mM) and 0.614 g/L Phenol red (1.63 mM). 50 mL PR and 50 mL buffer *exactly* were combined to create a 1:1 solution. 96-well assay plates were used, with an 8-channel multichannel pipette. 300 uL standards were pipetted into each channel, starting with 0 mg/L Br (8 replications per sample, 12 different samples per plate). 22.5 uL PR + buffer was added to each row of wells, followed by 22.5 uL CT. Plates were shaken gently and let color develop for 5-15 minutes. Plates were read by the Plate Reader at 590 nm wavelength, again developing a calibration curve from the standards against which to derive concentrations from samples.

V. Barak and Lepore (2009), macroscale. The following solutions were prepared: 0.69 g/L Chloramine-t (2.45 mM) and 0.614 g/L Phenol red (1.63 mM). Into each cuvette, 2 mL of each standard (starting with 0 mg/L Br-) was added, followed by 2 mL of each sample. Next, 75 uL buffer was added to each cuvette in turn. 75 uL PR solution was then added, followed by 150 uL CT. Cuvettes were shaken to initialize reaction. Cuvettes were read in triplicate in the spectrophotometer at 590 nm wavelength.

VI. APHA 4500. The following solutions were prepared: 500 mg/100mL Chloramine-t (17.75 mM), 21 mg/100mL Phenol red (0.557 mM), and 49.6 g/100 mL sodium thiosulfate. Into each cuvette, 1 mL of each standard and sample (starting with 0 mg/L Br-) was added. Next, 0.04 mL buffer was added to each cuvette in turn. 0.04 mL PR solution was then added, followed by 0.01 mL CT. Cuvettes were shaken to initialize reaction. After 20 minutes, 0.01 mL sodium thiosulfate was added. Each cuvette is read in triplicate on the Cary UV-Vis spectrophotometer to obtain absorbance at 590 nm wavelength. Known standard concentrations are plotted against absorbance to derive a calibration curve, from which concentrations of samples can be derived by using their absorbance values.

Instrumentation

The apparatus used were a Varian Cary 50 Uv-Visible Spectrophotometer (figure 6), Synergy H1 Hybrid Plate Reader from BioTek (figure 7), Varian Quartz Cuvettes, 10mm flowpath (Figure 8), Costar Assay Microwell plates, 96-well, Eppendorf Research Plus Pipettes (8 channel and 1 channel) (Figure 9 and 10), and a Dell computer operating Windows 95.

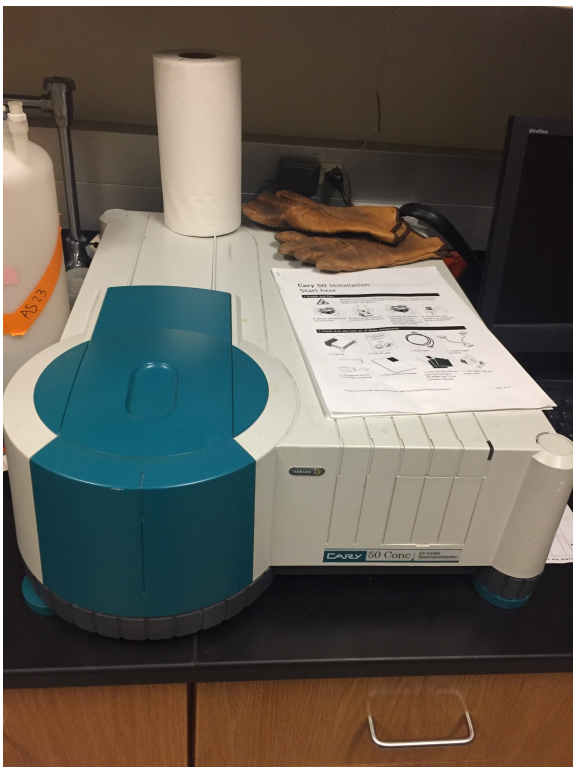

Figure 6: Varian Cary 50 Uv-Visible Spectrophotometer, Varian, Inc, certified to the ISO-9001

Figure 7: Synergy H1 Hybrid Plate Reader – BioTek

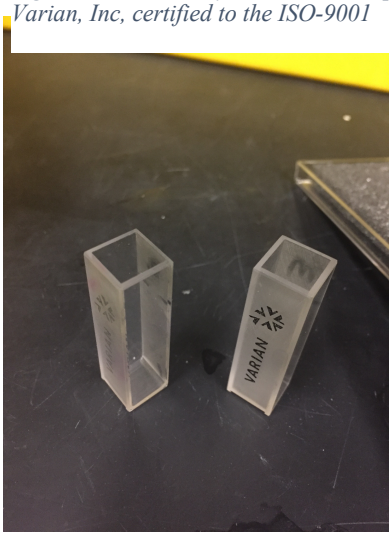

Figure 8: Varian Quartz Cuvettes, 10mm flowpath

Figure 9: Eppendorf Research plus Pipettes

Figure 10: Eppendorf Research plus Pipettes

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Analysis of stability of absorbance values over time with and without sodium thiosulphate

In this research, the distinction between results derived from samples to which sodium thiosulphate was added, and those to which it as not added, was sought. Below, Figure 11 displays the absorbance measured at 1 minute, 3 minutes, 5 minutes, 10 minutes, 15 minutes, and 30 minutes after addition of an ammonium acetate buffer, phenol red, and chloramine-t are added to standards of bromide at known concentrations 0.2 mg/L, 0.5 mg/L, and 0.8 mg/L to discern stability in solutions without sodium thiosulphate. Figure 12 displays the concentration standard 0.2 mg/L over the same intervals of time, with sodium thiosulphate added after exactly 20 minutes of reaction, with mixing. Per Barak and Lepore's findings, the thiosulfate had very little impact on stability of color with time, and there was even a slight increase in the readings of the sample to which thiosulfate was added. I decided to omit thiosulfate from my experimentation

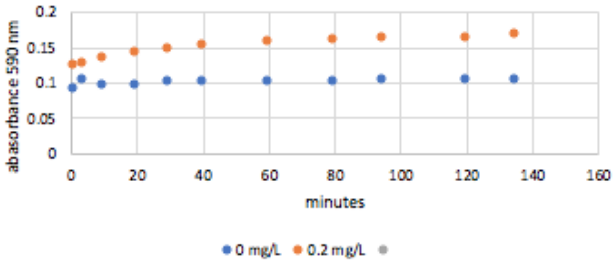


Figure 11 – Absorbance taken for standards of 0 mg/L and 0.2 mg/L bromide concentrations over 140 minutes, at regular intervals, after the addition of the APHA-method prescribed reagent concentrations and volumes to those samples. Sodium thiosulfate was not added to the solution to determine if degradation of absorbance due to oxidative bleaching was noted.

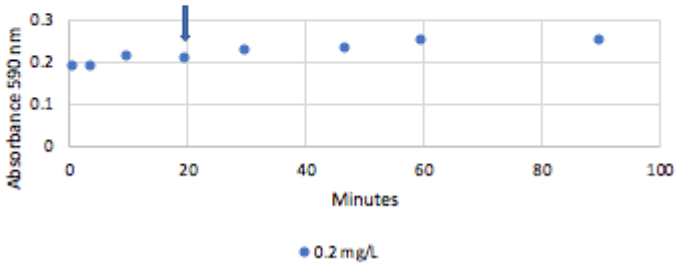


Figure 12 – Absorbance taken for a standard of 0.2 mg/L bromide concentration over 90 minutes, at regular intervals, after the addition of the APHA-method prescribed reagent concentrations and volumes to those samples. Sodium thiosulfate was added at 20 minutes of reaction time after phenol red and chloramine-t were added. These results were compared to those in Figure 11 to determine a difference in absorbance over time, with and without the addition of Sodium Thiosulfate.

Results

Jones (1993)

After increasing the initial concentrations to garner better results than were received in Figure 3, concentrations were measured against Gila River Injection 4 results. The concentrations found in Figure 6 were used.

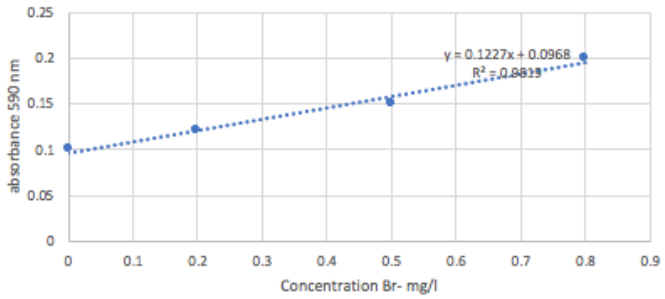


Figure 13: calibration curve derived using Jones (1993) method

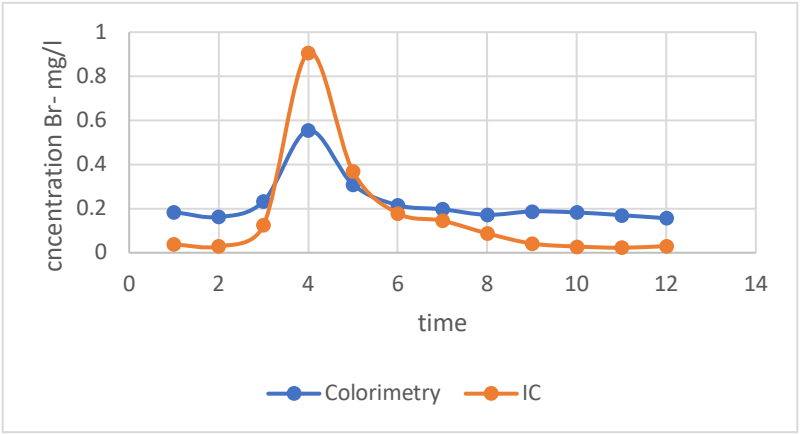


Figure 14: Comparison of bromide concentrations derived from same water samples, ion chromatography vs. colorimetry, using Jones (1993) method. Water samples from a Gila River Tracer Experiment called "Gila Injection 4" were used. Bottles were labeled numerically, not temporally, resulting in the x-axis written thusly.

The curve derived using Jones' method appears constrained within the range of values found by the IC, with the peak concentration found with colorimetry considerably lower than that found by the IC. The one-to-one analysis plotting colorimetry results (y-axis) by IC results (x-axis) yielded the plot below (figure 15). A potential error source is the high single point in the upper right corner, which can weight a regression curve and give higher R-square values. R-square is not always the ideal measure of fit.

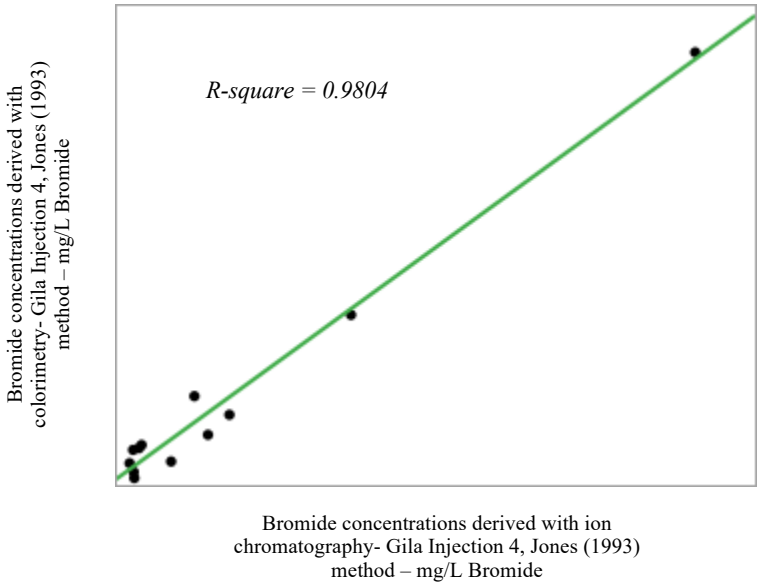
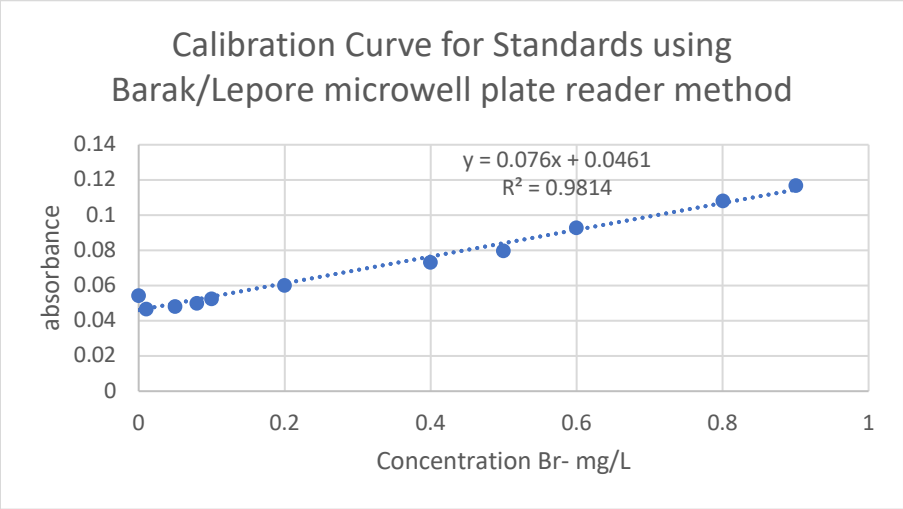


Figure 15: The one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived by colorimetry – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Barak and Lepore (2009), microscale



The calibration curve in Figure 16 was derived from known concentrations of Br- measured against absorbance at 590 nm wavelength. The linearity of the fit has an R^2 of 0.9814, perhaps owing to the high blank value – which could be attributable to the formation of chlorophenol blue.

