

Purdue University

Purdue e-Pubs

Department of Food Science Faculty
Publications

Department of Food Science

10-2018

Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution

Lisa J. Mauer

Purdue University, mauer@purdue.edu

Adrienne L. Voelker

Purdue University

Jenna Miller

Purdue University

Cordelia Running

Purdue University, crunning@purdue.edu

Lynne S. Taylor

Purdue University

Follow this and additional works at: <https://docs.lib.purdue.edu/foodscipubs>

Recommended Citation

Mauer, Lisa J.; Voelker, Adrienne L.; Miller, Jenna; Running, Cordelia; and Taylor, Lynne S., "Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution" (2018). *Department of Food Science Faculty Publications*. Paper 15.
<https://docs.lib.purdue.edu/foodscipubs/15>

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries.
Please contact epubs@purdue.edu for additional information.

This is the author copy of an accepted manuscript, posted to the Purdue University Repository after an embargo period as permitted by the publisher.

The published copy can be found at:

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

AL Voelker, J Miller, CA Running, LS Taylor, LJ Mauer
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

Adrienne Voelker¹, Jenna Miller¹, Cordelia A. Running², Lynne S. Taylor³, Lisa J. Mauer^{1*}

¹ Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, Indiana 47907, United States

Lisa J. Mauer: mauer@purdue.edu

Adrienne Voelker: avoelke@purdue.edu

² Department of Nutrition Science, Purdue University, 700 West State Street, West Lafayette, Indiana 47907, United States

Cordelia A. Running: crunning@purdue.edu

³ Department of Industrial and Physical Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, Indiana 47907, United States

Lynne S. Taylor: lstaylor@purdue.edu

*Corresponding author: Lisa J. Mauer; Department of Food Science, Purdue University; 745

Agriculture Mall Drive, West Lafayette, Indiana 47907, United States; Email:

mauer@purdue.edu; Phone: 765-494-9111

Declarations of interests: none

1

2

3 **Abstract**

4 Two types of thiamine (vitamin B₁) salts, thiamine mononitrate (TMN) and thiamine
5 chloride hydrochloride (TCIHCl), are used to enrich and fortify food products. Both of these
6 thiamine salt forms are sensitive to heat, alkali, oxygen, and radiation, but differences in stability
7 between them have been noted. It was hypothesized that stability differences between the two
8 thiamine salts could be explained by differences in solubility, solution pH, and activation
9 energies for degradation. This study directly compared the stabilities of TMN and TCIHCl in
10 solution over time by documenting the impact of concentration and storage temperature on
11 thiamine degradation and calculating reaction kinetics. Solutions were prepared containing five
12 concentrations of each thiamine salt (1, 5, 10, 20, and 27 mg/mL), and three additional
13 concentrations of TCIHCl: 100, 300, and 500 mg/mL. Samples were stored at 25, 40, 60, 70, and
14 80°C for up to 6 months. Degradation was quantified over time by high-performance liquid
15 chromatography, and percent thiamine remaining was used to calculate reaction kinetics. First-
16 order reaction kinetics were found for both TMN and TCIHCl. TMN degraded significantly
17 faster than TCIHCl at all concentrations and temperatures. For example, in 27mg/mL solutions
18 after 5 days at 80°C, only 32% of TMN remained compared to 94% of TCIHCl. Activation
19 energies and solution pHs were 21-25 kcal/mol and pH 5.36-6.96 for TMN and 21-32 kcal/mol
20 and pH 1.12-3.59 for TCIHCl. TCIHCl degradation products had much greater sensory
21 contributions than TMN degradation products, including intense color change and potent aromas,
22 even with considerably less measured vitamin loss. Different peak patterns were present in
23 HPLC chromatograms between TMN and TCIHCl, indicating different degradation pathways
24 and products. The stability of essential vitamins in foods is important, even more so when
25 degradation contributes to sensory changes, and this study provides a direct comparison of the

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

28

29 **Key Words**

30 Thiamine, vitamin B₁, chemical stability, degradation, reaction kinetics, activation energy, pH,
31 sensory, thiamine mononitrate, thiamine chloride hydrochloride

32 **1. Introduction**

33 Vitamin B₁, also known as thiamine (Figure 1), is an essential micronutrient in the
34 human diet that is found both naturally and as a fortification supplement in many foods.
35 Thiamine acts as a coenzyme for metabolism of carbohydrates and branched-chain amino acids
36 and has roles in digestion, the nervous system, and muscle contraction (Institute of Medicine,
37 1998). Thiamine deficiency persists in both developing and developed countries. In developing
38 countries, a lack of nutritious food or nutritional variety, which may occur when unfortified
39 grains such as polished rice are the main dietary component, are the main contributors to
40 thiamine deficiency, which is found in up to 25% of the population (Ball, 2006; Prinzo, 1999).
41 In developed countries, where fortification efforts have reduced overall rates of thiamine
42 deficiency to near 10%, deficiency is more likely found in alcoholics, people on strict weight
43 loss diets, and people avoiding consumption of fortified grain products, including those with
44 Celiac's disease (Ball, 2006; Shepherd & Gibson, 2013). Thiamine deficiency can cause both
45 minor symptoms, such as fatigue, insomnia, irritability, and other neurological indicators, as well
46 as severe diseases resulting from prolonged deficiency, e.g., Beriberi and Wernicke-Korsakoff
47 syndrome (Spitzer & Schweigert, 2007). Thiamine stores in the body are very small and last
48 only weeks, which contributes to the concern of deficiency (Baumgartner, Henderson, Fox, &
49 Gondi, 1997). The Recommended Dietary Allowance (RDA) and Daily Value (DV) for
50 thiamine in the U.S. are both 1.2 mg/day (Institute of Medicine, 1998; U.S. Food & Drug
51 Administration, 2018). To combat the likelihood of deficiency, thiamine salts are often used to
52 enrich and fortify many food and beverage products.

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

55 Additionally, two salt forms are used as food additives: thiamine mononitrate* (TMN) and
56 thiamine chloride hydrochloride (TCIHCl) (Figure 1). TMN is a mono-salt, with only one nitrate
57 anion present, and TCIHCl is a di-salt with two chlorides present. TCIHCl is often
58 interchangeably called ‘thiamine hydrochloride’ (Ash, 2008); however, it is important to note
59 that the molecular formula contains two chlorides ($C_{12}H_{17}ClN_4OS \cdot HCl$), as shown in Figure 1.
60 While thiamine has two pK_{aS} ($pK_{a1} = 4.8$ for the pyrimidine N1 and $pK_{a2} = 9.2$ for the thiazole
61 quaternary nitrogen (Edwards et al., 2017)), pK_{a1} is the only relevant pK_a for the majority of
62 food products. Solid state properties of TMN and TCIHCl differ widely from one another (Table
63 1). TMN is often used in dry food products due to its low hygroscopicity, and TCIHCl is often
64 used in liquid or beverage products due to its high solubility (Labuza & Kamman, 1982). The
65 higher solubility of TCIHCl compared to TMN is due to the higher free energy of the TCIHCl
66 crystalline salt form (Atkins & de Paula, 2006). The two salt forms also have substantial
67 stability differences that have been explained by different activation energies, reported as 22.4
68 kcal/mol for TCIHCl and 26.3 kcal/mol for TMN in solid state systems, with E_a decreasing as
69 water activity (a_w) increased (Labuza & Kamman, 1982).

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

* Abbreviations: TMN, thiamine mononitrate; TCIHCl, 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

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

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

94

95 **2. Materials and Methods**

96 **2.1 Materials:**

97 Two thiamine salt forms were studied: thiamine mononitrate (TMN), $C_{12}H_{17}N_4OS \cdot$
98 NO_3 , obtained from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ), and thiamine
99 chloride hydrochloride (TCIHCl), $C_{12}H_{17}ClN_4OS \cdot HCl$, obtained from Fisher Scientific (Fair
100 Lawn, NJ). For use in high performance liquid chromatography (HPLC), HPLC grade
101 acetonitrile was obtained from Fisher Scientific and HPLC grade trifluoroacetic acid was
102 obtained from Sigma-Aldrich Inc. (St. Louis, MO). Water used in all experiments was deionized
103 and purified using a Barnstead E-Pure ultrapure water purification system with a resistivity at
104 $25^\circ C$ greater than $17.5 M\Omega \cdot cm$ (ThermoScientific, Waltham, MA).

