INVESTIGATING STABILITY IN AMORPHOUS SOLID DISPERSIONS: A STUDY OF THE PHYSICAL AND CHEMICAL STABILITY OF TWO SALT FORMS OF THIAMINE AND THE PHYSICAL STABILITY OF CITRIC ACID

by

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To my newcomer

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ABSTRACT

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The majority of water soluble vitamin and organic acid food additives are distributed in their crystalline forms. However, when they are combined with water and other food ingredients and then exposed to a variety of unit operations, there is potential to solidify these initially crystalline ingredients in the amorphous state. Amorphous solids are generally less chemically and physically stable than their crystalline counterparts. To ensure nutrient delivery to the consumer and fulfill labeling laws, deterioration of nutrients due to unintentional amorphization is undesirable. Additionally, the potential for recrystallization of an amorphous ingredient may alter texture and redistribute water. Hence, solid state form is a critical factor dictating the stability of food formulations. Building on earlier work from my M.S. degree that demonstrated thiamine chloride hydrochloride could solidify in the amorphous state in the presence of a variety of polymers (Arioglu-Tuncil et al., 2017), a major goal of this study was to develop a comprehensive understanding of the physical and chemical stability of amorphous forms of two thiamine salts, thiamine chloride hydrochloride (TClHCl) and thiamine mononitrate (TMN), in comparison to their crystalline counterparts and each other. The objectives for this part of the work were to investigate amorphization/recrystallization tendencies of TMN and TClHCl in solid dispersions, as well as chemical stability of thiamine in the solid dispersions to understand the impact of vitamin form, physical state (amorphous vs. crystalline), polymer type and features (T_g, hygroscopicity, and ability for intermolecular interactions), storage conditions, proportion of vitamin to polymer,

and pre-lyophilized solution pHs on thiamine degradation and the physical stability of dispersions. Thiamine degraded more when in the amorphous form compared to in the crystalline state. Additionally, polymer type and vitamin proportion influenced thiamine degradation, where thiamine degraded more when it was present in lower concentrations (in dispersions that had higher T_gs), and it was chemically more stable when a polymer with greater intermolecular interactions with the vitamin was used. As storage RH increased, variably hygroscopicities of the polymers resulted in different thiamine degradation rates. The pre-lyophilization pHs of the solutions had a significant impact on thiamine stability in the solid dispersions. Similar to thiamine salts, citric acid is a commonly used food ingredient with a high crystallization tendency. Following similar experimental designs for documenting the recrystallization tendencies of citric acid in amorphous solid dispersions to those used in the thiamine studies, hydrogen bonding and/or ionic interactions between polymer and citric acid were found to be the main stabilizing factor for delaying recrystallization, more than polymer T_g and hygroscopicity. The findings of this dissertation provide a powerful prediction approach to physically and chemically stabilize the small compounds in the complex food matrices for the production of high quality food products and ensuring nutrient delivery to target populations.

CHAPTER 1. INTRODUCTION

1.1 Overview

Thiamine is an essential vitamin, which plays important role in several biological events including carbohydrate metabolism and neurotransmissions. Fatigue, irritability, short term memory loss, insomnia, beri beri and Wernicke's disease are some of the complaint and disorders that can occur due to thiamine deficiency. People suffering from the diseases of alcoholism, Celiac, diabetes, and AIDS as well as older adults are more susceptible to thiamine deficiency. Continues intake of thiamine is, therefore, necessary for a healthy life. Cereals are recognized as one of the most important dietary sources of thiamine. In cereals, majority of thiamine is present in the bran portion; thus, milling process to which the cereal grains are often undergone for the production of flours cause a significant reduction in the thiamine content. Nevertheless, thiamine loss can be compensated with fortification strategies using two synthetic salts form of thiamine, namely thiamine chloride hydrochloride and thiamine mononitrate. Although both salt forms are distributed in crystalline form, they can intentionally or unintentionally transform from the crystalline state to amorphous or semi-crystalline state during processing. Some processing conditions including freeze drying, spray drying, and milling could be contributing factors for crystalline lattice disruption. Co-formulated ingredients may also alter the behaviors of small molecules with high crystallization tendencies such as some water-soluble vitamins including thiamine. In terms of physical stability, recrystallization events during processing and storage may cause quality defects in the final products. Moreover, from the chemical point of view, amorphous forms of compounds are known to degrade more than their crystalline counterparts. Thus, deterioration of thiamine due to unintentional amorphization is undesirable, since ensuring nutrient delivery to the consumer and fulfilling labeling laws are important. However, there is a gap in the

food science literature for implications if and/or how the amorphous structure is a contributing factor to vitamin instability and quality problems. Therefore, in this fundamental research study, a comprehensive understanding of physical and chemical stability of amorphous thiamine was established. Specifically, the crystallization inhibitor effects of a variety of polymers with diverse features on the two salt forms of thiamine in different storage conditions (relative humidity and temperature) were investigated. For investigating thiamine chemical stability, the effects of thiamine physical state (amorphous vs. crystalline), different thiamine salt forms, food matrix (created by different polymers), pHs of pre-lyophilized solutions, and storage conditions on the chemical degradation of thiamine were documented in detail.

Similar to thiamine, citric acid is a small compound with a high crystallization tendency. Since citric acid in crystalline form is widely used in food and confectionary products along with other ingredients (such as hydrocolloids), like thiamine, the potential of citric acid to solidify into amorphous form is high. Although citric acid has been studied as a carrier for some other small molecules, there has been no research conducted to investigate the amorphization and amorphous stability of citric acid that could be affected due to interactions with polymers and different processing conditions. Therefore, in addition to thiamine, citric acid as a widely used food ingredients is of interest in this dissertation, and the amorphous stability of citric acid was investigated in a variety of storage conditions by accounting for the effect of polymers hygroscopicity, T_g and ability for intermolecular interactions.

1.2 Solid state

Among the different states of matter, the solid state gets special attention in food science and related areas, as the majority of food ingredients are found in the solid state. Based on the arrangement of atoms that solids are composed of, they are classified into two broad categories: 1)

crystalline solids, and 2) amorphous solids. Because of differences in the structural features in a molecular level, these two classes of solids behave differently, which has a great impact on final product quality. Therefore, in order to control and estimate the changes that could spontaneously occur in a complex food matrix throughout processing and storage, characterizing the different solid forms of the ingredients and understanding their stabilities and functionalities are essential. Furthermore, this information also allows us to ensure the required (by law and producer) and expected (by customer and producer) quality and sensory characteristics of end-products and intended nutrient delivery. More detailed information about crystalline and amorphous solids is given below.

1.2.1 Crystalline solids

Crystalline solids are composed of atoms, ions, or molecules which are arranged regularly as repeating units with the same direction in three dimensions (Ladd & Palmer, 2003a). Due to having the same orientation of repeating units of crystals, a pattern, known as 'crystal lattice', is formed with short- and long-range order. The entire lattice is constructed from the smallest identical portions, called unit cells, which are defined using lattice parameters: three vectors (length of the edges) *a*, *b* and *c*, and the interaxial angles between them (α, β and γ) as shown in **Fig. 1.1** (Waseda, Matsubara, & Shinoda, 2011). Seven different crystal systems are defined with 14 kinds of Baravais lattices (Ladd & Palmer, 2003b). Intermolecular interactions (Van der Waals, dipole-dipole, hydrogen bonding, etc.) in crystalline solids are maximum, thereby leading to crystals having minimum free volume. Because of having highly ordered molecular arrangements, crystals have relatively low free energy, which makes this solid form thermodynamically more favorable.

Different intermolecular forces could lead to various molecular packing motifs of the same chemical entity, which for crystals is known as ‘polymorphism’ (Bernstein, 2002). Unit cell parameters of crystal polymorphs are different than each other due to the difference in packing arrangement. Physicochemical properties of different polymorphic forms such as melting point, solubility, dissolution rate, physical stability, chemical reactivity, the Gibbs free energy, density, optical, mechanical strength, texture, and taste could be highly distinct to the polymorphic form. Moreover, differences in free energy cause conversion of one polymorphic form to a more thermodynamically stable form in different environmental conditions. Therefore, identification of polymorphism and assessment of possible polymorphic phase transformations during processing and storage are essential for optimizing manufacturing and storage practices when a specific polymorph is desired. It should be also noted that physical characteristics of polymorphs are identical when melted or dissolved in solution.

In addition to polymorphism, crystalline solids can exist as cocrystals and solvates. A crystalline solid is named a cocrystal when the same crystalline lattice consists of two or more different molecules and/or ions usually in a stoichiometric ratio (Bond, 2012). On the other hand, a crystal is a solvate if the crystalline lattice contains solvent molecules as a participant, which could exist in either stoichiometric or nonstoichiometric proportions. If the solvent is water, the crystal is then called as a hydrate. The stabilizing forces for water molecules in the crystal lattice are hydrogen bonding and/or Van der Waals interactions. Some examples of crystalline solids used in food applications that form crystal hydrates include: thiamine chloride hydrochloride, citric acid, glucose, trehalose, lactose, and sorbitol.

1.2.2 Amorphous solids

'Long range order' is a fundamental notion that differentiates crystalline solids from amorphous solids. Unlike crystalline solids, an amorphous solid is characterized by not having long range well defined three-dimensional order. Molecules are randomly distributed in a short-range order over a few molecular dimensions; thus, an amorphous solid lacks translational and rotational order (Bellantone, 2014; Zografi & Newman, 2015). Some energetic features of the amorphous and crystalline forms are compared in **Fig. 1.2**, which shows changes in enthalpy versus temperature (Hancock & Zografi, 1997). A small increase in enthalpy and volume of a crystal as a function of temperature is seen. When the temperature reaches to the melting point, a steep increase in enthalpy is observed in a DSC profile as the crystal melts. Upon cooling the melt to the melting point of the crystal, a first order phase transition from liquid to crystalline phase is expected. If the cooling is too fast below the melting point, molecules may not have sufficient time to rearrange themselves into an ordered structure, meaning that crystallization does not occur. In this case, the supercooled liquid is obtained and the free energy, enthalpy, and volume of the supercooled liquid decrease with increasing viscosity (Bellantone, 2014; Zografi & Newman, 2015). Upon further cooling, a phenomenon, known as 'glass transition', takes place and the supercooled liquid transforms into the glassy state where viscosity is higher (Bellantone, 2014; Zografi & Newman, 2015). The temperature where a change in the slope from supercooled liquid phase to the glassy phase is called the 'glass transition temperature' (T_g); which is actually a range rather than a constant temperature. Below T_g, an amorphous compound is considered as 'kinetically frozen', meaning that the molecular mobility is limited. However, the opposite is true when it is above T_g (Hancock & Zografi, 1997). It should also be noted that different cooling rates and thermal histories result in changes in T_g, as shown in **Fig 1.3** (Ediger, Angell, & Nagel, 1996).

In addition to supercooling of a melt, the amorphous form can be intentionally produced from by rapid precipitation from solution that could be achieved by freeze drying, spray drying and solvent evaporation, vapor condensation, milling and compaction of crystals (Hancock & Zografi, 1997; Zografi & Newman, 2015). On the other hand, unintentional amorphization may also occur in complex food matrices due to processing conditions and interactions with polymeric ingredients as has been documented for vitamin B and C (Arioglu-Tuncil, Bhardwaj, Taylor, & Mauer, 2017; Christina, Taylor, & Mauer, 2015). Amorphization is sometimes desired due to some of its superior properties over crystalline solids for some applications. Since amorphous compounds lack long range molecular order, the energetic barrier to enter solution is lower, leading to amorphous solids having a higher dissolution rate. Thus solubility of amorphous solids is significantly higher than their crystalline counterparts (Bellantone, 2014; Zografi & Newman, 2015). Because of being more soluble, there is a great interest for amorphization of poorly soluble smaller compounds to improve the bioavailability. There are many drugs in the market formulated in the amorphous form (Huang & Dai, 2014). In addition, the majority of solid food products are found either in semi-crystalline or amorphous states. Therefore, a mechanistic understanding of the physical and chemical stability of amorphous solids is required from a food science perspective to control quality and delivery of a target compound.

1.2.3 Recrystallization

Despite having higher dissolution rates, there is a thermodynamic driving force for amorphous solids to recrystallize. Recrystallization is the process in which molecules of an amorphous solid rearrange themselves to the most stable configuration, the crystalline form. Crystallization occurs in two major steps: nucleation and crystal growth (Janssens & Van den Mooter, 2010). Nucleation is further divided into two categories: primary nucleation and

secondary nucleation (Mullin, 2001a). Primary nucleation refers to crystalline nucleation in a system which does not have any crystalline material, whereas secondary nucleation occurs due to a preexisting crystal nuclei. Primary nucleation is further categorized as homogenous nucleation, which happens spontaneously, or heterogeneous nucleation, which is induced by the presence of an impurity or foreign particle (Mullin, 2001a). The second step of crystallization, crystal growth, refers to growth of the crystals formed in primary nucleation to larger sizes (Mullin, 2001b). Less energy is required for crystal growth than is needed for nucleation.

Amorphous solids could recrystallize into different crystal forms depending on processing, formulation and storage conditions. The conditions which have impact on different crystalline structure formation are temperature, RH%, time, drying method, presence and type of other substances (Haque & Roos, 2005). Since different crystalline forms are likely to possess distinct physicochemical features, many of which differ from the amorphous state, it is essential to control crystallization. Therefore, recrystallization of an amorphous material is usually undesirable. In order to prevent or delay recrystallization, a promising method known as 'amorphous solid dispersion' was developed.

1.3 Amorphous solids dispersions

The idea of solid dispersion was first coined by Sekiquchi and Obi in 1961 who attempted to reduce the particle size of sulfathiazole in order to improve its solubility (Sekiguchi & Obi, 1961). In the study, a eutectic mixture of sulfathiazole with a soluble and physiologically inert carrier was prepared by melting. The mixture was then rapidly solidified. As a result, higher absorption of sulfathiazole was obtained in a eutectic mixture compared to the control sulfathiazole. This was attributed to changes in the physical form of sulfathizole in the carrier upon melting and solidifying. This method was then improved and termed as 'solid dispersion'.

The term solid dispersion was defined by Chiou and Riegelman as ‘The dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting (fusion), solvent, or melting-solvent method’ (Chiou & Riegelman, 1971). The main goal of forming many amorphous solid dispersions is to prevent recrystallization of the amorphous smaller compound in order to improve its solubility, which is achieved by particle size reduction and surface area increase, hence increasing wetting properties (Craig, 2002). Polymers are generally preferred as carriers for creating solid dispersions. Solid dispersions have been widely used by many researchers mostly in pharmaceutical applications and primarily for increasing the solubility of poorly soluble compounds (Konno & Taylor, 2008; B. Li et al., 2013; Wegiel, Mosquera-Giraldo, Mauer, Edgar, & Taylor, 2015).

Although the onset of crystallization is notably delayed by solid dispersion formation, there is still a thermodynamic driving force for recrystallization, which is mainly due to the free energy difference between crystalline and amorphous forms. Therefore, it is essential to understand the stability of a compound in a solid dispersion and document its structural behaviors in processing and storage conditions in order to determine which polymers are most effective at delaying crystallization. Several mechanisms were proposed to explain the effect of a polymer as a stabilizer against recrystallization, which will be covered in the next sections in detail.

1.3.1 Methods for preparation of amorphous solid dispersions

Amorphous solid dispersions are prepared by several techniques: melting, solvent, and melting-solvent (Chiou & Riegelman, 1971). The melting method, also known also as the fusion method, is employed by melting of a physical blend of a smaller compound with a water-soluble carrier, followed by rapid cooling using an ice bath or liquid nitrogen. It is a simple and economic method for solid dispersion formation. However, the high temperature treatment applied for

melting may cause decomposition of the smaller compound or carrier. For the solvent method, the compound and carrier are dissolved in a common organic solvent and the solvent is then removed directly by evaporation. Since evaporation of organic solvents requires relatively lower temperatures than melting, thermal decomposition of the smaller compound is expected to be less than in dispersions formed by the melting method (Chiou & Riegelman, 1971; Serajuddin, 1999). However, high cost of operation as well as concerns about residual organic solvent are the disadvantages of the solvent method (Chiou & Riegelman, 1971; Serajuddin, 1999). Another commonly used method for removing the solvent is freeze drying (lyophilization), which is the central focus of this dissertation in order to form amorphous solid dispersions.

1.3.1.1 Lyophilization

Lyophilization is conducted in three main steps: 1) the freezing step in which samples are frozen below the freezing temperature, 2) the primary drying step where the ice crystals are removed by sublimation under reduced pressure, and 3) the secondary drying step in which unfrozen residual water trapped into solidified solution is removed by increasing the temperature at a controlled rate (Franks, 2007). Controlling three main parameters, namely chamber pressure, shelf temperature, and time, is important to obtain lyophilized products with high yield and quality (Franks, 2007). Mass transfer is governed by several factors: fill depth, total solid content, cake porosity, and surface area. Therefore, these factors need to be taken into consideration for process development to determine the resistance to sublimation (Franks, 2007). Collapse temperature (T_c), also called the T_g prime (T_g'; glass transition of the maximally freeze concentrated solution), is the most critical parameter to determine prior to establishing a freeze-drying method in order to obtain an acceptable cake structure. The temperature in the primary drying step must be below the T_g' of the sample, which will maintain the frozen structure. Otherwise, a desirable cake structure cannot

be formed and sample collapse/shrinkage occurs, which can be defined as loss in microstructure. When the sample collapses, a decrease in efficiency of sublimation of water vapor due to closure of pores (formed due to sublimation) in the first drying step is observed (Wang, Hey, & Nail, 2004), and the secondary drying step is also delayed due to decrease in surface area which leads to formation of lyophilized sample with high residual moisture (Wang et al., 2004). Thus, the collapse of lyophiles above T_g' causes some detrimental effects on the sample, leading to having low physical and chemical stability.

'Residual moisture' in lyophilized preparations, of which the amount depends on the method, product, and depth and surface area of the container, is a determinant factor affecting DSC measurements. In addition, residual moisture could also have some impact on physical and chemical stability. The main advantage of freeze drying is that water removal is achieved without applying heat. Therefore, lyophilized products generally are high in nutrient content, and volatile aroma compounds are well preserved. Such advantages make lyophilization popular in food science applications. As an example, it has been widely used to produce dehydrated berries for cereals, instant coffee, and space foods.

1.3.2 Stabilizing factors of amorphous solid dispersions against recrystallization

There has been a great appreciation for the fact that recrystallization of a smaller compound can be significantly delayed thermodynamically and kinetically by solid dispersion formation using a polymer as a carrier, as shown in **Fig. 1-4** (Harmon et al., 2009). The free energy difference between the crystalline state and its amorphous counterpart is the main thermodynamic driving force leading the amorphous compound to recrystallize, as depicted in **Fig. 1-4**. Reduction in free energy compared to the amorphous compound, however, is achieved upon solid dispersion formation, which stabilizes the unstable amorphous compound by lowering its free energy. There

is also an increase in activation energy barrier for crystallization due to relatively low molecular motion in the amorphous dispersion (**Fig. 1-4**). Recrystallization of a compound in an amorphous solid dispersion is a complex process affected by several factors including storage temperature (Miyazaki, Yoshioka, Aso, & Kojima, 2004), moisture content (Konno & Taylor, 2008), tendency of the smaller compound for crystallization alone (Marsac, Konno, & Taylor, 2006), and the type and proportion of polymer used (Konno & Taylor, 2008; Wegiel et al., 2015; Yoshioka, Hancock, & Zografi, 1995). Several mechanisms were proposed on how the onset of crystallization is prevented or delayed by creation of amorphous solid dispersions, including: reduction in molecular mobility (Crowley & Zografi, 2001; Hancock & Shamblin, 2001), destruction of interaction between the molecules of the smaller compound (Taylor & Zografi, 1997), and creation of specific interactions between the smaller compound and polymer (Khougaz & Clas, 2000; Tantishaiyakul, Kaewnopparat, & Ingkatawornwong, 1996; Taylor & Zografi, 1997). Reduction in molecular mobility could be achieved by elevating the T_g.

1.3.2.1 Glass transition temperature

Since T_g is directly related to the molecular mobility, it has been claimed that T_g has a considerable impact on the stability of amorphous solid dispersions. When two compounds are present in a solid dispersion, the T_g of the dispersion is likely to be in between the T_gs of the individual components. Thus, free volume will be lowered with respect to the lower molecular weight amorphous smaller compound, when it is dispersed into matrix of a polymer with a relatively higher T_g. The proportion of the components in the dispersion is directly related to the magnitude of change in T_g. It should also be noted that one T_g is obtained for the systems that are completely miscible. Immiscible dispersions result in phase separation, and exhibit more than one T_g.

The impact of T_g as a molecular mobility limiting factor on the physical stability of amorphous solid dispersions was studied by several authors (Van den Mooter, Augustijns, & Kinget, 1999; Van den Mooter et al., 2001). For example, amorphous solid dispersions of ketoconazole with PVP K25 were created and factors inhibiting the recrystallization of amorphous dispersions were investigated for 30 days at 298.1 K and both 0% and 52% RH (Van den Mooter et al., 2001). Solid dispersions were stable against recrystallization in the presence of PVP K25 for 30 days at the studied conditions, whereas amorphous ketoconazole crystallized right after blending physically with PVP K25. Strong and specific interactions between ketoconazole and polymer were not confirmed using ^{13}C NMR and FTIR. Therefore, it was claimed that the polymer acted as an anti-plasticizer, and amorphous stability was attributed to elevation in T_g, which resulted in decrease in molecular mobility and increase in viscosity (Van den Mooter et al., 2001).