Figure 16: Calibration Curve for Standards using Barak/Lepore microwell plate reader method

Water samples collected from two tracer experiments in the Gila River (called Injection 5 and Injection 6) were analyzed for concentrations of bromide, and compared against the results from ion chromatography (Figures 19 and 20).

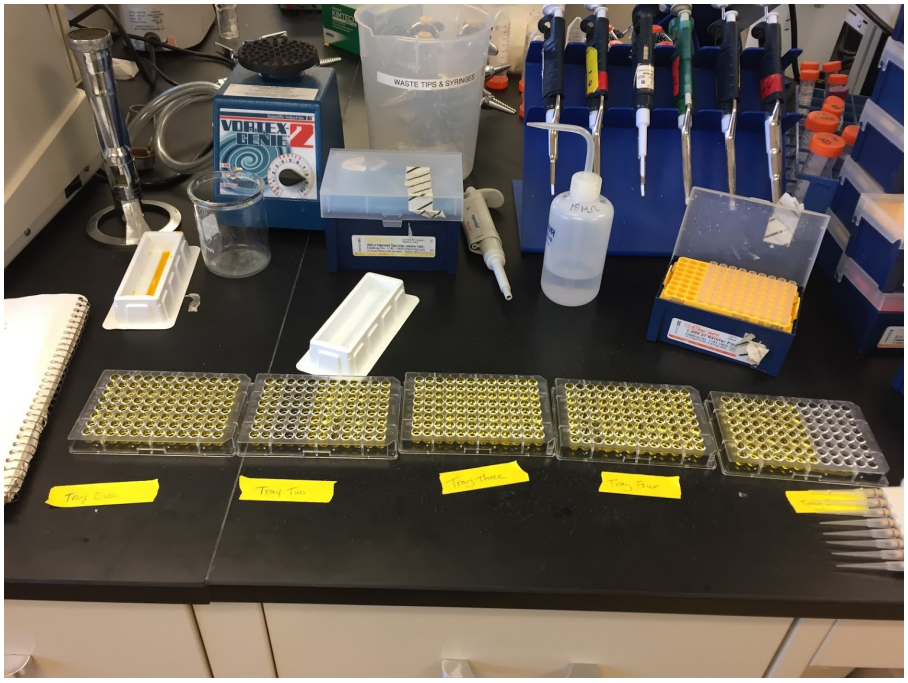


Figure 17: microwell plates with aliquots of samples and reagents added

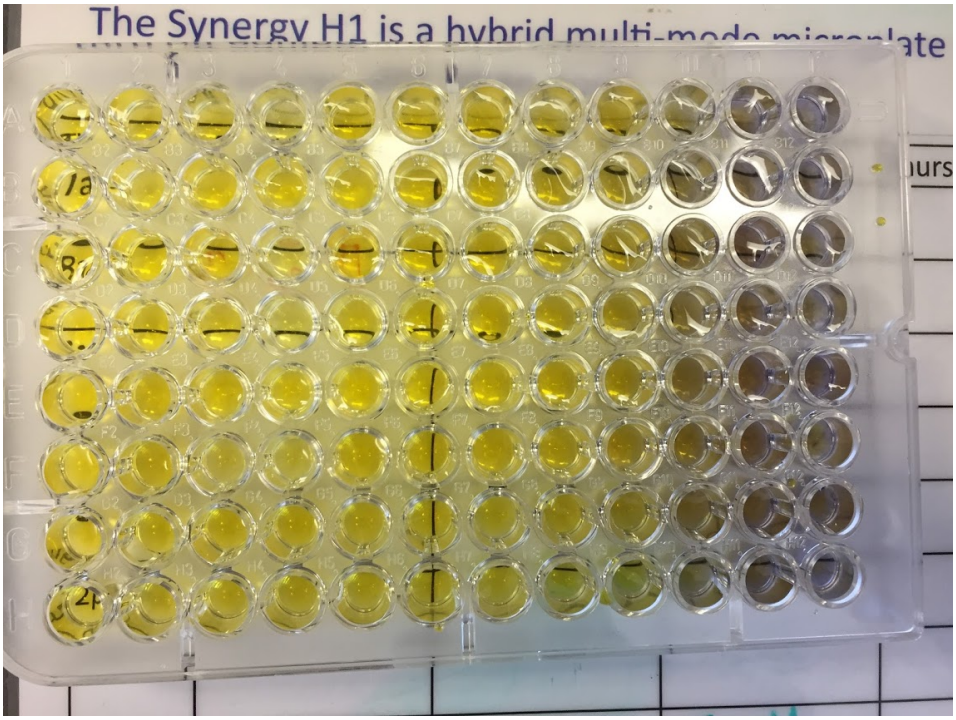


Figure 18: close-up of microwell plate

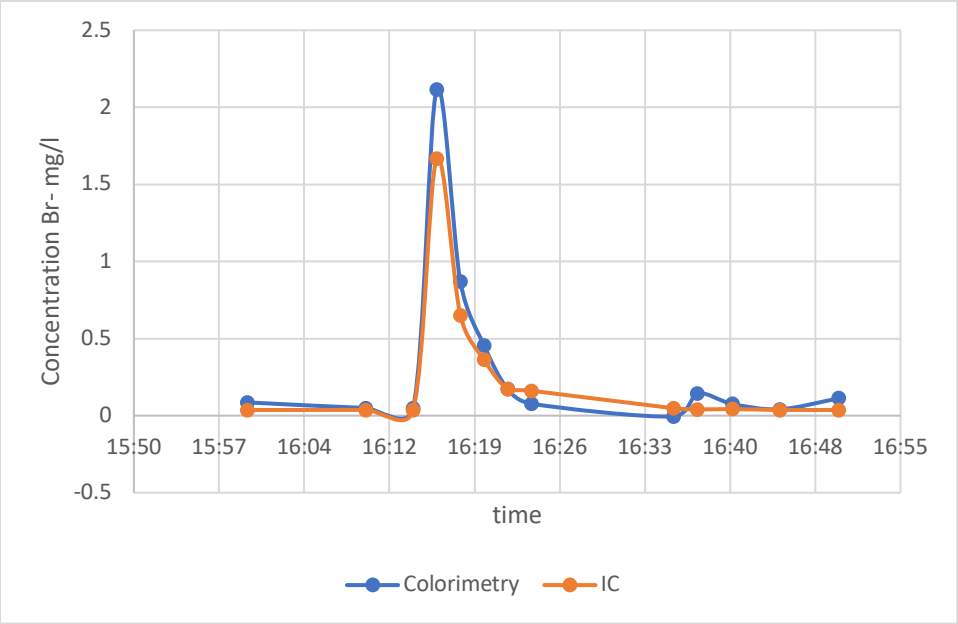


Figure 19: Gila Injection 5 - Colorimetric analysis of breakthrough curve (Barak/Lepore microwell method) vs. IC analysis

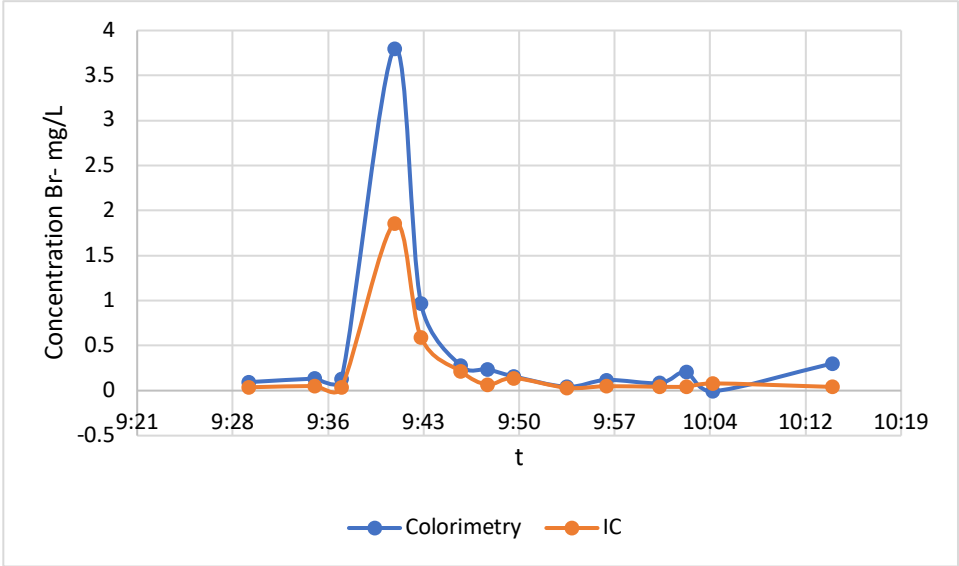


Figure 20: Gila Injection 6 - Colorimetric analysis of breakthrough curve (Barak/Lepore microwell method) vs. IC analysis

The low values appear to match closely with IC values, while the peaks in both Injection 5 and 6 appear higher than the peak concentration detected with ion chromatography.

Barak and Lepore (2009), macroscale

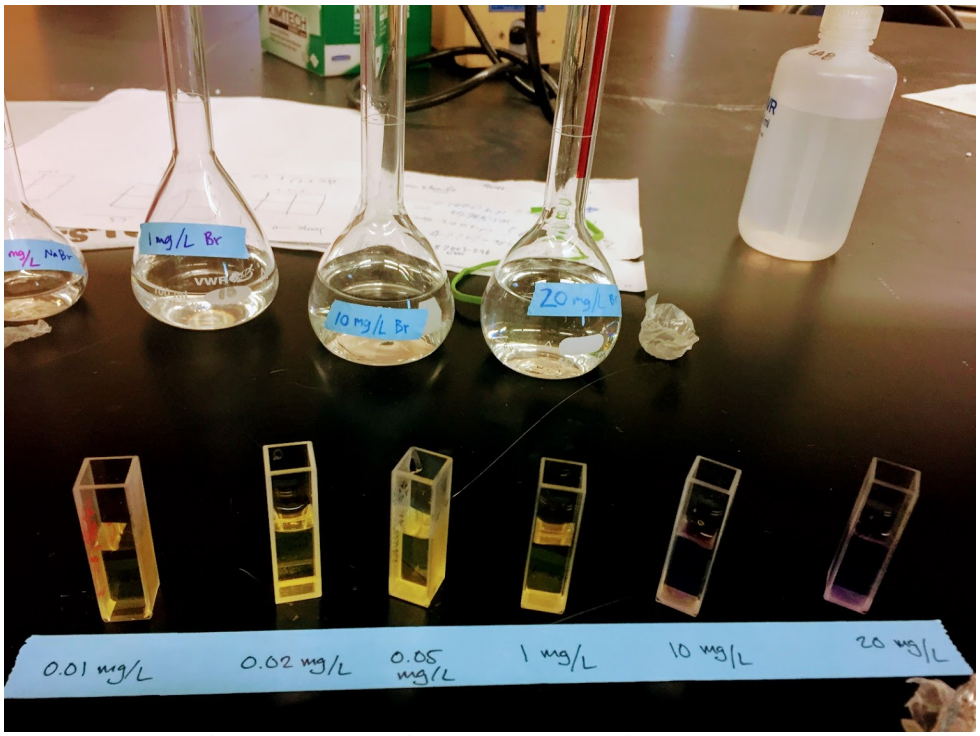


Figure 21: cuvettes filled with aliquots of sample and reagents for Barak and Lepore macro method

Figure 22 shows a calibration curve of standards for bromide concentration vs absorbance in 590 nm wavelength. The R^2 value in this case is 0.9954.

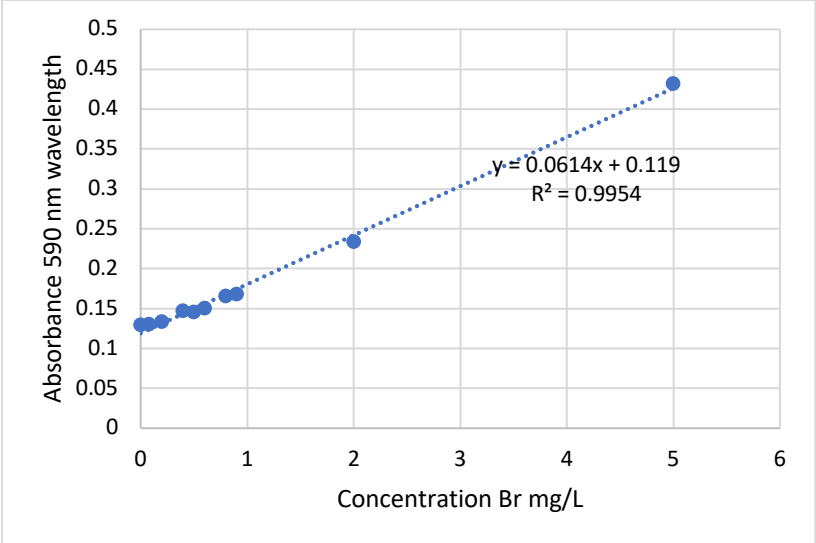


Figure 22: Barak and Lepore Methodology - calibration curve of standards for Bromide concentration vs absorbance (macroscale)

The Barak and Lepore macroscale experiment, though showing a strong calibration curve, yielded weak results when compared with IC concentration results (Figure 23 and 24).