105

106 **2.2 Solubility Measurement:**

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

115

116 **2.3 Sample Preparation:**

117 To understand the impact of thiamine concentration in solution on vitamin stability,
118 series of TMN and TCIHCl solutions were prepared containing 5 thiamine concentrations: 1, 5,
119 10, 20, and 27 mg/mL (the latter is just under the maximum solubility of TMN). Solutions

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

129

130 **2.4 Sample Storage:**

131 To monitor the effect of temperature on thiamine stability, solutions were stored at 5
132 temperatures: 25°C, 40°C, 60°C, 70°C, and 80°C. These temperatures were chosen to provide a
133 large range of temperatures for calculating temperature-dependent reaction kinetics. The 25°C
134 condition was used as an ambient temperature control and was maintained within $\pm 1^\circ\text{C}$ using a
135 temperature-controlled room (Commercial Fixture Company Inc., Indianapolis, IN). The 40°C,
136 60°C, and 70°C temperatures were maintained using Forma Scientific water-jacketed incubators
137 (Thermo Fisher Scientific Inc., Marietta, OH). The 80°C temperature was maintained using a
138 digital heatblock (VWR International, Radnor, PA). To monitor storage conditions, temperature
139 was confirmed by liquid-in-glass partial immersion thermometers. Solutions were stored in
140 controlled temperature environments for up to 6 months, depending on temperature and vitamin
141 form, and were analyzed for percent vitamin remaining at a minimum of 5 selected timepoints.

142

143 **2.5 Vitamin Quantification:**

144 The chemical stability of thiamine in solution was monitored by measuring vitamin
145 concentration over time using a high performance liquid chromatography (HPLC) method
146 adapted from Xia et al. (Xia et al., 2006). A Waters 2690 Separations Module (Waters Corp.
147 Milford, MA) equipped with a Waters 2996 Photodiode Array detector (Waters Corp.) was used
148 with a 100 mm x 3.9 mm, 3.5 μm particle size XTerra RP-C₁₈ column (Waters Corp.). The
149 wavelength scan used was 235-400 nm. Mobile phase A: 0.1% trifluoroacetic acid (TFA) in
150 water (v/v) and mobile phase B: acetonitrile (MeCN) were used with a flow rate of 1 mL/min and
151 the following gradient method: 100/0 at 0 min, 97/3 at 4 min (linear), 90/10 at 6 min (linear),
152 100/0 at 10 min (linear), and 100/0 at 15 min. Prior to analysis, solutions were removed from
153 controlled temperature storage, cooled in an ice bath, and diluted with mobile phase A to an
154 estimated thiamine concentration of 500 ppm, or 0.5 mg/mL. Standard curves of TMN and
155 TClHCl ($R^2 > 0.999$) at a concentration range of 10 ppm to 1000 ppm were prepared prior to
156 each day of analysis and used to calculate the concentration of each sample. Integration was
157 performed at 254 nm.

158

159 **2.6 Reaction Kinetics:**

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

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

$$168 \quad \ln \frac{x}{x_0} = -kt \quad (1)$$

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

$$172 \quad k = Ae^{\frac{-E_a}{RT}} \quad (2)$$

173 where k is the reaction rate constant (days^{-1}), A is the frequency factor of collision, E_a is the
174 activation energy (kJ/mol), R is the gas constant (8.3145 J/mol·K), and T is temperature (K).

175 Since some foods have multiple degradation patterns that may have different temperature
176 dependencies, it is possible to find non-linear Arrhenius plots (Gregory III, 2008), and therefore
177 nonlinear Arrhenius plots were also considered.

178

179 **2.7 pH Measurement:**

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

185

186 **2.8 Photography and color analysis:**

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

195

196 **2.9 Sensory Study of Odor Differences between Degraded Vitamin Solutions:**

197 Thiamine degradation is known to produce aromas and flavors (Buttery et al., 1984;
198 Dwivedi & Arnold, 1973). To determine if differences in the odors produced by degraded TMN
199 and TClHCl could be detected by untrained panelists, 5 mg/mL solutions of each vitamin salt
200 form were again prepared in the 20 mL amber vials with PE cone-lined caps, heated for 2 days at
201 80°C, and frozen until the day of the sensory test. These conditions were chosen as a
202 representation of the odor produced by each vitamin salt form, and the amount of thiamine
203 degradation in these samples was determined by HPLC.

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

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

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

223 Data were analyzed by GraphPad Software using a two-tailed binomial distribution with
224 $\alpha = 0.05$. Using a rearrangement of Abbott’s formula to adjust for chance (Lawless & Heymann,
225 2010), 75% of the participants needed to select the same sample as “stronger” in order to
226 conclude that participants found the aroma of one sample stronger than the other. This formula
227 was also used to determine the percentage of participants who were true discriminators.

228

229 **2.10 Statistical Analysis:**

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

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

238

239 **3. Results & Discussion**

240 **3.1 Effects of Concentration and Temperature on Stability of Thiamine in TMN Solutions:**

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

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

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

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

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

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

296

297 **3.2 Effects of Concentration and Temperature on Stability of Thiamine in TCIHCl**

298 **Solutions:**

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

302 temperatures. The pH of TCIHCl 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 TCIHCl in varying
304 concentrations of solution at 80°C is shown in Figure 5. Thiamine in solutions across all
305 concentrations of TCIHCl degraded in an exponential manner. A table containing all the
306 thiamine percent remaining data from all TCIHCl solution concentrations at all temperatures is
307 provided in the supplementary material (Table S2).

308 The thiamine degradation patterns found in all TCIHCl solution concentrations and
309 temperature treatments were consistent with those reported in the literature for TCIHCl (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 TCIHCl 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 TCIHCl 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 TCIHCl 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 TCIHCl 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 TCIHCl and TMN solutions. The main thiamine

325 degradation peaks found in TCIHCl solutions were at retention times of approximately 2.13,
326 4.05, 5.72, and 6.95 min. The L, a, and b values that documented the color of selected TCIHCl
327 solutions after storage are included in Table 4, and photographs of the color change are included
328 in the supplementary material (Figure S2). Unlike what was found in the TMN solutions, much
329 more color change was found in the TCIHCl solutions, even when less thiamine had degraded.
330 For example, when 56% of thiamine in TCIHCl 27 mg/mL solutions remained after 31 days at
331 80°C, the solutions were nearly black, compared to minimal color change when more thiamine
332 had degraded in a shorter timeframe in 27 mg/mL TMN solutions (31% thiamine remaining after
333 7 days at 80°C in solutions that were light yellow). After only 5 hours at 80°C, a 500 mg/mL
334 solution of TCIHCl in which no significant degradation of thiamine was found had a very similar
335 color to that same 27 mg/mL TMN solution with only 31% thiamine remaining. The color
336 changes found in solutions of TMN and TCIHCl at various points during degradation were
337 significantly different ($p < 0.05$). The difference in color change was attributed to the different
338 degradation products that were formed by the different thiamine salts, exemplified by their
339 differing HPLC chromatograms.

340

341 **3.3 Sensory Study of Odor Differences between Degraded Vitamin Solutions:**

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

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

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

365

366 **3.4 Comparison of Thiamine Stability in TMN and TCIHCl Solutions:**

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

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

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

387 It has also been reported that pH affects the degradation pathway of thiamine (Dwivedi &
388 Arnold, 1972). Thiamine has a pK_a of 4.8 (for the pyrimidine N1 nitrogen) (Edwards et al.,
389 2017). In acidic conditions ($pH < 6$), degradation occurs by cleavage of the methylene bridge to
390 release intact pyrimidine and thiazole moieties; while in conditions above pH 6, degradation
391 involves the same cleavage, but also further fragmentation of the thiazole ring (Gregory III,
392 2008). These varying pathways support the observation of different degradation products

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

400 Thiamine stability was significantly affected by TMN concentration, with thiamine
401 degradation rates increasing as the concentration of TMN increased. This observation was likely
402 more dependent on the changing solution pHs as TMN concentration increased rather than on the
403 solution concentration of the thiamine *per se*. It has been well-documented that there is a
404 dramatic decrease in stability of thiamine as pH reaches and exceeds pH 6.0 (Felicciotti &
405 Esselen, 1957; Mulley, Stumbo, & Hunting, 1975; Williams & Ruehle, 1935). This change in
406 stability is a result of the pK_a of thiamine (4.8). As illustrated in the speciation plot of thiamine
407 in Figure 9, the more stable protonated species of thiamine is present as a notable fraction in
408 acidic conditions up to approximately pH 6.0. As pH increases above 6.0, the less stable
409 unprotonated species of thiamine dominates, and the stability of thiamine dramatically decreases.
410 This noteworthy pH value (6.0) could be used to explain the dependence of thiamine stability on
411 TMN concentration since the pH values found for TMN solutions were between pH 5.36 and
412 6.96. Small increases in pH due to increases in TMN concentration would have led to major
413 changes in the fraction of protonated/unprotonated thiamine species present, which in turn would
414 have caused the large decrease in thiamine stability that was found to be so dependent on TMN
415 concentration. Conversely, in the pH range found in TClHCl solutions (from 1.12 to 3.59), the