There are several models developed to predict or estimate the T_g of a system composed of more than one compound. As a simple model, the Fox equation is used based on the contributions of weight fractions of each compound (Fox, 1956). The Fox equation is defined as:

$$\frac{1}{T_{gmix}} = \frac{W_1}{T_{g1}} + \frac{W_2}{T_{g2}} \qquad 1.1$$

where, W_1 and W_2 are the weight fractions of components 1 and 2, and T_{g1} and T_{g2} are the T_gs of each component. The Fox equation was established based on the assumption that components have similar density.

Another widely used model to calculate predicted T_g of a system comprised of more than one compound is Gordon-Taylor equation (Gordon & Taylor, 1952), which is written as:

$$\frac{1}{T_{gmix}} = \frac{W_1.T_{g1}+k.W_2.T_{g2}}{W_1+k.W_2} \qquad 1.2$$

where, W_1 and W_2 are the weight fractions of components 1 and 2, T_{g1} and T_{g2} are the T_gs of each component, and *k* is a constant that can be calculated by:

$$k = \frac{T_{g1} \cdot \rho_1}{T_{g2} \cdot \rho_2} \quad 1.3$$

where *p* refers to density of each component. The Gordon-Taylor equation was mainly developed for prediction of the T_gs of miscible blends of big molecules like polymers. The model assumes that components are completely miscible. In addition, the presence of intermolecular interactions between compounds was not taken into consideration in these models. If specific interactions (such as hydrogen bonding or ion-dipole interactions) occur between the compounds, deviation from predicted T_g value is likely. If a strong intermolecular interaction is present, positive deviation of T_g from that calculated by the Gordon-Taylor equation is observed due to having smaller free volume than expected, which reduces the molecular mobility.

1.3.2.2 Specific interactions possibly occurred between carrier and smaller compound

Although T_g is proposed as an important feature to maintain amorphous characteristic in solid dispersions by choosing storage conditions to maintain the system in the glassy state (Van den Mooter et al., 2001), many studies have shown that T_g is not the key factor to prevent recrystallization (Arioglu-Tuncil et al., 2017; Christina et al., 2015; Matsumoto & Zografi, 1999; Wegiel, Mauer, Edgar, & Taylor, 2013a).

The presence of strong intermolecular interactions between a smaller compound and a polymer via hydrogen bonding and/or ionic interactions was proven as the most important stabilizing factor. Even if the T_g of an amorphous dispersion is relatively higher than that of the amorphous compound itself, the system could still undergo recrystallization, when specific interactions between the compound and polymer do not exist or are not strong enough (Arioglu-

Tuncil et al., 2017; Christina et al., 2015; Khougaz & Clas, 2000; Matsumoto & Zografi, 1999; Tantishaiyakul et al., 1996; Taylor & Zografi, 1997; Wegiel et al., 2013a; Wegiel, Mauer, Edgar, & Taylor, 2013b; Wegiel, Zhao, Mauer, Edgar, & Taylor, 2014). Similarly, small molecule can be stabilized in amorphous state, although T_g of dispersion is lower than T_g of amorphous drug alone as long as a strong interaction between the small molecule and polymer exists. It was mainly achieved by disrupting the intermolecular interactions between the molecules of smaller compound in the crystalline lattice, coupling of molecular motions of small molecule to the polymer, and interacting directly at the nucleation sites. For example, in a recent study conducted in our laboratory, amorphous thiamine chloride hydrochloride stability was investigated in solid dispersions made by a variety of polymers with different ability for intermolecular interactions (Arioglu-Tuncil et al., 2017). The best polymers for inhibiting recrystallization of thiamine in these dispersions were the ones that had the ability to interact with thiamine via hydrogen bonding and ionic interactions. Moreover, there was no correlation observed between amorphous stability and T_g. In a different study, indomethacin-PVP amorphous solid dispersions were formed by a solvent evaporation method and specific interactions were identified using IR and FT-Raman spectra (Taylor & Zografi, 1997). Dimerization of indomethacin molecules was reported to be overcome first to prevent crystallization. Hydrogen bonding interactions between the polymer and indomethacin were postulated as the stabilizing factor that is thought be accomplished by disrupting dimerization of indomethacin, even when the antiplasticizing effect of the polymer was limited due to low polymer concentration used (Taylor & Zografi, 1997). No direct correlation between higher T_gs and enhanced physical stability was found in these additional studies. These comprehensive studies clearly indicate that intermolecular interactions between the carrier and

smaller compound appear to be very important and possibly the determinant factor dictating the amorphous stability towards recrystallization.

1.4 Citric acid

Citric acid is an organic acid naturally found in a variety of fruits and vegetables, predominantly in lemons, limes, and oranges. It has been used in food preparations and pharmaceutical applications for many purposes including adjusting the pH of solutions and tablets, and as an antioxidant, chelating agent, preservative, and flavor enhancer (Igoe, 2011; Sheskey, Cook, & Cable, 2017). Citric acid is a crystalline deliquescent ingredient, which exists in anhydrous and monohydrate forms. Allan and Mauer reported the RH-temperature phase diagram of citric acid for the first time (Allan & Mauer, 2017). Anhydrous to monohydrate conversion of citric acid was reported to occur at 60.3±0.1% RH at 25^{o}C, which is important information in order to design the processing and storage conditions. The deliquescence point of anhydrous and monohydrate citric acid were reported as 74-75% RH and 78% RH at 25^{o}C, respectively (Allan & Mauer, 2017; Salameh Adnan, Mauer Lisa, & Taylor Lynne, 2006).

Citric acid is a tricarboxylic acid, and two of these carboxylic acids are equivalent. A hydroxyl group is also present in the center of the citric acid molecule (**Fig. 1.5**). All these functional groups are involved in hydrogen bonding either as a donor or acceptor. Carboxylic acids potentially exist as dimers because of strong intermolecular interactions through hydrogen bonding, which also hold true for citric acid (Nordman, Weldon, & Patterson, 1960). Thus, self-association of citric acid dimers needs to be cleaved first in order to form hydrogen bonding with another compound.

Citric acid received much attention as a glass former due to being widely available, having GRAS status, and being a physiologically inert carrier. Therefore, it has been used as a carrier for

some poorly soluble smaller compounds in food and pharmaceutical applications to enhance their solubility (Chiou & Riegelman, 1969; Hoppu, Hietala, Schantz, & Juppo, 2009; Hoppu, Jouppila, Rantanen, Schantz, & Juppo Anne, 2010; Lu & Zografi, 1998; Summers, 1978; Summers & Enever, 1976; J. Wang et al., 2017). The reported T_g of citric acid in the dry state is 11^oC (Lu & Zografi, 1997). Although citric acid was used as a carrier in several studies, the amorphization of citric acid alone from its aqueous solutions was never achieved. This was attributed to its low T_g. As mentioned in the 'lyophilization' section, glass transition of the maximally freeze-concentrated solution (T_g') stands as the most important parameter to obtain a desirable cake structure for lyophilization applications. Thus, recrystallization of citric acid during the freeze drying process could be due to the temperature of first drying step being higher than the T_g' of citric acid. The T_g' of citric acid was reported as -53^oC (Lu & Zografi, 1997).

Solid dispersions of citric acid with other compounds were usually prepared using a melt-quenching method (Chiou & Riegelman, 1969; Hoppu et al., 2009; Hoppu et al., 2010; Lu & Zografi, 1998; Summers & Enever, 1976). Hoppu et al. investigated the physical stability of paracetamol: citric acid blends (prepared by melt-quenching) over time in the rubbery phase and found that the blends stored under dry conditions were stable for 27 weeks due to strong hydrogen bonding formation between citric acid and paracetamol (Hoppu et al., 2010).

Even though citric acid is used in food and confectionary products in the crystalline state, it can be solidified into the amorphous form due to processing conditions and possible interactions with a variety of ingredients (i.e polymers) found in the formulations. For example, citric acid is one of the most commonly used acidulants for confectionary products which are often desired to be in the amorphous state. Although citric acid has been used to amorphize some other small molecules including paracetamol, griseofulvin, loratadine, indomethacin, primidone and

barbiturates (Chiou & Riegelman, 1969; Hoppu et al., 2010; Lu & Zografi, 1998; Summers, 1978; Summers & Enever, 1976; J. Wang et al., 2017), to the best of our knowledge, there has been no study conducted investigating the physical stability of citric acid in amorphous form in the presence of a polymer as a carrier. In addition, it was also interest to investigate amorphous stability of citric acid in polymer dispersions to determine if the theory of stabilizing different small compounds via intermolecular interaction is valid for citric acid.

1.5 Food matrices: Polymers

Polymers are long chain substances consisting of monomeric units linked to each other via covalent bonds. Hydrocolloids, also known as gums, are hydrophilic polymers that are widely used in food formulations as thickeners, stabilizers, film formers, and binding, emulsifying, and gelling agents (Igoe, 2011). Once they are introduced into food matrices along with other ingredients (such as vitamins), their possible effects on formulation and physicochemical properties may alter. Thus, there is a need for advanced understanding of such properties in order to predict their behavior in complex food matrices.

Polymers have been widely used to create amorphous solid dispersions as carriers and thus, they are recognized as physical barriers which stabilize amorphous systems against recrystallization. As mentioned in section 1.5.2 in detail, the glass transition theory and formation of specific intermolecular interactions between polymer and smaller compound are the main mechanisms proposed for amorphous stability, although the storage conditions with respect to T_g are also likely to be important.

A variety of polymers possessing different physicochemical features (hygroscopicitiy, T_g, ability to interact via hydrogen bonding and/or ionic interactions) are the focus of this dissertation.

The polymers used are pectin, guar gum, gelatin, κ-carrageenan, PVP, HPMC and CMC-Na. Brief information regarding the chemical structures of the polymers is provided below.

Pectin is a heteropolysaccharide made of esterified D-galacturonic acid units joined by α-1, 4 glycosidic linkages in the backbone as shown in **Fig 1.6**. Pectin is mainly produced from citrus fruit and apple pomace, usually by acid hydrolysis (Thakur, Singh, Handa, & Rao, 1997). The hydrophilic nature of pectin makes it suitable for many food applications. It also has a great potential to be used in the preparation of solid dispersions due to the presence of functional groups available for hydrogen bonding. The functional groups of pectin available for hydrogen bonding are hydroxyl, carboxylic acid, ether, and ester.

Guar gum is a water soluble polymer made of linear chains of mannose units joint by β-1, 4 linkages and a single galactose unit as a branching side at every second mannose that are linked by α-1,6 linkages. It is produced from a guar plant known as *Cyamopsis tetragonolobus*. The chemical structure of guar gum is presented in **Fig 1.7**. Like pectin, due to the hydrophilic nature, guar gum can also be used for a variety of purposes in food and pharmaceutical applications. The functional hydroxyl and ether groups make guar gum highly suitable for its use in solid dispersion preparations as a carrier, because such groups are available for hydrogen bonding.

K-carrageenan is a linear polysaccharide comprising galactose units and 3,6-anhydrogalactose units that are connected by α-1,3 and β-1,4 glycosidic linkages, as depicted in **Fig. 1.8**. Red edible seaweeds are the main sources of κ-carrageenan. Hydroxyl, ether, and sulfate ester groups of κ-carrageenan could be involved in hydrogen bonding, thereby making κ-carrageenan suitable for being a carrier in the solid dispersion systems.

Hydroxypropyl methylcellulose (HPMC) and carboxymethyl cellulose sodium (CMC-Na) are cellulose derived polymers, and the structures are shown in **Figs. 1.9** and **1.10**. Hydroxyl and ether groups are the available functional groups of HPMC and CMC-Na for hydrogen bonding. Unlike the above mentioned polymers, gelatin is a protein-based polymer obtained mainly from bones, skin, muscle fibers, and connective tissues of animals like pig. It comprises from eighteen to twenty different amino acids. Carbonyl, carboxylic acid, amine, and sulfhydryl groups are the main functional groups that allow gelatin to be used in solid dispersion preparations where it acts as a carrier.

Polyvinyl pyrrolidone (PVP) is a synthetic polymer widely used particularly in pharmaceutical applications. As shown in **Fig. 1.11**, a linear 1-vinyl-2-pyrrolidinone unit forms the structure of PVP. It is available in different molecular weights depending on the degree of polymerization. PVP is a very hygroscopic polymer and it has a well-defined T_g. However, the T_g of PVP varies depending on the molecular weight. As seen from the **Fig. 1.11**, the only functional group available for hydrogen bonding is the amide group, which acts as a strong acceptor.

1.6 Chemical stability of amorphous materials

Powders are widely used for food formulations. Therefore, mechanistic understanding of solid state reactivity and determination of possible formulation and storage scenarios are crucial for nutrient delivery.

The degradation of nutrients is affected by formulations and is accelerated generally due to; 1) presence of other food ingredients (without involving in chemical reactions), 2) interactions with other constituents, and 3) processing conditions (Byrn, Xu, & Newman, 2001). Common solid state reactions are oxidation, cyclization, hydrolysis, deamidation, and interaction of

excipients in formulations (maillard, transacylation, solid state acid base reactions) (Byrn et al., 2001).

Food systems are usually either semi crystalline or completely amorphous, and therefore exhibit more chemical reactivity than if they were wholly crystalline. Water is recognized as one of the most common destabilizing factors, particularly for amorphous solids. The roles of water in solid state reactivity can be characterized as being plasticizer, reactant or product, and modifier of reaction media (Shalaev & Zografi, 1996). Although the impact of water on chemical reactivity is well-accepted, the underlying mechanisms for correlation between water and amorphous stability are yet to be completely understood (Shalaev & Zografi, 1996). The challenges are mainly caused by: 1) complexity of degradation pathways, which usually occur as parallel or serial processes where water could have different impacts for different reactions in the same system, 2) water formation as a by-product in a particular reaction which increases the water content (e.g Maillard reaction) (Shalaev & Zografi, 1996), and 3) implication of water on physical properties such as change in acidity (Sugimoto, Ishihara, Habata, & Nakagawa, 1981).

Increase in chemical reactivity is often associated with increase in molecular mobility which can be observed when elevating the temperature or the water content. Since T_g is a molecular mobility related factor, it has also been recognized to affect chemical stability. Moreover, correlation between chemical stability and $T-T_g$ has been tried to be established. The proposed theories claiming the T_g as a key parameter governing the stability and as a degradation rate limiting factor at temperatures where compounds are in the glassy state have not been successful. Lai et al., designed a study where two systems were similar in T_g and $T-T_g$, but differed in a_w and moisture content (Lai, Hageman, Schowen, Borchardt, & Topp, 1999). A higher degradation rate constant was observed for the system with higher a_w and moisture content, which led to the

conclusion that either a_w or moisture content was the predominant factor for chemical reactivity, rather than T_g (Lai et al., 1999). In another study, aspartame stability was assessed in two PVPs with different M_W (so, differ in T_g), but the a_w and moisture contents of both systems were similar (Bell & Hageman, 1994). Although the systems were present in different amorphous states (glassy vs. rubbery), similar degradation rate constants (k~0.05) were obtained, indicating that the relation of chemical reactivity with T_g cannot be oversimplified (Bell & Hageman, 1994; Lai et al., 1999; Li, Guo, & Zografi, 2002; Luthra, Shalaev, Medek, Hong, & Pikal, 2012).

Solution formation is another factor affecting the overall reactivity. The amorphous solids are likely to sorb significant amount of moisture over an extensive range of relative humidity. As water uptake increases, the rate of the reaction increases. Since amorphous solids absorb moisture into the bulk structure, after reaching a critical point it could lead to a situation where ingredients dissolve (Byrn et al., 2001; Hancock & Zografi, 1993; Shalaev & Zografi, 1996). This phenomenon causes a significant change in reaction rate constant. Therefore, implications of ingredients' hygroscopicity and the phenomena of synergistic moisture uptake on reactivity should be considered.

Solid state reactions are a major concern in food applications. A comprehensive understanding of potential mechanisms in the solid state is worthwhile to design approaches that prevent or minimize the detrimental effects of chemical changes during processing and storage.

1.7 Thiamine

Thiamine, also known as Vitamin B_1, was the first vitamin to be discovered and has many functions in the human body. Thiamine pyrophosphate is the biologically important form of thiamine and is mainly involved in carbohydrate metabolism as a cofactor of several enzymes including pyruvate dehydrogenase, transketolase, α-ketoglutarate dehydrogenase and α–keto acid

dehydrogenase, which have roles in glycolysis and the citric acid cycle (Lonsdale, 2006; McCandless, 2009a). Furthermore, thiamine plays an important role in neurotransmission (McCandless, 2009b). When considering its roles in energy metabolism and neurotransmission, it is expected that thiamine deficiency causes several serious disorders. Moderate deficiency of thiamine results in fatigue, irritability and insomnia; however, the consequences of prolonged deficiencies are more serious (McCandless, 2009b). Some of the clinical disorders including Wernicke's disease, Leigh's disease, African Seasonal Ataxia (ASA), and beri beri are directly associated with thiamine deficiency (McCandless, 2009b). It is unfortunate that the associated disorders were described many years before the thiamine discovery. Thiamine can be synthesized by plants, fungi, and microorganisms; however, the human body is not able to produce it. Therefore, thiamine is an essential vitamin, which is required to be taken through the diet. The recommended dietary allowances for thiamine for females and males are 1.1 and 1.2 mg/day, respectively (Bettendorff, 2013). Due to its role in carbohydrate metabolism, the dietary requirement for thiamine is mainly based on carbohydrate intake. Therefore, individuals whose diets consist primarily of carbohydrates are likely to suffer from thiamine deficiency more. Due to being a water soluble vitamin, the human body can reserve thiamine only for 2-4 weeks (Nath, Shope, & Koch, 2017); therefore, constant intake of thiamine via the diet is required.

Thiamine is naturally found in whole grains, yeast, dried legumes, meat (especially pork), beans, nuts, and egg yolk. Grains are good sources for thiamine; however, thiamine content is reduced in refined grains such as refined wheat flour and polished rice, as the majority of thiamine is found in the bran and germ portions of the grains (Prinzo, 1999). This further increases the risk of thiamine deficiency. To compensate for thiamine loss, two synthetic deliquescent salt forms of thiamine, chloride hydrochloride and mononitrate, are commonly used for food fortification.

Thiamine is also available as a dietary supplement. Some properties of these thiamine salts are compared in Table 1. Being more soluble makes thiamine chloride hydrochloride more suitable for enrichment of beverages, whereas thiamine mononitrate is mostly preferred in dry food products due to its less hygroscopic feature (Labuza & Kamman, 1982).

Thiamine is characterized as a white crystalline powder with a yeasty odor and salty/ nutlike flavor. Vitamin B_1 was first called 'thiamine'. The last 'e' was then omitted after discovery that it was not an amine. In current literature, thiamine is spelled in both ways based on the author's preference.

Thiamine is comprised of a pyrimidine nucleus and a thiazole ring joined by a methylene bridge. The p*K*a of thiamine is reported to be 4.75 (Hopmann, 1973). Thiamine is one of the least stable vitamins and the conditions affecting its chemical stability are discussed in the following section.

1.7.1 Degradation mechanism of thiamine

Thiamine is known as one of the least stable vitamins. The factors affecting its chemical degradation are heat, alkaline pH, oxidizing/reducing agents, light, radiation, inorganic bases, and presence of other ingredients (such as sulfate, some other inorganic bases, amino acids, carbohydrates, metal complexes and thiaminases) (Dwivedi & Arnold, 1973). In addition, thiamine loss is induced during processing, cooking, and storage, which further increase the challenge for its delivery.

Both natural and synthetic forms of thiamine are affected by higher relative humidity and temperature conditions (Bendix, Heberlein, Ptak, & Clifcorn, 1951; Hiatt, Ferruzzi, Taylor, & Mauer, 2008; Mauri, Alzamora, & Tomio, 1992). It was reported that 60-70% and 20-30% of thiamine degraded in meat and in vegetables, respectively, when heat was applied (Feliciotti &

Esselen, 1957). In addition, Bell and White investigated thiamine degradation in a variety of PVPs with different molecular weights as a model system in order to understand the effect of T_g on vitamin loss (Bell & White, 2008). They found that the degradation rate of thiamine increased above the T_g due to greater molecular mobility. Moreover, thiamine was shown to be involved in a Maillard-type reaction in the presence of glucose at 85^{o}C (Dwivedi & Arnold, 1973). As a result of its reaction with glucose at pHs lower than 4.0, 2-glucothiamines and some other unidentified compounds were found to be produced. Thiamine degradation causes off-flavor because of formation of sulfur containing products (Dreher, Rouseff, & Naim, 2003; Dwivedi & Arnold, 1973; Grosch & Zeiler-Hilgart, 1992). Although off-flavor formation is mostly undesirable, meaty/ brothy flavor could be acceptable for pork, chicken, and beef.

