10-2018

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Food research international 112, 443-456

https://doi.org/10.1016/j.foodres.2018.06.056

Chemical stability and reaction kinetics of two thiamine salts (thiamine mononitrate and thiamine chloride hydrochloride) in solution

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Declarations of interests: none

1

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Abstract

Two types of thiamine (vitamin B_1) salts, thiamine mononitrate (TMN) and thiamine chloride hydrochloride (TClHCl), are used to enrich and fortify food products. Both of these thiamine salt forms are sensitive to heat, alkali, oxygen, and radiation, but differences in stability between them have been noted. It was hypothesized that stability differences between the two thiamine salts could be explained by differences in solubility, solution pH, and activation energies for degradation. This study directly compared the stabilities of TMN and TClHCl in solution over time by documenting the impact of concentration and storage temperature on thiamine degradation and calculating reaction kinetics. Solutions were prepared containing five concentrations of each thiamine salt (1, 5, 10, 20, and 27 mg/mL), and three additional concentrations of TClHCl: 100, 300, and 500 mg/mL. Samples were stored at 25, 40, 60, 70, and 80°C for up to 6 months. Degradation was quantified over time by high-performance liquid chromatography, and percent thiamine remaining was used to calculate reaction kinetics. First-order reaction kinetics were found for both TMN and TClHCl. TMN degraded significantly faster than TClHCl at all concentrations and temperatures. For example, in 27mg/mL solutions after 5 days at 80°C, only 32% of TMN remained compared to 94% of TClHCl. Activation energies and solution pHs were 21-25 kcal/mol and pH 5.36-6.96 for TMN and 21-32 kcal/mol and pH 1.12-3.59 for TClHCl. TClHCl degradation products had much greater sensory contributions than TMN degradation products, including intense color change and potent aromas, even with considerably less measured vitamin loss. Different peak patterns were present in HPLC chromatograms between TMN and TClHCl, indicating different degradation pathways and products. The stability of essential vitamins in foods is important, even more so when degradation contributes to sensory changes, and this study provides a direct comparison of the

stability of the two thiamine salts used to fortify foods in environments relevant to the processing and shelf-life of many foods.

Key Words

Thiamine, vitamin B_1, chemical stability, degradation, reaction kinetics, activation energy, pH, sensory, thiamine mononitrate, thiamine chloride hydrochloride

1. Introduction

Vitamin B_1, also known as thiamine (Figure 1), is an essential micronutrient in the human diet that is found both naturally and as a fortification supplement in many foods. Thiamine acts as a coenzyme for metabolism of carbohydrates and branched-chain amino acids and has roles in digestion, the nervous system, and muscle contraction (Institute of Medicine, 1998). Thiamine deficiency persists in both developing and developed countries. In developing countries, a lack of nutritious food or nutritional variety, which may occur when unfortified grains such as polished rice are the main dietary component, are the main contributors to thiamine deficiency, which is found in up to 25% of the population (Ball, 2006; Prinzo, 1999). In developed countries, where fortification efforts have reduced overall rates of thiamine deficiency to near 10%, deficiency is more likely found in alcoholics, people on strict weight loss diets, and people avoiding consumption of fortified grain products, including those with Celiac's disease (Ball, 2006; Shepherd & Gibson, 2013). Thiamine deficiency can cause both minor symptoms, such as fatigue, insomnia, irritability, and other neurological indicators, as well as severe diseases resulting from prolonged deficiency, e.g., Beriberi and Wernicke-Korsakoff syndrome (Spitzer & Schweigert, 2007). Thiamine stores in the body are very small and last only weeks, which contributes to the concern of deficiency (Baumgartner, Henderson, Fox, & Gondi, 1997). The Recommended Dietary Allowance (RDA) and Daily Value (DV) for thiamine in the U.S. are both 1.2 mg/day(Institute of Medicine, 1998; U.S. Food & Drug Administration, 2018). To combat the likelihood of deficiency, thiamine salts are often used to enrich and fortify many food and beverage products.

Thiamine is found naturally in foods, such as meats, yeast, whole grains, nuts, pulses, and legumes, in a phosphorylated form, most commonly thiamine triphosphate (Gregory III, 2008).

Additionally, two salt forms are used as food additives: thiamine mononitrate* (TMN) and thiamine chloride hydrochloride (TClHCl) (Figure 1). TMN is a mono-salt, with only one nitrate anion present, and TClHCl is a di-salt with two chlorides present. TClHCl is often interchangeably called 'thiamine hydrochloride' (Ash, 2008); however, it is important to note that the molecular formula contains two chlorides ($C_{12}H_{17}ClN_4OS \bullet HCl$), as shown in Figure 1. While thiamine has two pK_as (pK_{a1} = 4.8 for the pyrimidine N1 and pK_a2 = 9.2 for the thiazole quarternary nitrogen (Edwards et al., 2017)), pK_{a1} is the only relevant pK_a for the majority of food products. Solid state properties of TMN and TClHCl differ widely from one another (Table 1). TMN is often used in dry food products due to its low hygroscopicity, and TClHCl is often used in liquid or beverage products due to its high solubility (Labuza & Kamman, 1982). The higher solubility of TClHCl compared to TMN is due to the higher free energy of the TClHCl crystalline salt form (Atkins & de Paula, 2006). The two salt forms also have substantial stability differences that have been explained by different activation energies, reported as 22.4 kcal/mol for TClHCl and 26.3 kcal/mol for TMN in solid state systems, with E_a decreasing as water activity (a_w) increased (Labuza & Kamman, 1982).

Thiamine is one of the most heat sensitive vitamins (Feliciotti & Esselen, 1957). It is often destroyed during thermal processing and, in addition to heat, is also sensitive to alkali, oxygen, radiation, sulfites, and the food matrix (Gregory III, 2008; Spitzer & Schweigert, 2007). Bis(2-methyl-3-furyl) disulfide, one possible degradation product of thiamine, delivers one of the lowest reported odor threshold values of any organic compound in water, at 0.02 parts per trillion

* Abbreviations: TMN, thiamine mononitrate; TClHCl, thiamine chloride hydrochloride; HPLC, high performance liquid chromatography; E_a, activation energy, k_{obs}, reaction rate constant; a_w, water activity; t_{90}, time when 90% of the initial concentration remains

(Buttery, Haddon, Seifert, & Turnbaugh, 1984). The presence of water has been shown to negatively impact the stability of thiamine in the solid state, with degradation rates increasing as relative humidity or a_w increase, especially when the deliquescence point is exceeded (Dennison, Kirk, Bach, Kokoczka, & Heldman, 1977; Hiatt, Ferruzzi, Taylor, & Mauer, 2008; Labuza & Tannenbaum, 1972). Many studies have also monitored the short-term stability of thiamine, primarily in its chloride hydrochloride form, in solution at very high temperatures, specifically as a function of pH (Dwivedi & Arnold, 1972; Farrer & Morrison, 1949; Feliciotti & Esselen, 1957; Williams & Ruehle, 1935). However, long term observations are lacking regarding the stability of thiamine in solution at temperatures to which foods are likely exposed, and few studies have directly compared the stability of TClHCl and TMN.

The objectives of this study were to: 1) investigate the impacts of concentration and storage temperature on the stability of thiamine in solutions prepared from TClHCl or TMN, 2) calculate activation energies of thiamine degradation using the temperature-dependent stability data collected from TClHCl and TMN solutions, 3) directly compare thiamine stability over time in solutions prepared from TMN and TClHCl, and 4) document if a difference in sensory impact exists in thiamine degraded in solutions prepared from TClHCl and TMN. The results of this study will provide a practical approach for understanding the delivery of thiamine salts in beverages and products containing varying amounts of water in which higher concentrations of thiamine could be found.

2. Materials and Methods

2.1 Materials:

Two thiamine salt forms were studied: thiamine mononitrate (TMN), $C_{12}H_{17}N_4OS \cdot NO_3$, obtained from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ), and thiamine chloride hydrochloride (TClHCl), $C_{12}H_{17}ClN_4OS \cdot HCl$, obtained from Fisher Scientific (Fair Lawn, NJ). For use in high performance liquid chromatography (HPLC), HPLC grade acetonitrile was obtained from Fisher Scientific and HPLC grade trifluoroacetic acid was obtained from Sigma-Aldrich Inc. (St. Louis, MO). Water used in all experiments was deionized and purified using a Barnstead E-Pure ultrapure water purification system with a resistivity at 25°C greater than 17.5 MΩ·cm (ThermoScientific, Waltham, MA).

2.2 Solubility Measurement:

The maximum solubility of each vitamin salt form in water at ambient temperature was determined, using a method adapted from Young (Young, 1957), to later use as a basis for preparing different solution concentrations of each sample. Beginning with 125 mg TMN or 50 mg TClHCl and 50 mL of water for each trial (based on reported solubility values), a mass balance was used to determine the saturation point by alternating additions of water (dropwise) and vitamin solid (1 mg). Saturation point was characterized by the inability of additional crystalline vitamin to be dissolved in solution. Volume was measured in a volumetric flask to quantify solubility in mg/mL of total solution.

2.3 Sample Preparation:

To understand the impact of thiamine concentration in solution on vitamin stability, series of TMN and TClHCl solutions were prepared containing 5 thiamine concentrations: 1, 5, 10, 20, and 27 mg/mL (the latter is just under the maximum solubility of TMN). Solutions

containing higher concentrations of TClHCl were also prepared (100, 300, and 500 mg/mL) to investigate behaviors in solutions nearing the saturation point of TClHCl. The range and number of concentrations chosen provided data for calculating reaction kinetics. The samples were prepared in terms of mass concentration rather than molar concentration, and although the two salt forms have slightly different molecular weights (Table 1), all calculations were done using percent remaining values, which account for this small discrepancy. Solutions (10 mL) containing each vitamin concentration were prepared in triplicate in 20 mL amber glass scintillation vials with PE cone-lined phenolic caps that were sealed with duct tape to prevent evaporation. Headspace in these vials was not modified prior to storage.

2.4 Sample Storage:

To monitor the effect of temperature on thiamine stability, solutions were stored at 5 temperatures: 25°C, 40°C, 60°C, 70°C, and 80°C. These temperatures were chosen to provide a large range of temperatures for calculating temperature-dependent reaction kinetics. The 25°C condition was used as an ambient temperature control and was maintained within ± 1°C using a temperature-controlled room (Commercial Fixture Company Inc., Indianapolis, IN). The 40°C, 60°C, and 70°C temperatures were maintained using Forma Scientific water-jacketed incubators (Thermo Fisher Scientific Inc., Marietta, OH). The 80°C temperature was maintained using a digital heatblock (VWR International, Radnor, PA). To monitor storage conditions, temperature was confirmed by liquid-in-glass partial immersion thermometers. Solutions were stored in controlled temperature environments for up to 6 months, depending on temperature and vitamin form, and were analyzed for percent vitamin remaining at a minimum of 5 selected timepoints.

2.5 Vitamin Quantification:

The chemical stability of thiamine in solution was monitored by measuring vitamin concentration over time using a high performance liquid chromatography (HPLC) method adapted from Xia et al. (Xia et al., 2006). A Waters 2690 Separations Module (Waters Corp. Milford, MA) equipped with a Waters 2996 Photodiode Array detector (Waters Corp.) was used with a 100 mm x 3.9 mm, 3.5 μm particle size XTerra RP-C_{18} column (Waters Corp.). The wavelength scan used was 235-400 nm. Mobile phase A: 0.1% trifluoroacetic acid (TFA) in water (*v/v*) and mobile phase B: acetonitrile (MeCN) were used with a flow rate of 1 mL/min and the following gradient method: 100/0 at 0 min, 97/3 at 4 min (linear), 90/10 at 6 min (linear), 100/0 at 10 min (linear), and 100/0 at 15 min. Prior to analysis, solutions were removed from controlled temperature storage, cooled in an ice bath, and diluted with mobile phase A to an estimated thiamine concentration of 500 ppm, or 0.5 mg/mL. Standard curves of TMN and TClHCl ($R^2 > 0.999$) at a concentration range of 10 ppm to 1000 ppm were prepared prior to each day of analysis and used to calculate the concentration of each sample. Integration was performed at 254 nm.

2.6 Reaction Kinetics:

To understand the kinetics of thiamine loss due to specific treatments, the data collected on the concentration of thiamine remaining in solution over time from the different initial solution concentrations and storage temperatures were applied to first-order reaction kinetic models, and the Arrhenius equation was used to model temperature-dependence of the reaction rate constants. Microsoft Excel 2016 (Redmond, WA) was used for the calculations.

Previous work has shown that thiamine degradation follows pseudo first-order reaction kinetics (Gregory III, 2008; Mauri, Alzamora, Chirife, & Tomio, 1989) wherein thiamine concentration is described by:

$$ln\frac{x}{x_0} = -kt \quad (1)$$

where x is the concentration of thiamine at time t (days), x_0 is the initial thiamine concentration, and k is the reaction rate constant (days^{-1}). The Arrhenius equation can be used to describe temperature dependence of rate constant k:

$$k = Ae^{\frac{-E_a}{RT}} \quad (2)$$

where k is the reaction rate constant (days^{-1}), A is the frequency factor of collision, E_a is the activation energy (kJ/mol), R is the gas constant (8.3145 J/mol·K), and T is temperature (K). Since some foods have multiple degradation patterns that may have different temperature dependencies, it is possible to find non-linear Arrhenius plots (Gregory III, 2008), and therefore nonlinear Arrhenius plots were also considered.