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

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

427

428 **3.5 Degradation Kinetics of Thiamine Salt Forms:**

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

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

443 It was reported previously that thiamine degradation in buffered solutions from 50°C to
444 110°C exhibited no deviation from Arrhenius behavior (Farrer & Morrison, 1949), but
445 temperatures below 50°C were not included in the study. In the current study, non-linear
446 Arrhenius plots were found to occur as the concentration of degradation products increased;
447 however, in the early stages of thiamine degradation linear Arrhenius plots were found. These
448 linear Arrhenius plots were used to calculate reaction kinetics. Using the k_{obs} values from
449 temperatures 25, 40, 60, 70, and 80°C, the TMN activation energies were consistent with
450 previous reports, ranging from 21-25 kcal/mol (88-105 kJ/mol), dependent on concentration. All
451 values are included in Table 5. Using the k_{obs} values from temperatures 60, 70, and 80°C,
452 TClHCl activation energies were found to range from 21-32 kcal/mol (90-135 kJ/mol). While
453 these values are slightly higher than those previously reported, the extremely low pH found in
454 the TClHCl solutions was not studied elsewhere. The low pH values (1.12-3.59) and
455 consequently the predominance of the more stable protonated form of thiamine (Figure 9) led to
456 the higher stability of thiamine in TClHCl solutions observed in this study (for example, 91% of
457 TClHCl remained in the 10 mg/mL solution after 7 days at 80°C compared to 38% TMN
458 remaining in the same conditions). Additionally, the high thiamine stability in TClHCl solutions
459 at 25°C and 40°C allowed the use of only 3 (higher) temperatures for the kinetics calculations,
460 rather than the preferred 5 temperatures. However, the R^2 values for the Arrhenius calculations
461 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. TCIHCl 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 TCIHCl 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 TCIHCl that have close

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

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

505

506 **4. Conclusions**

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

524

525 **5. Acknowledgements**

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

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

- 533 Ahmad, I., Mobeen, M. F., Sheraz, M. A., Ahmed, S., Anwar, Z., Shaikh, R. S., . . . Ali, S. M.
 534 (2018). Photochemical interaction of ascorbic acid and nicotinamide in aqueous solution:
 535 A kinetic study. *Journal of Photochemistry and Photobiology B: Biology*, 182, 115-121.
 536 doi: <https://doi.org/10.1016/j.jphotobiol.2018.04.011>
- 537 Ash, M. (2008). *Handbook of food additives* (3rd ed.. ed.). Endicott, NY: Endicott, NY : Synapse
 538 Information Resources.
- 539 Atkins, P., & de Paula, J. (2006). Phase equilibria. In P. Atkins & J. de Paula (Eds.), *Physical*
 540 *chemistry for the life sciences* (pp. 104-150). New York, NY: W. H. Freeman and
 541 Company.
- 542 Ball, G. F. M. (2006). *Vitamins in foods : analysis, bioavailability, and stability*. Boca Raton,
 543 FL: CRC/Taylor & Francis.
- 544 Baumgartner, T. G., Henderson, G. N., Fox, J., & Gondi, U. (1997). Stability of ranitidine and
 545 thiamine in parenteral nutrition solutions. *Nutrition*, 13(6), 547-553.
- 546 Bettendorff, L. (2012). Thiamin *Present Knowledge in Nutrition* (pp. 261-279): Wiley-
 547 Blackwell.
- 548 Buttery, R. G., Haddon, W. F., Seifert, R. M., & Turnbaugh, J. G. (1984). Thiamin odor and
 549 bis(2-methyl-3-furyl) disulfide. *J. Agric. Food Chem.*, 32(3), 674-676. doi:
 550 10.1021/jf00123a061
- 551 Cauvain, S. P., & Young, L. S. (2006). Ingredients and their influences. In S. P. Cauvain & L. S.
 552 Young (Eds.), *Baked products science, technology and practice*: Oxford : Blackwell.
- 553 ChemSpider. (2015). Thiamine: Royal Society of Chemistry.
- 554 Clark, J. (2017). Temperature dependence of the pH of pure water. *LibreTexts*
- 555 Dennison, D., Kirk, J., Bach, J., Kokoczka, P., & Heldman, D. (1977). Storage stability of
 556 thiamin and riboflavin in a dehydrated food system. *Journal of Food Processing and*
 557 *Preservation*, 1(1), 43-54.
- 558 Dhakal, S., Balasubramaniam, V. M., Ayvaz, H., & Rodriguez-Saona, L. E. (2018). Kinetic
 559 modeling of ascorbic acid degradation of pineapple juice subjected to combined pressure-
 560 thermal treatment. *Journal of Food Engineering*, 224, 62-70. doi:
 561 <https://doi.org/10.1016/j.jfoodeng.2017.12.016>
- 562 Dwivedi, B. K., & Arnold, R. G. (1972). Chemistry of thiamine degradation: mechanisms of
 563 thiamine degradation in a model food system. *Journal of Food Science*, 37(6), 886-888.
 564 doi: 10.1111/j.1365-2621.1972.tb03694.x
- 565 Dwivedi, B. K., & Arnold, R. G. (1973). Chemistry of thiamine degradation in food products and
 566 model systems: a review. [Review]. *Journal of Agricultural and Food Chemistry*, 21(1),
 567 54-60. doi: 10.1021/jf60185a004
- 568 Edwards, K. A., Tu-Maung, N., Cheng, K., Wang, B., Baeumner, A. J., & Kraft, C. E. (2017).
 569 Thiamine Assays—Advances, Challenges, and Caveats (Vol. 6, pp. 178-191).
- 570 Farrer, K. T. (1947). The thermal destruction of vitamin B1: the influence of the concentration of
 571 buffer salts on the rate of destruction of aneurin at 100 degrees. *The Biochemical Journal*,
 572 41(2), 167.

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

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

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

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

584 Institute of Medicine. (1998). *Thiamin Dietary reference intakes for thiamin, riboflavin, niacin,*
585 *vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin, and choline*. Washington, D.C.:
586 National Academy Press.

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

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

592 Labuza, T. P., & Kamman, J. F. (1982). Comparison of stability of thiamin salts at high
593 temperature and water activity. *Journal of Food Science*, 47(2), 664-665. doi:
594 10.1111/j.1365-2621.1982.tb10146.x

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

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

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

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

607 Mauri, L. M., Alzamora, S. M., Chirife, J., & Tomio, M. J. (1989). Review: kinetic parameters
608 for thiamine degradation in foods and model solutions of high water activity.
609 *International Journal of Food Science & Technology*, 24(1), 1-9. doi: 10.1111/j.1365-
610 2621.1989.tb00613.x

611 Mauri, L. M., Alzamora, S. M., & Tomio, J. M. (1992). Effect of electrolytes on the kinetics of
612 thiamine loss in model systems of high water activity. *Food Chemistry*, 45(1), 19-23. doi:
613 10.1016/0308-8146(92)90006-N

614 McIntire, F. C., & Frost, D. V. (1944). Thiamin stability: effect of amino acids and related
615 compounds and of thiamin concentration. *Journal of the American Chemical Society*,
616 66(8), 1317-1318. doi: 10.1021/ja01236a033

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

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

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

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

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

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

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

635 Williams, R. R., & Ruehle, A. E. (1935). Studies of crystalline vitamin B1: presence of
636 quaternary nitrogen. *Journal of the American Chemical Society*, 57(10), 1856-1860. doi:
637 10.1021/ja01313a029