As mentioned in previous sections, the solid state, amorphous versus crystalline, is an important factor dictating the physical and chemical stabilities of compounds. Vitamin loss, however, is further induced in solutions. The pH of a solution has a significant impact on thiamine degradation because its degradation was found to follow different pathways based on the pH (Pachapurkar & Bell, 2006). Moreover, thiamine loss is accelerated in basic solutions where more complex mechanisms were observed. At least four different reaction pathways were identified depending on pH under buffer free conditions (Dwivedi & Arnold, 1973; Windheuser & Higuchi, 1962): a) pH below 1, oxythiamine is produced (**Fig. 1.12a**), b) at pH between 1.0-6.0, 2-methyl-4-amino-5-hydroxymethylpyrimidine and 4-methyl-5-β-hydroxyethylthiazole is formed, as a result of water cleavage of protonated thiamine (**Fig. 1.12b**), c) at pH between 2-6, pyrimidine and thiazole fractions obtained in reaction b are formed upon hydroxyl ion catalysis of protonated thiamine (**Fig. 1.12c**), and d) pH above 6.5, 2-methyl-4-amino-5-aminomethylpyrimidine and thiamine thiothiazolone are produced (Windheuser & Higuchi, 1962) (**Fig. 1.12d**).

Formation of a derivative of thiamine with an opened thiazolium ring (I) and the compound (II) are observed upon alkali treatment of thiamine (**Fig. 1.12e**). Thiochromo formation as a result of oxidation of II is also possible. (Bettendorff, 2013; Dwivedi & Arnold, 1973) (**Fig. 1.12e**).

Cereal grains and bread products (where starches and gums are used) are commonly fortified with two salts form of thiamine which are present in crystalline form. Our recent finding revealed that processing conditions and interaction of thiamine with polymeric ingredients induce amorphization of thiamine (Arioglu-Tuncil et al., 2017). The main challenge with thiamine delivery, therefore, could be chemical stability differences between amorphous and crystalline form of thiamine with an amorphous compound being less chemically labile than its crystalline form. Moreover, as being one of the least stable vitamins (when considered its sensitivity to heat, alkaline conditions, presence of some other ingredients), amorphization of thiamine is expected to further decrease its chemical stability in formulations (such as in bread products, cereal grains, and gluten-free products). Although it is well appreciated that amorphous solids are less chemically stable than their crystalline counterparts, there is a gap in the literature about chemical stability of thiamine in amorphous form compared to its crystalline form. Moreover, there is no report found in the literature to compare stability of amorphous thiamine in two different salt forms which possess different physicochemical features (Thiamine could be expected to exhibit different stability traits in different salt forms due to having different physicochemical features). In order to ensure thiamine delivery and meet the labeling requirements, documenting the thiamine stability in the amorphous form compared to crystalline form is required. In addition, studying two salt forms of thiamine is necessary to identify the optimal form of thiamine for different formulations and storage treatments.

1.8 Analytical techniques for the characterization of solid state and chemical degradation

There are many techniques available to characterize solid state properties that include: diffraction methods, microscopic methods, spectroscopic methods, thermal analysis, sorption techniques, and chromatographic separation techniques. In this section, common analytical instruments will be discussed, many of which are utilized in the data collection presented in this dissertation.

Crystalline structure determination can be achieved by X-ray Powder Diffraction (XRPD), which is a well-appreciated gold standard analytical technique. XRD is a sensitive, reliable, and non-destructive analytical technique which can be used to determine the degree of crystallinity, polymorphism, phase purity, phase changes, crystallite size, number/type and positions of atoms in the unit cell, and size and shape of unit cell (Vogt, 2015). The intensity of X-ray diffraction as a function of diffraction angles is obtained. The information about three-dimensional structure of matter can be collected using this technique. XRD provides both qualitative and quantitative information. Each crystalline compound has a unique diffraction pattern; therefore, XRD is known as a 'fingerprint' technique. There are some databases available that provide information regarding the structures of compounds. The Cambridge Structural Database (CSD) is the most reliable one that contains the information of many reported crystalline compounds (https://www.ccdc.cam.ac.uk). Identification of some unknown materials is possible using the CSD and search and match method.

Microscopy is a rapid, versatile, and usually non-destructive tool that can be used to examine solid state properties of materials. The information gathered with this technique includes particle shape, particle size, optical properties, and crystallinity (Munson, 2009; Nichols, Luk, & Roberts, 2011). Different types of microscopy techniques, such as atomic force microscopy,

transmitted light microscopy, scanning electron microscopy, and polarized light microscopy, are available and these methods can be selected depending on the purpose of characterization (Nichols et al., 2011). Among these microscopy methods, polarized light microscopy has been widely used for initial screening of the samples. This is because it is rapid, cheap, and non-destructive. In addition, it is very convenient to handle a very small amount of crystals in this technique (Vogt, 2015). Crystalline and amorphous samples can be differentiated using polarized light microscopy due to differences in their optical properties against polarized light and crossed polarizers. As a result of interaction of the long range alignment of molecules (typical to crystals) with polarized light, birefringence occurs for many crystals. In contrast, the absence of birefringence can be used as an indicator that the compound is in the amorphous form.

Infrared (IR) spectroscopy is one of the most common vibrational spectroscopy techniques used for solid state characterization (Vogt, 2015). In IR spectroscopy, basically absorption, transmission, or reflectance of IR radiation is detected. It relies on the absorption of IR radiation with different frequencies by compounds. Molecules can vibrate in several modes, such as stretching (symmetrical and asymmetric stretch) and bending (scissoring, rocking, twisting and wagging) (Wehling, 2010), that lead to IR absorption. FTIR (Fourier Transform Infrared Spectroscopy) is one of the infrared spectroscopy methods in which simultaneous entrance of all wavelengths at the detector occurs, rather than dispersion of radiation, followed by converting the results into an IR spectrum. Vibrational frequencies of functional groups are affected by the presence of intermolecular interactions and the change in molecular conformation. Therefore, FTIR is a powerful analytical tool for investigation of intra- and intermolecular interactions and differentiation of polymorphs, hydrates, and salts. It is important to note here that if the stretching vibration of a molecule does not cause a change in net dipole moment, absorbance in IR region is

not observed; and these kinds of compounds are called ‘IR inactive’. In the FTIR spectrum, the existence and extent of intermolecular interactions can be determined based on shifts observed in the wavenumber regions where intermolecular interactions are formed or destroyed, compared to a control sample (reference). Moreover, the region between 1500- 400 cm^{-1} is known as the ‘fingerprint region’, which is characteristic to each compound. Therefore, unknown compounds can be identified by means of FTIR. Some advantages of FTIR include high signal to noise ratio and short measurement time. Requiring small sample size is another benefit of FTIR. Depending on the instrument, FTIR could be destructive or non-destructive and might require sample preparation.

Thermal analysis methods are widely used to investigate solid state features of compounds when heat is applied. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are commonly used thermo-analytical techniques (Munson, 2009; Vogt, 2015). Endothermic and exothermic events can be measured by DSC based on the difference of heat required to increase the temperature of a sample pan compared to a reference pan as a function of temperature. Melting, glass transition, crystallization, polymorphic transition, and solid-solid transition are the endothermic and exothermic events most commonly evaluated by DSC. The advantages of DSC are that it is sensitive, rapid, and easy to use. Moreover, it requires very small amount of samples (Munson, 2009).

Since water is ubiquitous, it is crucial to characterize the behavior of solids in the presence of moisture. Water can interact with solids via five major mechanisms: 1) Adsorption onto the solid surface, 2) deliquescence, 3) capillary condensation, 4) crystal hydrate formation, and 5) absorption into the bulk structure (Mauer & Taylor, 2010). Water vapor sorption techniques provide insight about hygroscopicity of materials and solid type. Moisture sorption isotherms

measure weight changes as a function of relative humidity at a constant temperature due to moisture sorption or desorption. Moisture sorption isotherms were categorized into 6 general types by IUPAC (from Type I to Type VI) (Rouquerol, Rouquerol, Sing, Maurin, & Llewellyn, 2014). Amorphous solids are mostly characterized by their sigmoidal moisture sorption isotherm shape (Type II), whereas J-shaped isotherms (Type III) refer to deliquescent crystalline solids in which adsorption and capillary condensation are observed until the deliquescence point.

High performance liquid chromatography (HPLC) is the most common separation technique to separate, identify, and quantify components based on their affinity with the stationary phase and the mobile phase. HPLC is a rapid and sensitive tool with high resolution (Reuhs & Rounds, 2010), which is widely used to determine chemical purity, chemical composition, and chemical degradation.

1.9 Hypothesis

As discussed above, this dissertation focused on solid states of two different small compounds: 1) citric acid, and 2) thiamine. Therefore, two specific hypotheses were developed for each compound used, as follows:

1) Physical stability of amorphous citric acid which has a low T_g (13^{o}C) could be maintained in solid dispersions exposed to various storage conditions by forming noncovalent intermolecular interactions with polymers by means of three carboxylic groups and hydroxyl group in its structure.
2) Thiamine is likely found in amorphous form in complex food systems due to processing treatments and interactions with polymeric ingredients and amorphous thiamine is more labile than its crystalline form due to having a higher energy state. The chemical stability of amorphous thiamine, however, could be dependent on the type of polymers used in solid

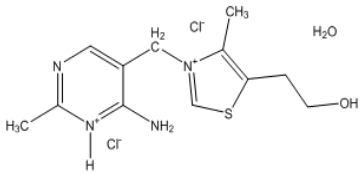
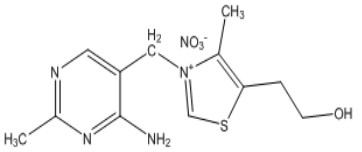
dispersions, with being more stable against degradation where intermolecular interaction between thiamine and polymer through hydrogen bonding is evident.

1.10 Dissertation objectives

The specific objectives of this dissertation include to:

1) Create and study amorphous solid dispersions of thiamine chloride hydrochloride, thiamine mononitrate, and citric acid in select polymers.
2) Determine the impact of structural features of the polymers (hygroscopicity, T_g, interaction ability via hydrogen bonding and/or ionic interaction) on the physical stability of citric acid amorphous solid dispersions.
3) Investigate the chemical stability of thiamine in the absence and presence of polymer, in the physical blends, and in the various solid dispersions containing select thiamine to polymer proportions stored at different environmental conditions.
4) Understand the effect of physical state (crystalline vs. amorphous), vitamin form (chloride hydrochloride vs. mononitrate), storage conditions, polymer, pHs of pre-lyophilized solutions, and proportions of vitamin to polymer on thiamine degradation.

Table 1.1 Properties of synthetic salt forms of thiamine commonly used for food fortifications.

Type of Thiamine Salt	**Molecular Structure**	**Molecular Weight (g/mol)**	**Melting Temperature: T_m (ºC)**	**Aqueous Solubility at 25ºC**	**Deliquescence Point (RH_0) at 25ºC**
Thiamine Chloride Hydrochloride	 (Voelker et al., 2018)	**337.25** (Al-Rashood, Al-Shammary, & Mian, 1990)	**248** (Al-Rashood et al., 1990)	**1g/1mL H_2O** (Al-Rashood et al., 1990)	**88** (Hiatt et al., 2008)
Thiamine Mononitrate	 (Voelker et al., 2018)	**327.37** (Macek, Feller, & Hanus, 1950)	**196-200** (Macek et al., 1950)	**2.7g/100 mL H_2O** (Macek et al., 1950)	**98.5** (Hiatt et al., 2008)

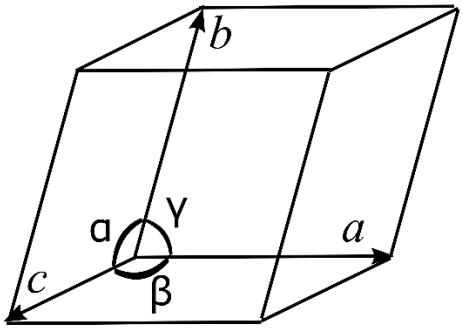


Figure 1.1 “Example of a unit cell.” Redrawn based on the reference (Waseda et al., 2011).

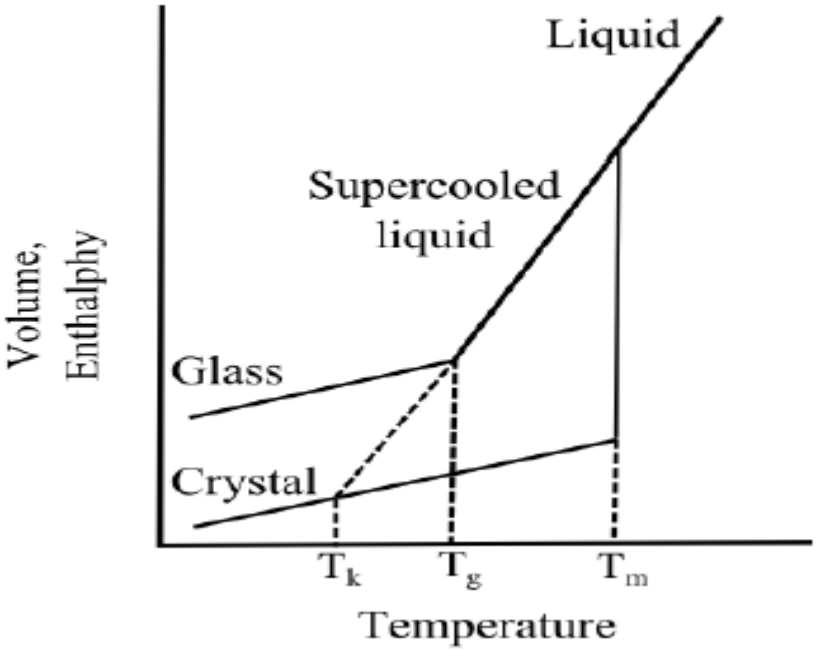


Figure 1.2 "Schematic depiction of the variation of enthalpy (or volume) with temperature." Redrawn based on the reference (Hancock & Zografi, 1997).

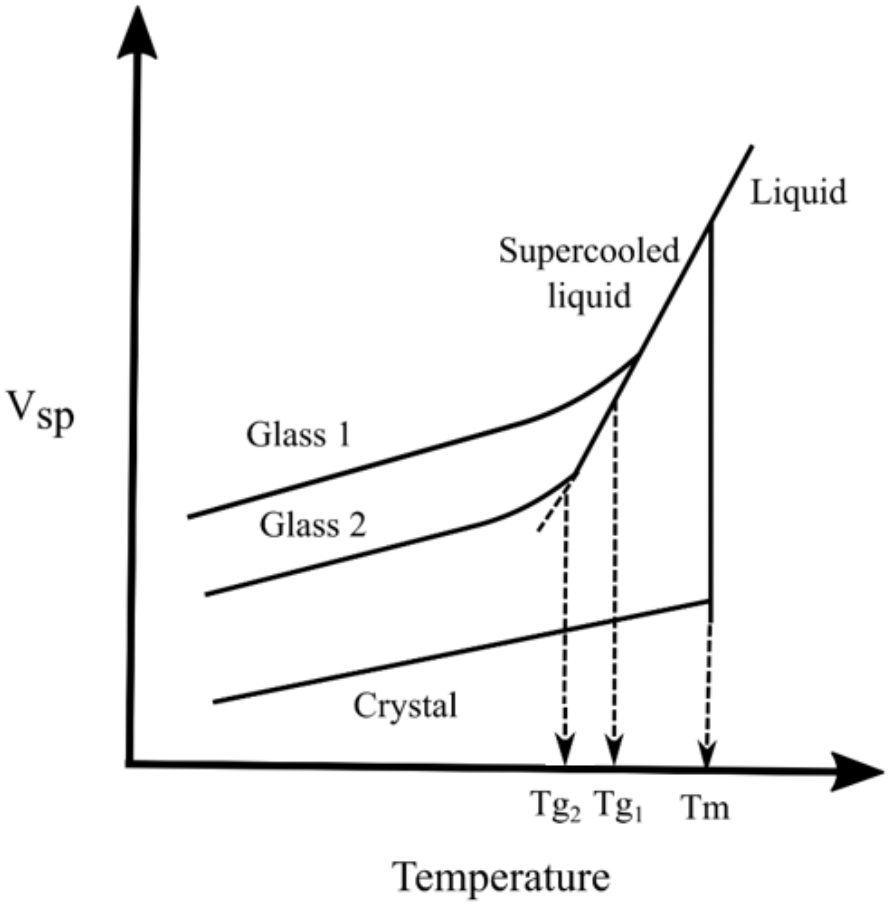


Figure 1.3 "Schematic representation of the specific volume as a function of temperature for a liquid which can both crystallize and form a glass. The thermodynamic and dynamic properties of a glass depend upon the cooling rate; glass 2 was formed with a slower cooling rate than glass 1. The glass transition temperature T_g can be defined by extrapolating V_{sp} in the glassy state back to the supercooled liquid line. T_g depends upon the cooling rate. Typical cooling rates in laboratory experiments are 0.1-100 K/min." Redrawn based on the reference (Ediger et al., 1996).

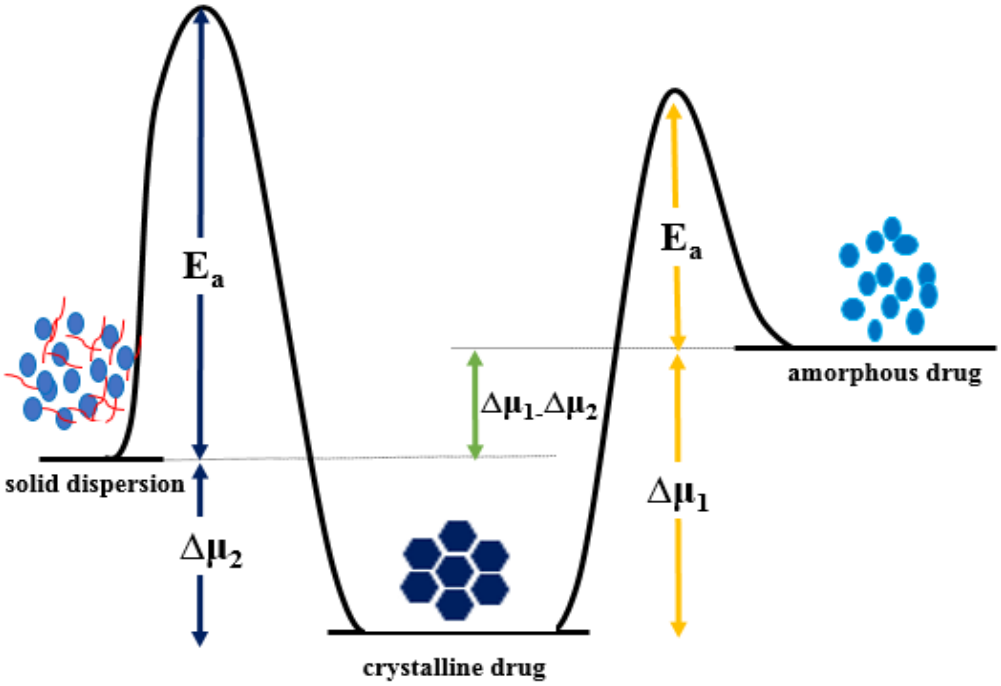


Figure 1.4 "Schematic hypothetical energy cartoon showing the amorphous drug, crystalline drug, and several single phase amorphous solid dispersions. μ represents the chemical potential of the drug and E_a represents the activation energy barrier for crystallization." Redrawn based on the reference (Harmon et al., 2009).

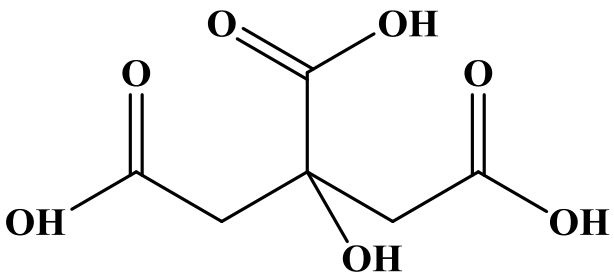


Figure 1.5 Chemical structure of citric acid.

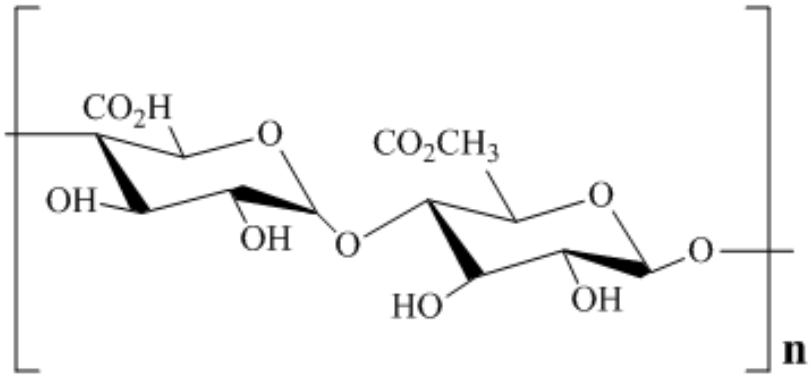


Figure 1.6 Chemical structure of pectin. Redrawn based on the reference (Bhatia, 2016b).