2.7 pH Measurement:

The pH of solutions containing both vitamin forms, at all concentrations, and at all temperatures, was measured to document how these variables affected the pH. The pH of each solution was measured in duplicate at all temperatures studied using an Orion pH probe (ThermoScientific) that had been calibrated from pH 5 to 7 for TMN and pH 1 to 4 for TClHCl using calibration standards obtained from Fisher Scientific.

2.8 Photography and color analysis:

The color of the TMN and TClHCl solutions was documented in solutions removed from the different storage temperatures. Samples were photographed at their endpoints in a Deep Professional LED Photography light box using an iPhone 6s camera. The Hunter L, a, and b color scale values of the solutions were determined by using the Color Companion iPhone application as described in Li et al. (Li, Taylor, Ferruzzi, & Mauer, 2013; Li, Taylor, & Mauer, 2014) to analyze the photographs. In this color scale, L represents lightness (in percent), a represents red (positive) vs. green (negative), and b represents yellow (positive) vs. blue (negative) colors.

2.9 Sensory Study of Odor Differences between Degraded Vitamin Solutions:

Thiamine degradation is known to produce aromas and flavors (Buttery et al., 1984; Dwivedi & Arnold, 1973). To determine if differences in the odors produced by degraded TMN and TClHCl could be detected by untrained panelists, 5 mg/mL solutions of each vitamin salt form were again prepared in the 20 mL amber vials with PE cone-lined caps, heated for 2 days at 80^{o}C, and frozen until the day of the sensory test. These conditions were chosen as a representation of the odor produced by each vitamin salt form, and the amount of thiamine degradation in these samples was determined by HPLC.

Eligibility requirements for participants in the sensory test included no food allergies or sensitivities, no known problems with sense of smell or taste, and no illness that may interfere with smelling capabilities. All procedures were approved by the Purdue University Human Subjects Research Protection Institutional Review Board as exempt under category 6 (taste and food quality evaluation and consumer acceptance studies). Samples (5 mL, in capped amber vials) were thawed at ambient temperature for 2 hours prior to the sensory analysis. The amber

vials prevented color changes from affecting responses, and 3-digit codes were used for blinding purposes. A two-alternative forced choice test was used to evaluate which sample smelled stronger. Participants were presented with two vials (one containing each vitamin form) in counterbalanced order and instructed to: *"Start with the sample on the left. Open the bottle and **smell the cap**. Then put the cap back on the bottle. Then open the bottle on the right and **smell the cap**. Then put the cap back on the bottle. Which sample smelled stronger? You may smell the samples again if you need to, but please smell just the cap."* Instructing participants to smell only the cap of the vials ensured that smelling techniques were more consistent across all participants.

After selecting the sample with the stronger smell, participants were given the option to describe the odor of the stronger smelling sample. This was done to surreptitiously determine if the participants found the samples to be unpleasant without biasing them for or against the "stronger" sample.

Data were analyzed by GraphPad Software using a two-tailed binomial distribution with $\alpha = 0.05$. Using a rearrangement of Abbott's formula to adjust for chance (Lawless & Heymann, 2010), 75% of the participants needed to select the same sample as "stronger" in order to conclude that participants found the aroma of one sample stronger than the other. This formula was also used to determine the percentage of participants who were true discriminators.

2.10 Statistical Analysis:

Samples were prepared and analyzed in triplicate for each time point of analysis. Single-variable ANOVA using SAS 9.4 (SAS Institute, Cary, NC) was used to determine significant differences in percent thiamine remaining between the initial solution and the degraded sample

over time, between varying concentrations of solution at each time point, between both salt forms, and between temperatures. Single-variable ANOVA was also used to determine significant differences in pH and color change. Regression analysis was used to determine 95% confidence intervals for k_{obs} values. Differences were determined using Tukey's post hoc test for multiple comparisons at a significance level of $\alpha = 0.05$.

3. Results & Discussion

3.1 Effects of Concentration and Temperature on Stability of Thiamine in TMN Solutions:

Both temperature and concentration significantly ($p < 0.05$) affected thiamine stability in TMN solutions. Typical degradation profiles of thiamine across varying TMN solution concentrations are shown in Figure 2. Increasing temperature increased thiamine degradation rates at all TMN concentrations. Thiamine degraded in an exponential manner for all concentrations of TMN solutions at all temperatures. Degradation patterns were related to the concentration of thiamine in solution, with more thiamine degradation occurring in solutions with higher TMN concentrations. As an example, in TMN solutions stored at 80°C, solutions containing the lowest TMN concentration, 1 mg/mL, had 48% thiamine remaining after 7 days (the least degradation), while solutions containing the most TMN (27 mg/mL) exhibited the greatest degradation (31% thiamine remaining) (Figure 2). A table containing all the thiamine percent remaining data from all TMN solution concentrations at all temperatures is included in the supplementary material (Table S1).

A clear trend was found at all temperatures that indicated there was a relationship between increasing concentration and decreasing stability of thiamine in TMN solutions. This finding conflicts with older reports that increasing thiamine concentrations in solutions adjusted

to pH 6 resulted in increasing thiamine stability (Farrer, 1947; McIntire & Frost, 1944). Differences between those studies and this one include: lower concentrations in the previous reports (the μg/mL scale rather than the mg/mL scale), and controlled pH versus unmodified pH. Controlling pH using a buffer system would be beneficial to better understand the dependency of TMN stability on pH independently from TMN concentration. However, this study did not explore buffer systems due to the possibility of thiamine interactions with the buffer affecting the degradation kinetics. The pH of TMN solutions in this study ranged from 5.36 to 6.96 due to the range of concentrations studied (Table 2). It is likely that pH, rather than concentration, was the main reason for differences in stability.

The thiamine degradation patterns found in all TMN solution concentrations and temperature treatments were consistent with those reported in previous TMN studies (Gregory III, 2008; Mauri et al., 1989), showing apparent first-order reaction kinetics (a typical example is shown in Figure 3). As expected, reactions proceeded faster as temperature increased. High correlations in linear regressions of the natural log of percent thiamine remaining over time for all TMN concentrations and temperature treatments were obtained (R^2 = 0.86-0.99). These results confirmed that the initial thiamine degradation in TMN solutions followed first-order reaction kinetics. Reaction rate constants, or k_{obs} values, were obtained using linear regressions and eq 1 (Arrhenius plots shown in Figure 4), and t_{90} values were calculated using each respective rate constant to describe the time it took for 10% of thiamine to degrade, or when 90% of the initial concentration of thiamine remained. The k_{obs} and t_{90} values are provided in Table 3. After the initial degradation which ended when the samples had approximately 40% TMN remaining, the first order reaction rate was lost. This was likely due to interactions of thiamine with increasing amounts of degradation products along with change in concentration (Ahmad et

al., 2018; Dhakal, Balasubramaniam, Ayvaz, & Rodriguez-Saona, 2018) . While kinetic parameters of thiamine degradation have been estimated using an endpoints method in food systems (Peleg, Normand, & Goulette, 2016), which would require a smaller number of experimental data points than used in this study and provide useful information on amount of thiamine remaining in the system, such an approach assumes first order reaction rate and thus could miss inflection points during the course of thiamine degradation when the first order reaction rate is lost.

HPLC chromatograms of TMN solutions before and after storage treatments (and degradation) are provided in the supplementary material (Figure S1) to facilitate comparisons of the number and retention time of degradation peaks between TMN and TClHCl solutions. The main thiamine degradation peaks in the TMN solutions were found at retention times of approximately 3.26, 4.08, 5.79, 8.15, and 8.28 min. L, a, and b values that documented the color of TMN 27 mg/mL solutions over time are included in Table 4, and photographs are included in the supplementary material (Figure S2). Little color change was found in TMN solutions wherein a large proportion of the thiamine had degraded. For example, when only 31% of thiamine remained in the TMN 27 mg/mL solution, after 7 days at 80°C, only a slightly yellow color in solution was present.

3.2 Effects of Concentration and Temperature on Stability of Thiamine in TClHCl Solutions:

Thiamine stability in TClHCl solutions was also significantly ($p < 0.05$) affected by temperature, with increasing temperature resulting in faster degradation. However, no trends were found between thiamine stability and the concentration of TClHCl in solution across all

302 temperatures. The pH of TClHCl solutions in this study ranged from 1.12 to 3.59, due to the
303 range of concentrations studied (Table 2). A typical degradation profile of TClHCl in varying
304 concentrations of solution at 80°C is shown in Figure 5. Thiamine in solutions across all
305 concentrations of TClHCl degraded in an exponential manner. A table containing all the
306 thiamine percent remaining data from all TClHCl solution concentrations at all temperatures is
307 provided in the supplementary material (Table S2).
308 The thiamine degradation patterns found in all TClHCl solution concentrations and
309 temperature treatments were consistent with those reported in the literature for TClHCl (Gregory
310 III, 2008; Mauri et al., 1989). Similar to the findings for thiamine stability in TMN solutions,
311 apparent first-order reaction kinetics were found for thiamine in TClHCl solutions (Figure 6),
312 and the first order reaction rate was lost after reactions had proceeded to approximately 40%
313 thiamine remaining due to possible interactions with new solution components (thiamine
314 degradation products) (Ahmad et al., 2018; Dhakal et al., 2018). The degradation of thiamine in
315 TClHCl solutions was slower than in the TMN solutions, and thus only values from 60°C, 70°C,
316 and 80°C were used for reaction kinetics calculations. High correlations in linear regressions of
317 the natural log of percent thiamine remaining over time for all TClHCl concentrations and
318 temperature treatments were obtained (R^2 = 0.79-0.99), which again confirmed the first-order
319 reaction kinetics of the initial thiamine degradation. Reaction rate constants, or k_{obs} values, were
320 obtained using linear regressions and eq 1 (Arrhenius plots are shown in Figure 7), and t_{90} values
321 were calculated to describe the time it took for 10% of thiamine to degrade, as shown in Table 3.
322 HPLC chromatograms of TClHCl solutions before and after storage treatments (and
323 degradation) are provided in the supplementary material (Figure S1) to facilitate the comparison
324 of the degradation peaks of thiamine in TClHCl and TMN solutions. The main thiamine

degradation peaks found in TClHCl solutions were at retention times of approximately 2.13, 4.05. 5.72, and 6.95 min. The L, a, and b values that documented the color of selected TClHCl solutions after storage are included in Table 4, and photographs of the color change are included in the supplementary material (Figure S2). Unlike what was found in the TMN solutions, much more color change was found in the TClHCl solutions, even when less thiamine had degraded. For example, when 56% of thiamine in TClHCl 27 mg/mL solutions remained after 31 days at 80°C, the solutions were nearly black, compared to minimal color change when more thiamine had degraded in a shorter timeframe in 27 mg/mL TMN solutions (31% thiamine remaining after 7 days at 80°C in solutions that were light yellow). After only 5 hours at 80°C, a 500 mg/mL solution of TClHCl in which no significant degradation of thiamine was found had a very similar color to that same 27 mg/mL TMN solution with only 31% thiamine remaining. The color changes found in solutions of TMN and TClHCl at various points during degradation were significantly different ($p < 0.05$). The difference in color change was attributed to the different degradation products that were formed by the different thiamine salts, exemplified by their differing HPLC chromatograms.

3.3 Sensory Study of Odor Differences between Degraded Vitamin Solutions:

Throughout the course of the thiamine degradation studies, differences in both the color and aroma of TMN and TClHCl solutions were noted by the investigators, in addition to documenting the differences in thiamine degradation rates and degradation product patterns in the HPLC chromatograms. Investigators had noticed an intense odor and color change in TClHCl solutions that occurred before thiamine degradation in the TClHCl solutions was even

statistically significant. In contrast, the investigators had also noticed that TMN solutions had not produced an intense smell or color change even when only ~30% of thiamine remained.

To further pursue these initial observations, a sensory study was completed to determine if a larger audience noted a difference in aromas produced by thiamine degradation in TMN and TClHCl solutions. Using the two-alternative forced-choice test, 51 of 68 panelists chose the TClHCl sample as having a stronger aroma than the TMN sample. Adjusting for chance, this was sufficient to conclude that the TClHCl sample had a stronger aroma than the TMN sample. From the adjusted Abbott's formula (Lawless & Heymann, 2010), 34 of the 68 panelists would be considered true discriminators, indicating that approximately 50% of people should truly find the TClHCl sample more potent. A two-tailed binomial test yielded $p < 0.0001$, again indicating that the TClHCl solution had a significantly stronger aroma than the TMN solution (see supplementary Figure S3). A cursory evaluation of the words used to describe the TClHCl solution odor indicated that subjects found the aroma unfavorable. Descriptive words used by panelists are provided in the supplementary material (Table S3). The percent thiamine remaining in each of these solutions, as determined by HPLC, was 66% thiamine remaining in the TMN solution with no significant degradation found in the TClHCl solution. Thus, it was concluded that the thiamine degradation products in TClHCl solutions had a significantly more potent odor than the degradation products in TMN solutions.

3.4 Comparison of Thiamine Stability in TMN and TClHCl Solutions:

There was a significant difference ($p < 0.05$) in thiamine stability between TMN and TClHCl solutions, as shown by the comparison graphs in Figure 8 and by k_{obs} and t_{90} values reported in Table 3. Thiamine in TMN solutions degraded faster than thiamine in TClHCl

solutions, with more substantial differences in stability manifesting as the temperature increased (Figure 8, Table S1, Table S2). The differences between the two salt forms were also exemplified by sensory implications, including aroma and color change (Table 4, Figure S2). Some possible degradation products that may contribute to differences in TMN and TClHCl solutions were identified by Dwivedi and Arnold (1973), including thiochrome, dihydrothiochrome, thioketones, pyrimidine and thiazole derivatives, and disulfides, among others.