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

641 Young, F. E. (1957). D-glucose-water phase diagram. *The Journal of Physical Chemistry*, 61(5),
642 616-619.

643

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

	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 (RH₀) 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 TCIHCl 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 ^{Ac}	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}
TCIHCl	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 (°C)		1 mg/mL	5 mg/mL	10 mg/mL	20 mg/mL	27 mg/mL
TMN	25	k_{obs}^* (day ⁻¹)	$3.4 \times 10^{-4} \pm 0.5 \times 10^{-4}$ Aa	$6.3 \times 10^{-4} \pm 0.6 \times 10^{-4}$ Abc	$8.1 \times 10^{-4} \pm 0.1 \times 10^{-4}$ Ac	$4.8 \times 10^{-4} \pm 0.5 \times 10^{-4}$ Aab	$7.2 \times 10^{-4} \pm 0.8 \times 10^{-4}$ 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)	$1.74 \times 10^{-3} \pm 0.7 \times 10^{-4}$ Aa	$3.1 \times 10^{-3} \pm 0.2 \times 10^{-3}$ Aa	$5.1 \times 10^{-3} \pm 0.4 \times 10^{-3}$ Ab	$7.6 \times 10^{-3} \pm 0.6 \times 10^{-3}$ Ac	$9.7 \times 10^{-3} \pm 0.1 \times 10^{-3}$ 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)	$1.7 \times 10^{-2} \pm 0.1 \times 10^{-2}$ Ba	$2.7 \times 10^{-2} \pm 0.1 \times 10^{-2}$ Ba	$3.9 \times 10^{-2} \pm 0.3 \times 10^{-2}$ Bb	$6.3 \times 10^{-2} \pm 0.6 \times 10^{-2}$ Ac	$7.7 \times 10^{-2} \pm 0.6 \times 10^{-2}$ 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)	$4.6 \times 10^{-2} \pm 0.5 \times 10^{-2}$ Ca	$7.2 \times 10^{-2} \pm 0.5 \times 10^{-2}$ Cab	$9.7 \times 10^{-2} \pm 0.6 \times 10^{-2}$ Cb	$1.4 \times 10^{-1} \pm 0.1 \times 10^{-1}$ Bc	$1.9 \times 10^{-1} \pm 0.2 \times 10^{-1}$ 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)	$1.17 \times 10^{-1} \pm 0.6 \times 10^{-2}$ Da	$1.4 \times 10^{-1} \pm 0.1 \times 10^{-1}$ Da	$2.0 \times 10^{-1} \pm 0.2 \times 10^{-1}$ Da	$4.6 \times 10^{-1} \pm 0.5 \times 10^{-1}$ Cb	$6 \times 10^{-1} \pm 1 \times 10^{-1}$ 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	
TCIHCl	60	k_{obs} (day ⁻¹)	$2.1 \times 10^{-3} \pm 0.2 \times 10^{-3}$ Abc	$1.9 \times 10^{-3} \pm 0.2 \times 10^{-3}$ Aabc	$1.8 \times 10^{-3} \pm 0.3 \times 10^{-3}$ Aabc	$1.5 \times 10^{-3} \pm 0.1 \times 10^{-3}$ Aa	$1.6 \times 10^{-3} \pm 0.2 \times 10^{-3}$ 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)	$1.7 \times 10^{-2} \pm 0.3 \times 10^{-2}$ Bc	$7.3 \times 10^{-3} \pm 0.8 \times 10^{-3}$ Bab	$7 \times 10^{-3} \pm 1 \times 10^{-3}$ Bab	$5.3 \times 10^{-3} \pm 0.7 \times 10^{-3}$ Ba	$4.4 \times 10^{-3} \pm 0.4 \times 10^{-3}$ 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)	$3.1 \times 10^{-2} \pm 0.1 \times 10^{-2}$ Cd	$1.5 \times 10^{-2} \pm 0.1 \times 10^{-2}$ Ca	$2.3 \times 10^{-2} \pm 0.3 \times 10^{-2}$ Cbc	$2.4 \times 10^{-2} \pm 0.1 \times 10^{-2}$ Cbc	$1.89 \times 10^{-2} \pm 0.09 \times 10^{-2}$ 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 TCIHCl 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 TCIHCl 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 ± 0.7% ^{AB}	-7.2 ± 0.4 ^E	6.2 ± 0.6 ^D
		7 days	82 ± 2% ^A	-11.6 ± 0.4 ^G	15.8 ± 0.2 ^B
TCIHCl	27 mg/mL	0 days	77 ± 1% ^{BC}	-5.3 ± 0.6 ^D	2.8 ± 0.3 ^F
		31 days	16 ± 1% ^E	2.9 ± 0.6 ^B	2.7 ± 0.4 ^F
	100 mg/mL	0 days	77 ± 2% ^{BC}	-5.8 ± 0.3 ^{DE}	3.1 ± 0.1 ^F
		31 days	15 ± 3% ^E	-2 ± 1 ^C	0 ± 1 ^G
	500 mg/mL	0 days	76 ± 1% ^C	-6.8 ± 0.3 ^{DE}	4.8 ± 0.4 ^E
		5 hours	80.7 ± 0.6% ^A	-9.3 ± 0.7 ^F	10.4 ± 0.9 ^C
31 days		40 ± 2% ^D	38 ± 2 ^A	35.7 ± 0.7 ^A	

Table 5. Calculated activation energies of TMN and TCIHCl 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
TCIHCl	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 25°C to 80°C.

Figure 5. Degradation profiles of thiamine chloride in TCIHCl 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 TCIHCl solutions at temperatures from 60°C to 80°C.

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

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

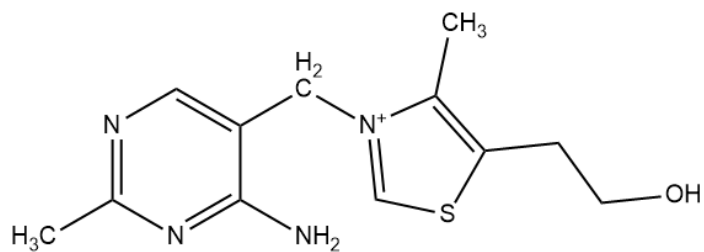
- TMN 1 mg/mL ○ TMN 5 mg/mL ■ TMN 10 mg/mL □ TMN 20 mg/mL ▲ TMN 27 mg/mL
- TCIHCl 1 mg/mL ○ TCIHCl 5 mg/mL ■ TCIHCl 10 mg/mL □ TCIHCl 20 mg/mL ▲ TCIHCl 27 mg/mL
- △ TCIHCl 100 mg/mL ◆ TCIHCl 300 mg/mL ◇ TCIHCl 500 mg/mL

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 TCIHCl and TMN samples, respectively.

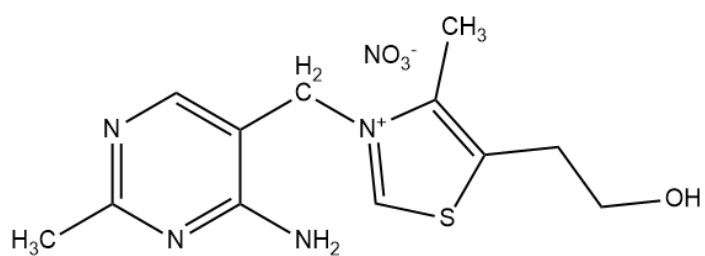
Figure 10. pH change with temperature of pure water (Clark, 2017), TMN, and TCIHCl for all concentrations studied.

Figure 1

A)



B)



C)

