Temperature Dependence of Indophenol Method Color Development: Monochloramine, Free Chlorine, and Free Ammonia Concentration Impacts

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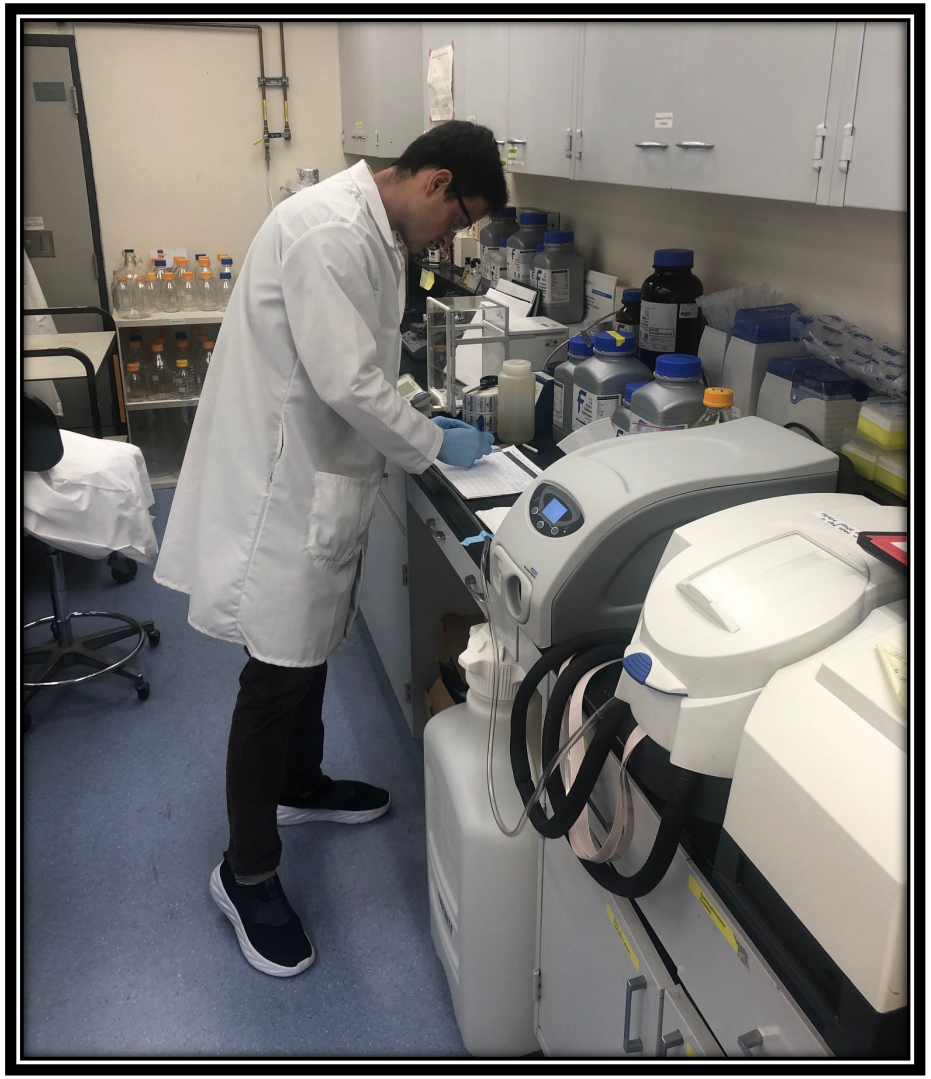


Background – EPA Method 127 Approval

- EPA Method 127 is a colorimetric method developed to measure monochloramine concentration in drinking water using commercially-available indophenol reagent and instrumentation.
  - Approved for determining disinfectant residual (i.e., both minimum and maximum residual at the entry point (EP) and in the distribution system (DS)) as required by the Surface Water Treatment Rule (SWTR) for systems using combined chlorine.
  - EPA Method 127 was approved through the Expedited Drinking Water Analytical Method Approval Process as an alternative to the total chlorine N,N-diethyl-p-phenylenediamine (DPD) colorimetric procedure described in Standard Method 4500-Cl G.
- For more information see *Monochloramine Measurement* column in October 2021 Journal AWWA or 2020 EPA Drinking Water Workshop.