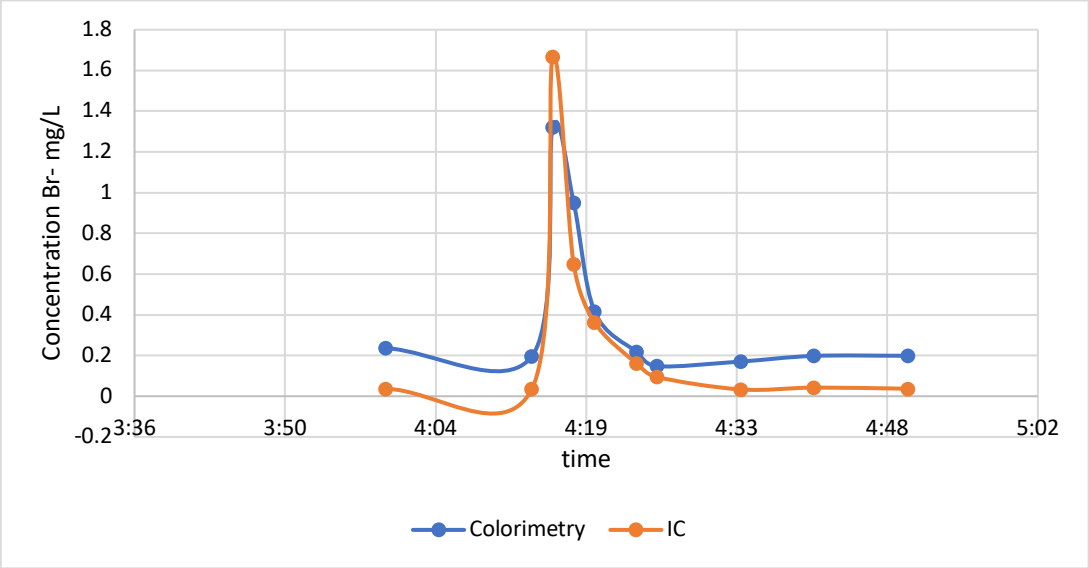


Figure 23: Gila Injection 5 - Barak/Lepore macroscale colorimetry method analyzing concentrations of bromide changing over the course of a breakthrough curve during a tracer injection experiment, vs. ion chromatography concentrations for the same samples

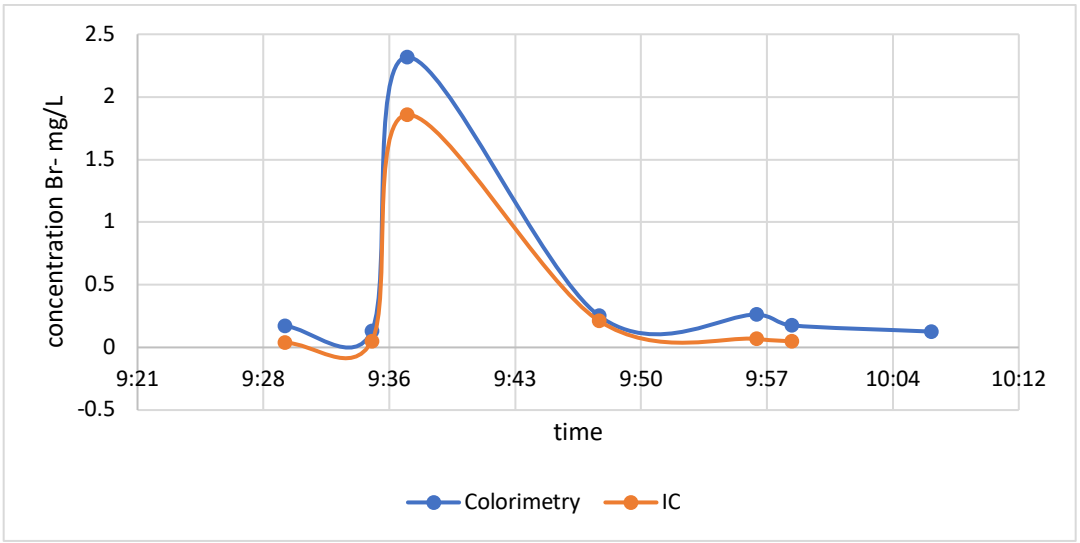


Figure 24: Gila Injection 6 - Barak/Lepore macroscale colorimetry method analyzing concentrations of bromide changing over the course of a breakthrough curve during a tracer injection experiment, vs. ion chromatography concentrations for the same samples

Once again, peaks are higher overall than peak concentrations calculated from ion chromatography in injection 6, yet lower (%) than the IC peak found in injection 5.

APHA 4500 Method

The first analysis using the APHA 4500 Method is distinguished from Jones by its higher initial chloramine-t concentration and lower phenol red concentration. The calibration curve and Gila Injections 5 and 6 can be found in Figures 25, 26, and 27, respectively.

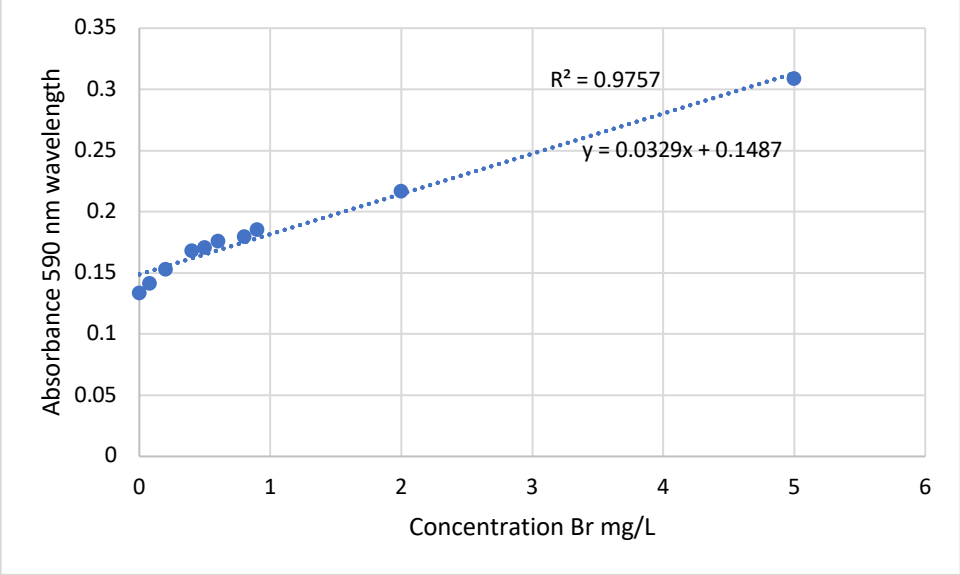


Figure 25: APHA Methodology - Calibration curve of standards for Bromide concentration vs absorbance

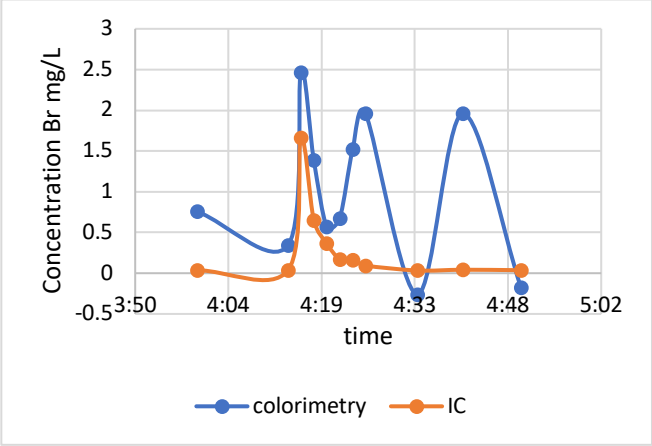


Figure 26: Gila Injection 5 - APHA colorimetry method analyzing concentrations of bromide changing over the course of a breakthrough curve during a tracer injection experiment, vs. ion chromatography concentrations for the same samples

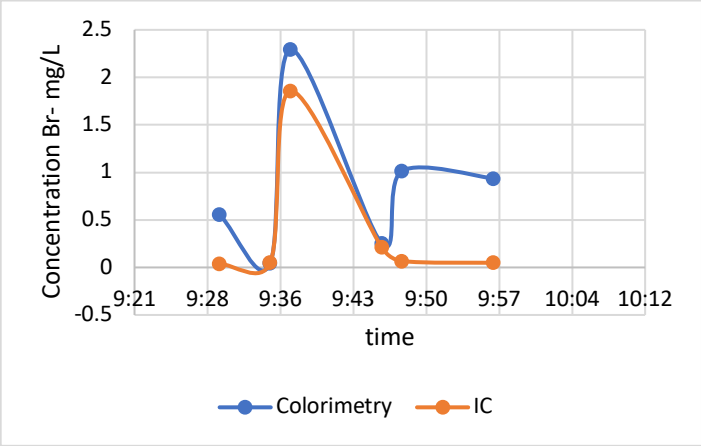


Figure 27: Gila Injection 6 - APHA colorimetry method analyzing concentrations of bromide changing over the course of a breakthrough curve during a tracer injection experiment, vs. ion chromatography concentrations for the same samples

The addition of sodium thiosulfate at 20 min before measuring absorbance was read was observed to make the measurements somewhat unstable and in fact increase, perhaps due to the decomposition of chloramine-t by sodium thiosulfate (Barak 2009). The APHA methodology describes detection at high levels, up to 100 – 800 mg/L, which are amounts rarely seen in nature. Industrial/oil-field brine discharges can make large contributions of bromide to water resources. Seawater intrusion and sea-spray

affected precipitation can foster an increase in bromide in natural waters – in general, bromide content in drinking water does not surpass 1 mg/L. An article by Earthworks reports: "Research by Carnegie Mellon University and Pittsburgh Water and Sewer Authority experts suggests that the natural gas industry has contributed to elevated levels of bromide in the Allegheny and Beaver Rivers. Bromides react with disinfectants used by municipal treatment plants to create brominated trihalomethanes, which have been linked to several types of cancer and birth defects (Earthworks)."

The Method Detection Limit (MDL) is described in the literature for phenol red methodology used in this research to be 0.1 mg/L Br- (Jones 1993, Barak 2009, APHA 2015). For negative values or absorbance values which attempt to measure known concentrations less than 0.1, future research will utilize methodology of plotting all those values as one-half of the MDL, or 0.05.

Gila Injections 5 and 6 – How did APHA and Barak & Lepore colorimetric methods compare with IC methods?

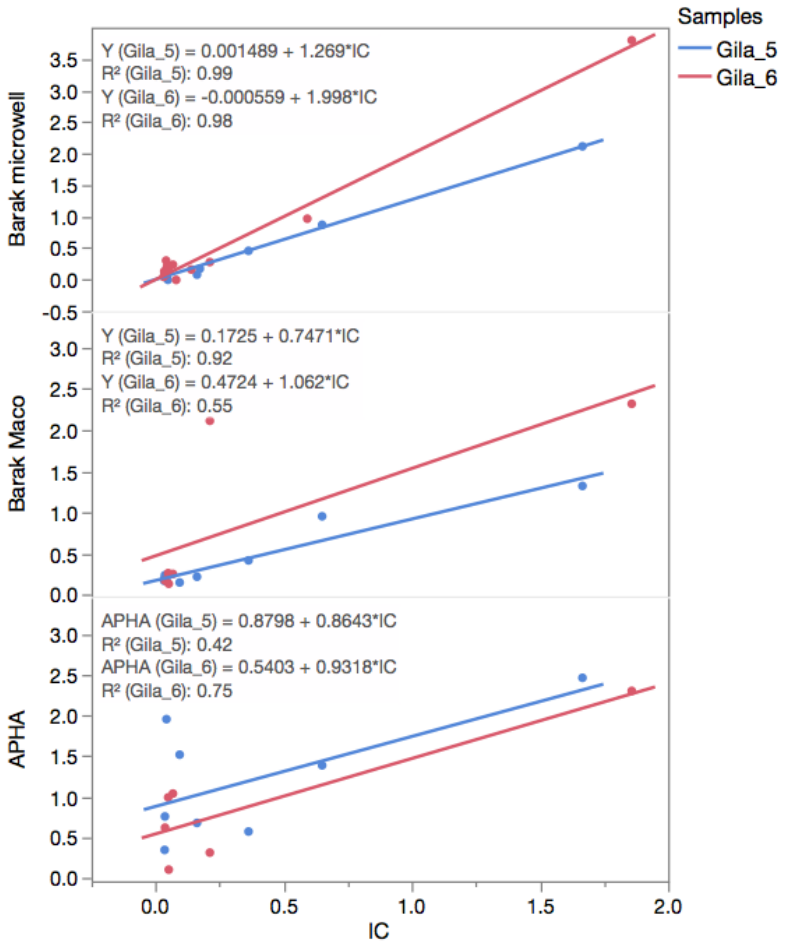


Figure 28: Created from a correlation matrix analyzing the success with which Barak & Lepore macro, Barak & Lepore micro, and APHA 4500 methods could produce bromide concentrations which accurately predict the bromide concentrations derived by IC analysis for the same samples. If the slope is greater than one, the spectrophotometric analysis overestimates IC concentrations. Slopes less than one underestimate IC concentrations.