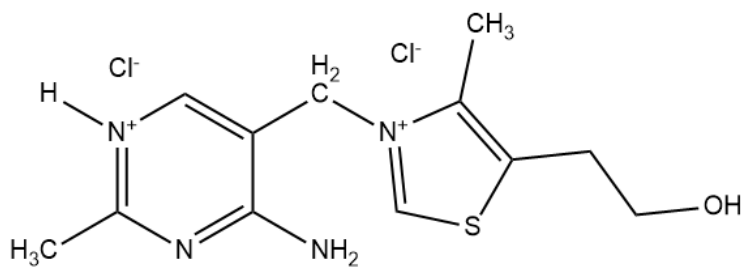


Figure 2

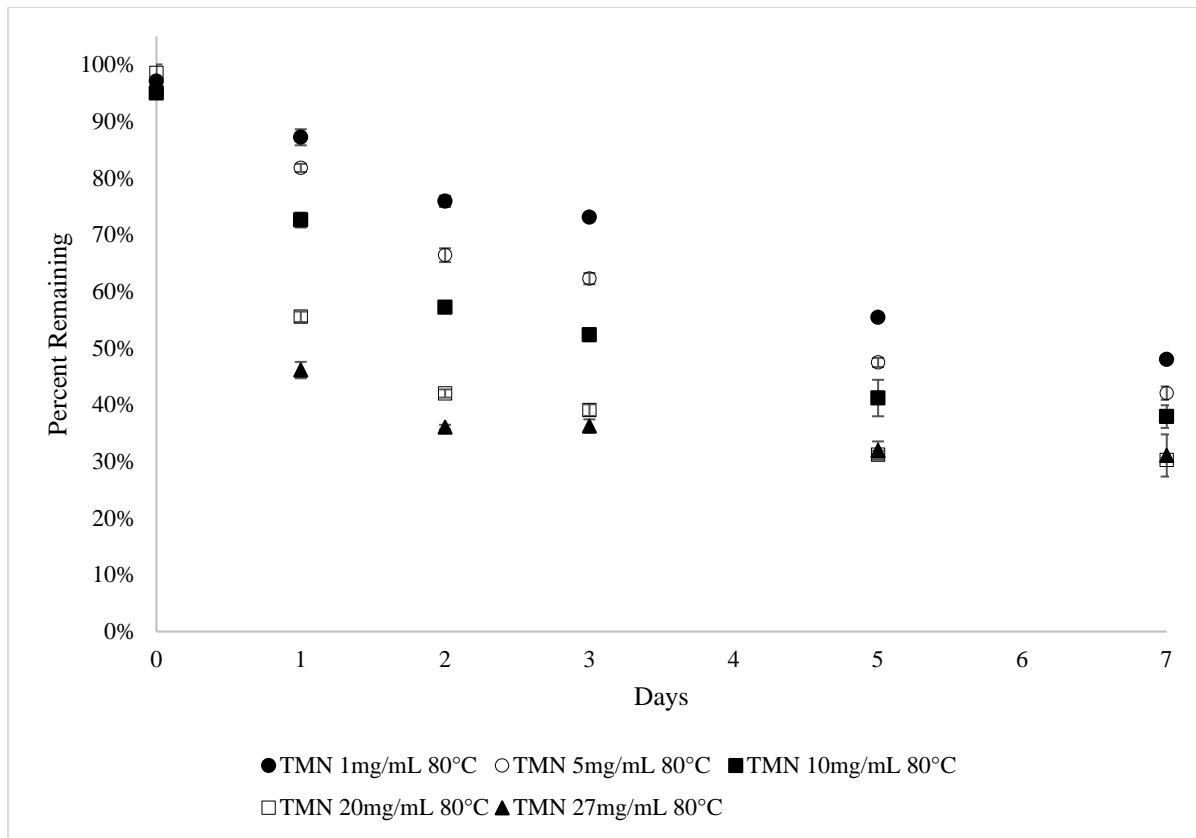


Figure 3

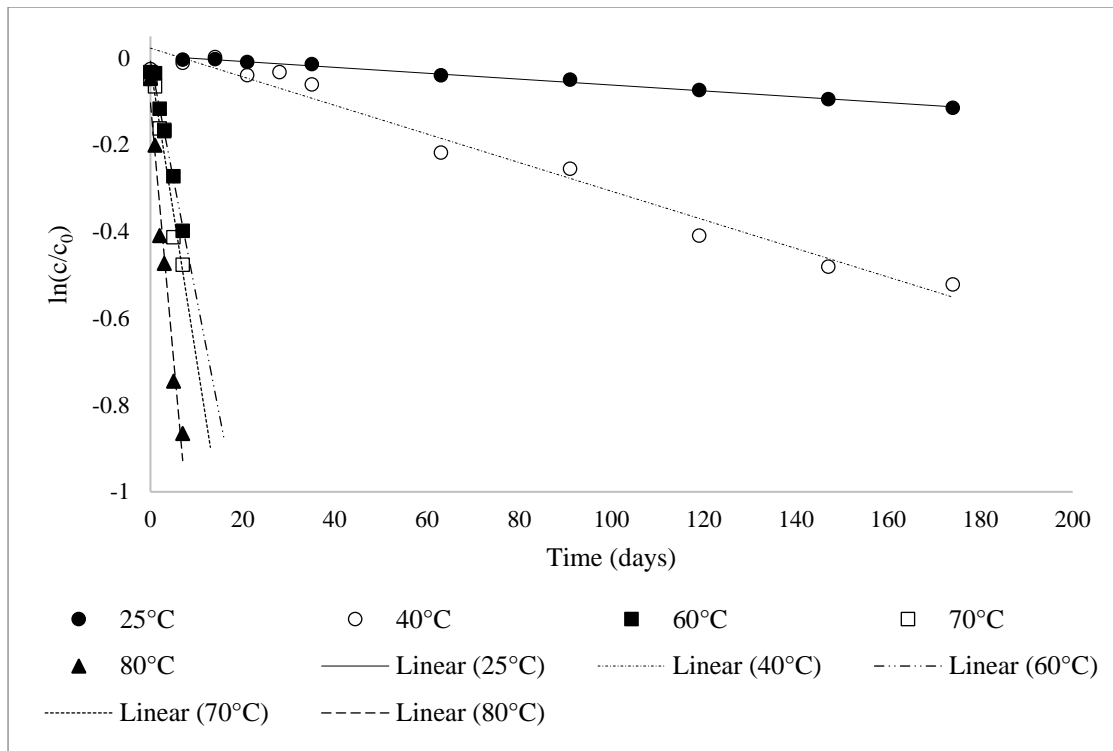


Figure 4

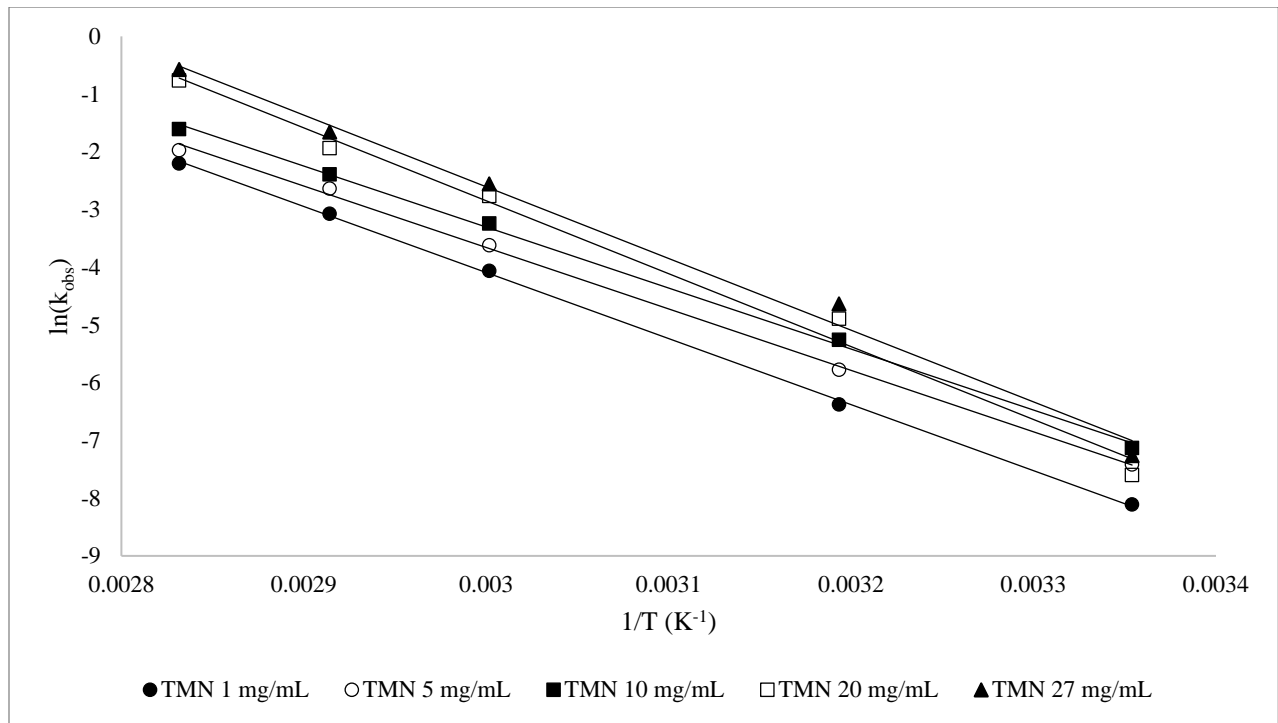


Figure 5