TMN and TClHCl salts dissociate in solution to become the thiamine cation (with one or two positive charges, depending on pH (Figure 9)) and the respective anions. The main differences in solution traits between these thiamine salt forms are the type of anion present and the resulting solution pH. The pH values of TMN and TClHCl solutions at all concentrations and temperatures studied are shown in Table 2. It has been well-documented that pH affects thiamine stability; specifically, thiamine is much more stable in acidic conditions than in approximately neutral or alkaline conditions (Dwivedi & Arnold, 1973; Farrer, 1947; Gregory III, 2008; McIntire & Frost, 1944). Thus, it was not surprising to find that thiamine in TClHCl solutions was much more stable than thiamine in TMN solutions, since the TClHCl formed more acidic solutions than the TMN.

It has also been reported that pH affects the degradation pathway of thiamine (Dwivedi & Arnold, 1972). Thiamine has a pK_a of 4.8 (for the pyrimidine N1 nitrogen) (Edwards et al., 2017). In acidic conditions (pH < 6), degradation occurs by cleavage of the methylene bridge to release intact pyrimidine and thiazole moieties; while in conditions above pH 6, degradation involves the same cleavage, but also further fragmentation of the thiazole ring (Gregory III, 2008). These varying pathways support the observation of different degradation products

formed in the close to neutral pH TMN solutions and the acidic TClHCl solutions, as noted in the HPLC chromatograms (Figure S1). By comparing the retention times of the thiamine degradation products in the HPLC chromatograms, common degradation products found in both TMN and TClHCl solutions had retention times of approximately 4.05 and 5.75 min, while differences were found in degradation products appearing at 3.26, 8.15, and 8.28 min in TMN solutions, and at 2.13 and 6.95 min in TClHCl solutions. These different degradation products likely caused the differences in color and aroma between the TMN and TClHCl solutions.

Thiamine stability was significantly affected by TMN concentration, with thiamine degradation rates increasing as the concentration of TMN increased. This observation was likely more dependent on the changing solution pHs as TMN concentration increased rather than on the solution concentration of the thiamine *per se*. It has been well-documented that there is a dramatic decrease in stability of thiamine as pH reaches and exceeds pH 6.0 (Feliciotti & Esselen, 1957; Mulley, Stumbo, & Hunting, 1975; Williams & Ruehle, 1935). This change in stability is a result of the pK_a of thiamine (4.8). As illustrated in the speciation plot of thiamine in Figure 9, the more stable protonated species of thiamine is present as a notable fraction in acidic conditions up to approximately pH 6.0. As pH increases above 6.0, the less stable unprotonated species of thiamine dominates, and the stability of thiamine dramatically decreases. This noteworthy pH value (6.0) could be used to explain the dependence of thiamine stability on TMN concentration since the pH values found for TMN solutions were between pH 5.36 and 6.96. Small increases in pH due to increases in TMN concentration would have led to major changes in the fraction of protonated/unprotonated thiamine species present, which in turn would have caused the large decrease in thiamine stability that was found to be so dependent on TMN concentration. Conversely, in the pH range found in TClHCl solutions (from 1.12 to 3.59), the

protonated species of thiamine would have been predominant, which was likely why thiamine was not only more stable in the TClHCl solutions but also exhibited no stability dependence on TClHCl concentration.

Over a large range of temperatures, pH is known to vary slightly (Clark, 2017): as temperature increases, pH decreases. As shown in Figure 10 and Table 2, this trend was found in the TMN and TClHCl samples. Although this is of interest to note, it is not likely that this temperature-dependent pH change significantly affected thiamine stability, especially since this stability trend is in opposition to the effect of temperature. However, K_w also changes with temperature (Clark, 2017), meaning that although pH changes, acidity/alkalinity does not change, which led to the conclusion that pH change with temperature was an inconsequential factor in this thiamine stability study.

3.5 Degradation Kinetics of Thiamine Salt Forms:

The degradation kinetics of thiamine in various matrices (different from the solutions studied here) have been reported, including solid state with varying water activities, controlling for pH, and in the presence of various humectants (Kamman, Labuza, & Warthesen, 1981; Labuza & Kamman, 1982; Mauri, Alzamora, & Tomio, 1992). Thiamine was generally reported to have an activation energy of 20-30 kcal/mol (80-125 kJ/mol) (Kamman et al., 1981; Mauri et al., 1992). When controlling for pH, the activation energy was reported to be 27.4 kcal/mol at pH 5.5 and 29 kcal/mol at pH 4.0 (Mauri et al., 1992). When specifically looking at the different salt forms, activation energy was reported as 22.4 kcal/mol for TClHCl and 26.3 kcal/mol for TMN, with the E_a decreasing as water activity increased (Labuza & Kamman, 1982). This difference in activation energies is the reason for the greater stability of TMN compared to

TClHCl in the solid state, but these values do not agree with the stability trends of thiamine in solution found in this study. In the current study, pH and vitamin form were assumed to influence activation energy in solution, with the main factor being pH change due to variations in the ionization of each thiamine salt in solution.

It was reported previously that thiamine degradation in buffered solutions from 50°C to 110°C exhibited no deviation from Arrhenius behavior (Farrer & Morrison, 1949), but temperatures below 50°C were not included in the study. In the current study, non-linear Arrhenius plots were found to occur as the concentration of degradation products increased; however, in the early stages of thiamine degradation linear Arrhenius plots were found. These linear Arrhenius plots were used to calculate reaction kinetics. Using the k_{obs} values from temperatures 25, 40, 60, 70, and 80°C, the TMN activation energies were consistent with previous reports, ranging from 21-25 kcal/mol (88-105 kJ/mol), dependent on concentration. All values are included in Table 5. Using the k_{obs} values from temperatures 60, 70, and 80°C, TClHCl activation energies were found to range from 21-32 kcal/mol (90-135 kJ/mol). While these values are slightly higher than those previously reported, the extremely low pH found in the TClHCl solutions was not studied elsewhere. The low pH values (1.12-3.59) and consequently the predominance of the more stable protonated form of thiamine (Figure 9) led to the higher stability of thiamine in TClHCl solutions observed in this study (for example, 91% of TClHCl remained in the 10 mg/mL solution after 7 days at 80ºC compared to 38% TMN remaining in the same conditions). Additionally, the high thiamine stability in TClHCl solutions at 25°C and 40°C allowed the use of only 3 (higher) temperatures for the kinetics calculations, rather than the preferred 5 temperatures. However, the R^2 values for the Arrhenius calculations for TClHCl solutions were high correlations (0.87-0.99). All E_a values are reported in Table 5.

462 Overall, the reaction kinetics found in the current study agree reasonably well with
463 previous reports. TClHCl was found to have a higher activation energy than TMN, presumably
464 due to the difference in pH values between the two salt forms in solution. The low pH
465 conditions in the TClHCl solutions studied caused the protonated thiamine species, the more
466 stable of the two species, to be predominately present in solution. The low pH samples had a
467 higher activation energy of thiamine degradation and were significantly ($p < 0.05$) more stable
468 than thiamine in the close to neutral pH TMN solutions.
469
470 **3.6 Potential Implications in Food Formulations:**
471 Although the concentrations of thiamine investigated in this study were higher than
472 concentrations found in most food products, the implications for trends in thiamine stability at
473 different pHs and temperatures are relevant for foods naturally containing or fortified with
474 thiamine. Many food products act as acidic environments that will protect thiamine stability,
475 including fruit products and energy drinks. In these acidic conditions, no significant thiamine
476 degradation was found at ambient temperature over the 6 month period of this study. However,
477 there are also many food sources of thiamine that are close to neutral pH or slightly alkaline,
478 including milk, teas, beans, eggs, peas, and peanuts. The higher pHs in these foods may
479 contribute to degradation of thiamine during storage. For example, in close to neutral pH or
480 slightly alkaline samples at ambient temperatures, the t_{90} was 130-310 days, depending on pH,
481 compared to t_{90} values that could not be calculated in acidic conditions due to lack of significant
482 degradation. While some products (e.g., fruits, yeast, meats, eggs, and legumes) naturally
483 contain thiamine, many other food products are enriched with the salt forms of thiamine
484 investigated in this study. Some of the products enriched with TMN or TClHCl that have close

to neutral pH or slightly alkaline pH include various dairy products, powdered or liquid infant formulas, dietary supplements, and enriched flour (Bettendorff, 2012). Enriched flours are commonly combined with leavening agents in baked goods formulations, and these leavening agents produce slightly alkaline conditions (Cauvain & Young, 2006) which, as shown in this study, provide an unstable environment for thiamine. Further heating these products, such as during baking, could contribute to more thiamine degradation. Additionally, common food products or dietary supplements with limited water but high thiamine content include nutritional yeast, dried milk, infant formula, dried seaweed, and vitamin B complex supplements (U.S. Department of Agriculture Agricultural Research Service, 2018). Since thiamine has the potential to begin to dissolve in small amounts of water and is known to degrade faster in solution than in the solid state (Hiatt et al., 2008), the thiamine found in these products may act more like the thiamine in this study at high concentrations in the water present.

Although thiamine is often found in the presence of excipients in supplements or other ingredients in food products that can improve (or worsen) chemical stability (Kandutsch & Baumann, 1953), the degradation kinetics found in this study for pure thiamine in solution provide valuable information on the fundamental behavior of thiamine. Analyzing thiamine stability in buffered solutions to control for pH or in the presence of co-formulated ingredients would extend the implications of this study to more representative food systems and provide useful information on additional factors that contribute to the stability and/or degradation of thiamine.

4. Conclusions

Degradation of thiamine in solution was dependent on the form of thiamine salt dissolved, the resulting solution pH, and the storage temperature. All thiamine degradation was found to follow first order reaction kinetics until degradation products were present in high concentrations (< 40% vitamin remaining), which were thought to alter the degradation pathway. Thiamine in TClHCl solutions was found to be much more stable in all conditions than thiamine in TMN solutions, which was attributed to the low pH of TClHCl solutions. Although acidic conditions delayed the degradation of thiamine in solution, the low pH also altered the degradation pathway and produced different degradation products than were found in close to neutral pH conditions. This was demonstrated by differing peak positions in HPLC chromatograms between solutions of TMN and TClHCl. Thiamine degradation products in TClHCl solutions also contributed a potent odor and intense color change even before degradation became significant ($p < 0.05$). However, even with very large amounts of thiamine degradation in TMN solutions, sensory impacts were minimal. This study developed shelf-life studies that directly compared the stabilities and reaction kinetics of the two most common salt forms of thiamine, used in dietary supplements and as food additives, as a function of concentration and temperature. The results can aid in improving the understanding of thiamine degradation in a variety of products that are enriched or fortified with thiamine.

5. Acknowledgements

The authors would like to acknowledge Ciera Crawford and Matt Allan for their generous assistance with the sensory studies.

Funding: This work was financially supported by the United States Department of Agriculture [grant number 2016-67017-24592].

References

Ahmad, I., Mobeen, M. F., Sheraz, M. A., Ahmed, S., Anwar, Z., Shaikh, R. S., . . . Ali, S. M. (2018). Photochemical interaction of ascorbic acid and nicotinamide in aqueous solution: A kinetic study. *Journal of Photochemistry and Photobiology B: Biology, 182*, 115-121. doi: https://doi.org/10.1016/j.jphotobiol.2018.04.011

Ash, M. (2008). *Handbook of food additives* (3rd ed.. ed.). Endicott, NY: Endicott, NY : Synapse Information Resources.

Atkins, P., & de Paula, J. (2006). Phase equilibria. In P. Atkins & J. de Paula (Eds.), *Physical chemistry for the life sciences* (pp. 104-150). New York, NY: W. H. Freeman and Company.

Ball, G. F. M. (2006). *Vitamins in foods : analysis, bioavailability, and stability*. Boca Raton, FL: CRC/Taylor & Francis.

Baumgartner, T. G., Henderson, G. N., Fox, J., & Gondi, U. (1997). Stability of ranitidine and thiamine in parenteral nutrition solutions. *Nutrition, 13*(6), 547-553.

Bettendorff, L. (2012). Thiamin *Present Knowledge in Nutrition* (pp. 261-279): Wiley-Blackwell.

Buttery, R. G., Haddon, W. F., Seifert, R. M., & Turnbaugh, J. G. (1984). Thiamin odor and bis(2-methyl-3-furyl) disulfide. *J. Agric. Food Chem., 32*(3), 674-676. doi: 10.1021/jf00123a061

Cauvain, S. P., & Young, L. S. (2006). Ingredients and their influences. In S. P. Cauvain & L. S. Young (Eds.), *Baked products science, technology and practice*: Oxford : Blackwell.

ChemSpider. (2015). Thiamine: Royal Society of Chemistry.

Clark, J. (2017). Temperature dependence of the pH of pure water. *LibreTexts*

Dennison, D., Kirk, J., Bach, J., Kokoczka, P., & Heldman, D. (1977). Storage stability of thiamin and riboflavin in a dehydrated food system. *Journal of Food Processing and Preservation, 1*(1), 43-54.