Background – Method Validation

- As part of the expedited method approval process, multiple validation studies were conducted to compare the performance of EPA Method 127 to Standard Method 4500-Cl G.
- One study evaluated the correlation between sample temperature and rate of color development using indophenol reagent, as detailed in a recent article published in the January 2021 issue of *Water Practice & Technology.*
- Although this study focused on EPA Method 127, the same indophenol reagent is used in other commercial monochloramine (NH_2Cl), free ammonia (free NH_3), and free chlorine (free Cl_2) methods.

Experimental Approach – Sample Preparation

- NH_2Cl stock solutions were prepared from sodium hypochlorite and ammonium sulfate stock solutions in ultra-pure water in chlorine demand-free (CDF) glassware.
- Both NH_2Cl and free Cl_2 stock solutions were standardized prior to use by spectrophotometry using known molar absorptivity.
- NH_2Cl sample solutions were prepared at 2.0 mg/L, buffered with 0.2 mM potassium phosphate monobasic and pH adjusted to 9.0 with sodium hydroxide. Both low and high ionic strength water matrices were evaluated.
- Aliquots of NH_2Cl sample solutions were transferred headspace-free into CDF amber glass vials and kept in a circulating water bath at the desired temperature prior to analysis.

Circulating Water Bath with Temperature Control

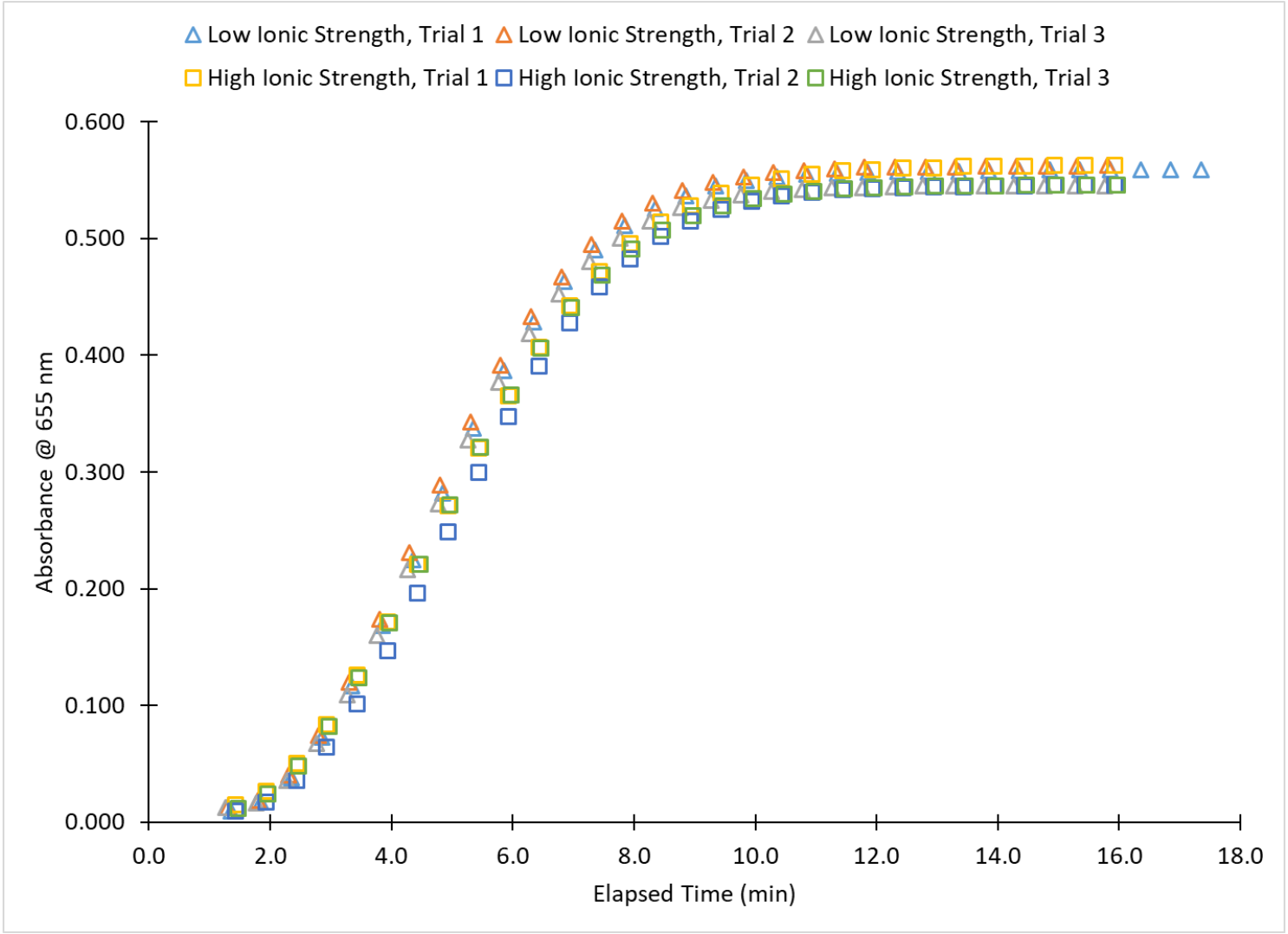
Experimental Approach – Sample Analysis

- The rate of indophenol reagent color development was evaluated at 5, 8, 10, 13, 15, 20, 25, and 30°C.
- A spectrophotometer equipped with a Peltier control and cooling unit was used to control sample temperature while measuring absorbance.
- After reagent was added to the sample, the amount of time taken to mix reagent and transfer to a cuvette (≈ 60 sec) was noted and added to the overall reaction time.
- Absorbance was continuously measured in 30-sec intervals at 655 nm until absorbance readings no longer increased (t_{100}) after five consecutive readings.
- To ensure quality control, the temperature of the water bath, sample, and Peltier unit were verified multiple times during each trial with a NIST-traceable thermometer.