In the above diagram (Figure 28), if slopes are greater than 1, the colorimetric method overestimates IC concentration and vice versa for slopes which are less than 1 and underestimate the IC concentration. In

general, the Barak/Lepore microwell method overestimates the IC concentrations for the same samples. The Barak/Lepore macro method is a closer approximation for Injection 5, and underestimates the IC concentration for Injection 6. Finally, the analysis showed that the APHA method underestimated IC concentrations and has the weakest R-square values.

Discussion

Further research to more closely approximate IC-derived values

Following the basic procedure of standard analysis, creation of standard calibration curves for each run, and analysis of natural water samples with trace bromide concentrations led to experimental approaches to improve the analytical method. These improvements included:

1. Correction of colorimetric concentration results by creating a calibration curve comparing IC concentrations of 3 samples against the absorbances of those same samples, and obtaining the trendline equation from the "IC concentration vs. spectrophotometer absorbance" graph. All the samples are quickly analyzed using spectrophotometric analysis, and the absorbances are converted to concentrations using the IC/spec regression from comparison of 3 samples.
2. Determination of quenching by measuring the absorbance of a sample of natural water with a known concentration of bromide, before and after reagents were added.
3. Correcting for the possible interference by chloride by using the standard addition method, described later, to create a "matrix effect" by combining samples with standards.
4. Creating a non-dimensional colorimetric curve and correcting it by values obtained with IC analysis of three or less select points.

A few factors may have contributed sources of error to the discrepancies between IC and colorimetric concentrations as shown with Gila Injections 4, 5, and 6, using Jones, Barak/Lepore, and APHA methodologies. With regard to the Gila Injections in particular, at some injection moments, other tracers (phosphate, nitrate, chloride) were injected simultaneously with bromide, but not in every case, adding unpredictability to the outcomes of samples.

Moving into a next phase of troubleshooting problems which arose, only the Barak and Lepore method was used due to preferable results yielded by that method.

Comparison of colorimetry data with IC data

Using Gila Injection 4 samples, I prepared all samples with the Barak & Lepore method for creating reagents and mixing reagents with samples. When the reaction was complete, I obtained absorbances for all the water samples to create a breakthrough curve and select the moments (samples taken at different locations along the breakthrough curve – rising limb, peak, falling limb, for example, to best capture the complexity of the full curve) for which to compare the corresponding IC concentrations. Absorbance values for three samples against those same three samples' concentrations derived from IC analysis were plotted, to obtain a regression analysis of the relationship between colorimetry absorbances and IC concentrations, seeking the goodness-of-fit relationship yielded by that analysis. The trendline equation was used to transform all absorbance values from spectrophotometric analysis into concentrations of bromide.

Using the Barak & Lepore method, the calibration curve from lab standards yielded the comparison of curves in figure 29, with a correlation (figure 30) having an R-square of 0.96.

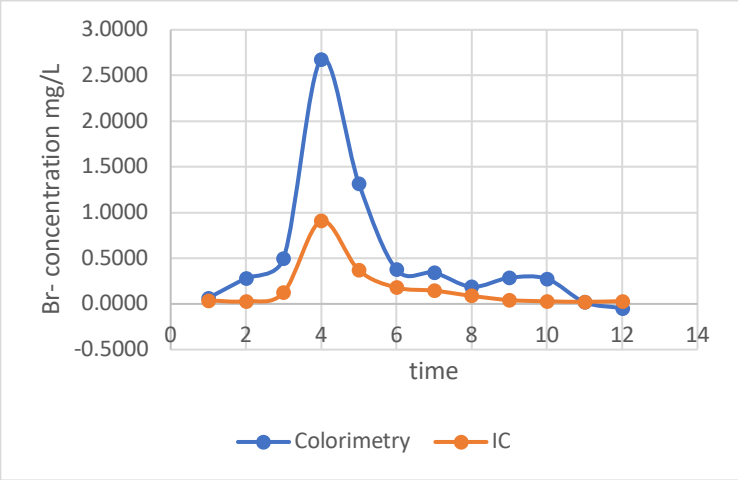


Figure 29: Colorimetric analysis of Gila Injection 4 using the Barak & Lepore macroscale method, analyzing concentrations of bromide changing over the course of a breakthrough curve during a tracer injection experiment, vs. ion chromatography concentrations for the same samples. Sample bottles were labeled numerically instead of temporally, reflected here on the x-axis.

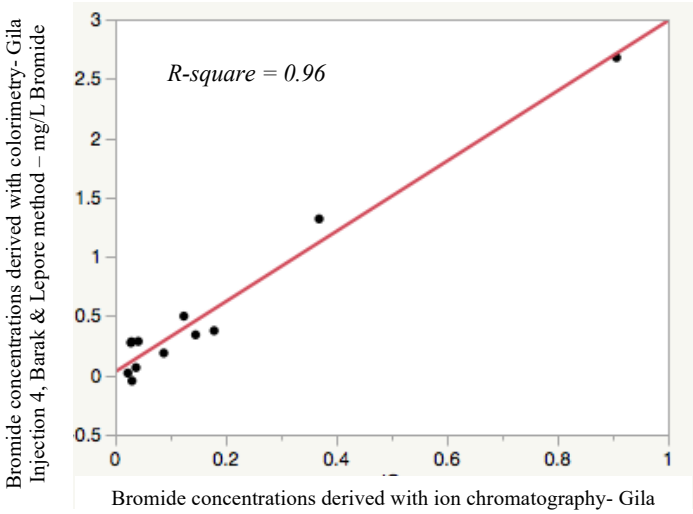


Figure 30: For the Barak & Lepore colorimetric method analysis of Gila Injection 4, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived by colorimetry – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Correcting that data (Gila Injection 4, Barak and Lepore) using three IC concentrations against the colorimetry absorbances to create an IC-derived calibration curve, and using the IC-derived calibration curve's trendline equation to transform all absorbance values into concentrations yielded the comparison of breakthrough curves in Figure 31. The IC corrected colorimetric values yielded the correlation found in figure 32, with an R-square of 0.97.

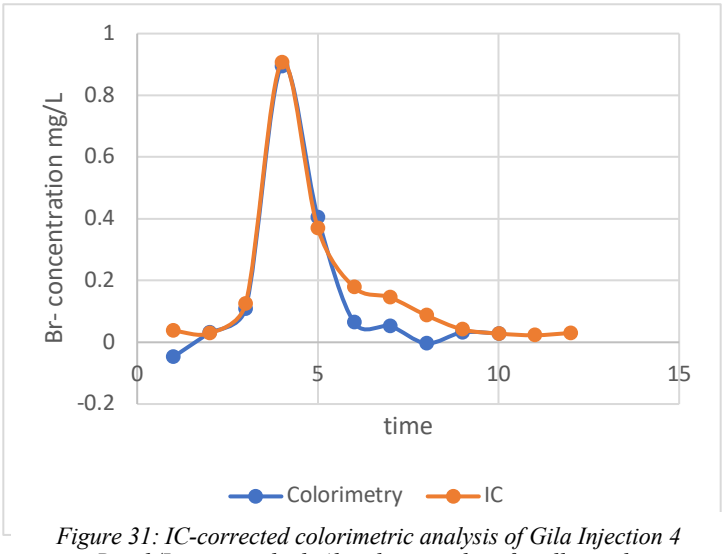


Figure 31: IC-corrected colorimetric analysis of Gila Injection 4 using Barak/Lepore method. Absorbance values for all samples were taken colorimetrically, and three points were chosen based on their distribution throughout the breakthrough curve to relate to their corresponding IC concentrations. Absorbance values for three samples against those same three samples' concentrations derived from IC analysis were plotted, to obtain a regression analysis of the relationship between colorimetry absorbances and IC concentrations, seeking the goodness-of-fit relationship yielded by that analysis. The trendline equation was used to transform all absorbance values from spectrophotometric analysis into concentrations of bromide.

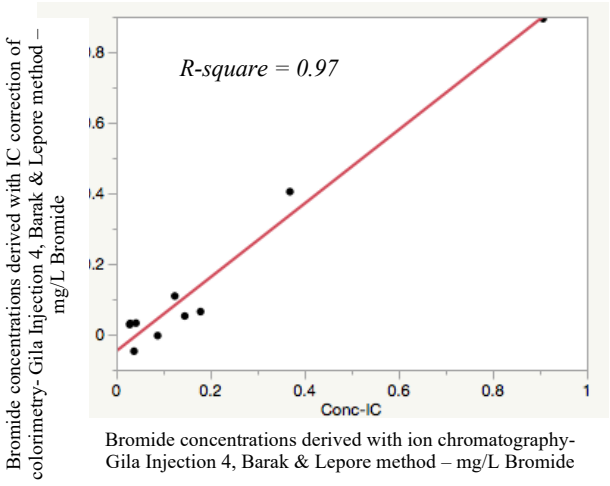


Figure 32: For the Barak & Lepore colorimetric method analysis of Gila Injection 4, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived from correcting absorbance to fit an IC-informed breakthrough curve – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Using the Jones method, the calibration curve from lab standards yielded the comparison of curves in Figure 14, shown previously.

Correcting that data (Gila Injection 4, Jones) using three IC concentrations against the colorimetry absorbances to create an IC-derived calibration curve yielded the comparison of breakthrough curves in Figure 33.

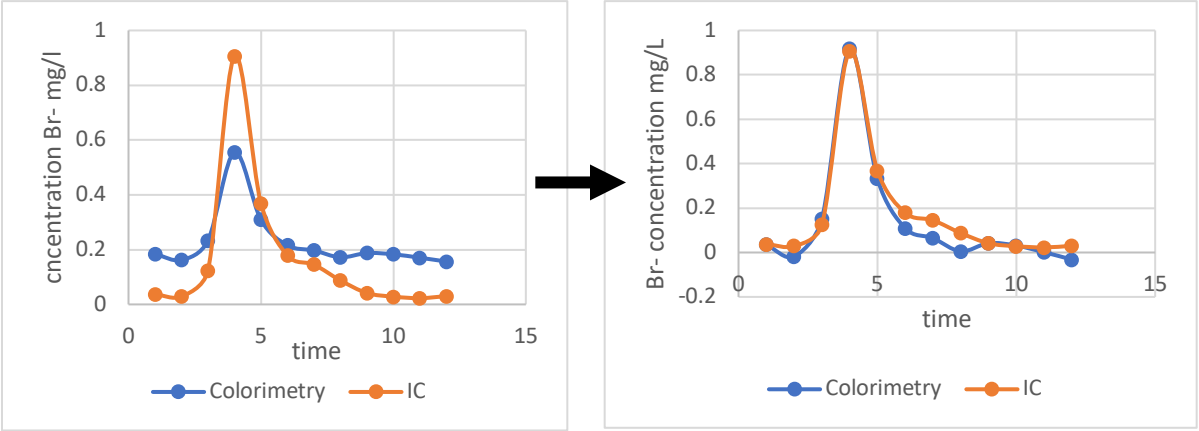


Figure 33: IC-corrected colorimetric analysis of Gila Injection 4 using Jones method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

I conducted the same analysis for the Barak & Lepore microscale results of Gila Injections 5 and 6, corrected the curve using a regression from three IC concentration results.

The two IC-corrected charts for the microscale Barak and Lepore method, for Gila injections 5 and 6 (Figure 34), yielded the one-to-one correlation in Figure 35, with an R-square of 0.989.