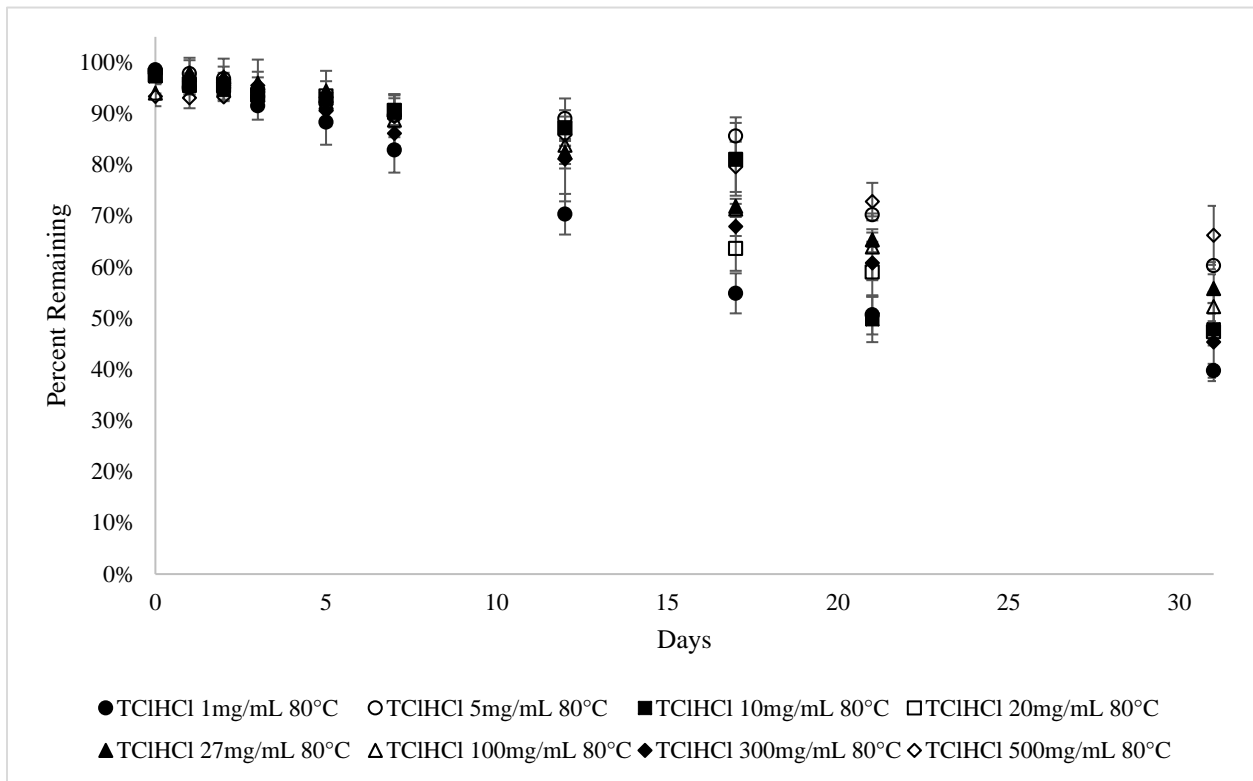


Figure 6

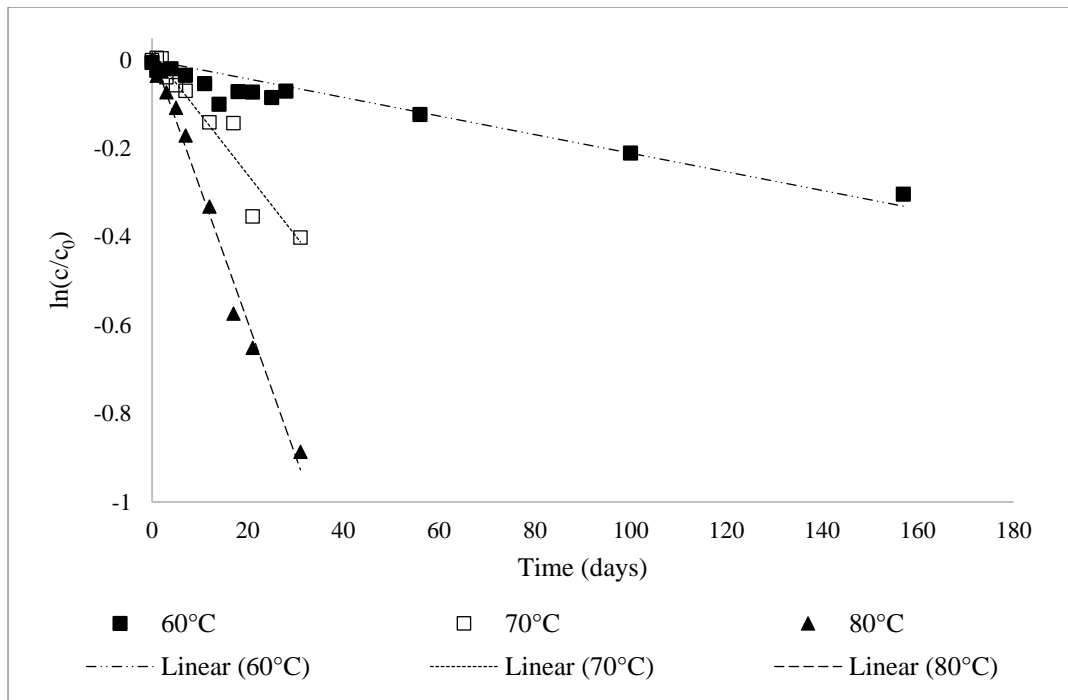


Figure 7

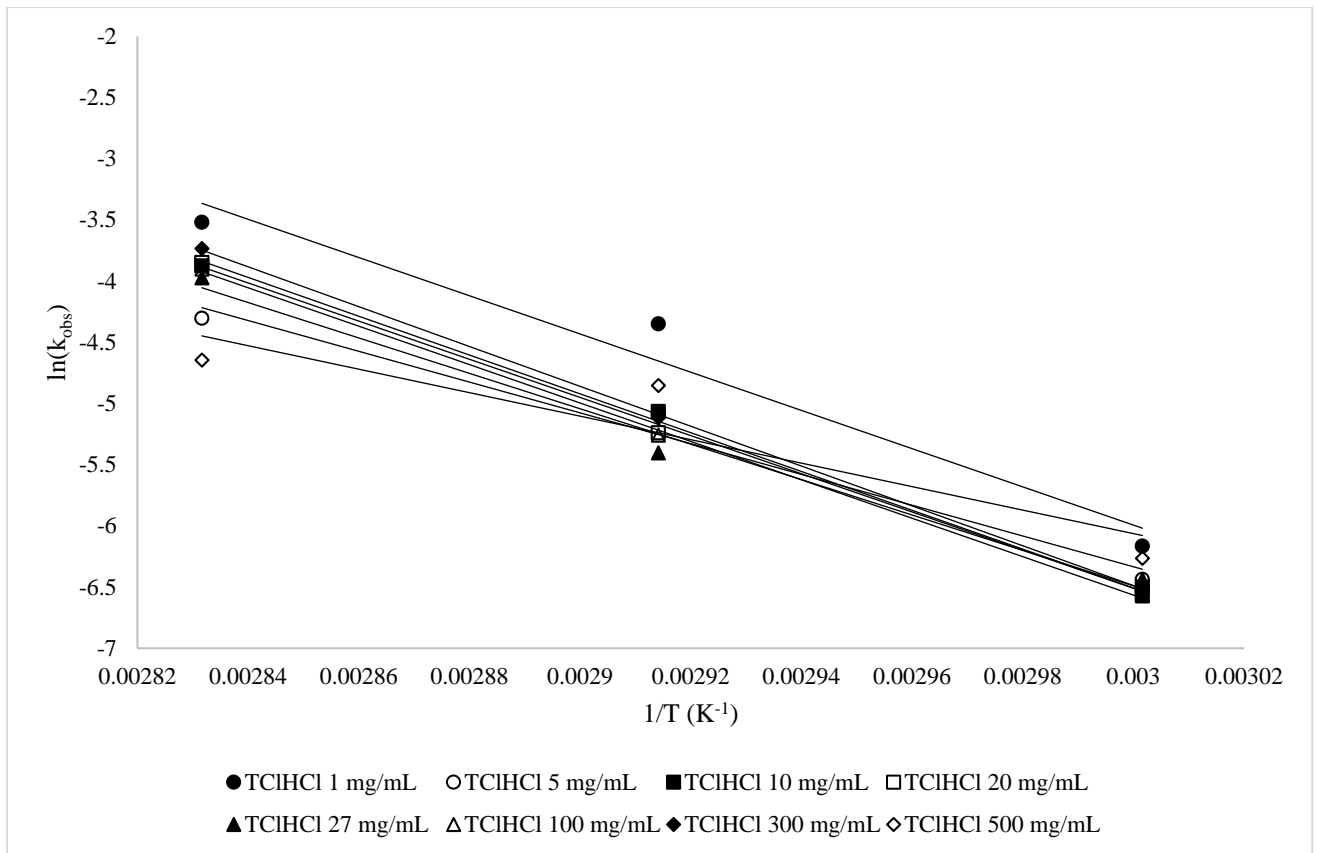
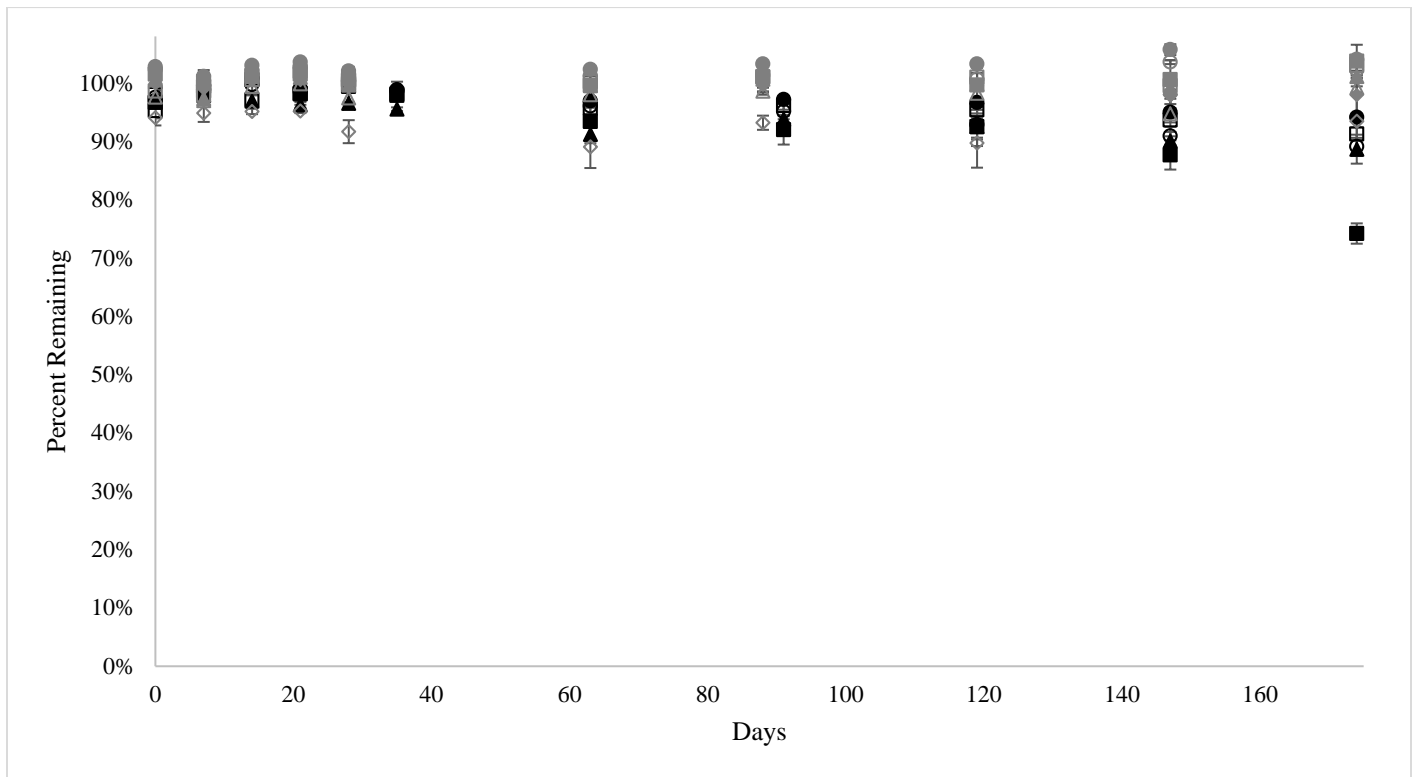
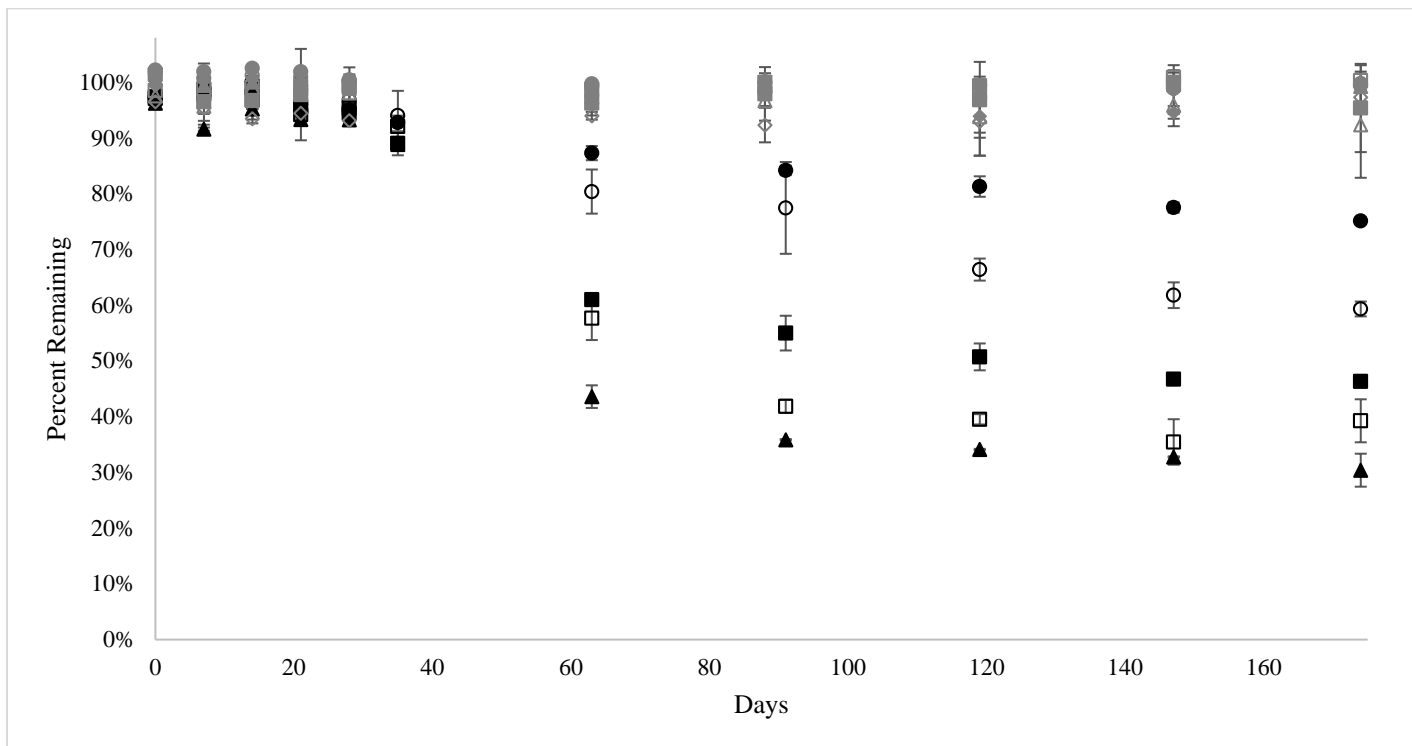