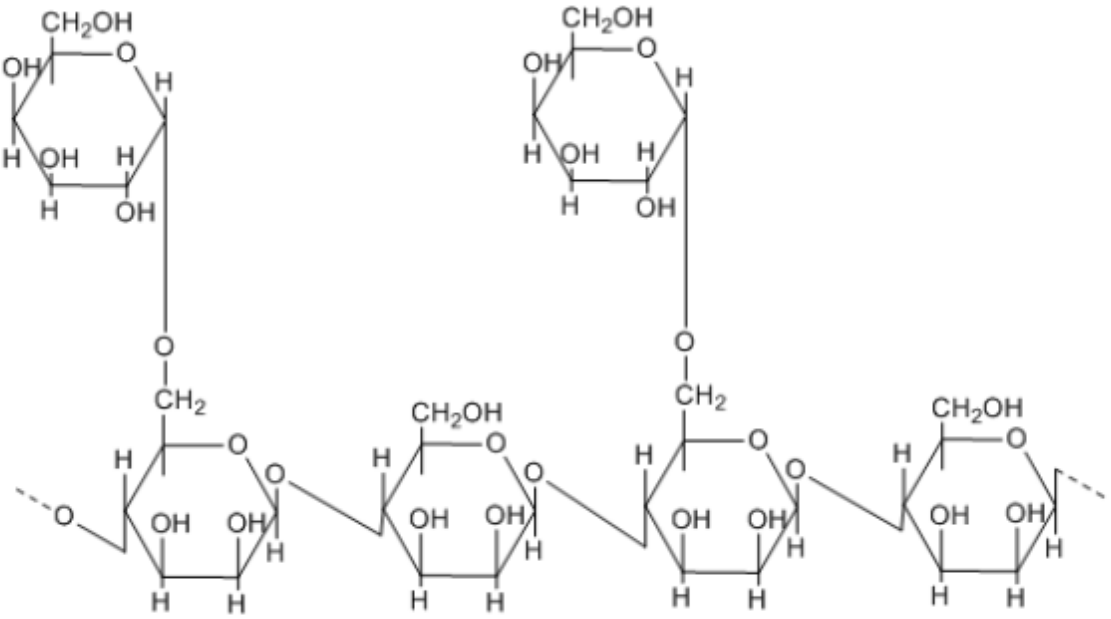


Figure 1.7 Chemical structure of guar gum. Redrawn based on the reference (Chudzikowski, 1971).

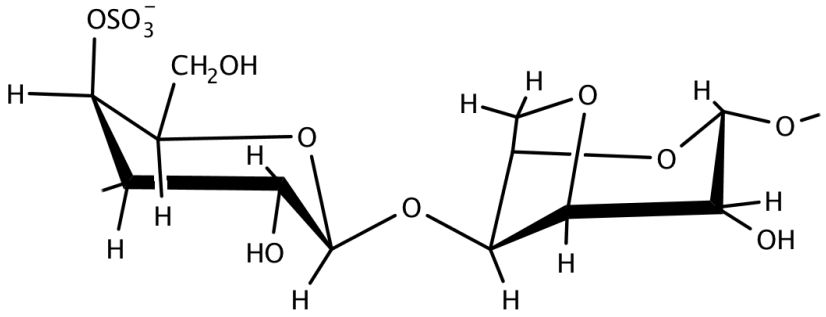


Figure 1.8 Chemical structure of κ-carrageenan. Redrawn based on the reference (Bhatia, 2016a).

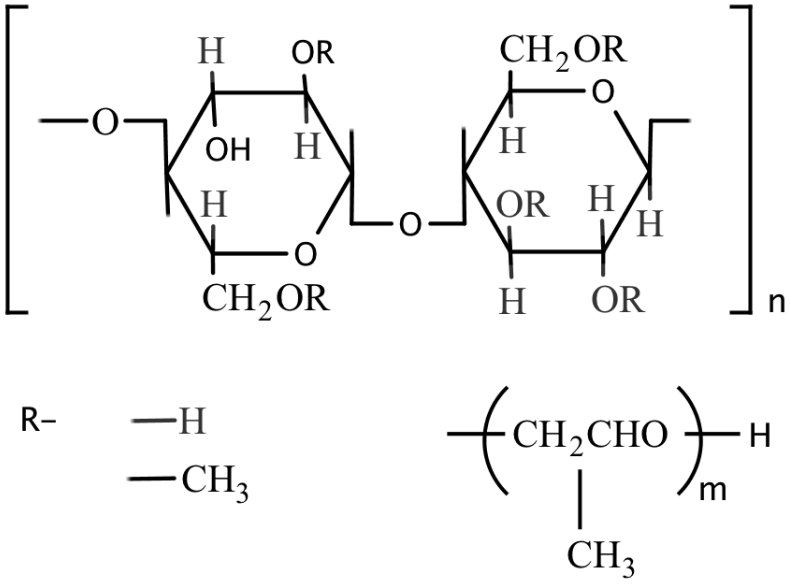


Figure 1.9 Chemical structure of HPMC.

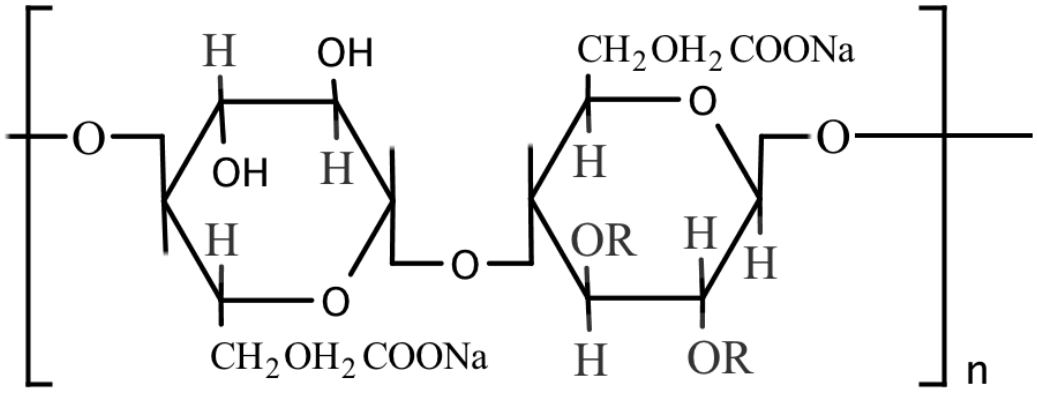


Figure 1.10 Chemical structure of CMC-Na. Redrawn based on the reference (Chen, Guo, Shen, Guo, & Ruan, 2012).

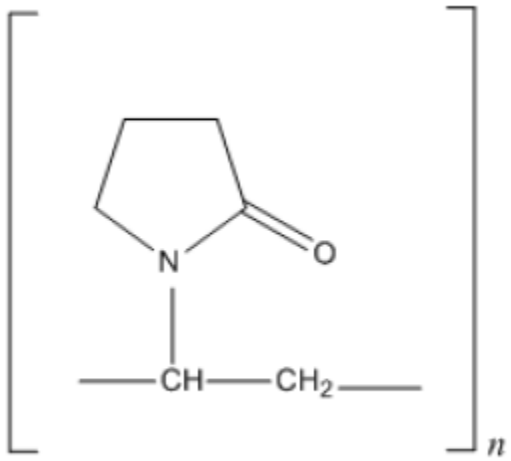
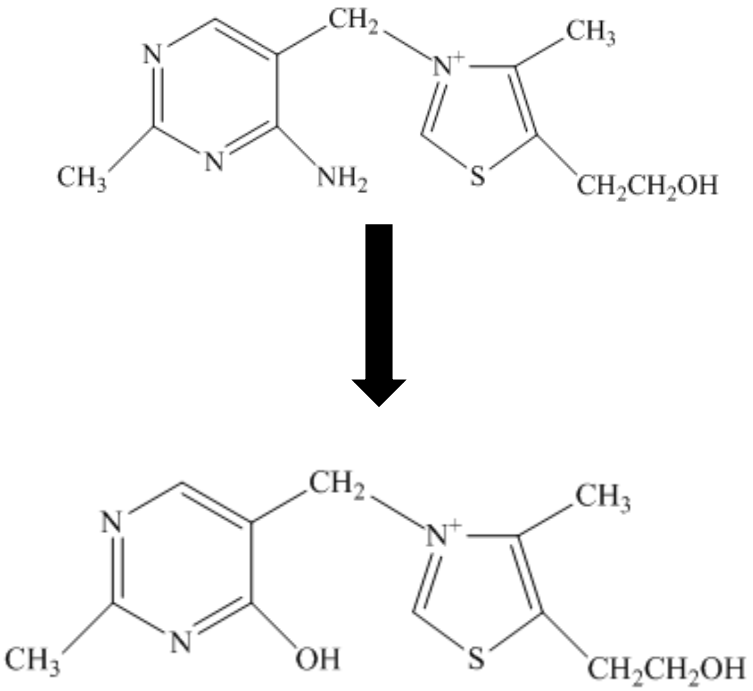


Figure 1.11 Chemical structure of PVP. Redrawn based on the reference (Brittain, 2003).

a)



b and c)

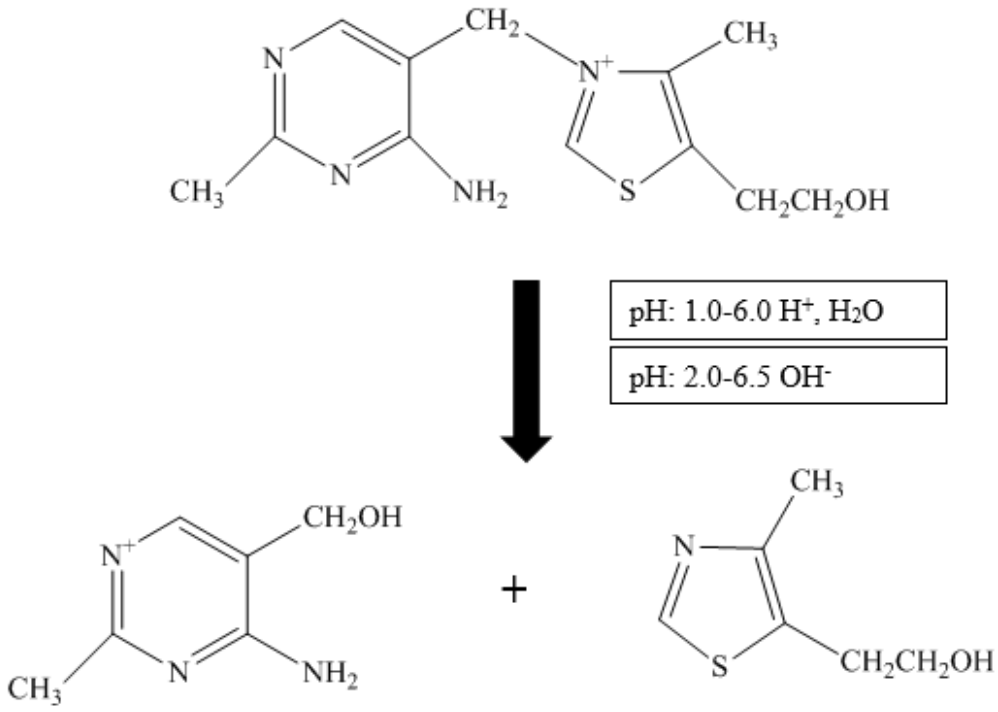


Figure 1.12 "Degradation mechanism of thiamine." Redrawn based on the reference (Dwivedi & Arnold, 1973; Windheuser & Higuchi, 1962). Continues on next pages.

Figure 1.12 Continued

d)

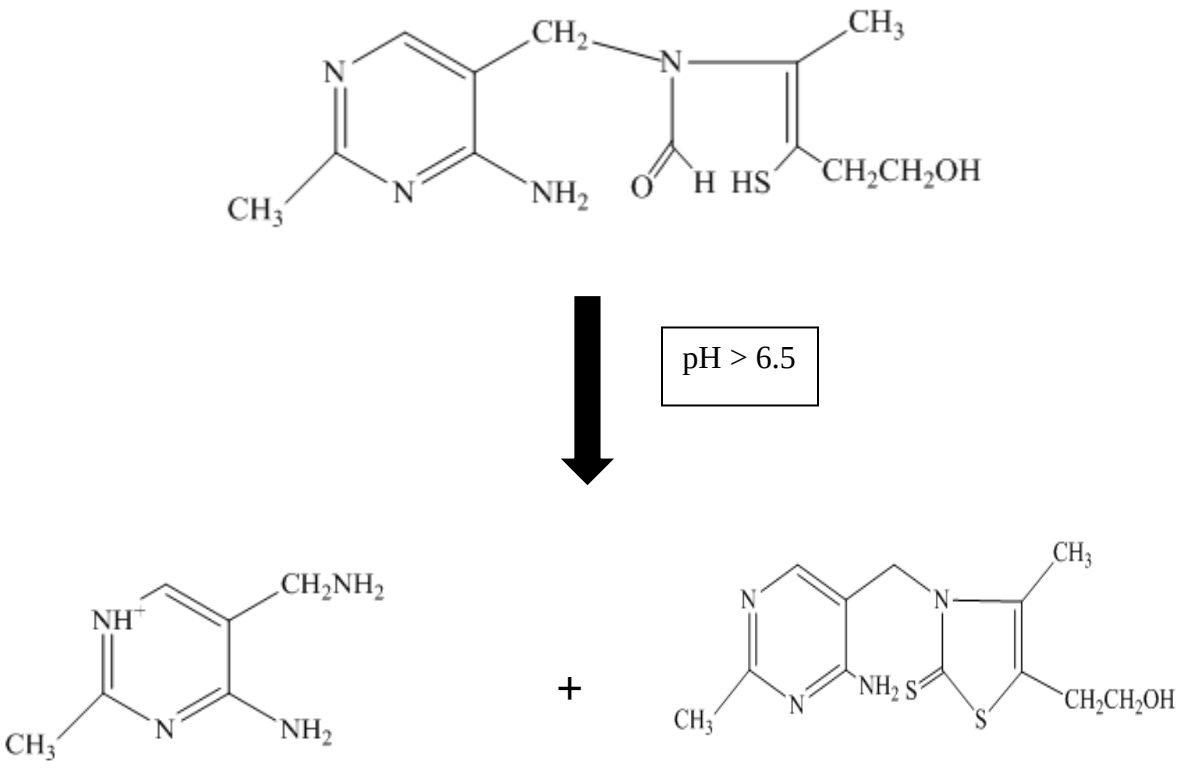
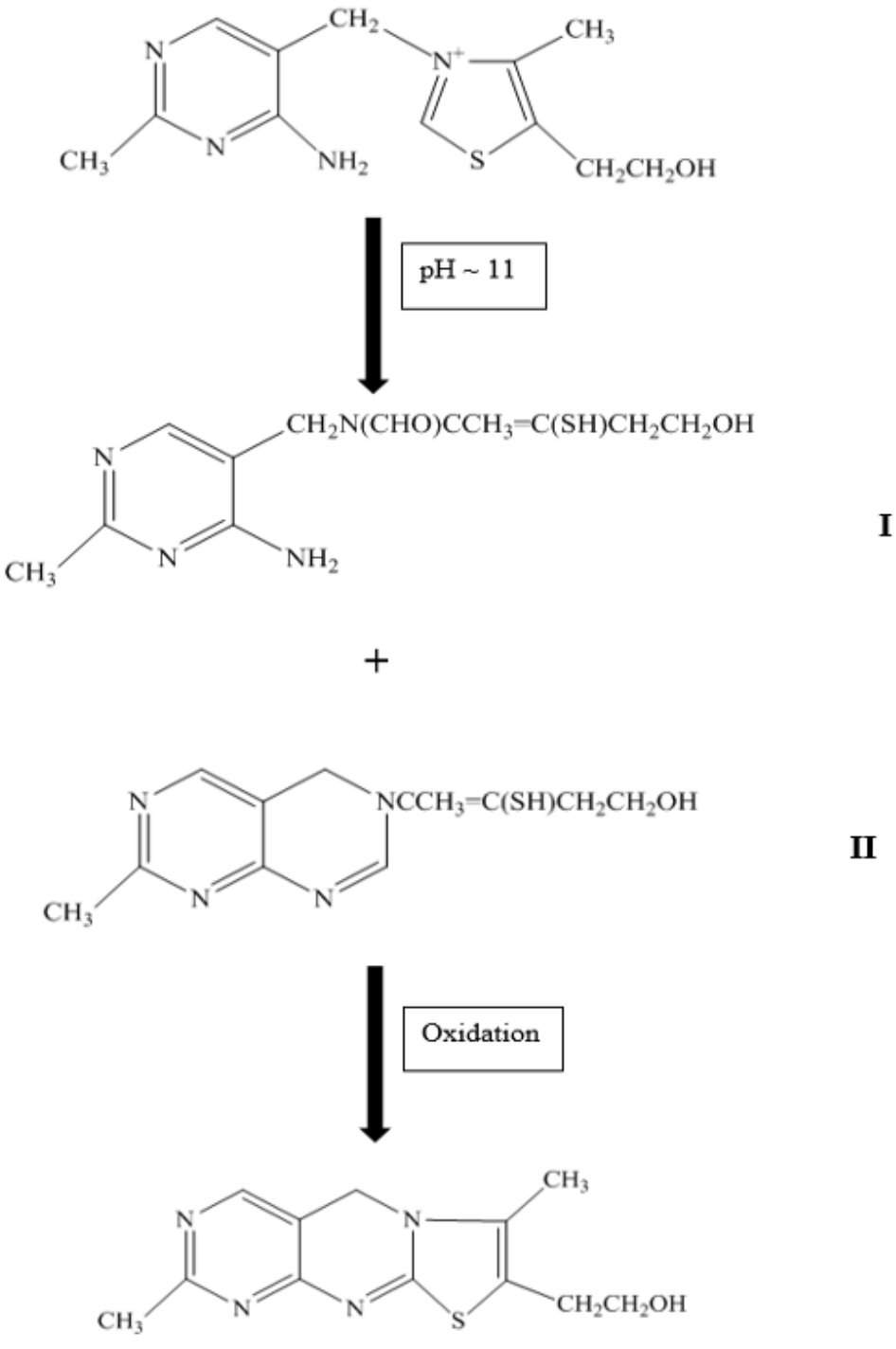


Figure 1.12 Continued

e)



References

Al-Rashood, K. A. M., Al-Shammary, F. J., & Mian, N. A. A. (1990). Analytical Profile of Thiamine Hydrochloride. In K. Florey, A. A. Al-Badr, G. A. Forcier, H. G. Brittain, & L. T. Grady (Eds.), *Analytical Profiles of Drug Substances* (Vol. 18, pp. 413-458): Academic Press.

Allan, M., & Mauer, L. J. (2017). RH-temperature phase diagrams of hydrate forming deliquescent crystalline ingredients. *Food Chemistry, 236*, 21-31. doi:https://doi.org/10.1016/j.foodchem.2017.02.110

Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International, 99*, 363-374. doi:https://doi.org/10.1016/j.foodres.2017.05.021

Bell, L. N., & Hageman, M. J. (1994). Differentiating between the Effects of Water Activity and Glass Transition Dependent Mobility on a Solid State Chemical Reaction: Aspartame Degradation. *Journal of Agricultural and Food Chemistry, 42*(11), 2398-2401. doi:10.1021/jf00047a007

Bell, L. N., & White, K. L. (2008). Thiamin Stability in Solids as Affected by the Glass Transition. *Journal of Food Science, 65*(3), 498-501. doi:10.1111/j.1365-2621.2000.tb16035.x

Bellantone, R. A. (2014). Fundamentals of Amorphous Systems: Thermodynamic Aspects. In N. Shah, H. Sandhu, D. S. Choi, H. Chokshi, & A. W. Malick (Eds.), *Amorphous Solid Dispersions: Theory and Practice* (pp. 3-34). New York, NY: Springer New York.

Bendix G, H., Heberlein D, G., Ptak L, R., & Clifcorn L, E. (1951). Factors Influencing the Stability of Thiamine During Heat Sterilization. *Journal of Food Science, 16*(1-6), 494-503. doi:10.1111/j.1365-2621.1951.tb17411.x

Bernstein, J. (2002). *Polymorphism in Molecular Crystals*. Oxford, UNITED KINGDOM: Oxford University Press USA - OSO.

Bettendorff, L. (2013). Thiamine. In *Handbook of Vitamins, Fifth Edition* (pp. 267-324): CRC Press.

Bhatia, S. (2016a). Marine Polysaccharides Based Nano-Materials and Its Applications. In S. Bhatia (Ed.), *Natural Polymer Drug Delivery Systems: Nanoparticles, Plants, and Algae* (pp. 185-225). Cham: Springer International Publishing.

Bhatia, S. (2016b). Plant Derived Polymers, Properties, Modification & Applications. In S. Bhatia (Ed.), *Natural Polymer Drug Delivery Systems: Nanoparticles, Plants, and Algae* (pp. 119-184). Cham: Springer International Publishing.

Bond, A. D. (2012). Chapter 2 Fundamental Aspects of Salts and Co-crystals. In *Pharmaceutical Salts and Co-crystals* (pp. 9-28): The Royal Society of Chemistry.

Brittain, H. G. (2003). Handbook of Pharmaceutical Excipients--4th Edition.(Book Review). *(Book Review), 27*(10), 172(171).