Dhakal, S., Balasubramaniam, V. M., Ayvaz, H., & Rodriguez-Saona, L. E. (2018). Kinetic modeling of ascorbic acid degradation of pineapple juice subjected to combined pressure-thermal treatment. *Journal of Food Engineering, 224*, 62-70. doi: https://doi.org/10.1016/j.jfoodeng.2017.12.016

Dwivedi, B. K., & Arnold, R. G. (1972). Chemistry of thiamine degradation: mechanisms of thiamine degradation in a model food system. *Journal of Food Science, 37*(6), 886-888. doi: 10.1111/j.1365-2621.1972.tb03694.x

Dwivedi, B. K., & Arnold, R. G. (1973). Chemistry of thiamine degradation in food products and model systems: a review. [Review]. *Journal of Agricultural and Food Chemistry, 21*(1), 54-60. doi: 10.1021/jf60185a004

Edwards, K. A., Tu-Maung, N., Cheng, K., Wang, B., Baeumner, A. J., & Kraft, C. E. (2017). Thiamine Assays—Advances, Challenges, and Caveats (Vol. 6, pp. 178-191).

Farrer, K. T. (1947). The thermal destruction of vitamin B1: the influence of the concentration of buffer salts on the rate of destruction of aneurin at 100 degrees. *The Biochemical Journal, 41*(2), 167.

Farrer, K. T., & Morrison, P. G. (1949). The thermal destruction of vitamin B1: the effect of temperature and oxygen on the rate of destruction of aneurin. *Australian Journal of Experimental Biology & Medical Science, 27*(5).

Feliciotti, E., & Esselen, W. (1957). Thermal destruction rates of thiamine in pureed meats and vegetables. *Food Technology, 11*(2), 77-84.

Gregory III, J. F. (2008). Vitamins. In S. Damodaran, K. L. Parkin & O. R. Fennema (Eds.), *Fennema's food chemistry* (4th ed., pp. 439-521). Boca Raton: CRC Press/Taylor & Francis.

Hiatt, A. N., Ferruzzi, M. G., Taylor, L. S., & Mauer, L. J. (2008). Impact of deliquescence on the chemical stability of vitamins B(1), B(6), and C in powder blends. [Article]. *Journal of Agricultural and Food Chemistry, 56*(15), 6471-6479. doi: 10.1021/jf800709f

Institute of Medicine. (1998). Thiamin *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline*. Washington, D.C.: National Academy Press.

Kamman, J. F., Labuza, T. P., & Warthesen, J. J. (1981). Kinetics of thiamin and riboflavin loss in pasta as a function of constant and variable storage conditions. *Journal of Food Science, 46*(5), 1457-1461. doi: 10.1111/j.1365-2621.1981.tb04197.x

Kandutsch, A. A., & Baumann, C. A. (1953). Factors affecting the stability of thiamine in a typical laboratory diet. *The Journal of nutrition, 49*(2), 209.

Labuza, T. P., & Kamman, J. F. (1982). Comparison of stability of thiamin salts at high temperature and water activity. *Journal of Food Science, 47*(2), 664-665. doi: 10.1111/j.1365-2621.1982.tb10146.x

Labuza, T. P., & Tannenbaum, S. R. (1972). Nutrient losses during drying and storage of dehydrated foods. *Critical Reviews in Food Science & Nutrition, 3*(2), 217-240.

Lawless, H. T., & Heymann, H. (2010). Similarity, equivalence testing, and discrimination theory. In H. T. Lawless & H. Heymann (Eds.), *Sensory evaluation of food: principles and practices* (2nd ed.. ed., pp. 101-122). New York: New York : Springer.

Li, N., Taylor, L. S., Ferruzzi, M. G., & Mauer, L. J. (2013). Color and chemical stability of tea polyphenol (−)-epigallocatechin-3-gallate in solution and solid states. *Food Research International, 53*(2), 909-921. doi: https://doi.org/10.1016/j.foodres.2012.11.019

Li, N., Taylor, L. S., & Mauer, L. J. (2014). The physical and chemical stability of amorphous (−)-epi-gallocatechin gallate: effects of water vapor sorption and storage temperature. *Food Research International, 58*, 112-123. doi: https://doi.org/10.1016/j.foodres.2014.01.043

Mauri, L. M., Alzamora, S. M., Chirife, J., & Tomio, M. J. (1989). Review: kinetic parameters for thiamine degradation in foods and model solutions of high water activity. *International Journal of Food Science & Technology, 24*(1), 1-9. doi: 10.1111/j.1365-2621.1989.tb00613.x

Mauri, L. M., Alzamora, S. M., & Tomio, J. M. (1992). Effect of electrolytes on the kinetics of thiamine loss in model systems of high water activity. *Food Chemistry, 45*(1), 19-23. doi: 10.1016/0308-8146(92)90006-N

McIntire, F. C., & Frost, D. V. (1944). Thiamin stability: effect of amino acids and related compounds and of thiamin concentration. *Journal of the American Chemical Society, 66*(8), 1317-1318. doi: 10.1021/ja01236a033

Mulley, E., Stumbo, C., & Hunting, W. (1975). Effect of pH and form of the vitamin on its rate of destruction. *Journal of Food Science, 40*(5), 989-992.

Peleg, M., Normand, M. D., & Goulette, T. R. (2016). Calculating the degradation kinetic parameters of thiamine by the isothermal version of the endpoints method. *Food Research International, 79*, 73-80. doi: http://dx.doi.org/10.1016/j.foodres.2015.12.001

Prinzo, Z. W. (1999). Thiamine deficiency and its prevention and control in major emergencies *Micronutrient series*: World Health Organization (WHO). Department of Nutrition for Health and Development; Office of the United Nations High Commissioner for Refugees (UNHCR).

Shepherd, S. J., & Gibson, P. R. (2013). Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease. *Journal of Human Nutrition and Dietetics, 26*(4), 349-358. doi: doi:10.1111/jhn.12018

Spitzer, V., & Schweigert, F. (2007). Vitamin basics the facts about vitamins in nutrition. *DSM Nutritional Products Ltd, Germany*.

U.S. Department of Agriculture Agricultural Research Service. (2018). USDA national nutrient database for standard reference. Retrieved March 2, 2018, from Nutrient Data Laboratory Home Page http://www.ars.usda.gov/nutrientdata

U.S. Food & Drug Administration. (2018). FDA vitamins and minerals chart

Williams, R. R., & Ruehle, A. E. (1935). Studies of crystalline vitamin B1: presence of quaternary nitrogen. *Journal of the American Chemical Society, 57*(10), 1856-1860. doi: 10.1021/ja01313a029

Xia, F., Hong, P., Alden, B., Boissel, C., Swanson, D., Chambers, E., . . . Walter, T. (2006). *Improvements in reversed-phase HPLC columns designed for polar compound*. Paper presented at the HPLC, San Francisco, CA.

Young, F. E. (1957). D-glucose-water phase diagram. *The Journal of Physical Chemistry, 61*(5), 616-619.

Table 1. Solid state property comparison between TMN and TClHCl.

	Thiamine Mononitrate	Thiamine Chloride Hydrochloride
Molecular weight [1]	327.36 g/mol	337.26 g/mol
Melting point [1]	196-200°C	248°C
Deliquescence point (RH_0) [2]	98.5% RH	88% RH
Aqueous solubility	30 mg/mL	570 mg/mL

[1] (ChemSpider, 2015)
[2] (Hiatt et al., 2008)

Table 2. pH values of **A)** pure water and **B)** TMN and TClHCl solutions at each concentration and temperature studied. Uppercase superscript letters on values denote statistical significance within temperatures for each vitamin salt form (down columns). Lowercase superscript letters on values denote statistical significance within concentration for each vitamin salt form (across rows).

A)

	25°C	30°C	40°C	50°C	100°C	Ref
Pure Water	7.00	6.92	6.77	6.63	6.14	(Clark, 2017)

B)

Vitamin Salt Form	Concentration (mg/mL)	25°C	40°C	60°C	70°C	80°C
TMN	1	6.42 ± 0.04^{Aa}	6.23 ± 0.09^{Aa}	5.95 ± 0.07^{Ab}	5.76 ± 0.03^{Ab}	5.46 ± 0.07^{ABc}
	5	6.6 ± 0.3^{Aa}	6.2 ± 0.4^{Aab}	5.9 ± 0.3^{Aab}	5.6 ± 0.2^{Aab}	5.36 ± 0.05^{Bb}
	10	6.8 ± 0.2^{Aa}	6.5 ± 0.2^{Aab}	6.1 ± 0.1^{Abc}	5.8 ± 0.1^{Ac}	5.5 ± 0.2^{ABc}
	20	6.93 ± 0.03^{Aa}	6.57 ± 0.09^{Ab}	6.14 ± 0.09^{Ac}	5.9 ± 0.1^{Acd}	5.61 ± 0.05^{ABd}
	27	6.96 ± 0.03^{Aa}	6.67 ± 0.03^{Aa}	6.24 ± 0.08^{Ab}	5.86 ± 0.09^{Ac}	5.8 ± 0.1^{Ac}
TClHCl	1	3.59 ± 0.03^{Aa}	3.2 ± 0.1^{Aab}	2.9 ± 0.1^{Abc}	2.6 ± 0.1^{Abc}	2.6 ± 0.2^{Ac}
	5	3.30 ± 0.01^{Ba}	2.8 ± 0.1^{Aab}	2.5 ± 0.1^{ABbc}	2.2 ± 0.1^{Bc}	2.2 ± 0.2^{ABc}
	10	3.17 ± 0.00^{Ca}	2.67 ± 0.08^{Bb}	2.3 ± 0.1^{BCc}	1.95 ± 0.08^{BCcd}	1.89 ± 0.08^{BCd}
	20	3.05 ± 0.00^{Da}	2.51 ± 0.00^{BCb}	2.19 ± 0.04^{BCc}	1.97 ± 0.07^{BCcd}	1.9 ± 0.1^{BCd}
	27	2.99 ± 0.01^{Da}	2.46 ± 0.02^{BCb}	2.14 ± 0.01^{BCc}	2.0 ± 0.1^{BCcd}	1.8 ± 0.1^{BCDd}
	100	2.77 ± 0.01^{Ea}	2.36 ± 0.02^{Cb}	1.9 ± 0.1^{CDc}	1.7 ± 0.1^{CDcd}	1.51 ± 0.03^{CDEd}
	300	2.53 ± 0.01^{Fa}	2.05 ± 0.06^{Db}	1.6 ± 0.1^{Dc}	1.44 ± 0.03^{DEcd}	1.28 ± 0.06^{DEd}
	500	2.35 ± 0.03^{Ga}	1.93 ± 0.01^{Db}	1.5 ± 0.2^{Dc}	1.3 ± 0.1^{Ecd}	1.12 ± 0.09^{Ed}

Vitamin Salt Form	Temperature (ºC)		1 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL	27 mg/mL
TMN	25	k_{obs}* (day^{-1})	$3.4x10^{-4} \pm 0.5x10^{-4}$ Aa	$6.3x10^{-4} \pm 0.6x10^{-4}$ Abc	$8.1x10^{-4} \pm 0.1x10^{-4}$ Ac	$4.8x10^{-4} \pm 0.5x10^{-4}$ Aab	$7.2x10^{-4} \pm 0.8x10^{-4}$ Ac
		R^2	0.8601	0.9560	0.8551	0.9259	0.9309
		t_{90}** (days)	310	167	130	220	146
	40	k_{obs} (day^{-1})	$1.74x10^{-3} \pm 0.7x10^{-4}$ Aa	$3.1x10^{-3} \pm 0.2x10^{-3}$ Aa	$5.1x10^{-3} \pm 0.4x10^{-3}$ Ab	$7.6x10^{-3} \pm 0.6x10^{-3}$ Ac	$9.7x10^{-3} \pm 0.1x10^{-3}$ Ad
		R^2	0.9858	0.9680	0.9629	0.9554	0.9321
		t_{90} (days)	60.6	34.0	20.7	13.9	10.9
	60	k_{obs} (day^{-1})	$1.7x10^{-2} \pm 0.1x10^{-2}$ Ba	$2.7x10^{-2} \pm 0.1x10^{-2}$ Ba	$3.9x10^{-2} \pm 0.3x10^{-2}$ Bb	$6.3x10^{-2} \pm 0.6x10^{-2}$ Ac	$7.7x10^{-2} \pm 0.6x10^{-2}$ ABd
		R^2	0.9840	0.9898	0.9832	0.9661	0.9762
		t_{90} (days)	6.20	3.90	2.70	1.67	1.37
	70	k_{obs} (day^{-1})	$4.6x10^{-2} \pm 0.5x10^{-2}$ Ca	$7.2x10^{-2} \pm 0.5x10^{-2}$ Cab	$9.7x10^{-2} \pm 0.6x10^{-2}$ Cb	$1.4x10^{-1} \pm 0.1x10^{-1}$ Bc	$1.9x10^{-1} \pm 0.2x10^{-1}$ Bd
		R^2	0.9577	0.9803	0.9837	0.9686	0.9611
		t_{90} (days)	2.29	1.46	1.09	0.753	0.555
	80	k_{obs} (day^{-1})	$1.17x10^{-1} \pm 0.6x10^{-2}$ Da	$1.4x10^{-1} \pm 0.1x10^{-1}$ Da	$2.0x10^{-1} \pm 0.2x10^{-1}$ Da	$4.6x10^{-1} \pm 0.5x10^{-1}$ Cb	$6x10^{-1} \pm 1x10^{-1}$ Cb
		R^2	0.9920	0.9764	0.9622	0.9819	0.9577
		t_{90} (days)	0.901	0.753	0.527	0.229	0.176
TClHCl	60	k_{obs} (day^{-1})	$2.1x10^{-3} \pm 0.2x10^{-3}$ Abc	$1.9x10^{-3} \pm 0.2x10^{-3}$ Aabc	$1.8x10^{-3} \pm 0.3x10^{-3}$ Aabc	$1.5x10^{-3} \pm 0.1x10^{-3}$ Aa	$1.6x10^{-3} \pm 0.2x10^{-3}$ Aab
		R^2	0.9304	0.9260	0.7922	0.9384	0.9283
		t_{90} (days)	50.2	55.5	58.5	70.2	65.9
	70	k_{obs} (day^{-1})	$1.7x10^{-2} \pm 0.3x10^{-2}$ Bc	$7.3x10^{-3} \pm 0.8x10^{-3}$ Bab	$7x10^{-3} \pm 1x10^{-3}$ Bab	$5.3x10^{-3} \pm 0.7x10^{-3}$ Ba	$4.4x10^{-3} \pm 0.4x10^{-3}$ Ba
		R^2	0.8215	0.9283	0.8745	0.8823	0.9498
		t_{90} (days)	6.20	14.4	15.1	19.9	23.9
	80	k_{obs} (day^{-1})	$3.1x10^{-2} \pm 0.1x10^{-2}$ Cd	$1.5x10^{-2} \pm 0.1x10^{-2}$ Ca	$2.3x10^{-2} \pm 0.3x10^{-2}$ Cbc	$2.4x10^{-2} \pm 0.1x10^{-2}$ Cbc	$1.89x10^{-2} \pm 0.09x10^{-2}$ Cab
		R^2	0.9873	0.9500	0.8928	0.9736	0.9857
		t_{90} (days)	3.40	7.02	4.58	4.39	5.57