Spectrophotometer Equipped with Peltier Temperature Control

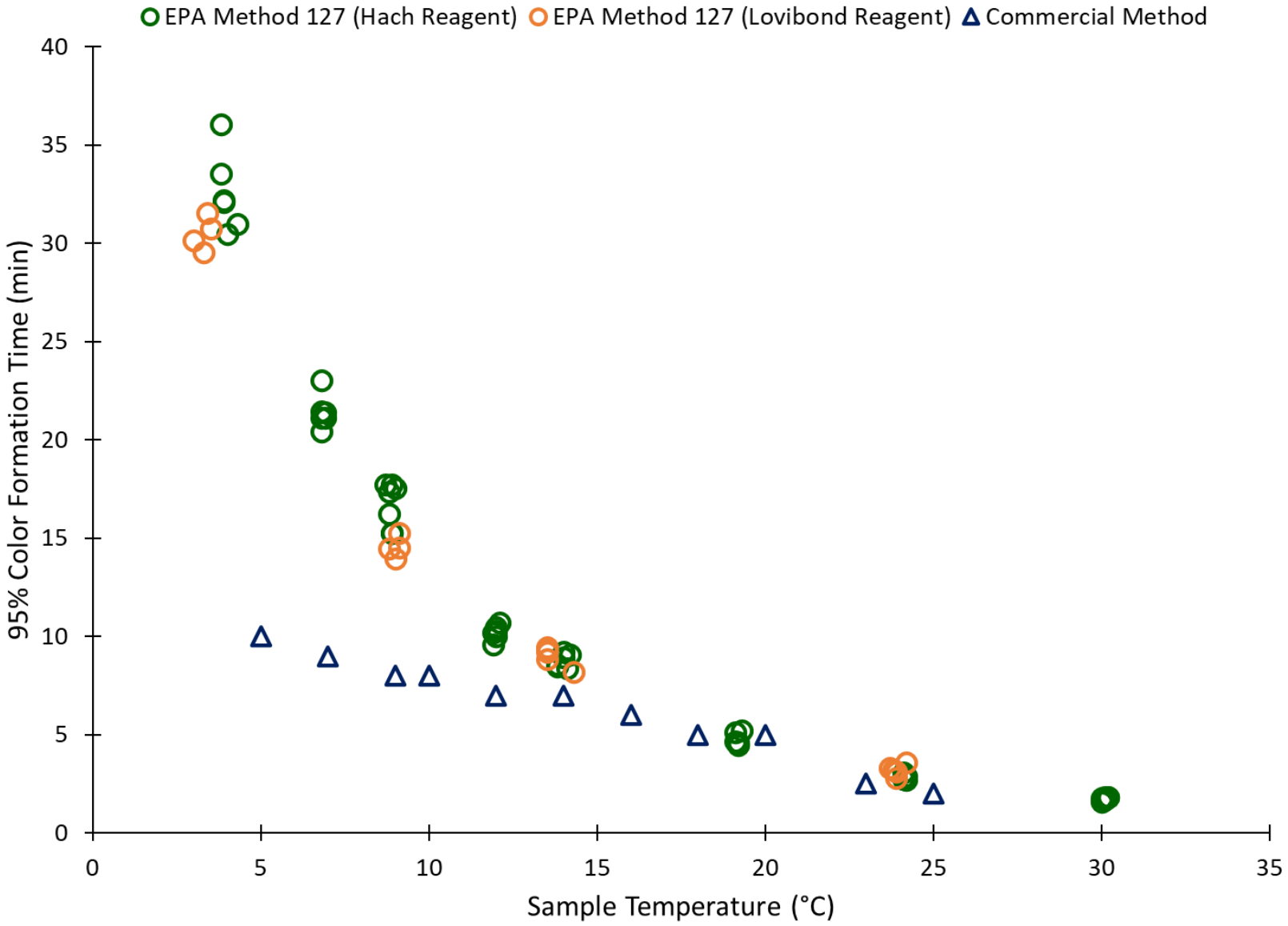
Results

- Experiments were conducted in triplicate in both low and high ionic strength matrices.
- Time series of absorbance readings from each experiment were generated.
- This figure shows data from the 15°C experiment, which is representative of all evaluated temperatures.