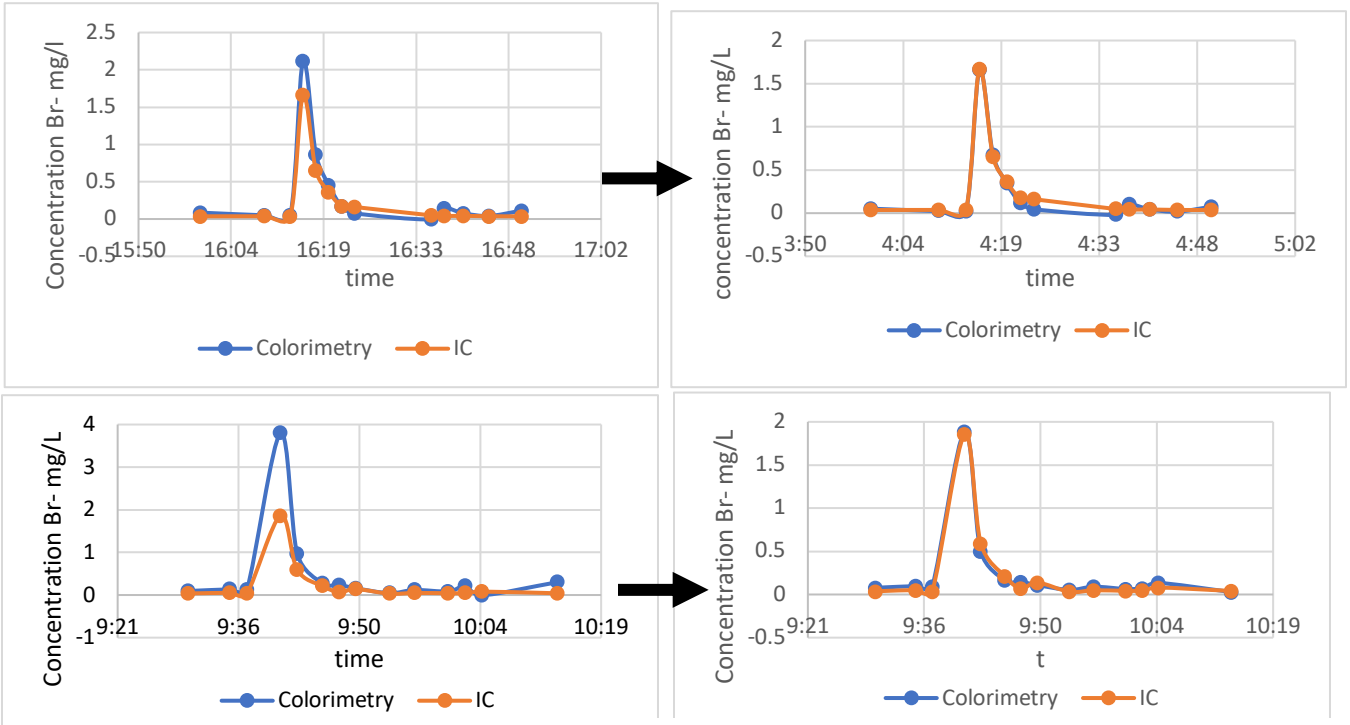


Figure 34: IC-corrected colorimetric analysis of Gila Injections 5 (top) and 6 (bottom) using Barak & Lepore microscale method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

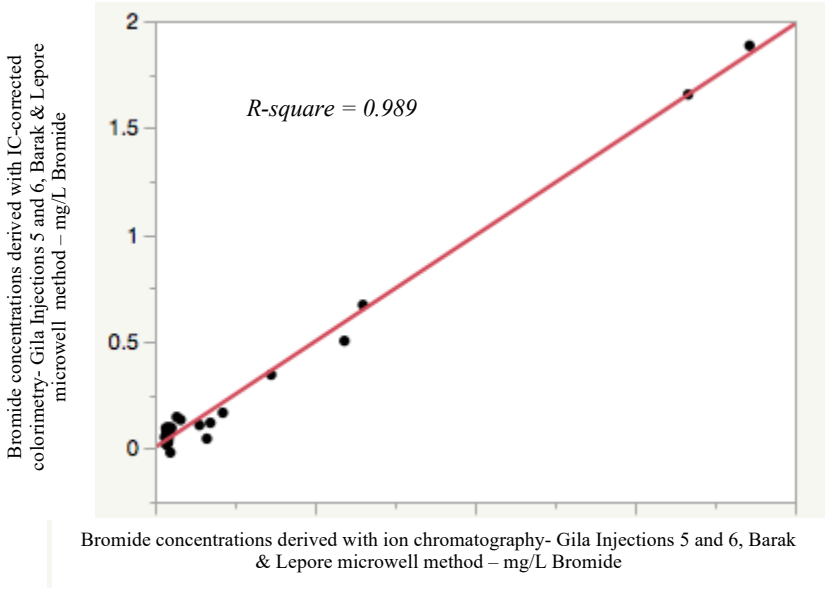


Figure 35: For the Barak & Lepore microwell colorimetric method analysis of Gila Injections 5 and 6, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived from correcting absorbance to fit an IC-informed breakthrough curve – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

A Rio Grande tracer experiment on October 19 was analyzed comparing 3 IC concentrations of water samples to the absorbances of the same 3 water samples, because the standards used by both the IC and myself for colorimetry contained an error and had to be remade. The original results, derived from the IC lab standard calibration curve, showed an extremely condensed breakthrough curve (figure 36):

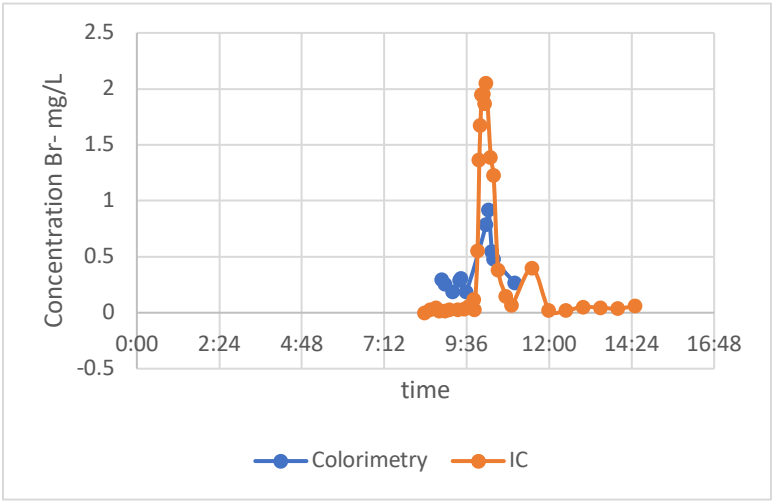


Figure 36: Barak & Lepore macroscale colorimetric method used to derive bromide concentrations along a breakthrough curve during a tracer injection experiment in the Rio Grande. Concentrations were derived from a calibration curve which had faulty standards, and outcomes are very weak. Concentrations are plotted here compared to IC concentrations for the same samples.

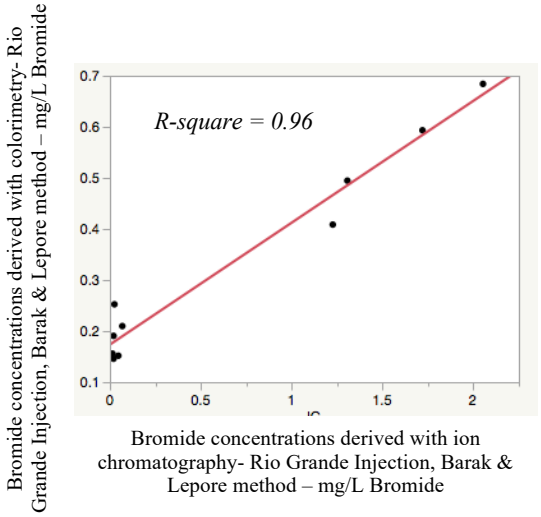


Figure 37: For the Barak & Lepore macroscale colorimetric method analysis of a Rio Grande Injection, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

The R-square from this comparison is 0.96.

Correcting the absorbances read in each sample with the corresponding IC concentration reading (regression found in figure 38), the breakthrough curves matched as follows (figure 39):

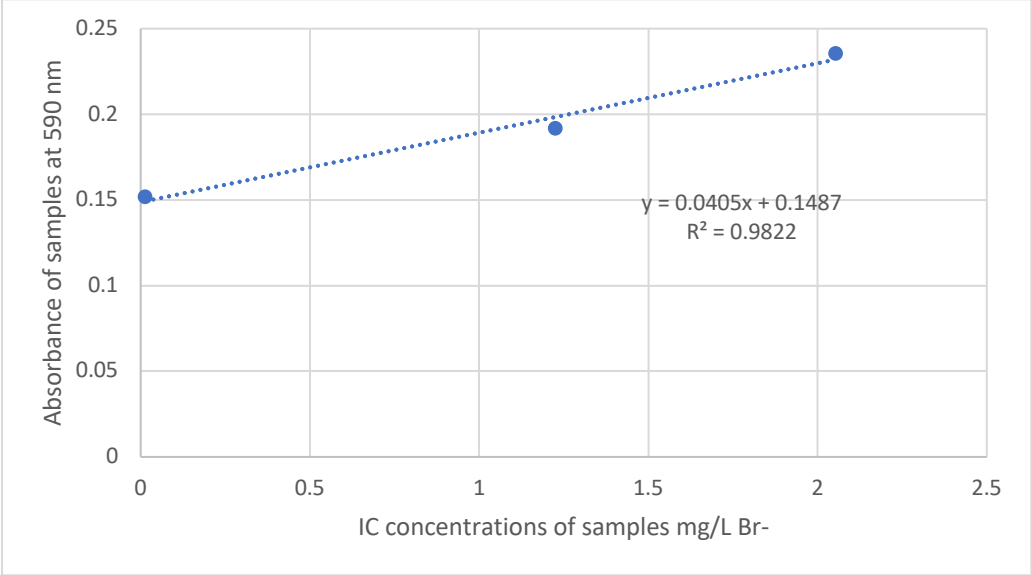


Figure 38: IC concentrations versus corresponding absorbances of three samples, Rio Grande tracer injection

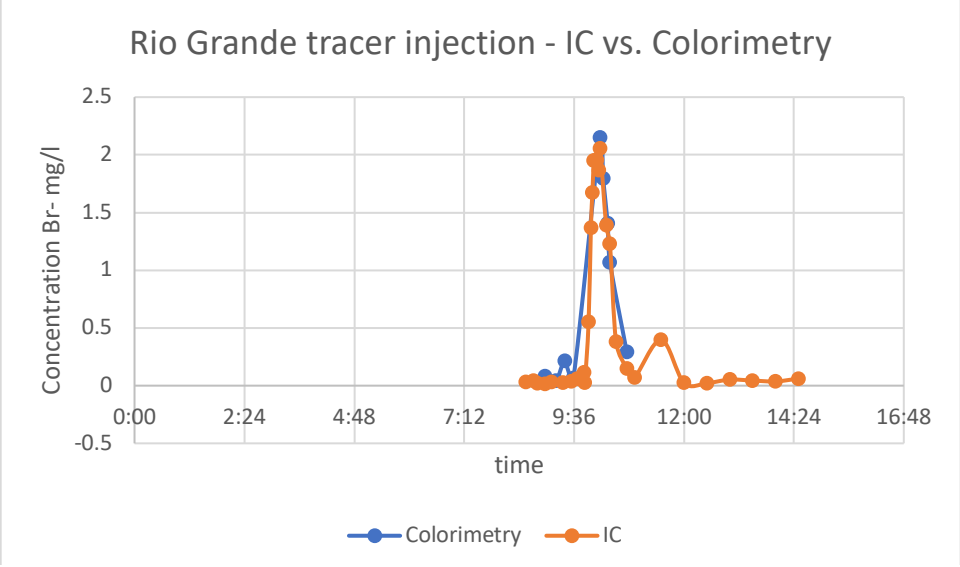


Figure 39: IC-corrected colorimetric analysis of Rio Grande Injection using Barak & Lepore macro method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

Because the APHA method performed badly in my Gila 5 and 6 experiments, I excluded it from the IC-correction round of data analysis. Because APHA calls for the preparation of standards up to 800 ppm,

that method is likely geared more towards analysis of waters containing extremely high concentrations of bromide – for example, wastewater treatment plant discharge analysis. Industrial/oil-field brine discharges make large contributions of bromide to water resources, and seawater intrusion and sea-spray affected precipitation can foster an increase in bromide in natural waters – in general, bromide content in drinking water does not surpass 1 mg/L.

The minimum detection limit described in the methodology is 0.1 mg/L. In that case, plotting concentration values which fall below the true minimum detection limit should be given a value of half MDL, since technically those concentrations would not be detectable, and negative concentrations are not possible.

Aliquot correction and standard addition method

Per recommendation by Bruce Thomson, I tested to ensure that the pipettes used were calibrated correctly to deliver the right amount of reagent to my cuvettes and microwells. This was done by pipetting ultrapure water onto a clean and dry petri dish, tared on a scale. 20 uL of water should weigh 20 mg, and correspondingly for the rest of the volumes. The pipette used to add amounts of 20, 75, and 80 uL of reagent did prove to deliver amounts very slightly lower than the amount desired: on average, a 20 uL pipette-ful weighed about 19.7599 mg. A different pipettor was then used for the remainder of the experiments. The pipette which measured 1000 uL, for adding aliquots of standard and sample, delivered exactly the correct amount of solution to the petri dish and was retained for use.

As mentioned above, I was concerned that the concentrations I derived from my calibration curves, created with standards using ultrapure water, were not very accurate when compared to the concentrations calculated through ion chromatographic analysis. Natural waters often contain constituents which have a certain absorbance of their own, which could falsely inflate the absorbances of the samples which I analyzed. Because of the tiny concentrations I was seeking to analyze, the addition of even seemingly negligible amounts of absorbance to samples could render them incomparable to IC samples. I followed the "standard addition method," wherein a sample of unknown concentration is added to different cuvettes in the same volume, followed by an aliquot of the same volume of DI water, followed by progressively higher standards in each (see figure 40). This should theoretically create a matrix by mixing standard and sample, offsetting any interference – for example, by the chloride interference of producing chlorophenol blue.