Table 3. Rate constants and t_{90} values for thiamine in solutions of TMN and TClHCl under all concentrations and temperatures studied. Uppercase superscript letters denote statistical significance within concentration for each vitamin salt form (down columns). Lowercase superscript letters denote statistical significance within temperature for each vitamin salt form (across rows).

* Error values indicated for k_{obs} values represent a 95% confidence interval

** t_{90} indicates time when 90% of the initial concentration of thiamine remains

Table 4. Color parameters L, a, and b values of selected TMN and TClHCl solutions at 80°C. Superscript letters on L, a, or b values denote statistical significance within their respective parameters.

Vitamin Form	Concentration	Time	L (0-100%, black-white)	a (negative=green, positive=red)	b (negative=blue, positive=yellow)
TMN	27 mg/mL	0 days	$80.0 \pm 0.7\%^{AB}$	-7.2 ± 0.4^{E}	6.2 ± 0.6^{D}
		7 days	$82 \pm 2\%^{A}$	-11.6 ± 0.4^{G}	15.8 ± 0.2^{B}
TClHCl	27 mg/mL	0 days	$77 \pm 1\%^{BC}$	-5.3 ± 0.6^{D}	2.8 ± 0.3^{F}
		31 days	$16 \pm 1\%^{E}$	2.9 ± 0.6^{B}	2.7 ± 0.4^{F}
	100 mg/mL	0 days	$77 \pm 2\%^{BC}$	-5.8 ± 0.3^{DE}	3.1 ± 0.1^{F}
		31 days	$15 \pm 3\%^{E}$	-2 ± 1^{C}	0 ± 1^{G}
	500 mg/mL	0 days	$76 \pm 1\%^{C}$	-6.8 ± 0.3^{DE}	4.8 ± 0.4^{E}
		5 hours	$80.7 \pm 0.6\%^{A}$	-9.3 ± 0.7^{F}	10.4 ± 0.9^{C}
		31 days	$40 \pm 2\%^{D}$	38 ± 2^{A}	35.7 ± 0.7^{A}

Table 5. Calculated activation energies of TMN and TClHCl as a function of temperature.

Vitamin Salt Form	Concentration (mg/mL)	E_A (kcal/mol)	E_A (kJ/mol)
TMN	1	22	94
	5	21	88
	10	21	88
	20	25	105
	27	25	103
TClHCl	1	32	133
	5	24	100
	10	30	124
	20	32	136
	27	29	120
	100	31	131
	300	32	135
	500	21	90

Figure Captions

Figure 1. Chemical structures of A) thiamine, B) thiamine mononitrate, and C) thiamine chloride hydrochloride.

Figure 2. Degradation profiles of thiamine in TMN solutions in varying concentrations (1-27 mg/mL) at 80°C over time.

Figure 3. First-order degradation regression lines of thiamine in 5 mg/mL TMN solutions at temperatures from 25°C to 80°C.

Figure 4. Arrhenius plots used to calculate temperature-dependent activation energy for TMN solutions (1-27 mg/mL) at temperatures from 25ºC to 80ºC.

Figure 5. Degradation profiles of thiamine chloride in TClHCl solutions at varying concentrations (1-500 mg/mL) at 80°C over time.

Figure 6. First-order degradation regression lines of thiamine chloride in 1 mg/mL TClHCl solutions at temperatures from 60°C to 80°C.

Figure 7. Arrhenius plots used to calculate temperature-dependent activation energy for TClHCl solutions (1-500 mg/mL) at temperatures from 25ºC to 80ºC.

Figure 8. Comparison of chemical stability over time of TMN and TClHCl in multiple concentrations of solution at A) 25°C, B) 40°C, C) 60°C, D) 70°C, and E) 80°C:

● TMN 1 mg/mL ○ TMN 5 mg/mL ■ TMN 10 mg/mL □ TMN 20 mg/mL ▲ TMN 27 mg/mL

● TClHCl 1 mg/mL ○ TClHCl 5 mg/mL ■ TClHCl 10 mg/mL □ TClHCl 20 mg/mL ▲ TClHCl 27 mg/mL

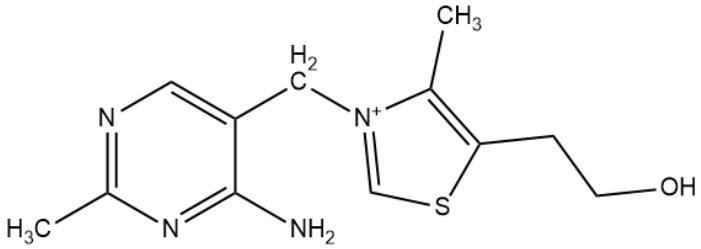
△ TClHCl 100 mg/mL ◆ TClHCl 300 mg/mL ◇ TClHCl 500 mg/mL

Figure 9. Speciation plot of thiamine as a function of pH prepared using only the pK_{a1} of thiamine (4.8) for the N1 nitrogen on the pyrimidine ring. Shaded areas indicate pH ranges of TClHCl and TMN samples, respectively.

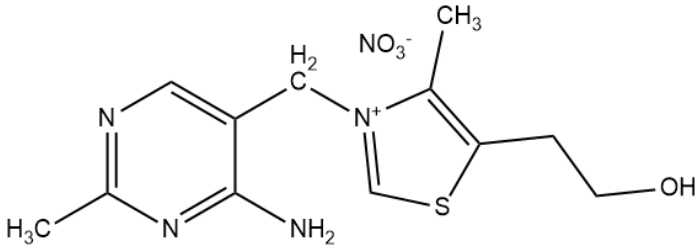
Figure 10. pH change with temperature of pure water (Clark, 2017), TMN, and TClHCl for all concentrations studied.

Figure 1

A)


CH3
H2
C
N
N+
OH
S
H3C
N
NH2

B)


CH3
NO3-
H2
C
N
N+
OH
S
H3C
N
NH2

C)

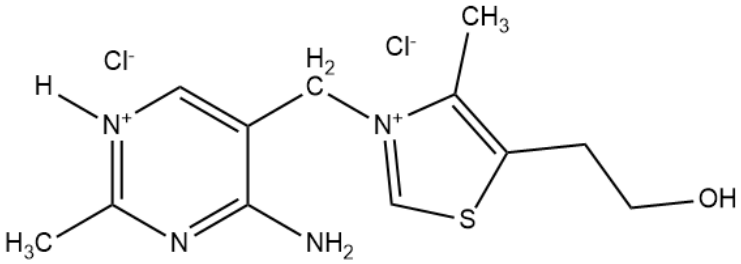

CH3
Cl-
Cl-
H2
C
H
N+
N+
OH
S
H3C
N
NH2

Figure 2

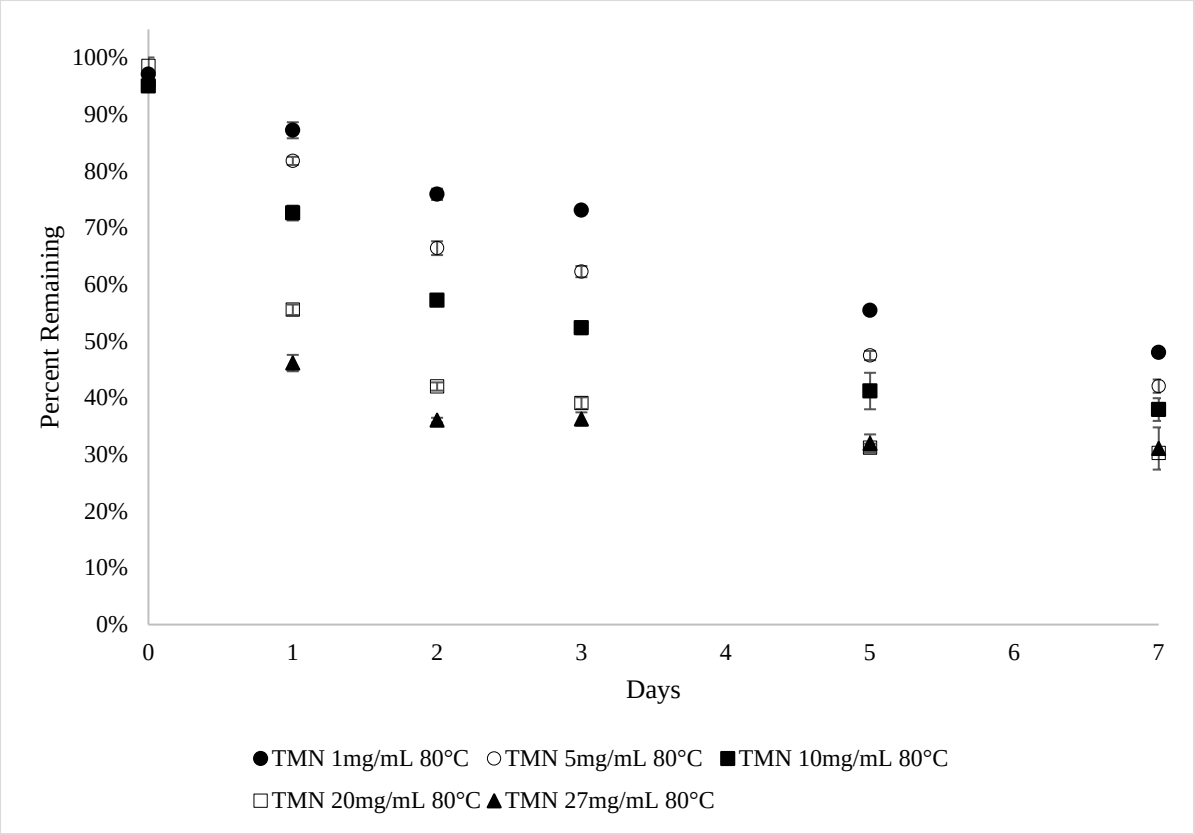

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
1
2
3
4
5
6
7
Days
●TMN 1mg/mL 80°C ○TMN 5mg/mL 80°C ■TMN 10mg/mL 80°C
□TMN 20mg/mL 80°C ▲TMN 27mg/mL 80°C

Figure 3

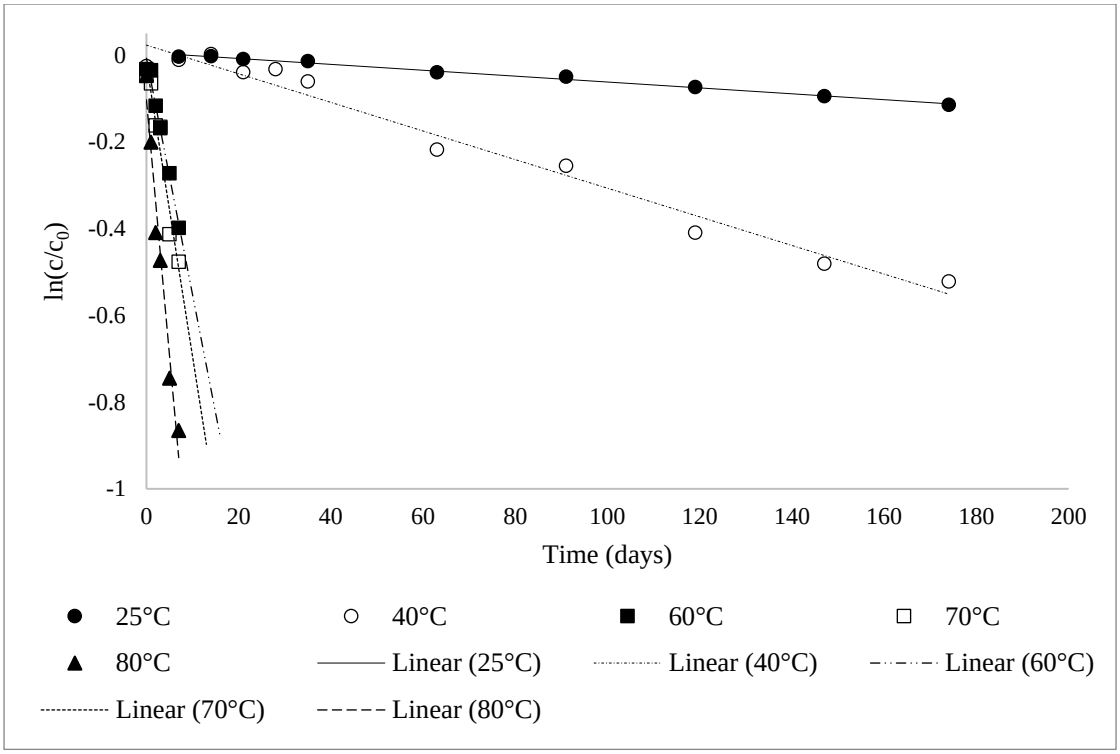

0
-0.2
-0.4
-0.6
-0.8
-1
ln(c/c0)
0
20
40
60
80
100
120
140
160
180
200
Time (days)
25°C
40°C
60°C
70°C
80°C
Linear (25°C)
Linear (40°C)
Linear (60°C)
Linear (70°C)
Linear (80°C)