Byrn, S. R., Xu, W., & Newman, A. W. (2001). Chemical reactivity in solid-state pharmaceuticals: formulation implications. *Advanced Drug Delivery Reviews, 48*(1), 115-136. doi:https://doi.org/10.1016/S0169-409X(01)00102-8

Chen, W. L., Guo, D. W., Shen, Y. Y., Guo, S. R., & Ruan, K. P. (2012). Effects of Highly Hygroscopic Excipients on the Hydrolysis of Simvastatin in Tablet at High Relative Humidity. *Indian Journal of Pharmaceutical Sciences, 74*(6), 527-534. doi:10.4103/0250-474X.110587

Chiou, W. L., & Riegelman, S. (1969). Preparation and Dissolution Characteristics of Several Fast-Release Solid Dispersions of Griseofulvin. *Journal of Pharmaceutical Sciences, 58*(12), 1505-1510. doi:https://doi.org/10.1002/jps.2600581218

Chiou, W. L., & Riegelman, S. (1971). Pharmaceutical Applications of Solid Dispersion Systems. *Journal of Pharmaceutical Sciences, 60*(9), 1281-1302. doi:https://doi.org/10.1002/jps.2600600902

Christina, B., Taylor, L. S., & Mauer, L. J. (2015). Physical stability of l-ascorbic acid amorphous solid dispersions in different polymers: A study of polymer crystallization inhibitor properties. *Food Research International, 76*, 867-877. doi:https://doi.org/10.1016/j.foodres.2015.08.009

Chudzikowski, R. J. (1971). Guar gum and its applications. *Journal of the Society of Cosmetic Chemists, 22*, 43-60.

Craig, D. Q. M. (2002). The mechanisms of drug release from solid dispersions in water-soluble polymers. *International Journal of Pharmaceutics, 231*(2), 131-144. doi:https://doi.org/10.1016/S0378-5173(01)00891-2

Crowley, K. J., & Zografi, G. (2001). The use of thermal methods for predicting glass-former fragility. *Thermochimica Acta, 380*(2), 79-93. doi:https://doi.org/10.1016/S0040-6031(01)00662-1

Dreher, J. G., Rouseff, R. L., & Naim, M. (2003). GC−Olfactometric Characterization of Aroma Volatiles from the Thermal Degradation of Thiamin in Model Orange Juice. *Journal of Agricultural and Food Chemistry, 51*(10), 3097-3102. doi:10.1021/jf034023j

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

Ediger, M. D., Angell, C. A., & Nagel, S. R. (1996). Supercooled Liquids and Glasses. *The Journal of Physical Chemistry, 100*(31), 13200-13212. doi:10.1021/jp953538d

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

Fox, T. G. (1956). Influence of diluent and of copolymer composition on the glass temperature of a polymer system. *Bulletin of the American Physical Society, 1*(2), 123-135.

Franks, F. (2007). The Process Sequence in Summary. In *Chapter 2: Freeze-drying of Pharmaceuticals and Biopharmaceuticals*: Royal Society of Chemistry.

Gordon, M., & Taylor, J., S. (1952). Ideal copolymers and the second-order transitions of synthetic rubbers. i. non-crystalline copolymers. *Journal of Applied Chemistry, 2*(9), 493-500. doi:10.1002/jctb.5010020901

Grosch, W., & Zeiler-Hilgart, G. (1992). Formation of Meatlike Flavor Compounds. In *Flavor Precursors* (Vol. 490, pp. 183-192): American Chemical Society.

Hancock, B. C., & Shamblin, S. L. (2001). Molecular mobility of amorphous pharmaceuticals determined using differential scanning calorimetry. *Thermochimica Acta, 380*(2), 95-107. doi:https://doi.org/10.1016/S0040-6031(01)00663-3

Hancock, B. C., & Zografi, G. (1993). The Use of Solution Theories for Predicting Water Vapor Absorption by Amorphous Pharmaceutical Solids: A Test of the Flory–Huggins and Vrentas Models. *Pharmaceutical Research, 10*(9), 1262-1267. doi:10.1023/A:1018901325842

Hancock, B. C., & Zografi, G. (1997). Characteristics and Significance of the Amorphous State in Pharmaceutical Systems. *Journal of Pharmaceutical Sciences, 86*(1), 1-12. doi:https://doi.org/10.1021/js9601896

Haque, M. K., & Roos, Y. H. (2005). Crystallization and X-ray diffraction of spray-dried and freeze-dried amorphous lactose. *Carbohydrate Research, 340*(2), 293-301. doi:https://doi.org/10.1016/j.carres.2004.11.026

Harmon, P., Li, L., Marsac, P., Craig, M., Variankaval, N., & Xu, W. (2009). Amorphous solid dispersions: Analytical challenges and oppurtunities. *AAPS Newmagazine*(September), 14-20.

Hiatt, A. N., Ferruzzi, M. G., Taylor, L. S., & Mauer, L. J. (2008). Impact of Deliquescence on the Chemical Stability of Vitamins B1, B6, and C in Powder Blends. *Journal of Agricultural and Food Chemistry, 56*(15), 6471-6479. doi:10.1021/jf800709f

Hopmann, R. F. (1973). pK of thiamine C(2)H. *Nature: New Biology, 246*(153), 157.

Hoppu, P., Hietala, S., Schantz, S., & Juppo, A. M. (2009). Rheology and molecular mobility of amorphous blends of citric acid and paracetamol. *European Journal of Pharmaceutics and Biopharmaceutics, 71*(1), 55-63. doi:https://doi.org/10.1016/j.ejpb.2008.06.029

Hoppu, P., Jouppila, K., Rantanen, J., Schantz, S., & Juppo Anne, M. (2010). Characterisation of blends of paracetamol and citric acid. *Journal of Pharmacy and Pharmacology, 59*(3), 373-381. doi:10.1211/jpp.59.3.0006

Huang, Y., & Dai, W.-G. (2014). Fundamental aspects of solid dispersion technology for poorly soluble drugs. *Acta Pharmaceutica Sinica B, 4*(1), 18-25. doi:https://doi.org/10.1016/j.apsb.2013.11.001

Igoe, R. S. (2011). *Dictionary of Food Ingredients* (5th ed.): Boston, MA : Springer US.

Janssens, S., & Van den Mooter, G. (2010). Review: physical chemistry of solid dispersions. *Journal of Pharmacy and Pharmacology, 61*(12), 1571-1586. doi:10.1211/jpp.61.12.0001

Khougaz, K., & Clas, S. D. (2000). Crystallization Inhibition in Solid Dispersions of MK-0591 and Poly(vinylpyrrolidone) Polymers. *Journal of Pharmaceutical Sciences, 89*(10), 1325-1334. doi:https://doi.org/10.1002/1520-6017(200010)89:10<1325::AID-JPS10>3.0.CO;2-5

Konno, H., & Taylor, L. S. (2008). Ability of Different Polymers to Inhibit the Crystallization of Amorphous Felodipine in the Presence of Moisture. *Pharmaceutical Research, 25*(4), 969-978. doi:10.1007/s11095-007-9331-3

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

Ladd, M., & Palmer, R. (2003a). Crystal Morphology and Crystal Symmetry. In M. Ladd & R. Palmer (Eds.), *Structure Determination by X-ray Crystallography* (pp. 1-50). Boston, MA: Springer US.

Ladd, M., & Palmer, R. (2003b). Lattices and Space-Group Theory. In M. Ladd & R. Palmer (Eds.), *Structure Determination by X-ray Crystallography* (pp. 51-116). Boston, MA: Springer US.

Lai, M. C., Hageman, M. J., Schowen, R. L., Borchardt, R. T., & Topp, E. M. (1999). Chemical stability of peptides in polymers. 1. Effect of water on peptide deamidation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) matrixes. *Journal of Pharmaceutical Sciences, 88*(10), 1073-1080. doi:https://doi.org/10.1021/js980227g

Li, B., Konecke, S., Harich, K., Wegiel, L., Taylor, L. S., & Edgar, K. J. (2013). Solid dispersion of quercetin in cellulose derivative matrices influences both solubility and stability. *Carbohydrate Polymers, 92*(2), 2033-2040. doi:10.1016/j.carbpol.2012.11.073

Li, J., Guo, Y., & Zografi, G. (2002). The Solid-State Stability of Amorphous Quinapril in the Presence of β-Cyclodextrins. *Journal of Pharmaceutical Sciences, 91*(1), 229-243. doi:https://doi.org/10.1002/jps.10014

Lonsdale, D. (2006). A Review of the Biochemistry, Metabolism and Clinical Benefits of Thiamin(e) and Its Derivatives. *Evidence-based Complementary and Alternative Medicine, 3*(1), 49-59. doi:10.1093/ecam/nek009

Lu, Q., & Zografi, G. (1997). Properties of Citric Acid at the Glass Transition. *Journal of Pharmaceutical Sciences, 86*(12), 1374-1378. doi:https://doi.org/10.1021/js970157y

Lu, Q., & Zografi, G. (1998). Phase Behavior of Binary and Ternary Amorphous Mixtures Containing Indomethacin, Citric Acid, and PVP. *Pharmaceutical Research, 15*(8), 1202-1206. doi:10.1023/A:1011983606606

Luthra, S. A., Shalaev, E. Y., Medek, A., Hong, J., & Pikal, M. J. (2012). Chemical Stability of Amorphous Materials: Specific and General Media Effects in the Role of Water in the Degradation of Freeze-Dried Zoniporide. *Journal of Pharmaceutical Sciences, 101*(9), 3110-3123. doi:https://doi.org/10.1002/jps.23128

Macek, T. J., Feller, B. A., & Hanus, E. J. (1950). Pharmaceutical Studies with Thiamine Mononitrate. *Journal of the American Pharmaceutical Association (Scientific ed.), 39*(7), 365-369. doi:https://doi.org/10.1002/jps.3030390702

Marsac, P. J., Konno, H., & Taylor, L. S. (2006). A Comparison of the Physical Stability of Amorphous Felodipine and Nifedipine Systems. *Pharmaceutical Research, 23*(10), 2306-2316. doi:10.1007/s11095-006-9047-9

Matsumoto, T., & Zografi, G. (1999). Physical Properties of Solid Molecular Dispersions of Indomethacin with Poly(vinylpyrrolidone) and Poly(vinylpyrrolidone-co-vinyl-acetate) in Relation to Indomethacin Crystallization. *Pharmaceutical Research, 16*(11), 1722-1728. doi:10.1023/A:1018906132279

Mauer, L. J., & Taylor, L. S. (2010). Water-Solids Interactions: Deliquescence. *Annual Review of Food Science and Technology, 1*(1), 41-63. doi:10.1146/annurev.food.080708.100915

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:https://doi.org/10.1016/0308-8146(92)90006-N

McCandless, D. W. (2009a). Early Chemistry. In D. W. McCandless (Ed.), *Thiamine Deficiency and Associated Clinical Disorders* (pp. 1-7). Totowa, NJ: Humana Press.

McCandless, D. W. (2009b). Early Thiamine Deficiency. In D. W. McCandless (Ed.), *Thiamine Deficiency and Associated Clinical Disorders* (pp. 9-16). Totowa, NJ: Humana Press.

Miyazaki, T., Yoshioka, S., Aso, Y., & Kojima, S. (2004). Ability of Polyvinylpyrrolidone and Polyacrylic Acid to Inhibit the Crystallization of Amorphous Acetaminophen. *Journal of Pharmaceutical Sciences, 93*(11), 2710-2717. doi:https://doi.org/10.1002/jps.20182

Mullin, J. W. (2001a). 5 - Nucleation. In *Crystallization (Fourth Edition)* (pp. 181-215). Oxford: Butterworth-Heinemann.

Mullin, J. W. (2001b). 6 - Crystal growth. In *Crystallization (Fourth Edition)* (pp. 216-288). Oxford: Butterworth-Heinemann.

Munson, E. J. (2009). Chapter 3 - Analytical Techniques in Solid-state Characterization A2 - Qiu, Yihong. In Y. Chen, G. G. Z. Zhang, L. Liu, & W. R. Porter (Eds.), *Developing Solid Oral Dosage Forms* (pp. 61-74). San Diego: Academic Press.

Nath, A., Shope, T. R., & Koch, T. R. (2017). Chapter 51 - Thiamine (Vitamin B1) After Weight Loss Bariatric Surgery A2 - Preedy, Victor R. In R. Rajendram & C. R. Martin (Eds.), *Metabolism and Pathophysiology of Bariatric Surgery* (pp. 479-489). Boston: Academic Press.

Nichols, G., Luk, S., & Roberts, C. (2011). Microscopy. In A. R. Storey & I. Ym é n (Eds.), *Solid State Characterization of Pharmaceuticals* (pp. 287-355): John Wiley & Sons Ltd.

Nordman, C. E., Weldon, A. S., & Patterson, A. L. (1960). X-ray Crystal analysis of the substrates of aconitase . II.Anhydrous citric acid. *Acta Crystallographica, 13*(5), 418-426. doi:10.1107/s0365110x60000996

Pachapurkar, D., & Bell Leonard, N. (2006). Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions. *Journal of Food Science, 70*(7), c423-c426. doi:10.1111/j.1365-2621.2005.tb11463.x

Prinzo, Z. W. (1999). *Thiamine Deficiency and Its Prevention and Control in Major Emergencies*: Micronutrient series; World Health, Organization (WHO). Department of Nutrient for Health and Development; Office of the United Nations High Commissioner for Refugees (UNHCR).

Reuhs, B. L., & Rounds, M. A. (2010). High-Performance Liquid Chromatography. In S. S. Nielsen (Ed.), *Food Analysis* (pp. 499-512). Boston, MA: Springer US.

Rouquerol, F., Rouquerol, J., Sing, K. S. W., Maurin, G., & Llewellyn, P. (2014). 1 - Introduction. In *Adsorption by Powders and Porous Solids (Second Edition)* (pp. 1-24). Oxford: Academic Press.

Salameh Adnan, K., Mauer Lisa, J., & Taylor Lynne, S. (2006). Deliquescence Lowering in Food Ingredient Mixtures. *Journal of Food Science, 71*(1), E10-E16. doi:10.1111/j.1365-2621.2006.tb12392.x

Sekiguchi, K., & Obi, N. (1961). Studies on Absorption of Eutectic Mixture.1. Comparison of Behavior of Eutectic Mixture of Sulfathiazole and That of Ordinary Sulfathiazole in Man. *Chemical & Pharmaceutical Bulletin, 9*(11), 866.

Serajuddin, A. T. M. (1999). Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs. *Journal of Pharmaceutical Sciences, 88*(10), 1058-1066. doi:https://doi.org/10.1021/js980403l

Shalaev, E. Y., & Zografi, G. (1996). How Does Residual Water Affect the Solid-state Degradation of Drugs in the Amorphous State? *Journal of Pharmaceutical Sciences, 85*(11), 1137-1141. doi:https://doi.org/10.1021/js960257o

Sheskey, P. J., Cook, W. G., & Cable, C. G. (2017). *Handbook of pharmaceutical excipients* (Eighth ed.): London : Pharmaceutical Press ; Washington, DC : APhA.

Sugimoto, I., Ishihara, T., Habata, H., & Nakagawa, H. (1981). Stability of lyophilized sodium prasterone sulfate. *Journal Of Parenteral Science And Technology: A Publication Of The Parenteral Drug Association, 35*(3), 88.

Summers, M. P. (1978). Glass Formation in Barbiturates and Solid Dispersion Systems of Barbiturates with Citric Acid. *Journal of Pharmaceutical Sciences, 67*(11), 1606-1610. doi:https://doi.org/10.1002/jps.2600671126

Summers, M. P., & Enever, R. P. (1976). Preparation and Properties of Solid Dispersion System Containing Citric Acid and Primidone. *Journal of Pharmaceutical Sciences, 65*(11), 1613-1617. doi:https://doi.org/10.1002/jps.2600651113

Tantishaiyakul, V., Kaewnopparat, N., & Ingkatawornwong, S. (1996). Properties of solid dispersions of piroxicam in polyvinylpyrrolidone K-30. *International Journal of Pharmaceutics, 143*(1), 59-66. doi:https://doi.org/10.1016/S0378-5173(96)04687-X

Taylor, L. S., & Zografi, G. (1997). Spectroscopic Characterization of Interactions Between PVP and Indomethacin in Amorphous Molecular Dispersions. *Pharmaceutical Research, 14*(12), 1691-1698. doi:10.1023/A:1012167410376

Thakur, B., Singh, R., Handa, A., & Rao, M. A. (1997). Chemistry and uses of pectin — A review. In (Vol. 37, pp. 47-73): Taylor & Francis Group.

Van den Mooter, G., Augustijns, P., & Kinget, R. (1999). Stability prediction of amorphous benzodiazepines by calculation of the mean relaxation time constant using the Williams–Watts decay function. *European Journal of Pharmaceutics and Biopharmaceutics, 48*(1), 43-48. doi:https://doi.org/10.1016/S0939-6411(99)00013-2

Van den Mooter, G., Wuyts, M., Blaton, N., Busson, R., Grobet, P., Augustijns, P., & Kinget, R. (2001). Physical stabilisation of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25. *European Journal of Pharmaceutical Sciences, 12*(3), 261-269. doi:https://doi.org/10.1016/S0928-0987(00)00173-1

Vogt, G. F. (2015). Solid-State Characterization of Amorphous Dispersions. In A. Newman (Ed.), *Pharmaceutical Amorphous Solid Dispersions* (pp. 117-178): John Wiley & Sons, Inc.

Wang, D. Q., Hey, J. M., & Nail, S. L. (2004). Effect of Collapse on the Stability of Freeze-Dried Recombinant Factor VIII and α-amylase. *Journal of Pharmaceutical Sciences, 93*(5), 1253-1263. doi:https://doi.org/10.1002/jps.20065

Wang, J., Chang, R., Zhao, Y., Zhang, J., Zhang, T., Fu, Q., Zeng, A. (2017). Coamorphous Loratadine-Citric Acid System with Enhanced Physical Stability and Bioavailability. *AAPS PharmSciTech, 18*(7), 2541-2550. doi:10.1208/s12249-017-0734-0

Waseda, Y., Matsubara, E., & Shinoda, K. (2011). Geometry of Crystals. In Y. Waseda, E. Matsubara, & K. Shinoda (Eds.), *X-Ray Diffraction Crystallography: Introduction, Examples and Solved Problems* (pp. 21-66). Berlin, Heidelberg: Springer Berlin Heidelberg.

Wegiel, L. A., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2013a). Crystallization of Amorphous Solid Dispersions of Resveratrol during Preparation and Storage—Impact of Different Polymers. *Journal of Pharmaceutical Sciences, 102*(1), 171-184. doi:https://doi.org/10.1002/jps.23358

Wegiel, L. A., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2013b). Mid-infrared spectroscopy as a polymer selection tool for formulating amorphous solid dispersions. *Journal of Pharmacy and Pharmacology, 66*(2), 244-255. doi:10.1111/jphp.12079

Wegiel, L. A., Mosquera-Giraldo, L. I., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2015). Phase Behavior of Resveratrol Solid Dispersions Upon Addition to Aqueous media. *Pharmaceutical Research, 32*(10), 3324-3337. doi:10.1007/s11095-015-1709-z

Wegiel, L. A., Zhao, Y., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2014). Curcumin amorphous solid dispersions: the influence of intra and intermolecular bonding on physical stability. *Pharmaceutical Development and Technology, 19*(8), 976-986. doi:10.3109/10837450.2013.846374

Wehling, R. L. (2010). Infrared Spectroscopy. In S. S. Nielsen (Ed.), *Food Analysis* (pp. 407-420). Boston, MA: Springer US.

Windheuser, J. J., & Higuchi, T. (1962). Kinetics of Thiamine Hydrolysis. *Journal of Pharmaceutical Sciences, 51*(4), 354-364. doi:https://doi.org/10.1002/jps.2600510415

Yoshioka, M., Hancock, B. C., & Zografi, G. (1995). Inhibition of indomethacin crystallization in poly(vinylpyrrolidone) coprecipitates. *Journal of Pharmaceutical Sciences, 84*(8), 983-986. doi:https://doi.org/10.1002/jps.2600840814

Zografi, G., & Newman, A. (2015). Introduction to Amorphous Solid Dispersions. In A. Newman (Ed.), *Pharmaceutical Amorphous Solid Dispersions* (pp. 1-41): John Wiley & Sons, Inc.

CHAPTER 2. AMORPHIZATION OF THIAMINE: A STUDY OF THE CHEMICAL STABILITY OF THIAMINE IN THIAMINE CHLORIDE HYDROCHLORIDE AMORPHOUS SOLID DISPERSIONS

2.1 Abstract

Thiamine is one of the most unstable vitamins, being highly sensitive to heat, alkaline pH, and processing/storage conditions. Two crystalline salts of thiamine, chloride hydrochloride (TClHCl) and mononitrate, are used for food fortification. Previously, we showed that TClHCl interacts with a variety of polymers, enabling its solidification in the amorphous state and indicating the likelihood of thiamine being in the amorphous state in many formulated foods. Amorphous solids are generally less chemically stable than their crystalline counterparts; however, no publications have documented the degradation of thiamine in the amorphous state. The objective of this study was, therefore, to directly compare thiamine stability in various forms (crystalline TClHCl, crystalline TClHCl physically blended with select polymers, and a variety of amorphous solid dispersions containing TClHCl and polymers), accounting for the impact of thiamine form (crystalline vs. amorphous), environmental conditions, and some physicochemical properties of the polymers on thiamine degradation. Solutions containing TClHCl with polymers (~% 1 w/v) (pectin and PVP (polyvinylpyrrolidone)) (50TClHCl:50/60 polymer and 5TClHCl:95 polymer) and fourteen different solutions varying from the concentrations of TClHCl and PVP were lyophilized. Control crystalline TClHCl and physical mixtures of crystalline TClHCl with polymers were also prepared. The samples were then stored in controlled temperature (30, 40, 50, 60^{o}C) and relative humidity (11% and 75%RH) conditions in desiccators for 8 weeks and monitored periodically by X-ray diffraction (to document physical state) and high performance liquid chromatography (to track degradation). Moisture sorption isotherms of all samples were

also generated, and T_gs were determined using differential scanning calorimetry. Significantly more degradation was found in amorphous samples at higher temperatures and lower RHs than in crystalline samples. For example, 25% of thiamine had degraded in amorphous TClHCl:PVP (5:95) dispersions stored at 11% RH and 60^{o}C on day 56, during which there was no measurable degradation in the crystalline control thiamine. Furthermore, significantly more thiamine degraded in PVP dispersions containing low concentrations of thiamine than in those containing higher proportions of the vitamin (which had lower T_gs). Understanding the effects of the solid state form and presence of other ingredients (and their proportions) on vitamin degradation in complex food matrices will inform decisions for improving the stability of thiamine in food products. In addition, a general fundamental knowledge could be gained for the stability of small compounds in the formulations containing polymeric ingredients and solid state strategies could be developed and applied to some other similar systems to maintain nutrient delivery and product quality.