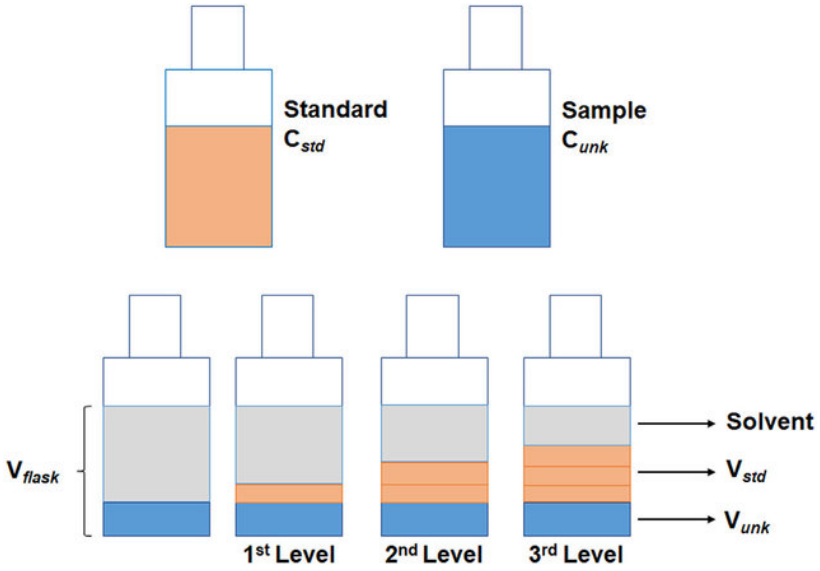
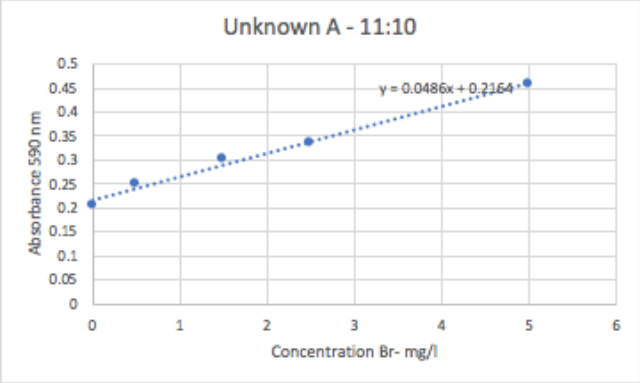


Figure 40: Schema of the standard addition method, wherein the standard is added directly to the aliquots of analyzed sample, This method is "used in situations where sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach" – for example, where inference by chloride might render standard and sample absorbances dissimilar for the same concentrations. (Zellmer 1998, Harris 2003)

Using four samples of unknown concentrations from a tracer injection experiment performed in the Rio Grande on October 19, 2018, I added in succession to each cuvette:

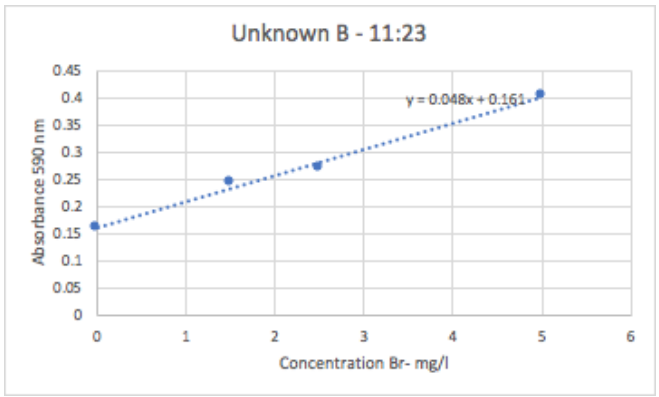
1. 0.5 mL sample A, B, C, and D
2. 0.5 mL standards of the following concentrations (resulting in five "A" cuvettes with successively increasing concentrations, and five cuvettes for each B, C, and D samples:
    a. 0 mg/L
    b. 0.5 mg/L
    c. 1.5 mg/l
    d. 2.5 mg/L
    e. 5 mg/L
3. 37.6 uL Phenol Red + buffer and 37.6 uL Chloramine-t

The following new standard calibration curves were derived, with the absolute value of their x-intercepts and corresponding IC concentrations, from prior analysis, below each.



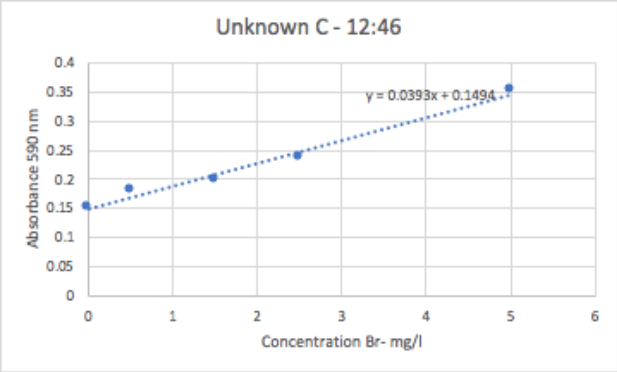
Extrapolated x-intercept absolute value, Unknown A sample at 11:10 (assigned concentration of sample)	4.452674897
IC value – concentration of 11:10 sample, Br- mg/L	1.44

Figure 41: calibration curve created with standard addition method, unknown concentration A, a sample taken during the Rio Grande tracer injection experiment at 11:10



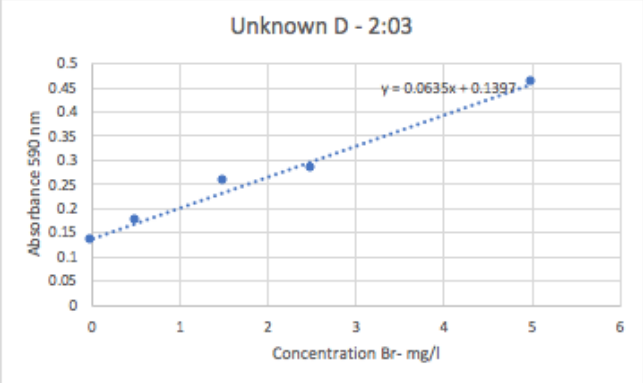
Extrapolated x-intercept absolute value, Unknown B sample at 11:23 (assigned concentration of sample)	3.3541666
IC value – concentration of 11:23 sample, Br- mg/L	0.777

Figure 42: calibration curve created with standard addition method, unknown concentration B, a sample taken during the Rio Grande tracer injection experiment at 11:23



Extrapolated x-intercept absolute value, Unknown C sample at 12:46 (assigned concentration of sample)	3.8015
IC value – concentration of 12:46 sample, Br- mg/L	0.048

Figure 43: calibration curve created with standard addition method, unknown concentration C, a sample taken during the Rio Grande tracer injection experiment at 12:46



Extrapolated x-intercept absolute value, Unknown D sample at 2:03 (assigned concentration of sample)	2.2
IC value – concentration of 2;03 sample, Br- mg/L	0.054

Figure 44: calibration curve created with standard addition method, unknown concentration D, a sample taken during the Rio Grande tracer injection experiment at 2:03

The absolute value of the x-intercept is the concentration of Br- in the unknown samples, if the trendline were extended left of the y-axis to reach the point of interception on the x-axis, as in figure 45.

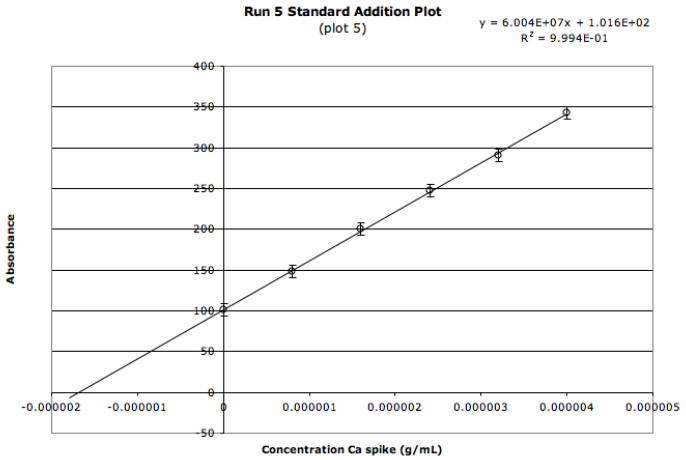


Figure 45: An example standard addition plot (Source: WikiFiles). The point at zero concentration (y-intercept) is the reading of the unknown, and the point of x-interception, when the absolute value is taken, should be the concentration of the unknown..

The standard addition method yielded far higher concentrations than were present in the unknown samples being analyzed, perhaps due to the mentioned cumulative errors – tiny aliquots of reagent, interference by chloride, and bromide concentrations below the level of detection, possibly.

Response factor determination and application

To determine what background of bromide is present in a baseline sample and how that affects absorbance's relation to the ultrapure standard calibration curve, I prepared a response factor determination experiment, using the Barak & Lepore method to prepare reagents.

Original results for the following analysis of a Rio Grande tracer injection experiment showed the customary inflation of concentration values for a tracer experiment performed in the Rio Grande on October 19, 2018 (figure 46).

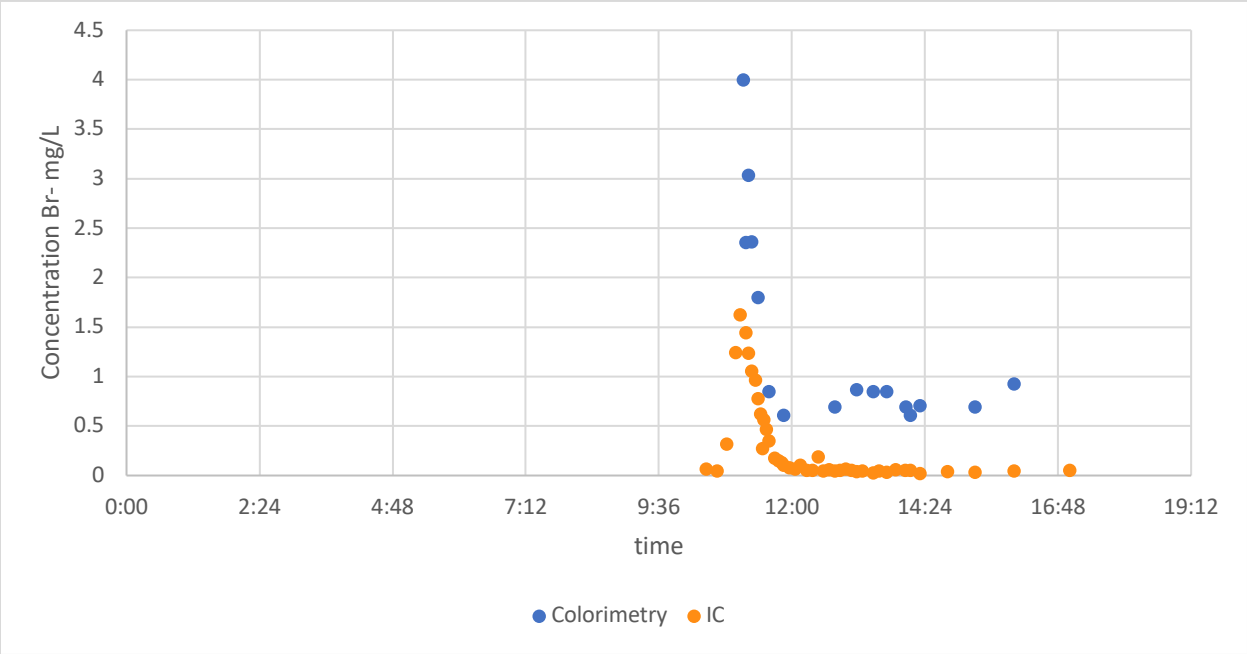


Figure 46: Barak & Lepore macroscale colorimetric method used to derive bromide concentrations along a breakthrough curve during a tracer injection experiment in the Rio Grande. Concentrations are plotted here compared to IC concentrations for the same samples.

The determination of a "correction," or response factor procedure was as follows:

- Read the absorbance of 5 standards at 590 nm wavelength
- Derive calibration curve (Figure 47)

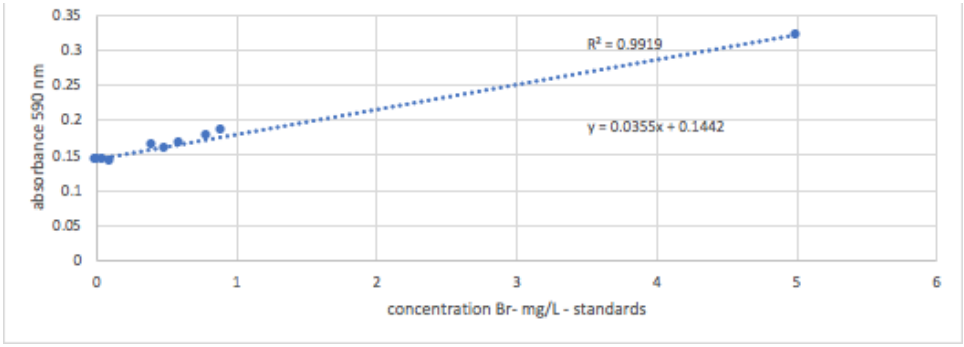


Figure 47: Calibration curve created from ultrapure standards of known bromide concentratin

- Read the absorbance of a sample of filtered river water with a known quantity of bromide (1 mg/L), plot on calibration curve. No reagents were added during this step, to set the instrument to zero.
- Add reagents (ammonium acetate buffer, phenol red, chloramine-t, following the Barak & Lepore method), mix and let color develop for 20 minutes.
- Read new absorbance of sample.
- Plot absorbance of sample on calibration curve to derive concentration.
- Compare calibration curve concentration with known concentration.

average absorbance (reagents added)	known concentration	concentration from calibration curve
0.2389	1 mg/L	2.698591549

- Take ratio of the known quantity absorbance to the blank absorbance.
  - 1/2.699 = 0.37
- Apply correction factor to all absorbances and derive new concentrations (Figure 48).