Figure 4

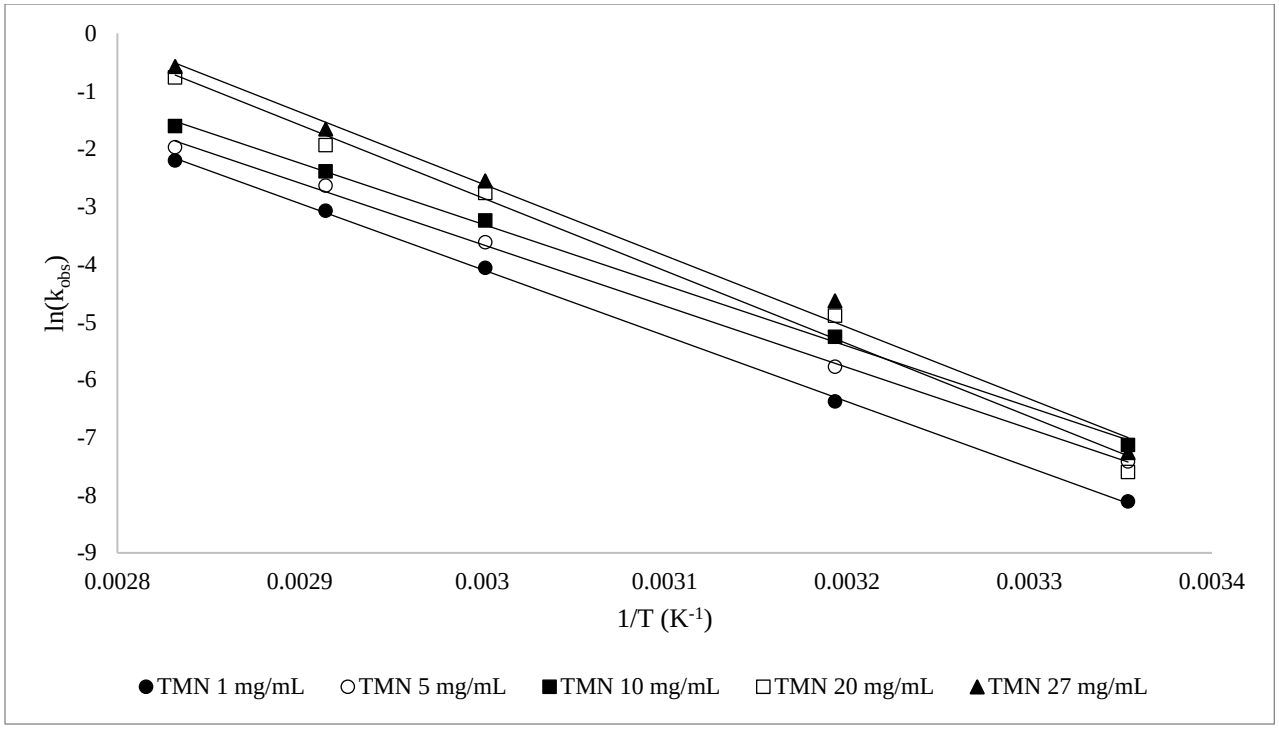

0
-1
-2
-3
-4
-5
-6
-7
-8
-9
ln(k_obs)
0.0028
0.0029
0.003
0.0031
0.0032
0.0033
0.0034
1/T (K-1)
TMN 1 mg/mL
TMN 5 mg/mL
TMN 10 mg/mL
TMN 20 mg/mL
TMN 27 mg/mL

Figure 5

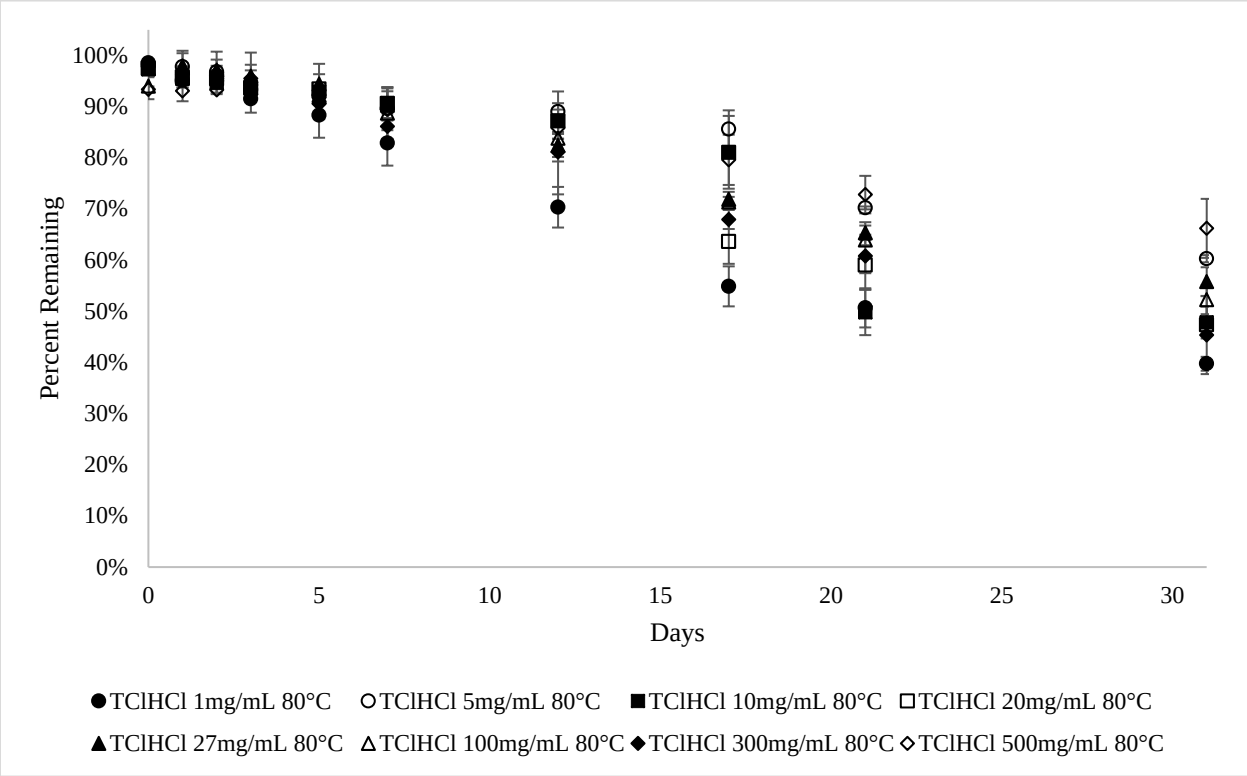

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
5
10
15
20
25
30
Days
TClHCl 1mg/mL 80°C
TClHCl 5mg/mL 80°C
TClHCl 10mg/mL 80°C
TClHCl 20mg/mL 80°C
TClHCl 27mg/mL 80°C
TClHCl 100mg/mL 80°C
TClHCl 300mg/mL 80°C
TClHCl 500mg/mL 80°C

Figure 6

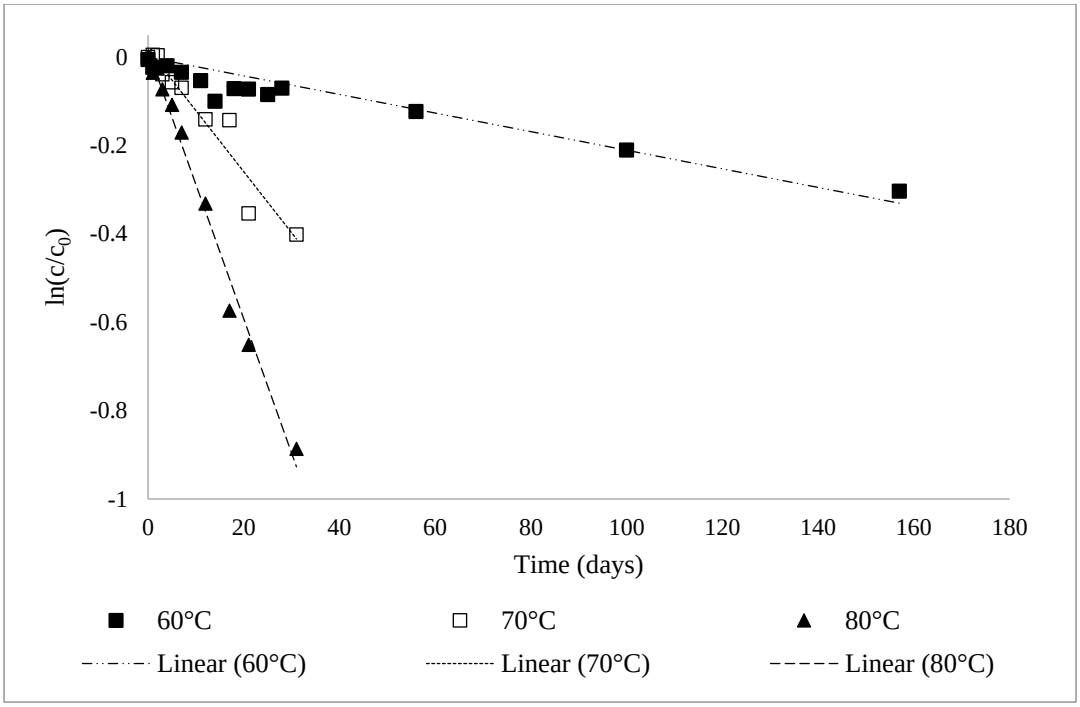

0
-0.2
-0.4
-0.6
-0.8
-1
ln(c/c0)
0
20
40
60
80
100
120
140
160
180
Time (days)
60°C
70°C
80°C
Linear (60°C)
Linear (70°C)
Linear (80°C)