2.2 Introduction

Thiamine (Vitamin B_1), the first vitamin to be discovered, is an essential nutrient which is involved in many physiological activities in the human body including, but not limited to, energy metabolism, synthesis of several neurotransmitters, and tissue, brain, and organ functions (Nath, Shope, & Koch, 2017). As a result of being a water-soluble vitamin, thiamine is held in reserve in the body only for 2-3 weeks (Nath et al., 2017); therefore, continuous intake of thiamine from the diet is important for maintaining health. The recommended daily thiamine intake for Americans 4 years of age or older is 1.2 mg (U.S. Food & Drug Administration, 2018). When considering the physiological functions of thiamine, it is not surprising that its deficiency results in serious disorders, evident within ten days as irritability, fatigue, weight loss, confusion, and blurred vision, with the potential to lead to more severe Wernicke-Korsakoff syndrome, beriberi, Leigh syndrome,

or death (McCandless, 2010). Deficient intake for 12 weeks results in development of thiamine deficiency, and subjective symptoms can be seen in 2-3 weeks (Prinzo, 1999). Overall rates of thiamine deficiencies in developed countries have been reduced due to fortification programs; however, alcoholics and people suffering from Celiac disease still face thiamine deficiencies (Prinzo, 1999). On the other hand, in developing countries, rates of thiamine deficiencies are still high, and even the severe deficiency symptom rates go up to 25% (Prinzo, 1999). This is likely attributed to different eating habits of the people living in these nations, the majority of which consume carbohydrate rich, non-nutritious meals and refined-unfortified grains. Diets high in carbohydrates accelerate thiamine depletion, because it is used as a cofactor in carbohydrate metabolism (Lonsdale, 2006).

A challenge for delivering thiamine via the diet is that thiamine is one of the most unstable vitamins due to its sensitivity to heat, radiation, alkaline pH, other ingredients (such as sulfites), and processing and storage conditions (Dwivedi & Arnold, 1973). The whole food sources of thiamine are yeast, meat (especially pork), whole grains, beans, soy beans, nuts, and egg yolk (McCandless, 2010). Cereal grains are good sources for thiamine, however most is lost during the production of white flour and polished rice (Pourcel, 2013). To compensate for this loss of nutrients, a synthetic form of thiamine is often used for enrichment or fortification, with thiamine chloride hydrochloride (TClHCl) and thiamine mononitrate being the most common additive forms, both of which have GRAS status in the USA (FDA codes: 21CFR184.1875 for chloride hydrochloride and 21CFR184.1878 for mononitrate). Both natural and synthetic forms of thiamine are known to degrade (Feliciotti & Esselen, 1957). Thiamine is comprised from a thiazole and a pyrimidine ring linked via a methylene bridge. Thiamine can, moreover, be involved in oxidation/reduction reactions and interact with other ingredients, which consequently cause a

degradation in its structure (Connors, 1986; Dwivedi & Arnold, 1973). Upon degradation, thiamine develops off-flavors. It has been reported that high temperature treatment at 100^{o}C for 24 hours in the dry state does not diminish the activity of thiamine (Connors, 1986). On the other hand, in solution state, pH is the main cause for degradation and thiamine loss proceeds faster (56% thiamine loss was reported at pH 6.67 and 40^{o}C on day 63) (Voelker, Miller, Running, Taylor, & Mauer, 2018). Different degradation pathways at different pH conditions were observed for thiamine (Pachapurkar & Bell, 2006). For example, thiamine is quite stable at pHs between 2.0-4.0, but it is highly sensitive to neutral and alkaline pH conditions. At strong acidic conditions (pH <1), the amino group attached to the pyrimidine ring is replaced by a hydroxyl group which results in oxythiamine formation (Windheuser & Higuchi, 1962). As pH increases toward less acidic and neutral conditions, the methylene bridge between the thiazole and pyrimidine ring is cleaved because of hydrolysis (Dwivedi & Arnold, 1973). Above pH 6.5, thiamine is found in the thiol form and water cleavage was reported to cause formation of a pyrimidine diamine and some unknown compounds (Windheuser & Higuchi, 1962). The degradation mechanisms of thiamine in different pH conditions were provided in **Fig. 1.12** (Chapter 1).

A recent study documented that TClHCl interacts with a variety of polymers, enabling its solidification in the amorphous state (Arioglu-Tuncil, Bhardwaj, Taylor, & Mauer, 2017). Solid state (amorphous vs. crystalline) is known to influence stability, with the chemical reactivity of amorphous solids being higher than their crystalline counterparts in many environments, often attributed to their greater molecular mobility and hygroscopicity (Roos & Drusch, 2016). Despite the possibility that thiamine is present in an amorphous form in some foods, no publications were found that documented the chemical degradation of thiamine confirmed to occur in the amorphous state. The objectives of this study were therefore: 1) to document thiamine degradation (monitored

as thiamine loss) in a variety of amorphous solid dispersions containing TClHCl and polymers selected based on their molecular structures, hydrogen bonding ability, T_g, and hygroscopicity traits, and 2) to compare the degradation of thiamine in the amorphous dispersions to that of crystalline thiamine physically blended with the same polymers.

2.3 Materials and methods

2.3.1 Materials

Thiamine chloride hydrochloride (TClHCl), pectin (PEC, from citrus peel with a ~61% degree of esterification), and poly (vinylpyrrolidone) (PVP, MW 40,000) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). The two polymers were chosen based on previous work conducted in our laboratory (Arioglu-Tuncil et al., 2017), in which PEC showed the best performance as a crystallization inhibitor for TClHCl while PVP was the poorest inhibitor. Specific relative humidity (RH) conditions (reported here at 25°C) were created using saturated salt solutions of magnesium chloride ($MgCl_2$, 32% RH), and sodium chloride (NaCl, 75% RH) (Sigma-Aldrich Inc., St. Louis, MO). Lithium chloride obtained from EMD Millipore (Billerica, MA) was used to create 11% RH conditions. HPLC grade trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich Inc. (St. Louis, MO), and acetonitrile was purchased from Fisher Scientific Co., LLC (Pittsburgh, PA). Deionized and purified water was used in the study. Barnstead E-Pure ultrapure water purification system (ThermoScientific, 98 Waltham, MA) was used with a resistivity ~17.5 MΩ·cm for deionization of water.

2.3.2 Preparations of THClHCl solid dispersions via lyophilization

TClHCl solid dispersions were prepared in triplicate using a range of vitamin:polymer mass ratios (50TClHCl:50PEC; 40TClHCl:60PVP; 5TClHCl:95polymer). A total of 100mg

solids containing the desired vitamin:polymer ratio was dissolved in 10 mL of deionized water in a 20 mL amber glass vial and mixed with a Roto-Shake Genie® SI-1100 (Scientific Industries, Inc., Bohemia, NY) for 10 minutes. In addition, fourteen different mass ratios of TClHCl to PVP (1%, 3%, 5%, 7%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) were prepared while keeping the total solids content constant in order to study the influence of proportion of TClHCl to PVP on vitamin loss. An Orion Star A211 pH meter (Thermo Fisher Scientific Inc., Waltham, MA) was used to measure the pH of the solutions. The solutions were then frozen overnight at -20^{o}C prior to lyophilization. After removal from the freezer, samples were placed into a VirTis Genesis 25ES shelf freeze dryer (SP Scientific, Stone Ridge, NY) and frozen for 6 hours at -40^{o}C and 300 mTorr. For the primary drying step, high vacuum was applied (150 mTorr) at -40^{o}C for 24 hours to remove the bulk of water via sublimation. Secondary drying was then achieved by holding the samples for 9 hours each at 10^{o}C intervals from -40^{o}C to 20^{o}C. Lastly, the samples were held for 6 hours at 25^{o}C at 300mTorr. The lyophilized samples were immediately placed into RH-controlled desiccators. Physical blends of TClHCl with polymers (5TClHCl:95polymer) were also prepared by weighting each ingredient separately, followed by simply mixing in amber vials.

2.3.3 Storage treatments

The following temperature and RH conditions were chosen as storage treatments: 11%RH and 30^{o}C, 40^{o}C, 50^{o}C and 60^{o}C; 32% RH and 25^{o}C; and 75%RH and 40^{o}C. Different temperature conditions at 11% RH were chosen in order to be able to calculate reaction kinetics of thiamine degradation. A higher RH and temperature condition (75% RH and 40^{o}C) was chosen to determine the effect of more severe storage conditions on the chemical stability of thiamine and because it is the condition recommended by ICH guidelines for accelerated conditions. RH was controlled by

using saturated salt solutions in desiccators (with water activity verified by measurement using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, WA), and the desiccators were placed into water jacketed incubators (Forma Scientific, Inc., Marietta, OH) to control the temperature. Samples were stored for up to eight weeks, with a subset removed biweekly for HPLC analysis that was then discarded after analysis.

2.3.4 Powder X-ray diffraction (PXRD)

Powder X-ray diffractograms of the starting ingredients and dispersions at set storage intervals were obtained using a Rigaku Smartlab^{TM} diffractometer (Rigaku Americas, Texas, USA) equipped with a Cu-Kα radiation source and D/ tex ultra-detector. Samples were scanned from 5 to 40 degrees 2-theta with an increment of 0.02° and a rate of 4° per minute. Structural distinction between amorphous and crystalline TClHCl was determined based on the PXRD patterns, in which samples exhibiting a diffuse halo were considered to be XRD amorphous, and those containing sharp peaks greater than 2 standard deviations above the baseline in their diffractograms were considered to be crystalline.

2.3.5 Chemical stability determination with HPLC

TClHCl was quantified in the absence and presence of polymers throughout the study using a Waters 2690SM (Waters Corp., Milford, MA) HPLC with a Waters Xselect HSS T3 (3.5µm, 4.6x100mm) column and a Waters 2996 photodiode array detector. Prior to each analysis, standard curves of TClHCl at a concentration range from 0.005 to 1 mg/mL were prepared (r^2=0.9997-1.0000). Samples were diluted with solvent to a final estimated vitamin concentration of 0.25 and 0.5 mg/mL for TClHCl: PEC samples and for TClHCl: PVP samples, respectively, and filtered through a 0.2 µm syringe filter. Since PEC dispersions formed very viscous solutions, it was hard filter the samples. Therefore, more solvent was added to TClHCl:PEC dispersions and final

concentration of vitamin was adjusted to 0.25 mg/mL. The mobile phase containing solvent A (acetonitrile) and solvent B (water and 0.1% TFA) was used with the following gradient procedure adapted from (Xia et al., 2006): 0/100 at 0 min (immediate), 3/97 at 4 min (linear), 10/90 at 6 min (linear), 0/100 at 10 min (linear), and 0/100 from 10 to 15 min (immediate), for a total chromatographic run time of 15 min. The flow rate was 1 mL/min, and the samples were scanned between 235-400 nm. Integration was conducted at 247 nm using Masslynx V4.1 software (Waters Corp., Milford, MA).

2.3.6 Dynamic vapor sorption profile measurements

A SPSx-1 μ Dynamic Vapor Sorption Analyzer (Project Messtechnik, Ulm, Germany) was used to generate moisture sorption profiles of the individual ingredients, solid dispersions, and physical blends at 25°C. Approximately 100-300 mg of each sample was weighted into the 18 mm aluminum sample pan (Mettler Toledo) in a 24-ring sample holder. Data were collected using an equilibrium criterion of a weight change of 0.001% in 30 min and a maximum step time of 12 hours. Samples were exposed to 0% RH for 12 hours and then analyzed from 5 to 95% with a 5% RH step size. The differences between the polymers and solid dispersions (φ) for moisture sorption were calculated as follows;

$$\varphi = m_{dispersions} - cm_{polymer} \quad 2.1$$

Where m represent the % moisture content (w/w) and c is the proportion of the polymer in dispersion. The moisture content of select samples was also determined right after freeze drying using a method adapted by Arabshahi and Lund (Arabshahi & Lund, 1988). Briefly, solid dispersions were weighted into aluminum pans and placed in the vacuum oven. Then, the samples were exposed to 45°C under the vacuum for 48 hours. To prevent degradation of thiamine, using

a higher temperature was avoided. Moisture content (%) was calculated based on wet weight basis using the formula provided below (Mauer & Bradley, 2017):

$$\%\ Moisture\ ({}^{wt}/_{wt}) = \frac{wt\ of\ wet\ sample - wt\ of\ dry\ smaple}{wt\ of\ wet\ sample} \times 100 \qquad 2.2$$

2.3.7 Differential scanning calorimetry (DSC)

Thermal analyses of the starting ingredients and lyophiles were carried out using a Discovery DSC equipped with a refrigerated cooling accessory (TA Instruments, New Castle, DE). Nitrogen (50 mL/min) was used as the purge gas. Samples were weighed (7-12 mg) and hermetically sealed into Tzero pans (TA Instruments). To determine the T_gs of the samples, the samples were heated from -20°C to a temperature 20-30°C higher than the expected T_gs at a rate of 20°C/min. Samples were then cooled to -20°C at 10°C/min, followed by heating to 150°C at a rate of 20°C/min. To determine onset T_g' of the samples, approximately 25 µL of solutions were pipetted into Tzero pans and sealed (TA Instruments). Solutions were first cooled to -80°C, held for 5 min at this temperature, followed by heating to 0°C at a rate of 10°C/min. The onset glass transition temperature of the second heating step was reported as T_g unless otherwise stated. TRIOS software was used to determine the T_g (TA Instruments). Briefly, before the baseline shift occurred in the second heating step, the first tangent was drawn on the straight line. Then, the second tangent was placed on the slope, and the cross point was calculated by TRIOS software and reported as onset T_g.

2.3.8 Reaction kinetics calculations

Chemical degradation of thiamine has been reported to follow first order reaction kinetics (Labuza T & Kamman J, 1982; Pachapurkar & Bell Leonard, 2006; Windheuser & Higuchi, 1962). Therefore, reaction rate constants were calculated based on the equation provided below:

$$\ln \frac{x}{x_0} = -kt \qquad 2.3$$

where x is thiamine concentration at t (day), x_0 is the initial concentration of thiamine, and k is defined as a reaction rate constant (days^{-1}). The following equation was used for shelf life estimation to calculate t_{90} which is defined as the time where 90% of the initial concentration of thiamine is left:

$$t_{90} = \frac{ln\left(\frac{x_0}{0.9x_0}\right)}{k} \qquad 2.4$$

where x_0 is the initial concentration of thiamine and k is the reaction rate constant (days^{-1}).

2.3.9 Statistical analysis

All the HPLC analyses were performed in triplicate, and data are presented as mean±SD. SAS Software Version 9.4 (SAS Institute, Cary, NC) was used to conduct statistical analyses. Analysis of variance (ANOVA) was performed at α = 0.05 significance level to determine differences among the samples and controls. Tukey's multiple comparison test (α=0.05) was used to test whether samples were statistically different.

2.4 Results and discussions

2.4.1 Chemical stability of thiamine: The effect of physical state on thiamine degradation

No significant degradation was observed in control samples where thiamine was in the crystalline state (**Fig. 2.1A**). This result was expected even for the samples stored at high RH and temperature condition (75% RH and 40^{o}C), since it was well below the RH_0 of TClHCl (88% RH at 25^{o}C). Similarly, thiamine was quite stable in 50TClHCl:50PEC and 40TClHCl:60PVP amorphous dispersions, where only less than 10% vitamin loss was observed (**Figs. 2.1B-C)** over 77 days of storage at 75%RH and 40^{o}C. It needs to be noted here that TClHCl samples

recrystallized at 75% RH and 40^{o}C from both PEC and PVP dispersions within one week, as confirmed by PXRD.

In order to be able to investigate thiamine stability in the situations where thiamine remained amorphous during the study, thiamine to polymer proportion was altered to 5:95. Amorphous structure of TClHCl: PEC dispersion was maintained for 77 days at 75% RH and 40^{o}C by altering the ratio of TClHCl to 5% (w/w) in the dispersion; in contrast, storing 5TClHCl:95PVP dispersions in this condition led to formation of solution where both PVP and TClHCl dissolved due to moisture sorption into the bulk dispersion structure. When compared to 50TClHCl:50PEC dispersions in which thiamine crystallized within a week, 12% more thiamine degraded in 5TClHCl:95PEC amorphous solid dispersions at 75% RH and 40^{o}C at the end of 77 days as presented in **Fig. 2.1D**, which was statistically significant ($p < 0.05$). This result clearly indicates that thiamine degrades more when it is in the amorphous state. Moreover, thiamine degradation proceeded faster in 95% PVP dispersions. For example, 27% thiamine degraded in the first two weeks of storage at 75% RH and 40^{o}C and thiamine loss continuously increased with time, reaching 40% loss in 77 days (**Fig 2.1D**). The reason of faster degradation of thiamine in this sample was the fact that presence of higher amount of PVP at high RH condition brought significant amount of moisture to the system where the samples formed solution, and compounds are known to degrade faster in the solution state. Additionally, as being amorphous initially, thiamine also had an impact on the amount of moisture sorbed.

A low RH (11% RH) condition with four different temperatures (30, 40, 50, and 60^{o}C) was selected to store TClHCl dispersions made with 95% PVP and PEC. These conditions allowed to maintain amorphous state (due to low RH) and enhance degradation by increasing the storage temperature at the same RH; therefore, Arrhenius plots could be applied in order to calculate the

activation energy. As expected, the solid dispersions remained amorphous at the conditions studied regardless of the polymer type (**Figs. 2.2**). No significant degradation was found for thiamine in 95% PEC dispersions as shown in **Fig. 2.3A**. Similarly, thiamine was quite stable in the 95% PVP dispersions stored at 11%RH and 30^{o}C and 40^{o}C (less than 7% thiamine degraded by day 56) (**Fig. 2. 3B**). Increasing the temperature to 50^{o}C and 60^{o}C resulted in increased amounts of thiamine degradations (**Fig. 2.3B**) with 12% thiamine degrading at 50^{o}C and 25% degrading at 60^{o}C over the 8 weeks. The significant difference in thiamine loss occurred between 11% RH 50^{o}C and 60^{o}C was attributed to storage temperatures used, in where dispersions were present above the T_g at 60^{o}C and below the T_g at 50^{o}C, as discussed in detail in T_g section (**Table 2.3**). Since thiamine was relatively stable at temperatures below 60^{o}C, degradation rate constants could not be calculated and Arrhenius equation could not be applied.

As a second control, thiamine loss was investigated in physical blends of crystalline TClHCl with 95% polymers stored at 11%RH-60^{o}C and 75% RH-40^{o}C (**Fig. 2.4A**) and the results were compared with the dispersions (**Fig. 2.4B**). There was no significant difference ($p < 0.05$) found for thiamine loss between amorphous solid dispersions and physical blends of 5TClHCl:95PEC samples stored at 11% RH and 60^{o}C (**Fig. 2.4B**) with both exhibiting 4% thiamine loss on day 56. At higher RH (75% RH and 40^{o}C), 8% thiamine degraded in the 5TClHCl:95PEC physical blends on day 56, whereas thiamine loss was around 15% in PEC amorphous dispersions. Similar to 5TClHCl:95PEC physical blends, 95% of thiamine remained in PVP: TClHCl physical mixtures at 11% RH and 60^{o}C at the end of 56 days; however, 25% of thiamine degraded in 5TClHCl:95PVP dispersions in the same conditions where thiamine was amorphous throughout the study (56 days) (**Fig. 2.4B**). These results indicate that thiamine degradation proceeded more rapidly when it was in the amorphous state.