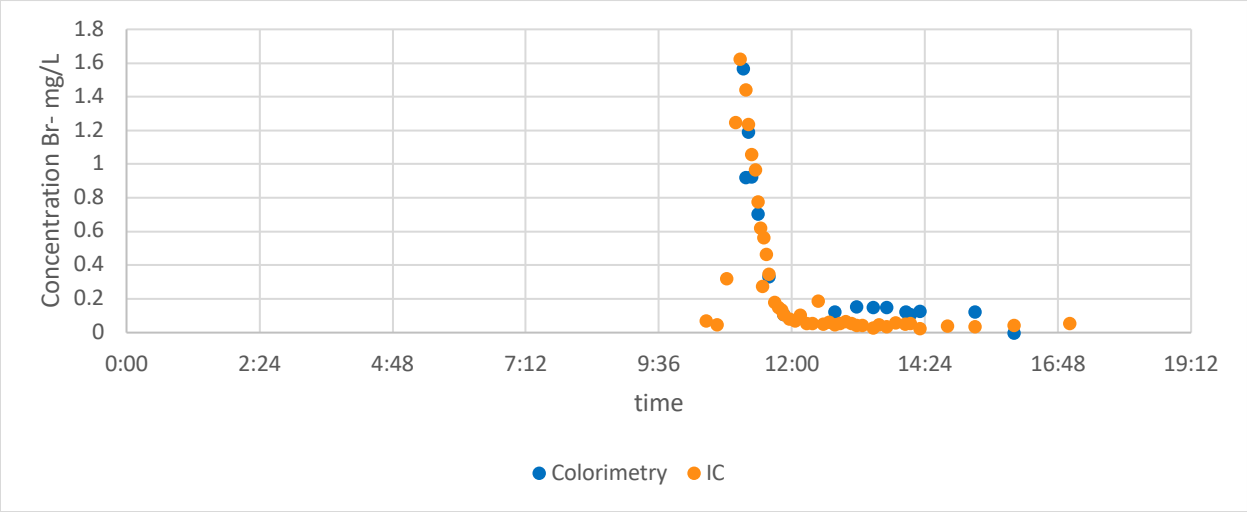


Figure 48: Breakthrough curve from Rio Grande tracer injection, colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

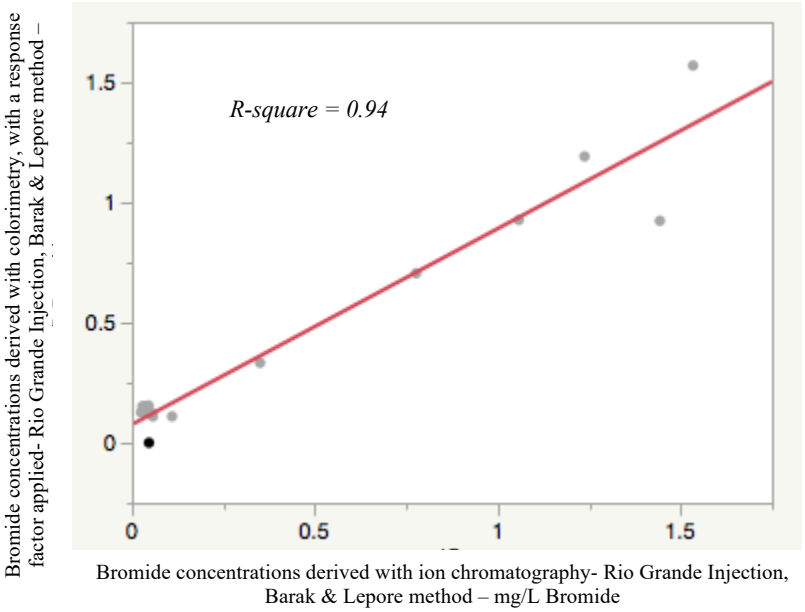


Figure 49: For the Barak & Lepore macroscale colorimetric method analysis of a Rio Grande Injection, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Because the falling limb concentrations remained above 1 mg/L even with a correction factor, the R-square of fitting colorimetry by IC was 0.94.

Another experiment aiming to determine an offset – or "normalize" data, was done with the re-running of Gila Injection 4 with the Barak and Lepore macro method and a filtered river water sample with known concentration of 0.9 mg/L (figure 50) - the resulting R-square was 0.96.

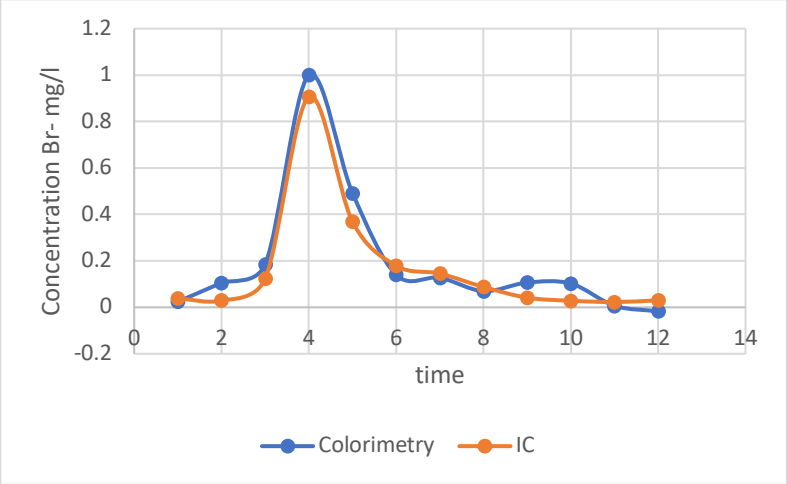


Figure 50: Breakthrough curve from Gila River Injection 4 tracer injection, showing colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

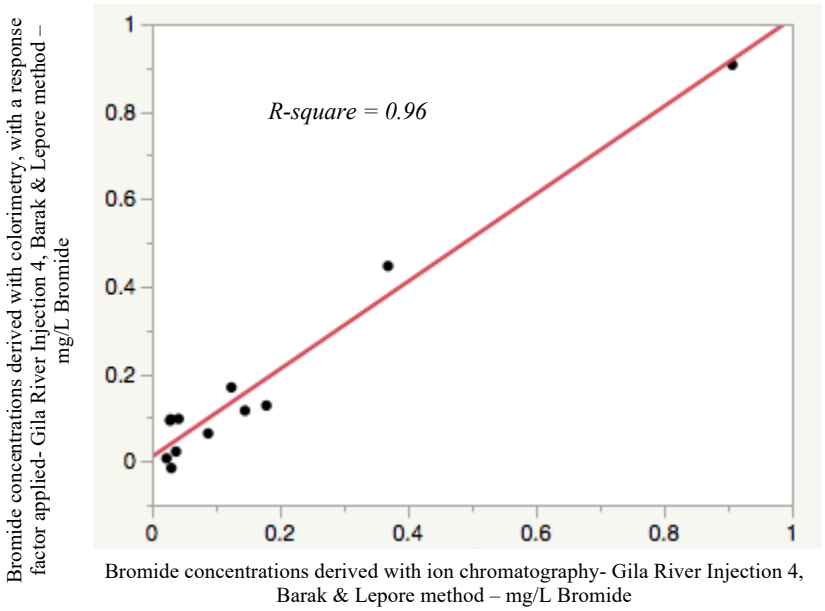


Figure 51: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 4, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis, containing concentrations from colorimetric analysis of tracer injection samples, corrected with a "response" factor which takes into account the offset of colorimetric values, to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Finally, a determination of the response factor experiment was performed with Barak and Lepore's method using Gila Injection 5 (figure 52):

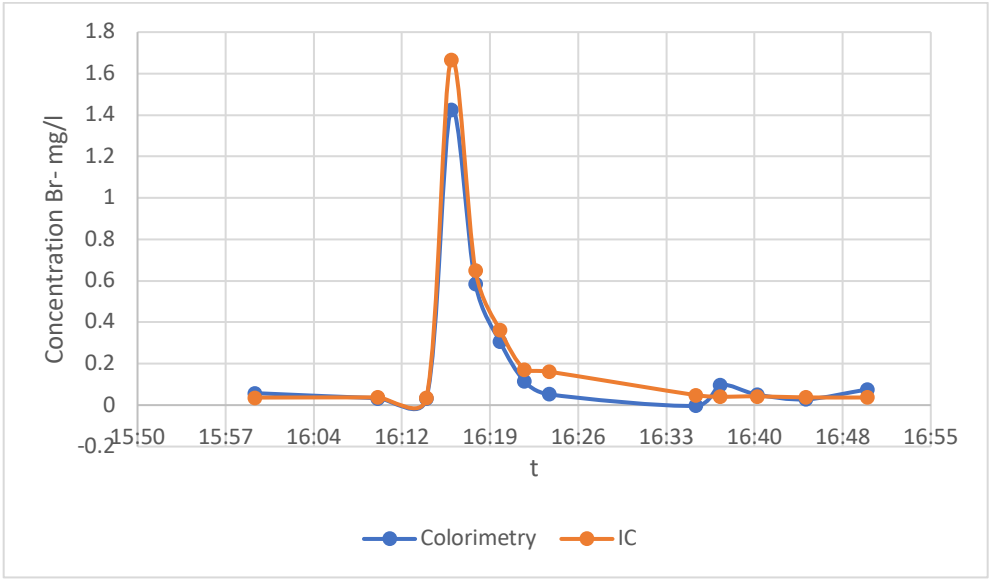


Figure 52: Breakthrough curve from Gila River Injection 5 tracer injection, showing colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

Combined, an x fit to y analysis of the approximation of colorimetric concentrations to IC concentrations for this method shows an R-square of 0.99.

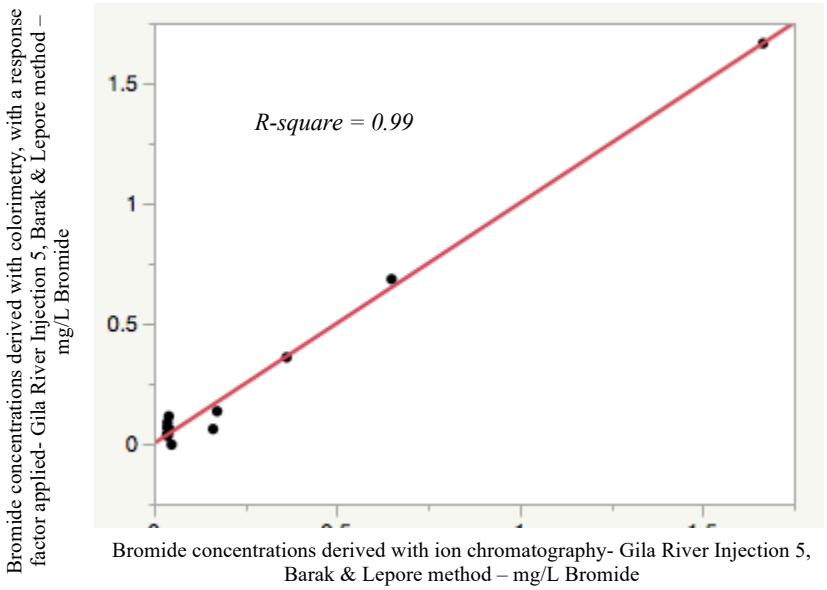


Figure 51: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis, containing concentrations from colorimetric analysis of tracer injection samples, corrected with a "response" factor which takes into account the offset of colorimetric values, to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Creating a non-dimensional absorbance breakthrough curve

An addition option to use colorimetry to predict IC concentration values when detecting bromide concentration levels is to transform a breakthrough curve, created with absorbance values, to a non-dimensional curve, with the peak at 1. To do this, each value of the curve should be divided by the maximum value of the curve (peak concentration). An example of this first step is shown in Figure 52, using absorbance values from Gila Injection 5, the Barak & Lepore method in the microscale.