Figure 7

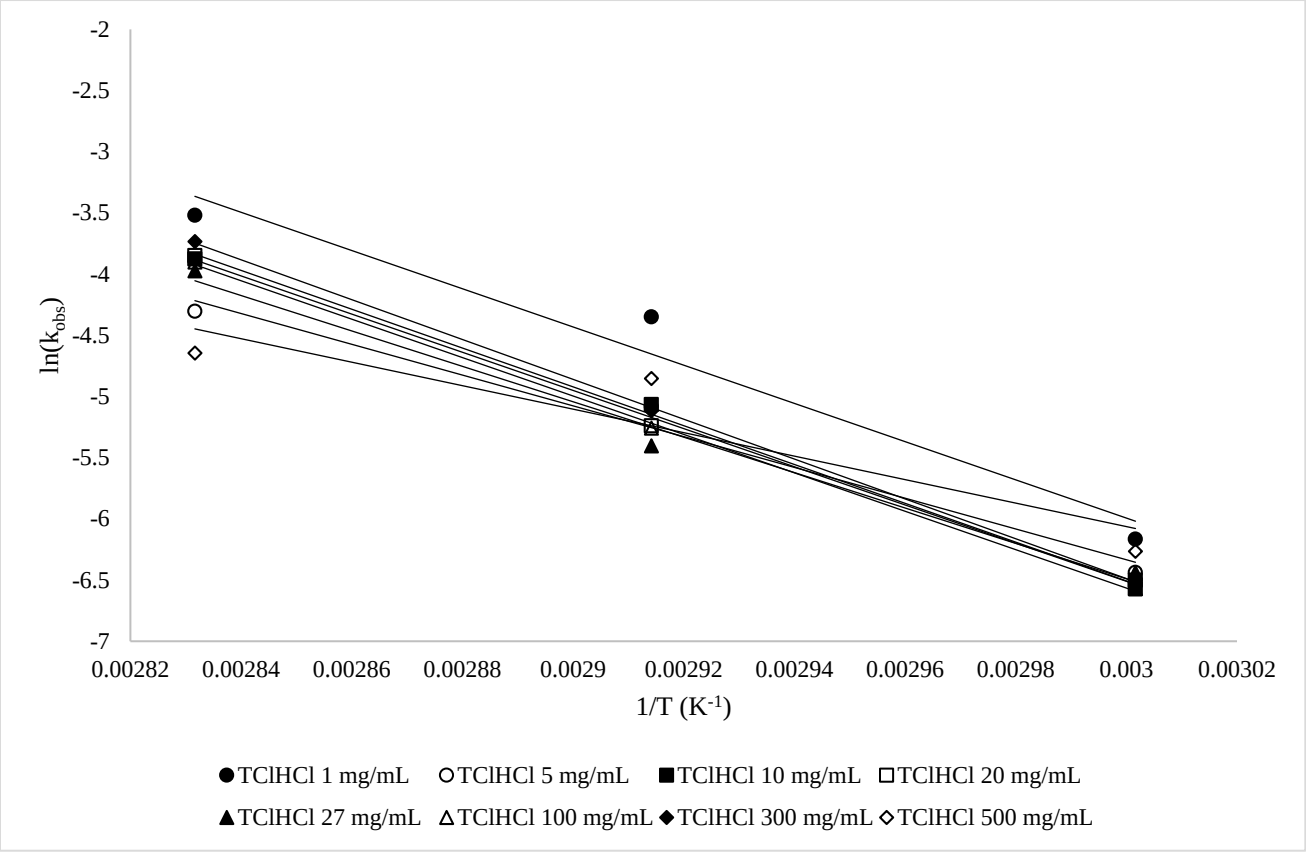
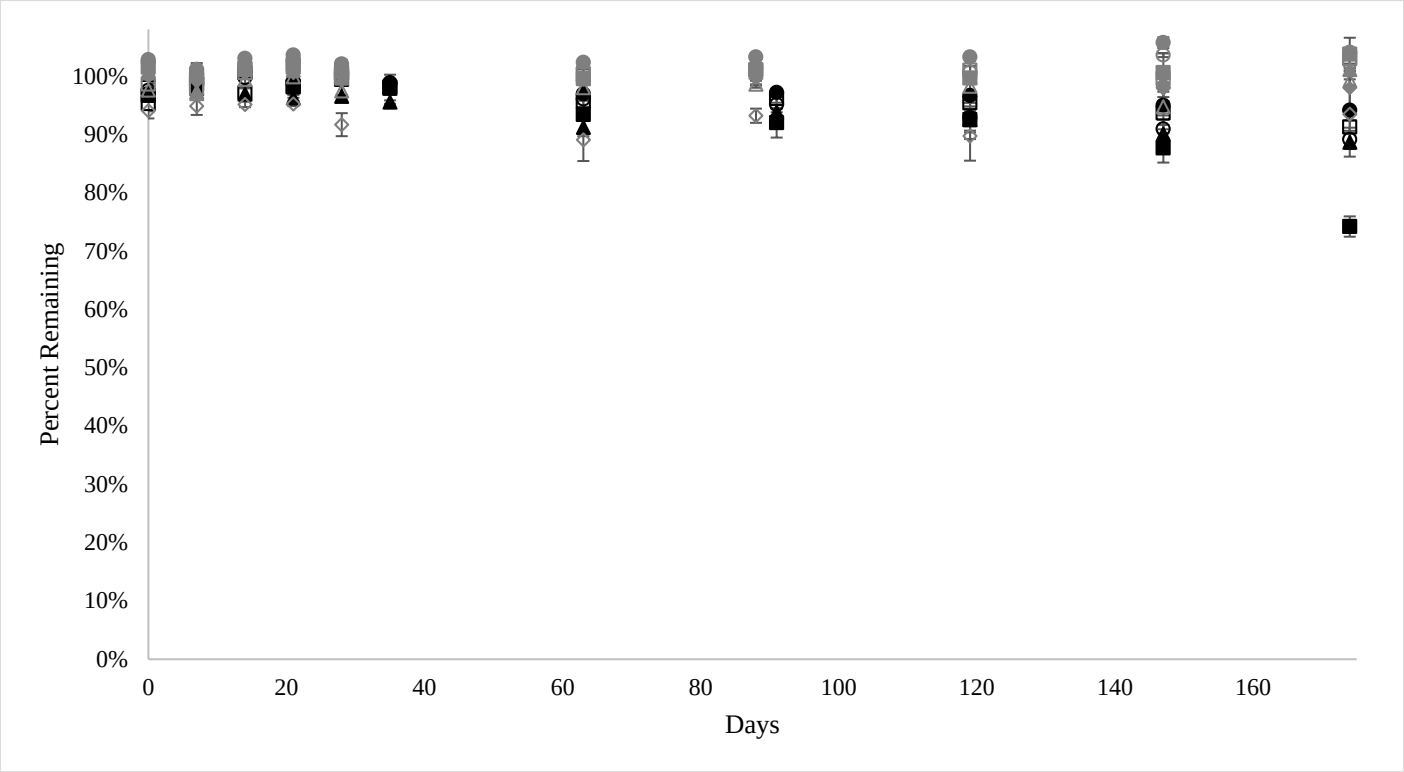
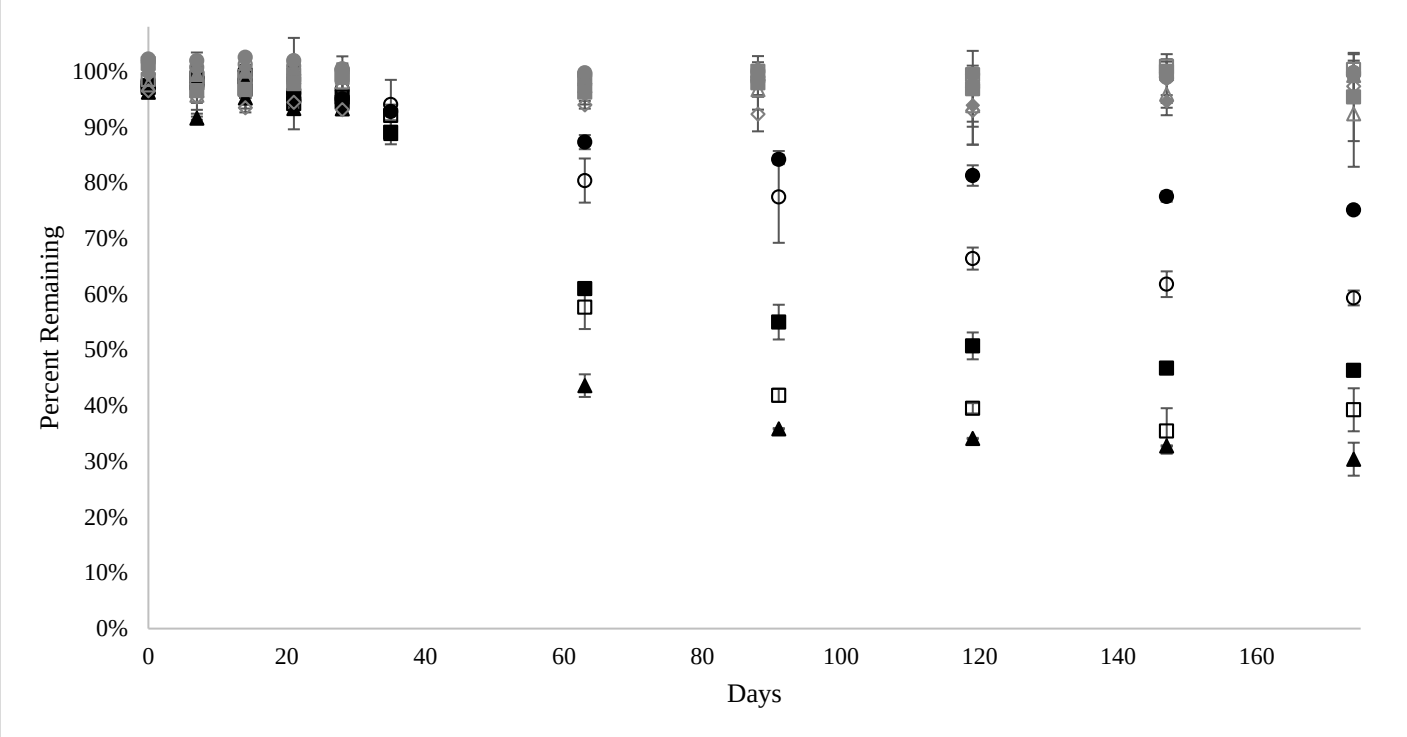

-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-6.5
-7
ln(kobs)
0.00282
0.00284
0.00286
0.00288
0.0029
0.00292
0.00294
0.00296
0.00298
0.003
0.00302
1/T (K-1)
TClHCl 1 mg/mL
TClHCl 5 mg/mL
TClHCl 10 mg/mL
TClHCl 20 mg/mL
TClHCl 27 mg/mL
TClHCl 100 mg/mL
TClHCl 300 mg/mL
TClHCl 500 mg/mL

Figure 8

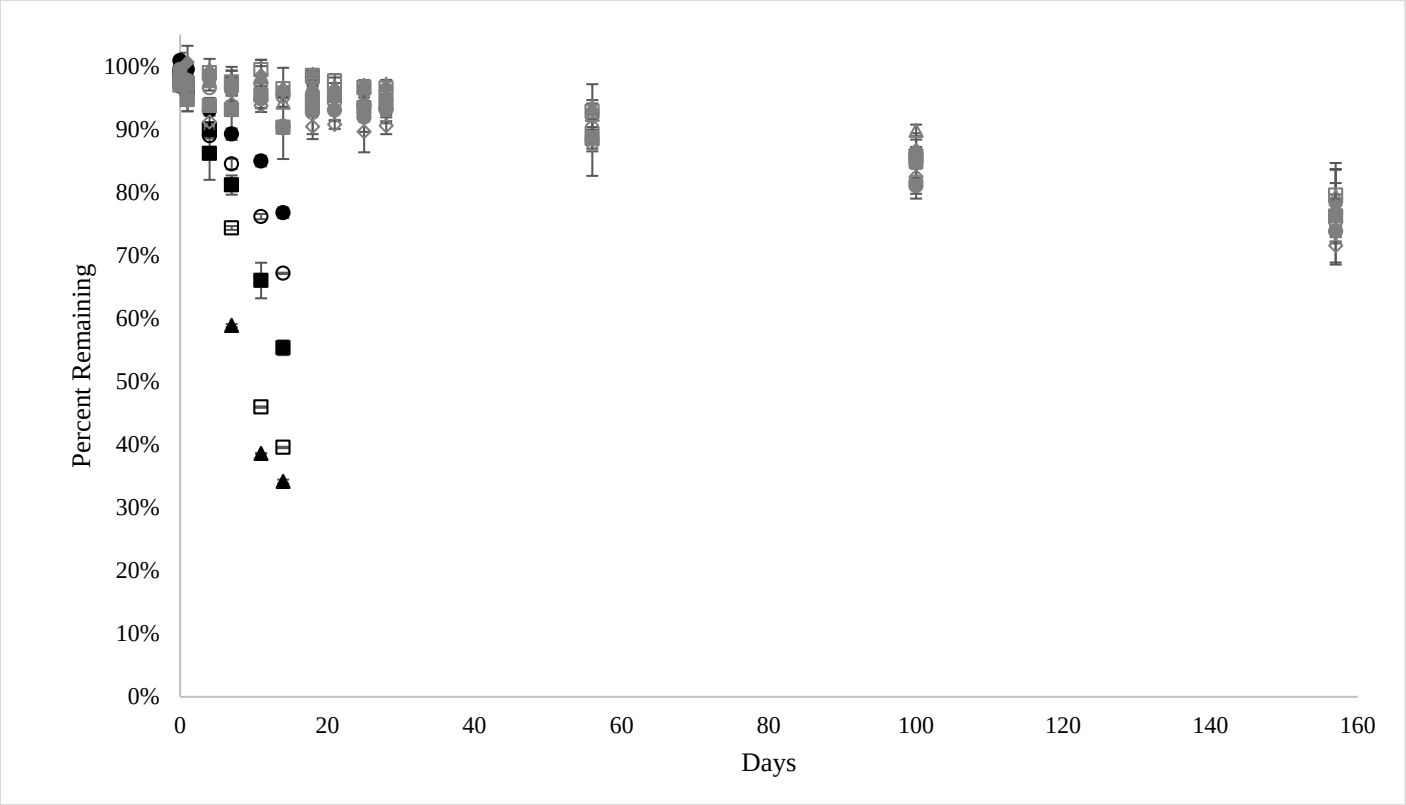
A)


Percent Remaining
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
20
40
60
80
100
120
140
160
Days