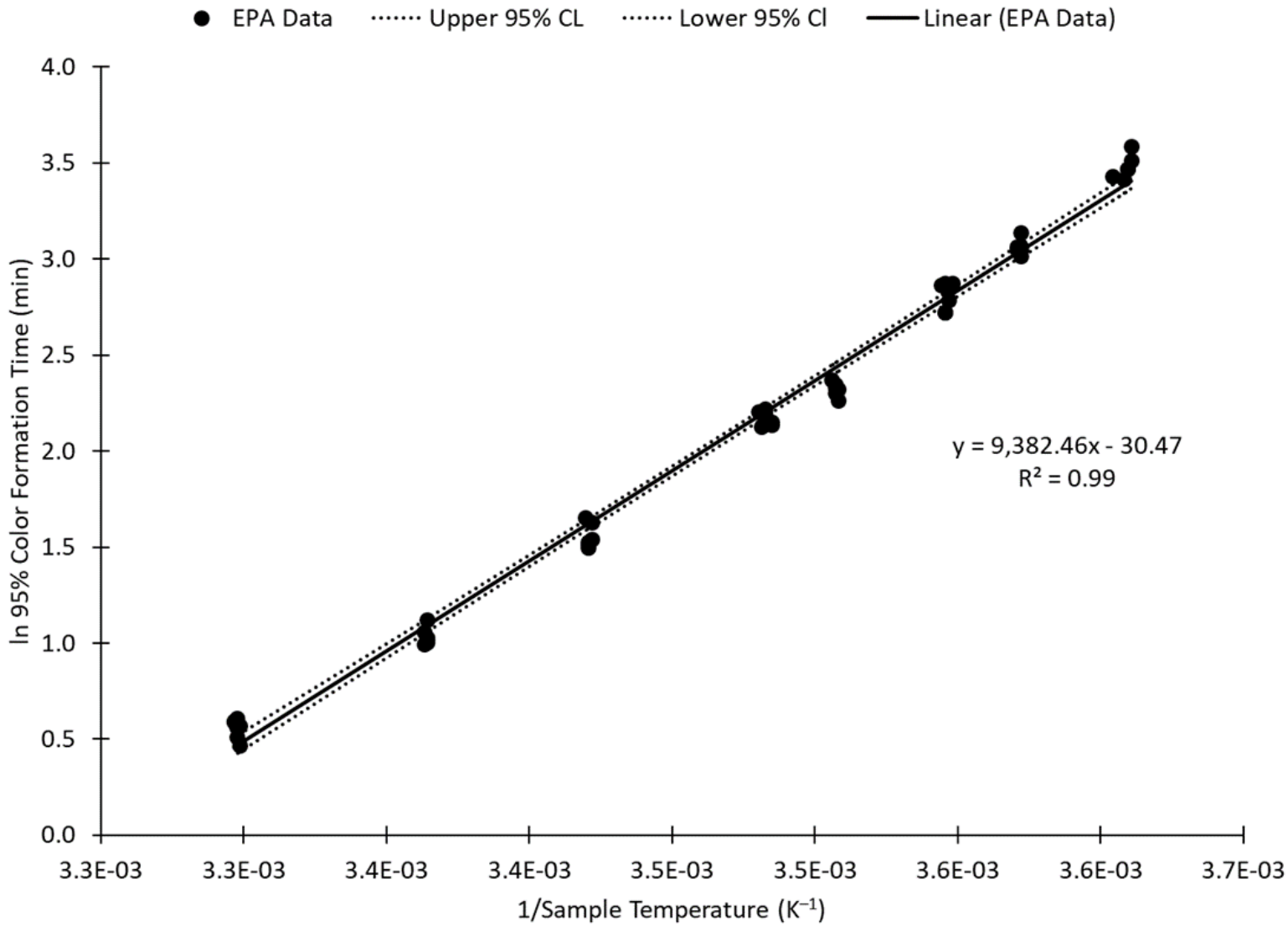
Results

- Color formation time differed below ≈20°C.
- Difference between observed (EPA) and suggested (commercial) reaction times increased as temperature decreased.
- Evaluated indophenol reagent from two manufacturers (Hach and Lovibond)
- Temperature and reaction time relationship varied:
  - EPA → exponential function
  - Commercial → linear function



Results

- Linear regression between the natural logarithm of 95% color formation time in units of minutes (t_{95}) and the reciprocal of *T* in units of Kelvin was used to calculate the required color formation time over the desired temperature range.