Figure 8

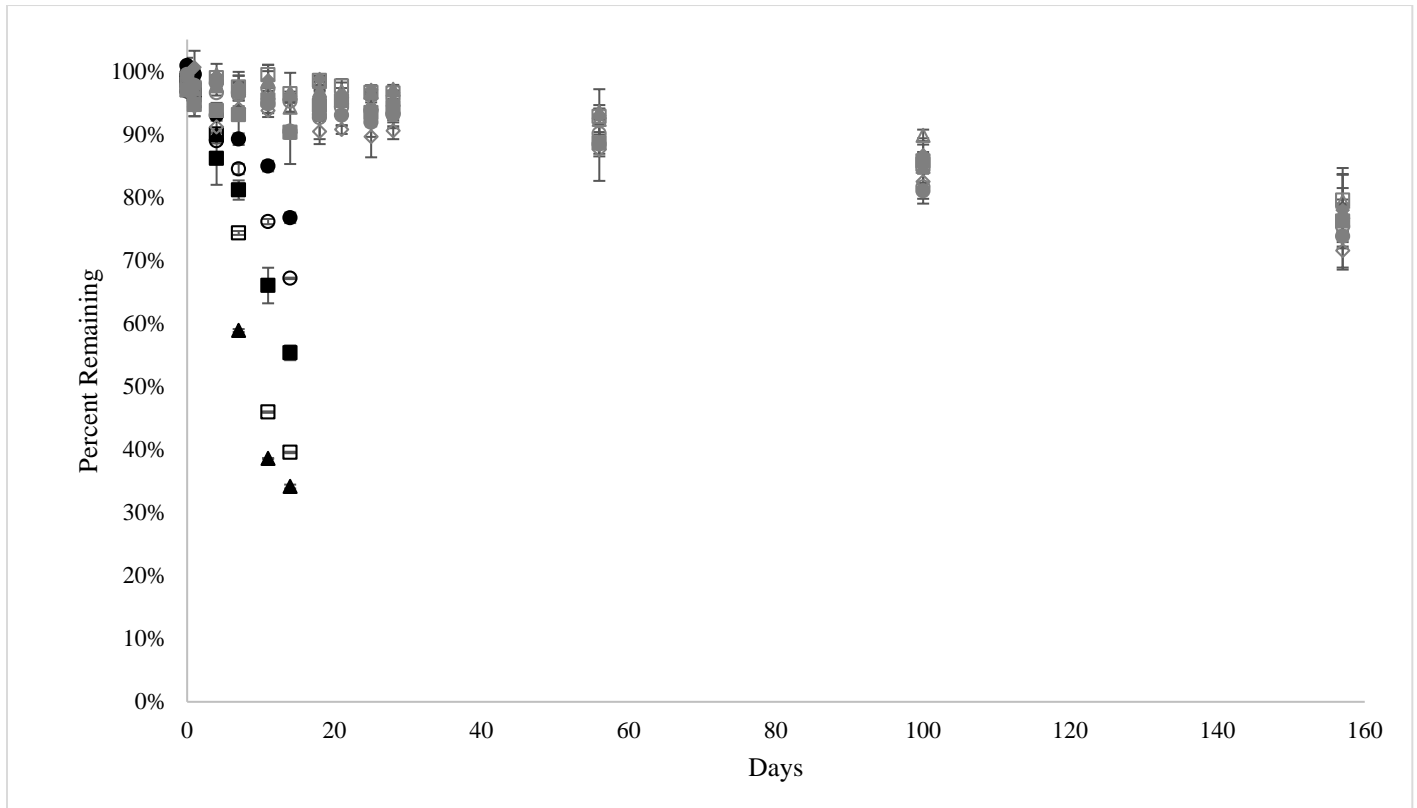
A)