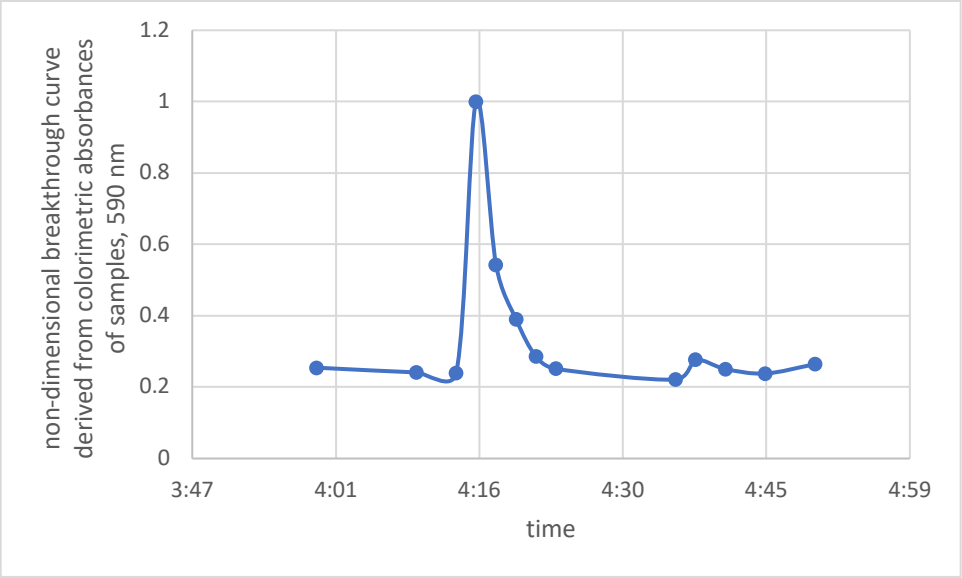


Figure 52: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the non-dimensional breakthrough curve derived by dividing each absorbance obtained per sample by the maximum of those absorbances. The peak of the curve is exactly "1."

Next, the IC-derived concentrations at the peak is measured, as well as the IC measurements at two more points on the rising and falling limbs of the breakthrough curve, and those concentrations are plotted against the non-dimensional figures corresponding to them. The equation from the trendline is then used to transform the non-dimensional breakthrough curve into one which reflects the real-world concentrations, relying on just three accurate point from the IC (Figure 53).

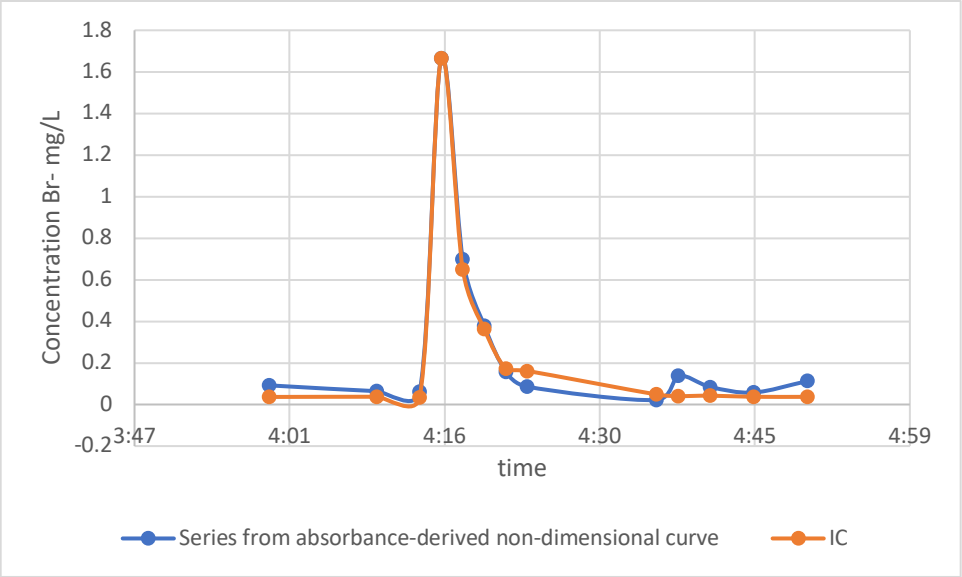


Figure 53: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the breakthrough curve derived by plotting non-dimensional points against IC concentrations, using the trendline equation to transform non-dimensional values in bromide concentrations.

Conclusion

Because of the repeated attempts to approximate the concentrations determined by IC analysis were not, in my estimation, sufficiently proximal, the strongest alternative to fulling using ion chromatography for every sample taken in the field would be to correlate three or four IC concentrations with the absorbances read from the corresponding samples in the spectrophotometer. With each sample running about 20 minutes in the IC, and a full collection of samples (40-60 samples) taking approximately 40 minutes to complete, plus an hour and a half, on average, for reagent and sample prep time, analysis could be completed within three hours. An average lamp fee on UNM's campus is $25 per hour, and running samples in the IC costs about $10 per sample. This combination of methods would also cost far less than running 60 samples on the IC, coming to the range of $55 to $75.

Using the correction factor/quenching determination yielded good results for the three trials to which it was applied; however, the colorimetric analysis of some sets of samples yielded results which were not uniformly offset from IC values in one direction alone – for example, the macro Barak and Lepore method for Gila Injection 5 yielded a peak concentration lower than the IC peak concentration, and rising/falling limbs higher than those of the IC. This "normalization" method could also depend heavily on the reliability of the check standard (natural water spiked with bromide which is measured before and after reagents are added), and would present an opportunity for error.

The tenuous relationship between the IC and colorimetric concentrations may originate from the interference of chloride, which, with bromide, is a member of the halide family – also including fluorine (F), iodine (I), and astatine (At). These halogens are lacking one electron in their outer shell, possessing seven – lending them the trait of combining readily with other elements. When a halogen bonds with another agent – for example, sodium chloride (NaCl) - a halide is formed.

While occasionally good agreement was reached, the presence of other constituents in the water – for example, the additional phosphate, nitrate, and chloride tracers used in conjunction with bromide, might decrease agreement for a injection experiment on the same reach of river.

Chloride, in particular, can majorly interfere in spectrophotometric analysis due to – as discussed – the formation of chlorophenol blue, which can have an absorbance of its own high enough to offset the absorbance readings for bromophenol blue. The addition and omission of sodium thiosulfate, which should theoretically mask chloride interference, may not sufficiently mask the background present in the samples analyzed in this study. Further methods to eliminate chloride interference are discussed in the following section highlight future areas of potential study.

A final source of error may arise from the use of 590 nm wavelength to measure the absorbance of light into the samples being analyzed colorimetrically. 590 nm falls along the "visible" light spectrum – a much broader spectrum than ultraviolet light, for example, which has a much sharper range (figure 54). Absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. This broader spectrum could invite the interference of other constituents with their own absorbance.

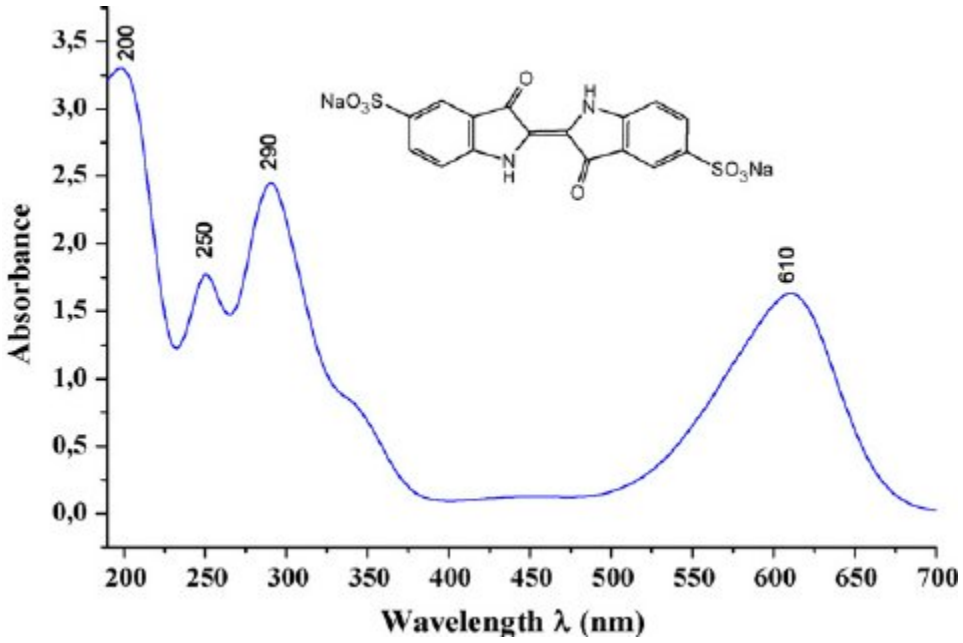


Figure 54: Structure and UV–vis spectrum of indigo carmine dye. This displays the width of light waves, with the visible light wave (at 610 nm) being the broadest.

Potential future research

Due to the presumed interference from chloride, as mentioned, an approach to eliminate the chloride present in the sample is the precipitation of chloride from sample, with the addition of Silver Nitrate (AgNO3) to react with chloride and turn into Silver Chloride – AgCl2. The procedure would then include the filtration of the solution through a 0.45 micron filter, and then adding the phenol red and chloramine-t reagents. An alternative method using silver's reaction with chloride, which would be effective for smaller numbers of samples, would be filtration through silver cartridges – priced at about $1.50 each. In labs where an ion chromatography instrument is not available, this procedure could prove effective in eliminating interference with chloride.

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List of Figures

Figure 32: For the Barak & Lepore colorimetric method analysis of Gila Injection 4, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived from correcting absorbance to fit an IC-informed breakthrough curve – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 33: IC-corrected colorimetric analysis of Gila Injection 4 using Jones method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

Figure 34: IC-corrected colorimetric analysis of Gila Injections 5 (top) and 6 (bottom) using Barak & Lepore microscale method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

Figure 35: For the Barak & Lepore microwell colorimetric method analysis of Gila Injections 5 and 6, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis – the concentrations derived from correcting absorbance to fit an IC-informed breakthrough curve – to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 36: Barak & Lepore macroscale colorimetric method used to derive bromide concentrations along a breakthrough curve during a tracer injection experiment in the Rio Grande. Concentrations were derived from a calibration curve which had faulty standards, and outcomes are very weak. Concentrations are plotted here compared to IC concentrations for the same samples.

Figure 37: For the Barak & Lepore macroscale colorimetric method analysis of a Rio Grande Injection, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 38: IC concentrations versus corresponding absorbances of three samples, Rio Grande tracer injection

Figure 39: IC-corrected colorimetric analysis of Rio Grande Injection using Barak & Lepore macro method. Absorbance values for three samples and their corresponding IC concentrations were plotted against each other in a calibration curve, and the equation from the resulting trendline is used to calculate concentrations from absorbances.

Figure 40: Schema of the standard addition method, wherein the standard is added directly to the aliquots of analyzed sample, This method is “used in situations where sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach” – for example, where inference by chloride might render standard and sample absorbances dissimilar for the same concentrations. (Zellmer 1998, Harris 2003)

Figure 41: calibration curve created with standard addition method, unknown concentration A, a sample taken during the Rio Grande tracer injection experiment at 11:10

Figure 42: calibration curve created with standard addition method, unknown concentration B, a sample taken during the Rio Grande tracer injection experiment at 11:23

Figure 43: calibration curve created with standard addition method, unknown concentration C, a sample taken during the Rio Grande tracer injection experiment at 12:46

Figure 44: calibration curve created with standard addition method, unknown concentration D, a sample taken during the Rio Grande tracer injection experiment at 2:03

Figure 45: An example standard addition plot (Source: WikiFiles). The point at zero concentration (y-intercept) is the reading of the unknown, and the point of x-interception, when the absolute value is taken, should be the concentration of the unknown.

Figure 46: Barak & Lepore macroscale colorimetric method used to derive bromide concentrations along a breakthrough curve during a tracer injection experiment in the Rio Grande. Concentrations are plotted here compared to IC concentrations for the same samples.

Figure 47: Calibration curve created from ultrapure standards of known bromide concentration

Figure 48: Breakthrough curve from Rio Grande tracer injection, colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

Figure 49: For the Barak & Lepore macroscale colorimetric method analysis of a Rio Grande Injection, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 50: Breakthrough curve from Gila River Injection 4 tracer injection, showing colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

Figure 51: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 4, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis, containing concentrations from colorimetric analysis of tracer injection samples, corrected with a "response" factor which takes into account the offset of colorimetric values, to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 52: Breakthrough curve from Gila River Injection 5 tracer injection, showing colorimetry derived bromide concentration vs. ion chromatography derived concentrations. Colorimetric concentrations have been multiplied by a response factor which takes into account the offset which inflated colorimetric concentrations

Figure 51: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the one-to-one regression analyzing the line of best fit, fitting the y (response) axis, containing concentrations from colorimetric analysis of tracer injection samples, corrected with a "response" factor which takes into account the offset of colorimetric values, to the x (independent) axis, which has the concentrations of the same samples derived from the IC.

Figure 52: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the non-dimensional breakthrough curve derived by dividing each absorbance obtained per sample by the maximum of those absorbances. The peak of the curve is exactly "1."

Figure 53: For the Barak & Lepore macroscale colorimetric method analysis of Gila River Injection 5, this figure displays the breakthrough curve derived by plotting non-dimensional points against IC concentrations, using the trendline equation to transform non-dimensional values in bromide concentrations.

Figure 54: Structure and UV–vis spectrum of indigo carmine dye. This displays the width of light waves, with the visible light wave (at 610 nm) being the broadest.