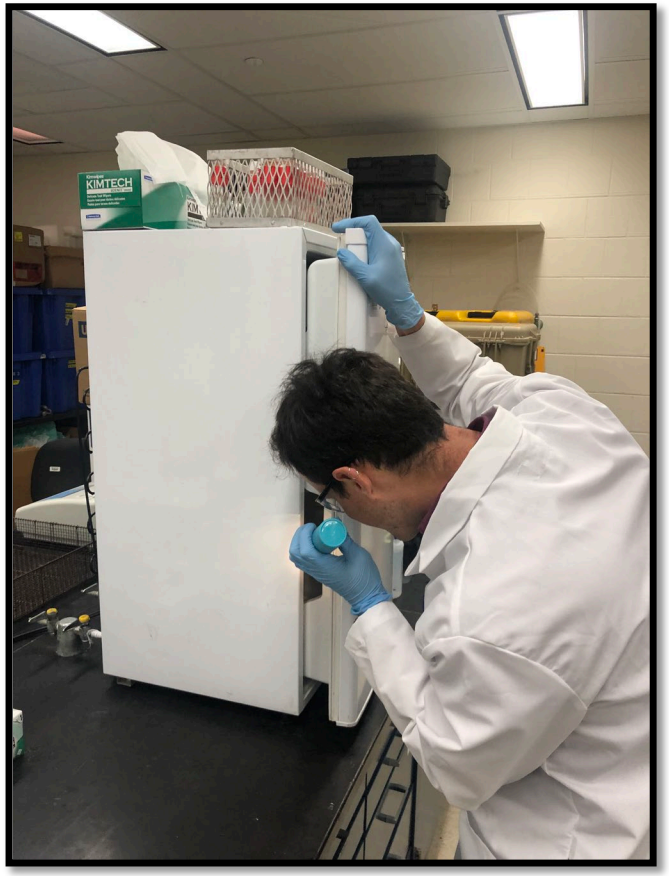
Results – Color Development Time

Sample Temperature (°C)	Sample Temperature (°F)	EPA Method 127 Color Development Time (min.)	Commercial Method Color Development Time (min.)
5	41	28	10
7	45	22	9
9	48	17	8
10	50	15	8
12	54	12	7
14	57	10	7
16	61	8	6
18	64	6	5
20	68	5	5
23	73	4	2.5
25	77	3	2
30	86	2	2

Portable Parallel Analyzer (PPA) Evaluation

- EPA Method 127 allows the use of a PPA.
- The influence of sample temperature on reaction time using a PPA was evaluated.
- According to the manufacturer, method reaction times and internal temperature control are fully automated.