2.4.2 Chemical stability of thiamine: The effect of different proportions of TClHCl to PVP on thiamine degradation

Observing differences in thiamine degradation rate for two ratios (5% TClHCl vs. 50-60% TClHCl) led us to investigate more the influence of thiamine proportion to polymer on its chemical degradation. Therefore, fourteen different ratios of TClHCl to PVP were prepared (From 100% TClHCl to 1% TClHCl). PVP as a polymer and 11% RH and 60^{o}C as a storage condition were selected since thiamine degradation was accelerated at higher temperature by maintaining the amorphous structure at 11% RH. Physical (crystalline vs. amorphous) characterizations determined by the PXRD patterns revealed that the samples prepared using 50% or less PVP contained crystalline TClHCl after lyophilization, whereas when incorporating 60% or higher PVP resulted in a formation of amorphous TClHCl in the lyophiles (**Figs. 2.5**). Moreover, the samples made with different ratios of PVP to TClHCl maintained their initial physical states during 56 days of storage at 11% RH and 60^{o}C. This also enabled us to investigate the chemical stability of thiamine in amorphous form. After one week of storage, 5% of thiamine degraded in the freeze-dried thiamine (FD TClHCl) which was crystalline and no more degradation was observed in the rest of the storage (**Fig. 2.6**). On the other hand, nearly 15% of thiamine degraded within one week in the samples composed of 1% TClHCl, which was significantly higher ($p < 0.05$) than the samples containing >7% TClHCl. As storage time increased, thiamine degradation proceeded more rapidly in the samples containing ≤%10 TClHCl than those that remained amorphous but contained more TClHCl and those that crystallized. For example, after 56 days of storage, thiamine loss was 35% in dispersions composed of 1% TClHCl and 99% PVP, and thiamine loss in this sample was significantly higher than in all the vitamin: polymer ratios studied on 4, 6, and 8 weeks. More specifically, the thiamine lost in dispersions containing 3% TClHCl and 97% PVP was 29%; 24% in dispersions made of 5% TClHCl and 95% PVP; 21% in dispersions constituting 7%

TClHCl and 93% PVP; 18% in dispersions constituting 10% TClHCl and 90% PVP (**Fig. 2.6**). Moreover, in the samples composed of ≥ 20% TClHCl, 12-15% of thiamine degradation was observed (**Fig. 2.6**). In contrast, as TClHCl proportion increased to 40% or higher (where thiamine is in the crystalline state), only ≤ 10% thiamine was lost on day 56 in all the samples. These results indicated that chemical reactivity was lowered in the samples where thiamine was crystalline, and the crystalline form of thiamine was chemically more stable than its amorphous counterpart.

Thiamine degradation was reported to follow first-order kinetics (Labuza & Kamman, 1982; Pachapurkar & Bell Leonard, 2006). Thus, reaction rate constants were calculated for the samples prepared with different TClHCl to PVP ratios using first-order kinetics model and presented in **Table 2.1**. It was observed that experimental data obtained from amorphous TClHCl:PVP dispersions containing ≤ 20% TClHCl fit the first order kinetics model with high R^2 values (**Table 2.1**). However, reaction rate constants for the rest of the data could not be calculated due to very low reaction rates. Based on the calculated reaction rate constants (k), thiamine stability was found to be influenced by the proportion of PVP used to form solid dispersion, with "k" increasing as the proportions of PVP increased (**Fig. 2.7**). Estimated shelf life, based on the t_{90} value where 10% of degradation is reached was calculated based on the Equation 4 (**Table 2.1**). As TClHCl concentration decreased in the solid dispersions, reaction rates increased and t_{90} values decreased. For example, the estimated shelf life of thiamine in 20TClHCl:80PVP dispersions was calculated to be 70 days, whereas it was only 17 days in 1TClHCl:99PVP dispersions (**Table 2.1**). Bis(2-methyl-3-furyl) disulfide is one of the reported degradation products which causes odor with an odor threshold of 0.02 parts per trillion in water (Buttery, Haddon, Seifert, & Turnbaugh, 1984). Since food products are fortified with relatively low amount of thiamine and some degradation

products of thiamine have a very low odor threshold, the shelf life predictions (**Table 2.1**) are important to ensure thiamine delivery and to obtain vitamin activity in the body.

2.4.3 Chemical stability of thiamine: The reason behind observing more degradation in the samples containing higher proportions of PVP

Thiamine in samples containing higher proportions of PVP was more stable than in those containing less polymer. For example, solid dispersion containing 20% TClHCl and 80% PVP was more stable than that composed of 1% TClHCl and 99% PVP, with a t_{90} of 70 days versus 17 days, respectively. The reason of observing more degradation when the proportion of TClHCl in PVP dispersions decreased was initially thought to be due to pH affect in the solid state because TClHCl is more acidic itself, and thus, an increase in the PVP proportion causes an increase in the pH, which could lead rapid degradation of thiamine [its stability decreases as pH increases (Dwivedi & Arnold, 1973)]. On the other hand, another study performed as a part of this dissertation in which chemical stability of thiamine in thiamine mononitrate (TMN) solid dispersions containing different ratios of PVP was studied revealed that although an increase in PVP concentration increased the pH, these changes in pH did not result in higher stability (Chapter 3). Because of these discrepancies in the findings, we conclude that pH does not explain the phenomena of being less stable in the presence of higher ratio of PVP. This is also in an agreement with another independent study done by our laboratory in which the chemical stability of ascorbic acid in PVP dispersions was investigated (Sanchez et al., 2018). Specifically, they found that degradation of ascorbic acid found in amorphous form increased at 11% RH and 60^{o}C as the relative proportion of PVP increases (Sanchez et al., 2018). Moreover, they attributed this observation to a kinetic model coined by Waterman et al. (Waterman, Gerst, & Dai, 2012) based on drug and excipient relation for solid state degradation. This kinetic model proposes that if the number of drug and excipient particles are comparable to each other, the impact of excipient on drug degradation rate

is negligible. In contrast, if the excipient particles are found in excess in the system, the effect of excipient on drug degradation rate is significant, which is due to greater surface area of contact between drug and the excipient (degradation rate increases with increasing contact surface area) (Waterman et al., 2012). This proposed mechanism also explains very well the reason of the increases in degradation rate of thiamine in TClHCl:PVP solid dispersions, as the number of PVP particles increased relative to that of TClHCl. Chemical stability of thiamine in the systems containing different proportions of thiamine to polymer was appeared to be mainly dictated by the extent of surface area of contact between thiamine and polymer.

2.4.4 Chemical stability of thiamine: The effect of polymer type and intermolecular interaction on thiamine degradation

When the degradation rate of thiamine was compared in PVP and PEC matrices, it was observed that thiamine was chemically more stable in PEC dispersions. In order to understand the reason for the differences in thiamine degradation, chemical structures and properties of these two polymers and TClHCl were considered. Structures of thiamine and the polymers were extensively reviewed by Arioglu-Tuncil et al., (2017) in terms of functional groups (and hydrogen bond acceptor group strengths based on the pK_{BHX} scale), which are potentially involved in hydrogen bonding (Arioglu-Tuncil et al., 2017). Thiamine is comprised from a thiazole and pyrimidine ring, which are connected to each other via methylene bridges. Free hydroxyl and NH_2 groups of TClHCl can act as both hydrogen bond donor (HBD) and acceptor (HBA). In addition, nitrogen group of pyrimidine ring can interact via hydrogen bonding as a weak HBA. PEC possessed a variety of functional groups, including hydroxyl, carboxylic acid, ether, and ester, which form hydrogen bonding. On the other hand, the only functional group for hydrogen bonding that PVP carries is the amide carbonyl group which acts as a strong hydrogen bond acceptor (PVP does not have any hydrogen bond donor group). As proved by FTIR, TClHCl was found to form strong

hydrogen bonding with PEC (Arioglu-Tuncil et al., 2017). In hydroxyl region of FTIR spectra, a peak shift to the lower wavenumber was found compared to PEC alone and the magnitude of the shift was dependent on the polymer proportion. For example, a shift of 32 cm^{-1} (towards to lower wavenumber) occurred in 10TClHCl:90PEC dispersions. Moreover, a shift to lower wavenumber was also observed in the carbonyl region of TClHCl:PEC dispersions. In addition to hydrogen bonding, ionic interaction between TClHCl and PEC was also proposed, which is even stronger. In contrast to PEC, a shift of 1 cm^{-1} to the lower wavenumber (relative to PVP alone) was seen in 10TClHCl:90PVP dispersions, indicating the lack of hydrogen bonding between TClHCl and PVP in dispersions. PEC was observed to be the best crystallization inhibitor for amorphous TClHCl in dispersions which stabilized the amorphous dispersion for more than 120 days of storage at 0-23% RH and 25-40^{o}C, whereas PVP was reported to be the poorest crystallization inhibitor. The physical stability of TClHCl in PEC dispersions was attributed to hydrogen bonding and/or ionic interaction formation between TClHCl and PEC (Arioglu-Tuncil et al., 2017). As occurred in the physical stability study of amorphous TClHCl (Arioglu-Tuncil et al., 2017), these interactions were proposed as the main determinant to stabilize TClHCl:PEC amorphous solid dispersions and protected thiamine against chemical degradation by restricting the molecular motion of TClHCl. Due to lack of interactions between TClHCl and PVP, thiamine degradation was significantly more in PVP dispersions than PEC dispersions.

As mentioned before, the lowest amount of polymers needed to amorphize TClHCl were 40% and 60% for PEC and PVP, respectively. In order to create amorphous TClHCl, the crystal lattice needs to be disrupted. It could be achieved by formation of a new intermolecular interaction between TClHCl and polymer, mainly via hydrogen bonding. Arioglu-Tuncil et al., 2017 showed that amorphous TClHCl was created when hydrogen bonding formation between TClHCl and

polymers was evident. As confirmed with FTIR, intermolecular hydrogen bonding was observed to be formed when at least 40% of PEC and 60% of PVP were present in TClHCl solid dispersions (Arioglu-Tuncil et al., 2017).

2.4.5 Chemical stability of thiamine: Moisture sorption profiles

In order to provide a better understanding of the effect of moisture on TClHCl degradation, moisture sorption isotherms of individual ingredients, TClHCl solid dispersions, and physical blends of TClHCl with polymers generated at 25^{o}C were compared (**Figs. 2.8-11)**. TClHCl underwent adsorption and capillary condensation below its deliquescence point (RH_0) (88% RH at 25^{o}C), and deliquesced to form a solution once this RH was exceeded (**Fig. 2.8**). Below the deliquescence point of TClHCl, polymers absorbed significantly more water than TClHCl ($p < 0.05$). This is attributed to their amorphous nature. Significantly more moisture was absorbed by PVP, compared to PEC, especially above 30% RH, and the difference in sorbed moisture increased as RH increased. For example, at 75% RH, the moisture amount gained by PVP was 33%, whereas PEC absorbed only around 18% of moisture (**Fig. 2.8**). This clearly indicates that PVP is more hygroscopic than PEC.

Moisture sorption profiles of solid dispersions containing 50, 60, and 95 % of polymer are shown in **Fig. 2.9**. TClHCl dispersions showed a sigmoidal moisture sorption shape which is typical for amorphous compounds, thereby confirming the PXRD results. Solid dispersions followed the same general trend as individual polymers for moisture sorption, where PVP dispersions sorbed significantly more moisture than PEC dispersions, regardless of the polymer amount used. This can be attributed to the more hygroscopic nature of PVP, compared to PEC.

On the other hand, the proportion of TClHCl used in PVP solid dispersions had a significant impact on the amount of moisture sorbed at RHs above 35% RH (**Fig. 2.9**). The

moisture sorption isotherms of TClHCl solid dispersions composed of 60% or 95% PVP were almost identical up to 35% RH. However, TClHCl recrystallization in 60% PVP dispersions was evident as an inflection point at 35% RH in the moisture sorption isotherm. Crystallization of amorphous compounds can result in mass loss. This finding further confirmed the PXRD data, where onset of TClHCl crystallization was observed in 60% PVP dispersions stored at 32% RH. The difference in moisture gain between dispersions composed of either 95% or 60% PVP increased as RH% increased until the deliquescence point of TClHCl. This is mainly due to 1) higher proportion of PVP in 5TClHCl:95PVP dispersions, which makes the system more hygroscopic, and 2) amorphous state of TClHCl in 95% PVP dispersion, whereas TClHCl was partially crystalline in dispersions composed of 60% PVP. Similar to PVP dispersions, the moisture sorption profiles of TClHCl dispersions made with 50% and 95% PEC were almost identical until the RH reached 45% RH. Above this RH value, the effect of PEC amount on the moisture sorption behavior of dispersions was more pronounced. For example, at 75% RH, dispersions composed of 95% PEC and 5% TClHCl absorbed 6% more moisture than those made of 60% PEC. This is mainly due to the higher amount of polymer present in 95% PEC dispersion that increase the hygroscopic behavior of the dispersion.

When the moisture sorption behaviors of PVP dispersions were compared to PEC dispersions, the difference in the amount of moisture sorbed by 60% PVP and 95% PEC dispersions was insignificant until 70% RH, and above this point, 60% PVP dispersions sorbed significantly more moisture than 95% PEC dispersions. This can be attributed to very hygroscopic feature of PVP compared to PEC, even though the polymer amount was quite lower and TClHCl was present in semi-crystalline form in 60% PVP dispersion.

Moisture sorption isotherms of physical blends of 50-60% and 95% of polymers with crystalline TClHCl are shown in **Figs. 2.10A.** As polymer proportion in the physical blends increased, the amount of moisture gained by the systems also gradually increased. For example, at 75% RH, 9% more moisture was absorbed by 95% PVP physical blends compared to 60% PVP. Similarly, 5TClHCl:95PEC physical mixtures sorbed 6% more moisture than the mixtures containing 50% of PEC. In addition, moisture sorption isotherms of physical mixtures made by PEC and PVP followed the same trend with solid dispersions, where physical blends of TClHCl with PVP sorbed more moisture than that of PEC. The effect of amorphous structure of TClHCl on moisture sorption are given in **Fig. 2.10B,** where amorphous solid dispersions of 50TClHCl:50PVP was compared to its physical blends. The differences in moisture sorptions of two samples were between 0.05-0.2% up to 25% RH. Above 25% RH, amorphous dispersion of TClHCl sorbed more moisture than its physical blends, and the difference in weight gain increased as RH increased. For example, 50TClHCl:50PEC amorphous dispersions sorbed 1% more moisture at 35%RH and this value reached up to 4% at 75% RH due to amorphous structure of TClHCl. Compared to polymer alone, the contribution of vitamin on moisture sorption was also determined by calculating the phi values for dispersions (**Fig. 2.10C**). Positive phi values were obtained for all dispersions, indicating that hygroscopicity of dispersions increased compared to polymers due to amorphous TClHCl. The effect of amorphous TClHCl was more pronounced in 50TClHCl:50PEC dispersions than 40TClHCl:60PVP dispersions, especially above 35% RH, since TClHCl crystallized in 40TClHCl:60PVP dispersions at this RH. Moreover, the contribution of amorphous TClHCl on hygroscopicity of 5TClHCl:95polymer dispersions were less because of the low proportion of TClHCl present in the dispersions **(Fig. 2.10C)**.

Moisture sorption isotherms of polymers, 5% TClHCl solid dispersions, and 5% physical mixtures of crystalline TClHCl with the polymers are compared in **Figs. 2.11**. The weight gain due to moisture sorption was not significant between PEC, TClHCl:PEC dispersion and physical blend until 35% RH (**Fig. 2.11A**). Above 35% RH and below the deliquescence point of TClHCl (88%), 95% PEC dispersion sorbed 0.8-3% more moisture than physical mixture of TClHCl and PEC, and polymer itself. Unlike PEC samples, PVP, 95% PVP dispersions and physical blends (TClHCl and PEC) absorbed indistinguishable amount of water at all the RH values examined **(Fig. 2.11B)**, indicating that moisture sorption trends of dispersions containing lower amount of TClHCl were mainly governed by the polymers, particularly in case the polymer is very hygroscopic like PVP.

Based on the findings explained above, no direct relation found between chemical stability of thiamine and hygroscopicity of polymers/dispersions at low RH conditions studied., For example, significantly more thiamine (20% more) degraded in 5TClHCl:95PVP dispersions than 5TClHCl:95PEC dispersions at 11% RH and 60^{o}C, although moisture gained by the dispersions of 5TClHCl:95PVP and 5TClHCl:95PEC were not significantly different than each other at 11% RH.

2.4.6 Chemical stability of thiamine: The effect of glass transition temperature on thiamine degradation

In order to avoid collapse, the initial temperature of freeze drying protocol must be below the T_g' values (Franks, 2007). Thus, the onset T_g' values of select samples were measured to see whether they were exposed to temperatures below their T_g's during lyophilization. T_g' of TClHCl and PVP solutions were measured to be -49.4±0.2^{o}C and -25.29±0.09^{o}C, respectively, suggesting that the dispersions prepared using different proportions of PVP must be between -25.29^{o}C and -49.4^{o}C and should decreases as TClHCl to PVP ratio increases. Moreover, the onset T_g' values

were measured to be -23.7°C, -26.1±0.5°C, -27.16±0.09°C, -36.27±0.04°C, -41.3±0.3°C, and -41.8±0.5°C for the solutions of 1TClHCl:99PVP, 5TClHCl:95PVP, 10TClHCl:90PVP, 50TClHCl:50PVP, 80TClHCl:20PVP, and 90TClHCl:10PVP, respectively (DSC graphs of all the solutions were provided in **(Fig. 2.12**). These results collectively showed that the dispersions containing ≥80 TClHCl were prone to collapse. Although the dispersions containing higher proportions of PVP had T_g's which were well above the temperature applied during the primary drying step (-40°C), these samples were chemically less stable than the samples containing lower amount of PVP with T_g's around/below the primary drying temperature (-40°C).

Using the lyophilization technique described in this study, amorphous TClHCl in the absence of a polymer could not be created due to its T_g' (-49.4±0.2°C) which was below the primary drying step of freeze drying protocol (-40°C). Therefore, the experimental T_g of amorphous TClHCl in the absence of a polymer could not be measured. In addition, the melt-quench method to determine the T_g would not be applicable for TClHCl, since it degrades by melting (Al-Rashood, Al-Shammary, & Mian, 1990). The estimated T_g of TClHCl, however, was calculated based on the Boyer-Beamen rule (Beaman, 1952). This rule states that multiplying the melting temperature of a compound, in Kelvin, by 2/3 gives the T_g in Kelvin. According to Boyer-Beamen rule, the calculated T_g of TClHCl was found to be 74°C. On the other hand, the reported T_g values for PVP and PEC were 134 ± 0.2 and 90 ± 2, respectively (Arioglu-Tuncil et al., 2017). So, the calculated T_g of TClHCl is well below the T_g of PVP.

In this study, dry 'T_gs' of solid dispersions were increased towards to T_g of PVP with increasing the proportion of PVP relative to TClHCl. Onset T_gs of the solid dispersions containing 1% TClHCl to 40% TClHCl were provided in **Table 2.2**. These results were measured right after lyophilization without exposing the samples any further drying method. Due to remaining residual

moisture, the values were below the estimated T_gs (**Table 2.2**). As expected, the T_gs increased as the amount of PVP in dispersions increased. The T_g values provided in **Table 2.2** indicated that samples containing ≤ 80PVP were stored at the temperature (11% RH and 60ºC) above the T_gs. Moreover, 5TClHCl:95PVP solid dispersions were equilibrated at 11% RH and 30ºC, and 60ºC, followed by T_g measurement (**Table 2.3**). The results showed that the storage condition of 11% RH and 30ºC was below the T_g of 5TClHCl:95PVP dispersions, whereas samples (5TClHCl:95PVP) stored at 11% RH and 60ºC were found to be stored above the T_g (**Table 2.3**). Since the solid dispersions prepared by different proportions of TClHCl to PVP were also stored at 11% RH and 60ºC for eight weeks, the results shown in **Table 2.3** for 5TClHCL:95PVP dispersions indicated that the dispersions containing ≤5 TClHCl were also stored above their T_gs. Although solid dispersions prepared by ≤ 40TClHCl:60PVP were found to be in the supercooled liquid state when stored at 11% RH and 60ºC, all the samples remained amorphous throughout the timeframe of the study (8 weeks). On the other hand, 40TClHCl:60PVP dispersion had the lowest T_g among the amorphous dispersions created by different ratios of TClHCl to PVP. Despite having the lowest T_g, thiamine was chemically the most stable in 40TClHCl:60PVP dispersions compared the samples containing ≤30TClHCl.

On the other hand, T_g was observed as an important parameter affecting thiamine stability in 5TClHCl:95PVP dispersions when thiamine loss was compared to each other at different storage conditions (11% RH and 30-60ºC). For example, among the 5TClHCl:95PVP dispersions stored at 11% RH and different temperatures (30-60ºC), the dispersions exposed to 60ºC showed the highest degradation of thiamine and it was attributed to the fact that the samples were stored above the T_g. All these observations about the effect of T_g on thiamine stability suggested that the general assumption of 'samples stored below the T_g are stable or vice versa' could be misleading, unless

the samples compared to each other are identical. If the samples are not identical to each other (e.g samples prepared by different proportions of polymer, etc.), other factors (e.g intermolecular interactions, increasing the contact surface area between compounds) rather than T_g could be determinant for chemical stability. T_gs of TClHCl:PEC dispersions could not be measured using the setting parameters described in this study due probably to the heterogeneity of pectin samples.

The effect of T_g on chemical stability of amorphous dispersions has also been debating in the literature. Some studies has been reported the T_g as a main parameter governing the chemical stability of amorphous solids due to its direct correlation with molecular mobility (Hancock & Zografi, 1997; Slade, Levine, & Reid, 1991). In contrast, many other reports proved that the assumption of having higher T_g results in higher chemical stability is unsuccessful (Bell & Hageman, 1994; Lai, Hageman, Schowen, Borchardt, & Topp, 1999; Luthra, Shalaev, Medek, Hong, & Pikal, 2012; Sanchez et al., 2018). For example, Bell et al. investigated the aspartame stability in two different PVPs possessing different T_gs (Bell & Hageman, 1994). The moisture contents and a_ws of both systems were designed to be similar. Although T_g values were different, no difference in degradation rate constants for aspartame was found (Bell & Hageman, 1994). Moreover, a recent study showed that ascorbic acid stability was higher in the samples with lower T_gs (Sanchez et al., 2018). Thus, based on the findings of this study and the recent reports from the literature, the assumption of T_g as a chemical stability dictating factor has been proved to be not always true.