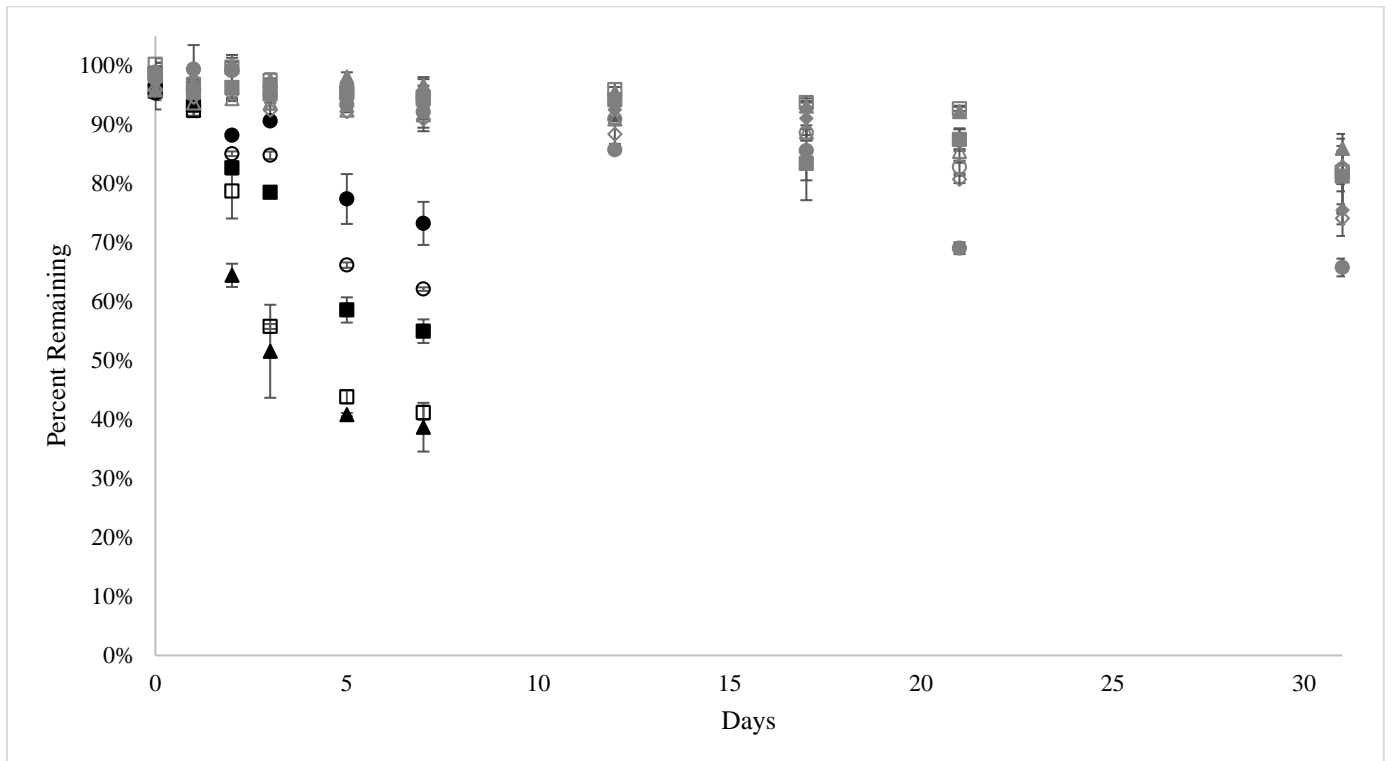
B)



C)



D)



E)

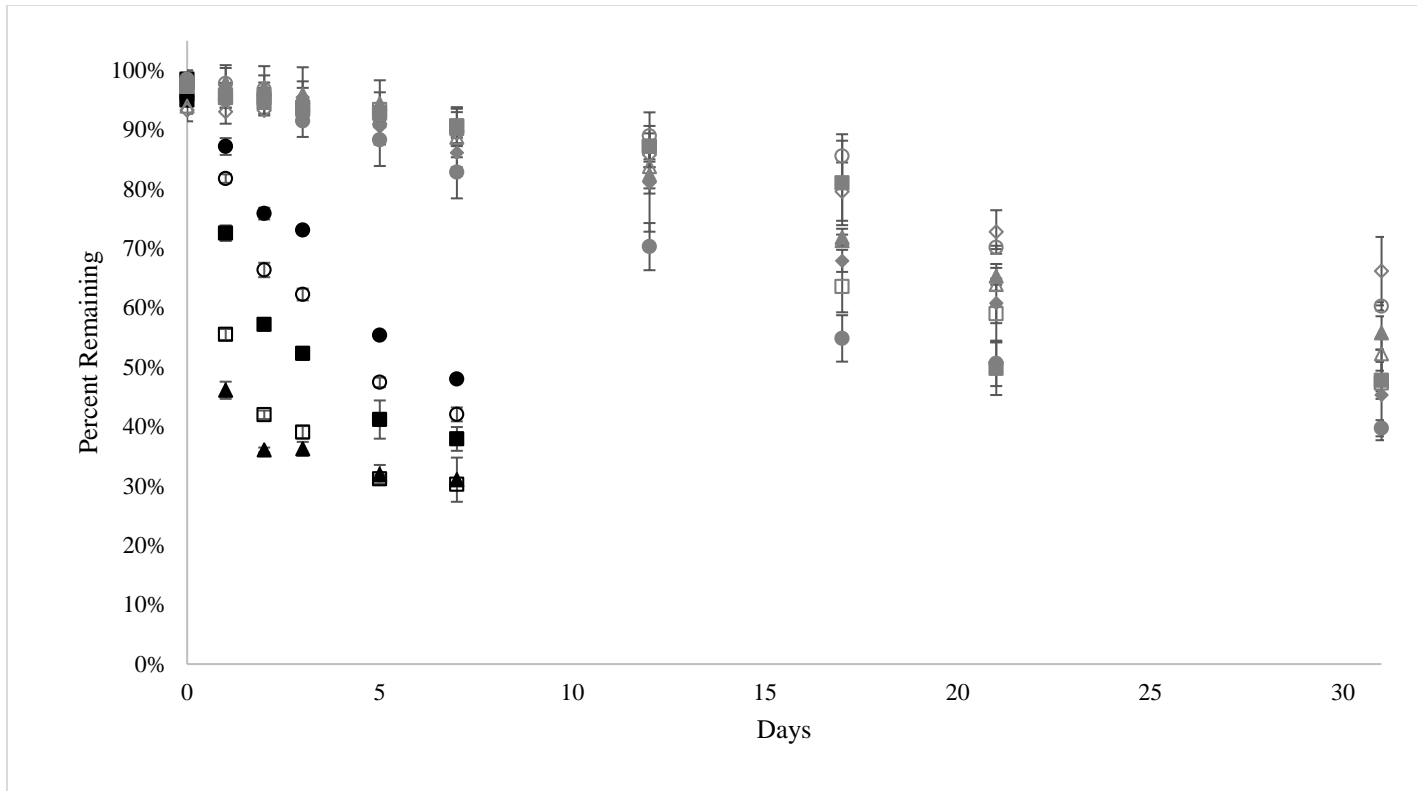


Figure 9

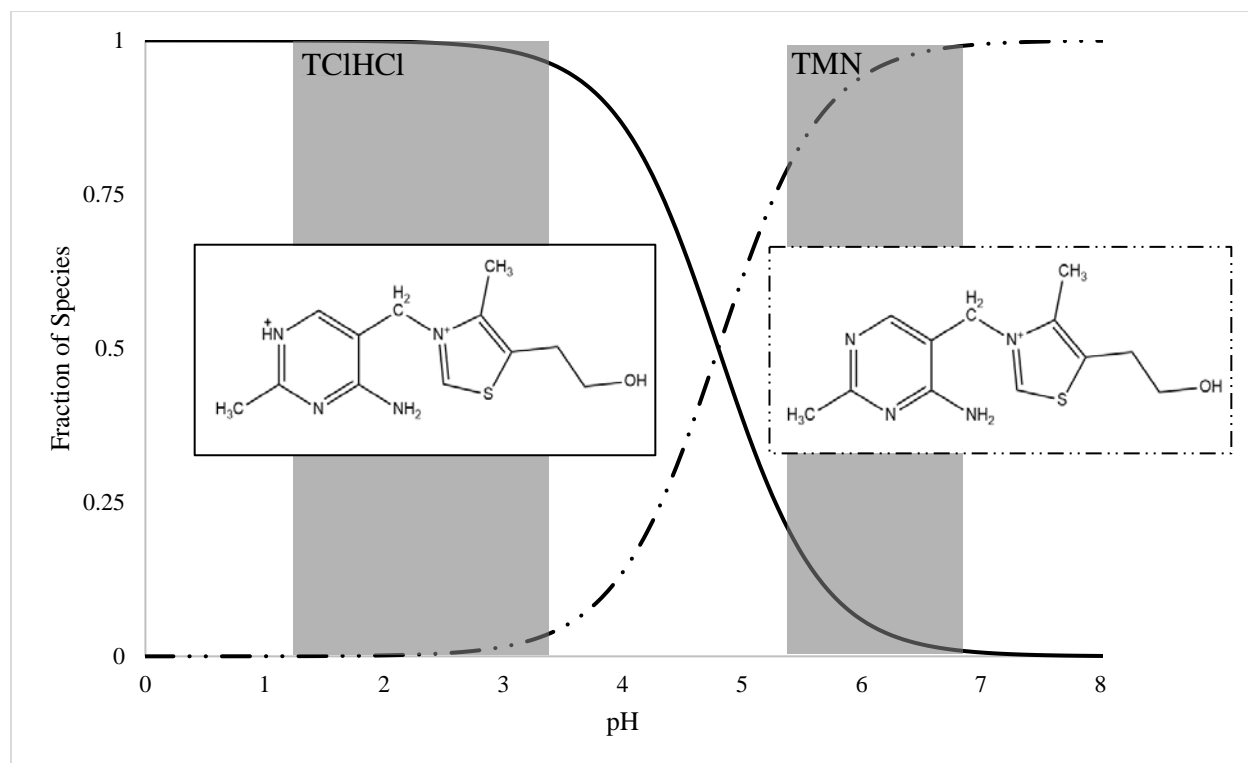


Figure 10