Portable Parallel Analyzer (PPA) Evaluation



- A PPA and reagents were stored in a refrigerator (≈5°C) for approximately 12 hours prior to analysis to allow temperatures to stabilize.
- Another PPA and reagents were left at room temperature (≈20°C) as a control.
- NH_2Cl samples were prepared at 2.0 mg/L in both low ionic strength (LIS) and high ionic strength (HIS) water matrices at ≈5°C and placed in the same refrigerator.
- The refrigerated samples were then analyzed using both PPAs (i.e., one at ≈5°C and the other at ≈20°C).

Portable Parallel Analyzer (PPA) Evaluation

Sample	Monochloramine (mg/L) Measured at ≈5°C	Monochloramine (mg/L) Measured at ≈20°C	Sample Reaction Time (mm:ss) ≈5°C	Sample Reaction Time (mm:ss) ≈20°C
LIS #1	2.02	2.02	6:40	6:40
LIS #2	2.03	2.01	6:50	6:50
LIS #3	2.03	2.00	6:40	6:40
HIS #1	2.03	1.93	6:40	6:40
HIS #2	2.01	1.97	6:40	6:40
HIS #3	2.03	2.00	6:50	6:50

- Sample reaction times were the same under both conditions (≈5°C and ≈20°C).
- Results suggest that the internal temperature control of the PPA controlled the influence of sample temperature on reaction time.

Indophenol Free Cl_2 and Free NH_3 Methods

- Indophenol free Cl_2 and free NH_3 methods were not directly evaluated in this study but are likely influenced by sample temperature because they use the same reagent.
- Free Cl_2 Method Summary (at 20°C)
  - Sample Cell #1 (Blank) – Add indophenol reagent (wait 5-min)
  - Sample Cell #2 (Sample) – Add ammonia solution to convert free Cl_2 to NH_2Cl (wait 5-min), then add indophenol reagent (wait 5-min)
- Free NH_3 Method Summary (at 20°C)
  - Sample Cell #1 (Blank) – Add indophenol reagent (wait 5-min)
  - Sample Cell #2 (Sample) – Add chlorine solution to convert free NH_3 to NH_2Cl (wait 5-min), then add indophenol reagent (wait 5-min)

Practical Implications on Free NH_3

- Reaction times presented in commercial indophenol methods are too short at temperatures < 20°C, which could result in variable free NH_3 measurements (either biased low or high).
- Consider a sample with 2.0 mg/L NH_2Cl and 0.25 mg/L free NH_3 at 15°C:

Scenario	Did blank reach full color development?	Did sample reach full color development?	"Measured"† Free NH_3 Concentration (mg/L)	Percent of Sample Free NH_3 Concentration
#1	No	No	0.17	68% (0.17/0.25)
#2	Yes	No	0.06	24% (0.06/0.25)
#3	No	Yes	0.35	140% (0.35/0.25)
#4	Yes	Yes	0.24	96% (0.24/0.25)

No = Indicates using commercial method reaction time of 6 minutes at 15°C.
Yes = Indicates using EPA Method 127 reaction time of 9 minutes at 15°C.
† = Theoretically determined based on stoichiometry and experimentally determined rate of color formation at 15°C .

Practical Implications on NH_2Cl and Free Cl_2

- Reaction times presented in commercial indophenol methods are too short at temperatures < 20°C, which could under quantify actual NH_2Cl and free Cl_2 concentrations.
  - Although unlikely, the presence of NH_2Cl in the blank could over quantify actual free Cl_2.
- Two factors may increase sample temperatures when conducting outdoor field analysis at ambient temperatures < 20°C:
  - Sample analysis may occur in a field vehicle at a warmer ambient temperature;
  - Physical handing of samples.
- If low sample temperature is a concern (< 20°C) and/or analysis is conducted in cold weather, consider measuring the prepared sample with reagent again 5 to 10 minutes after the specified reaction time to confirm color formation is complete.

Future Work

- Directly evaluate the impact of sample temperature on indophenol free chlorine and free ammonia methods.

Summary

- Sample reaction time for full color development in colorimetric indophenol methods are strongly influenced by sample temperature, regardless of reagent manufacturer.
- Below 20°C, times needed to reach full color development were greater than reported in commercial methods, reaching nearly three times longer at 5°C.
- To avoid measurement errors of samples analyzed below 20°C, use of reaction times determined in this study is recommended for commercial indophenol methods.

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Questions?

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