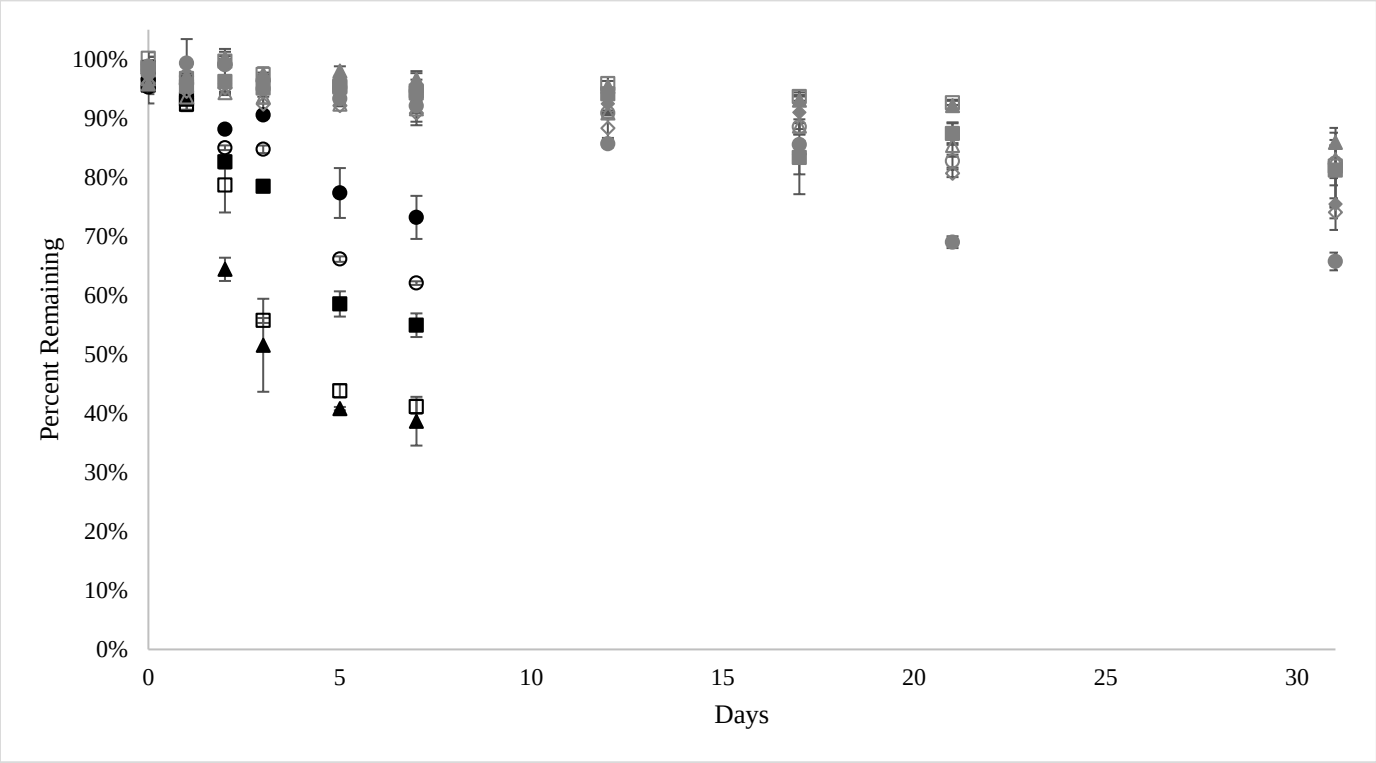
B)


Percent Remaining
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
20
40
60
80
100
120
140
160
Days

C)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
20
40
60
80
100
120
140
160
Days

D)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
5
10
15
20
25
30
Days

E)

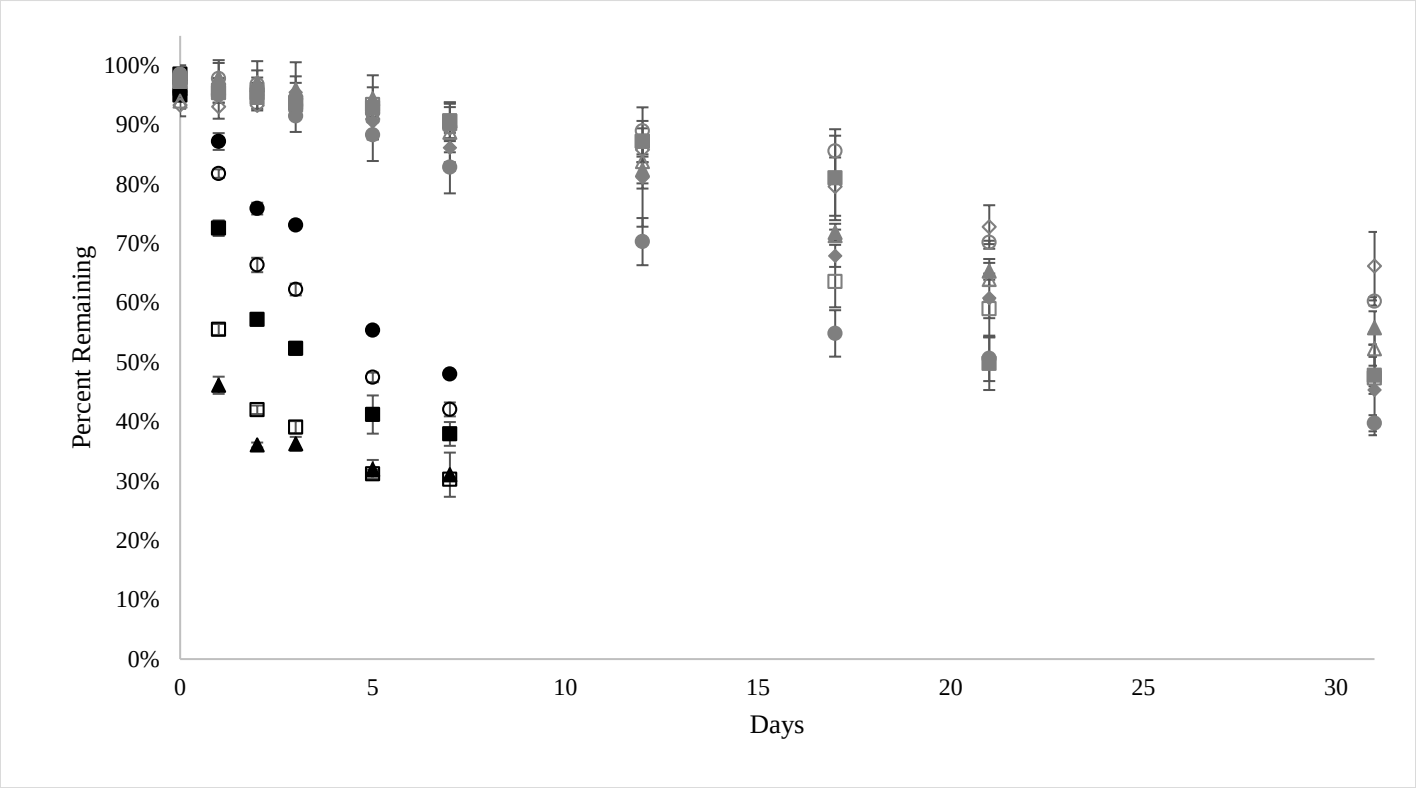

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
5
10
15
20
25
30
Days

Figure 9

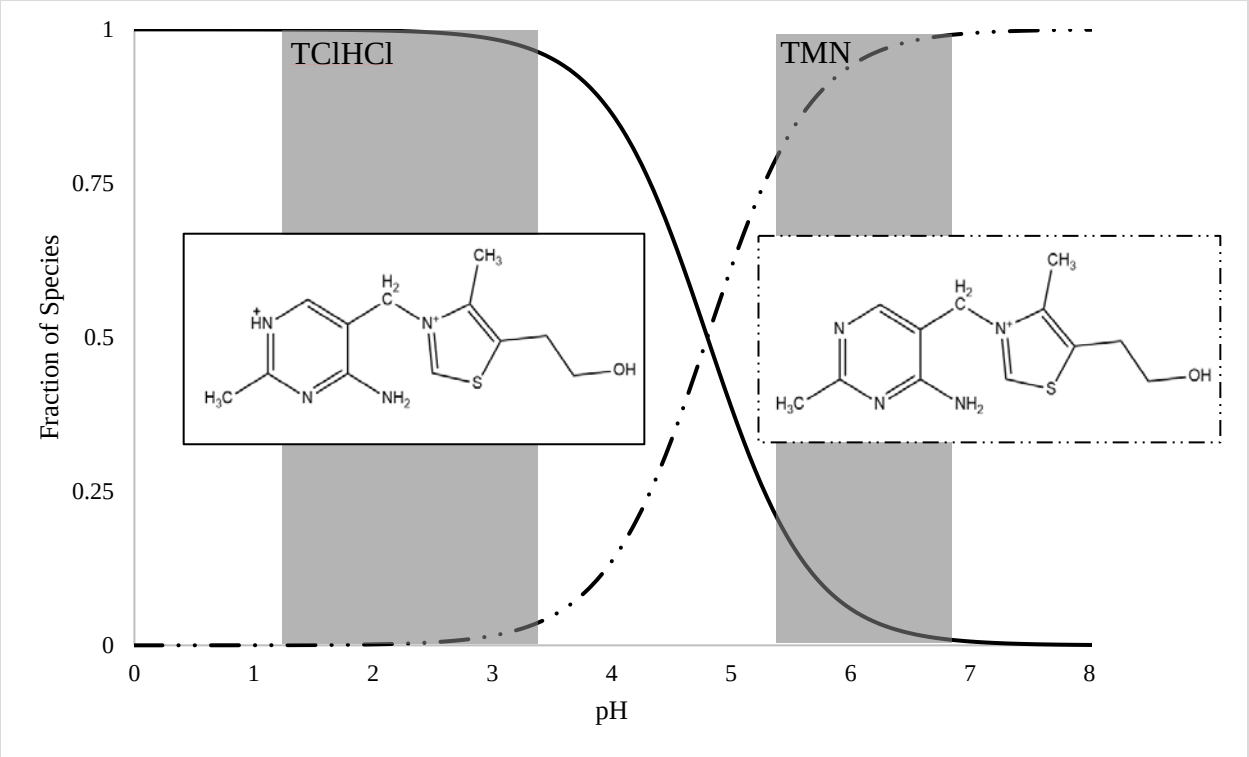
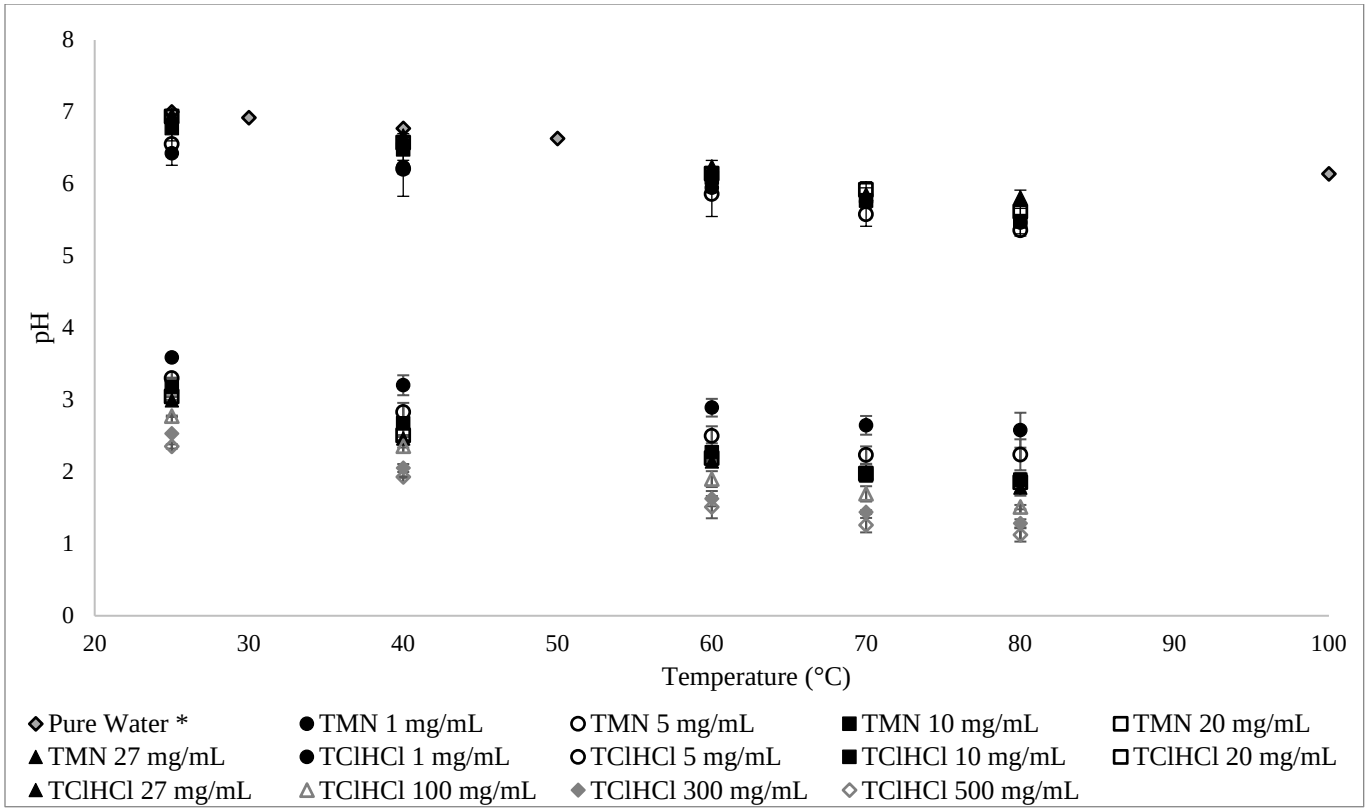

TClHCl
TMN
Fraction of Species
pH
1
0.75
0.5
0.25
0
0
1
2
3
4
5
6
7
8
CH3
H2
C
HN
N+
S
OH
H3C
N
NH2
CH3
H2
C
N
N+
S
OH
H3C
N
NH2

Figure 10


8
7
6
5
4
3
2
1
0
pH
20
30
40
50
60
70
80
90
100
Temperature (°C)
Pure Water *
TMN 1 mg/mL
TMN 5 mg/mL
TMN 10 mg/mL
TMN 20 mg/mL
TMN 27 mg/mL
TClHCl 1 mg/mL
TClHCl 5 mg/mL
TClHCl 10 mg/mL
TClHCl 20 mg/mL
TClHCl 27 mg/mL
TClHCl 100 mg/mL
TClHCl 300 mg/mL
TClHCl 500 mg/mL