2.5 Conclusion

Thiamine was quite stable against degradation when it was in the crystalline state. In addition, the presence of a polymer did not affect thiamine degradation in the physical blends, wherein TClHCl was crystalline. In contrast, significantly more thiamine degraded in amorphous

form, which was also highly affected by type and the proportion of polymers and storage conditions used. Amorphous thiamine was more stable in dispersions made by PEC than these of PVP at any storage treatments. No direct correlation found between chemical stability of thiamine and hygroscopicity and T_g of polymers in solid dispersions. The most important factor dictating the stability of amorphous thiamine in dispersions made by different polymers was the formation of intermolecular interactions between TClHCl and polymer, leading TClHCl:PEC dispersions being more stable than PVP dispersions. Thiamine degradation was significantly higher in samples containing low concentrations of thiamine relative to PVP, which was mainly governed by the extent of surface area of contact between TClHCl and PVP. The significant point to be taken from this study is that physical form of TClHCl (amorphous vs. crystalline), excipient type (e.g. pectin vs. PVP), relative proportions of ingredients, excipient properties, and storage conditions were important for thiamine stability, with intermolecular interaction formation between TClHCl and polymer being the most determinant one to ensure chemical stability of thiamine. All these factors need to be considered and carefully selected for improving the stability of thiamine in food fortified with TClHCl and pharmaceutical formulations.

Table 2.1 Rate constants and t_{90} values for thiamine in solid dispersions prepared by different proportions of PVP, upon storage at 11%RH and 60^{o}C.

	1TClHCl:99PVP	**3TClHCl:97PVP**	**5TClHCl:95PVP**	**7TClHCl:93PVP**	**10TClHCl:90PVP**	**20TClHCl:80PVP**
k_{obs} (day^{-1})	0.0062	0.0047	0.0038	0.0032	0.0025	0.0015
R^2	0.9637	0.9537	0.9519	0.9315	0.9495	0.9161
t_{90} (days)	16.99363	22.41713	27.72645	32.92516	42.14421	70.24034

*t_{90}: Time where 90% of the ini
tial concentration of thiamine is left.

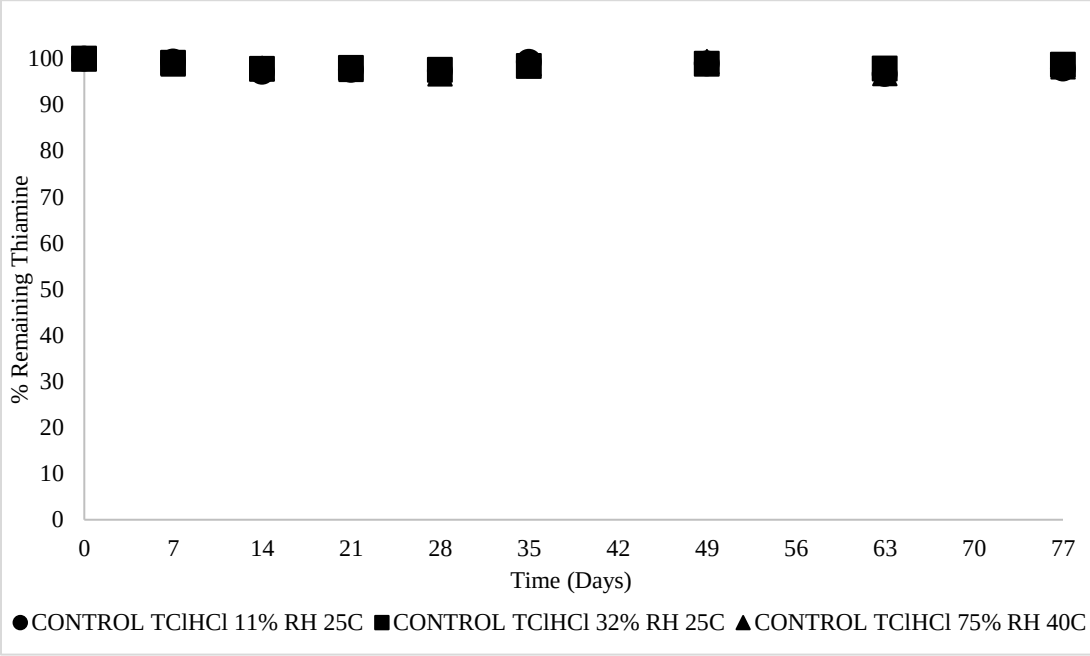
Table 2.2 Onset glass transition temperatures and moisture contents of amorphous solid dispersions with different TClHCl to PVP ratios after lyophilization.

SAMPLE	**Onset T_g ('As is' after lyophilization)**	**%Moisture Content**
1TClHCl:99 PVP SD	**60.8 ±1.9**^{A}	**2.1±0.5**^{a}
5TClHCl :95 PVP SD	**60.6±1.7**^{A}	**1.8±0.1**^{a}
20TClHCl:80 PVP SD	**54.3±6.7**^{BA}	**1.3±0.7**^{a}
30TClHCl:70 PVP SD	**55.0±3.8**^{BA}	**1.39±0.07**^{a}
40TClHCl:60 PVP SD	**41.5±5.4**^{B}	**0.8±0.2**^{a}

Table 2.3 Onset glass transition temperatures of amorphous solid dispersions equilibrated at identified conditions.

SAMPLE	**Condition**	**Onset T_g**
5TClHCl: 95PVP SD	**11% RH and 30ºC**	**53.6±0.1**^{A}
5TClHCl:95PVP SD	**11% RH and 60ºC**	**47.4±1**^{B}

A)



B)

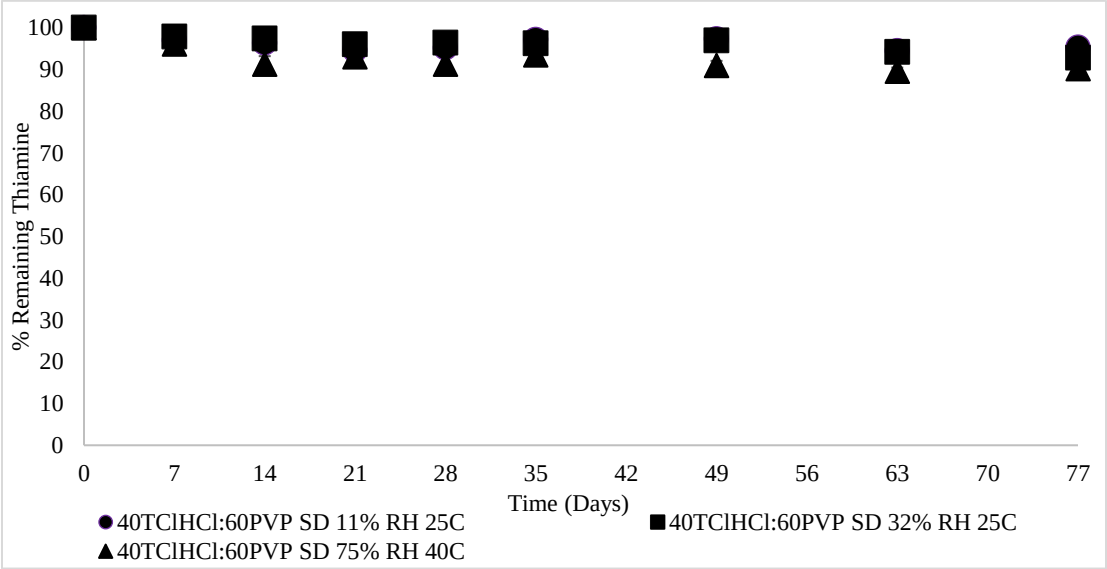
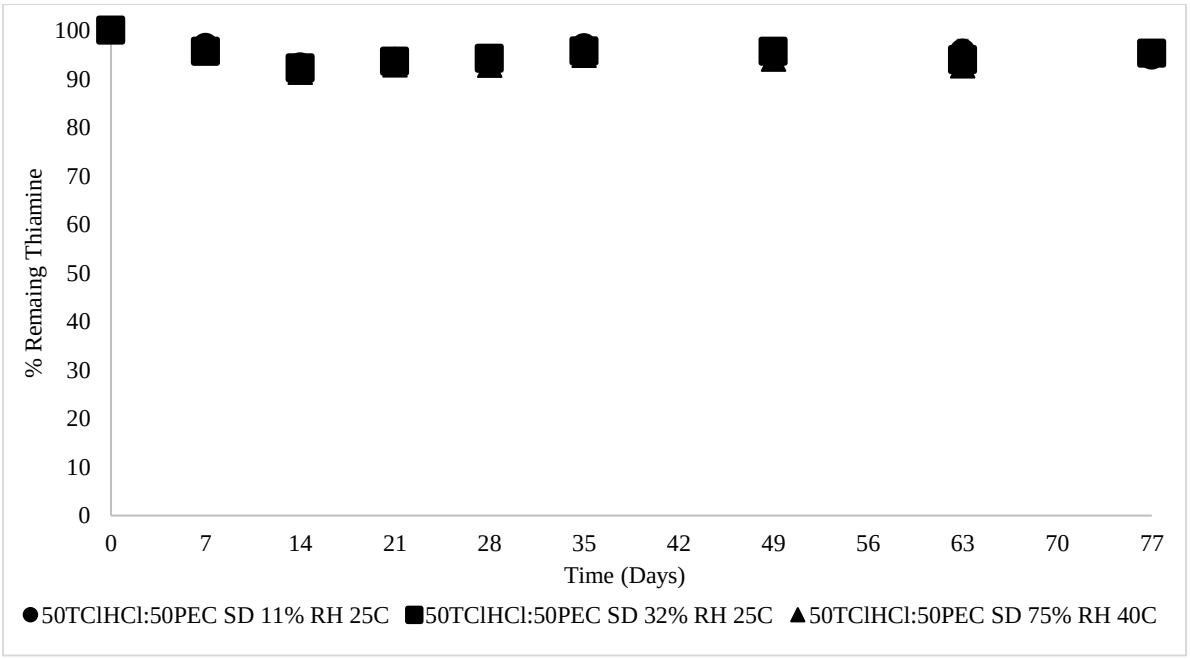


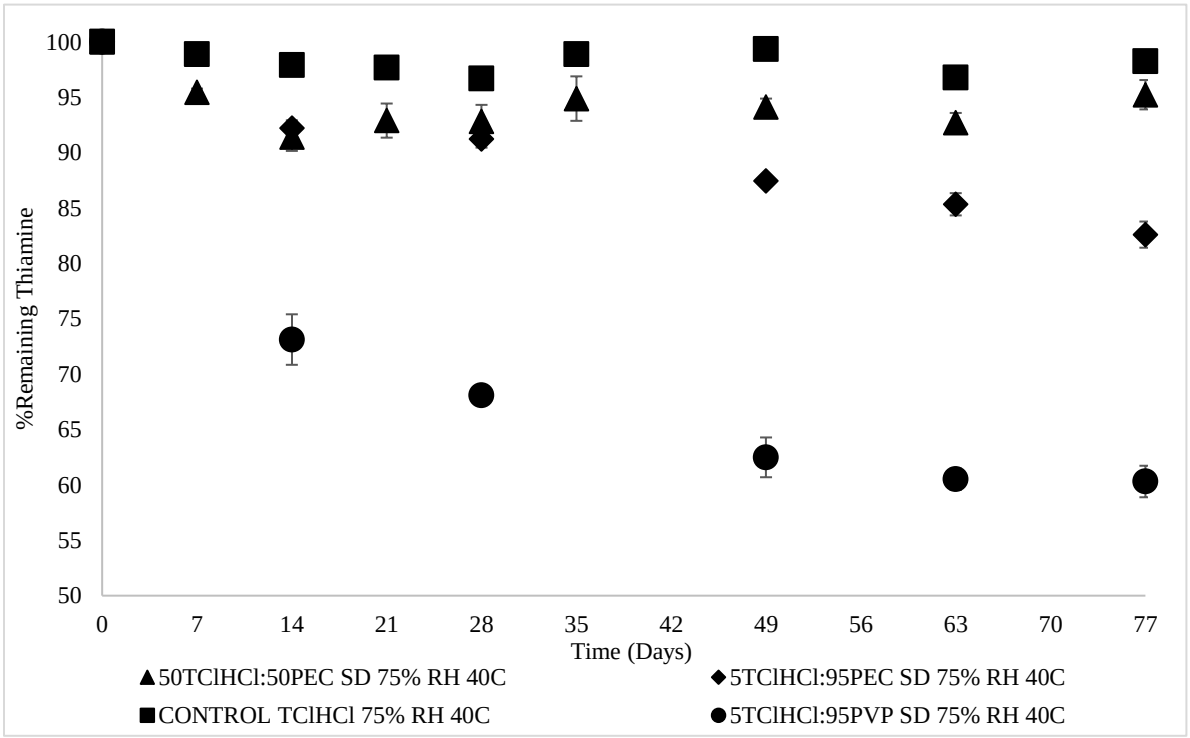
Figure 2.1 Chemical stability of thiamine **A)** in crystalline TClHCl form, **B)** in 40TClHCl:60PVP solid dispersions (SD), **C)** in 50TClHCl:50PEC solid dispersions (SD) stored at 11% RH and 25ºC, 32%RH and 25ºC, and 75%RH and 40ºC for 77 days**,** and **D)** in crystalline TClHCl, 50TClHCl:50PEC, and 5TClHCl:95PEC and PVP dispersions stored at 75%RH and 40ºC for 77 days.

Figure 2.1 continued

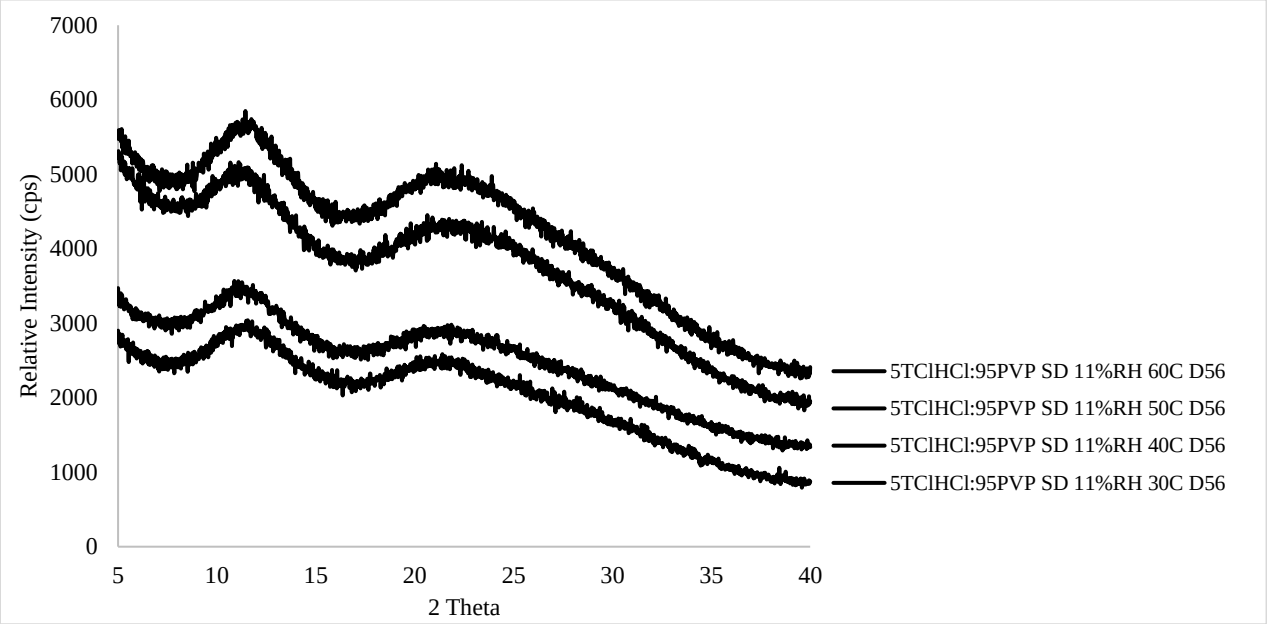
C)



D)



A)



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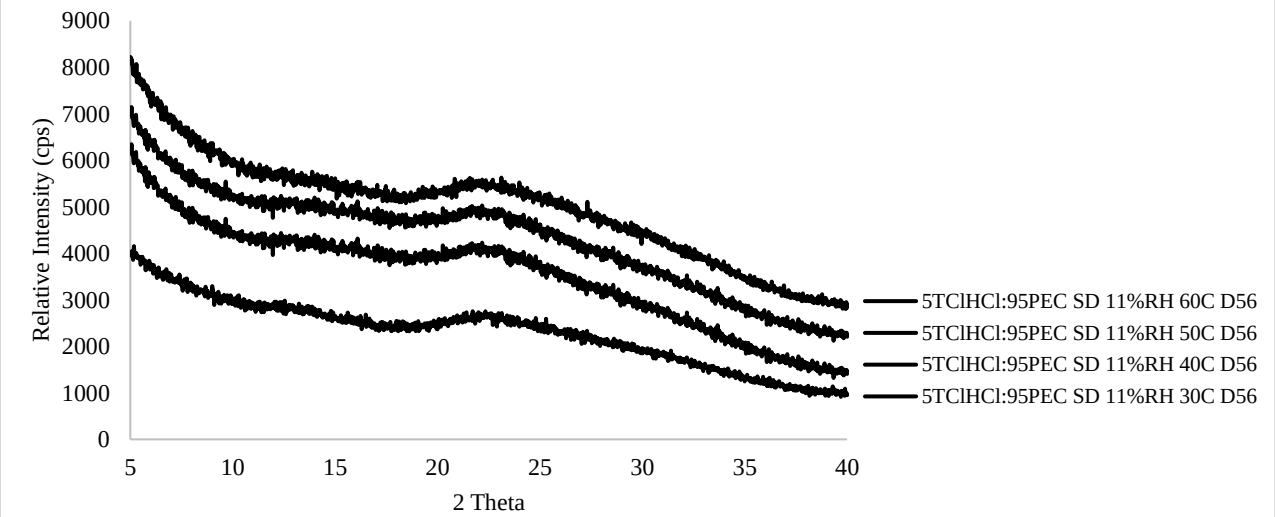
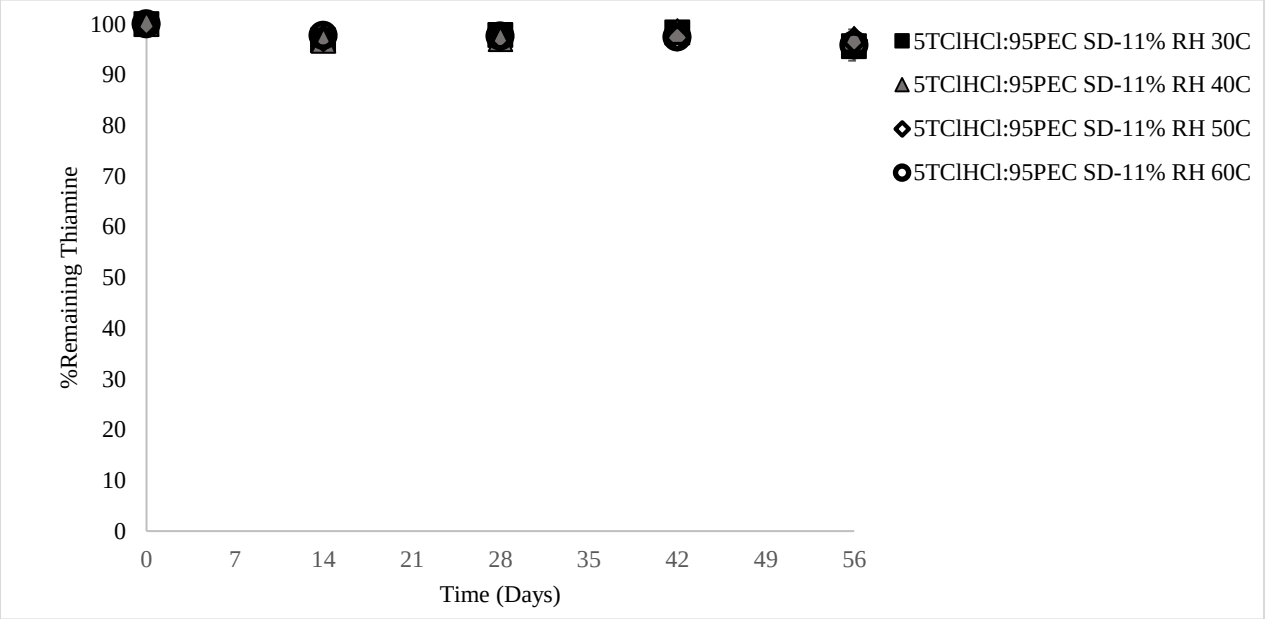


Figure 2.2 X-ray powder diffraction patterns of **A)** 5TClHCl:95PVP and **B)** 5TClHCl:95PEC solid dispersions (SD) stored at 11% RH and 30ºC-60ºC on day 56.

A)



B)

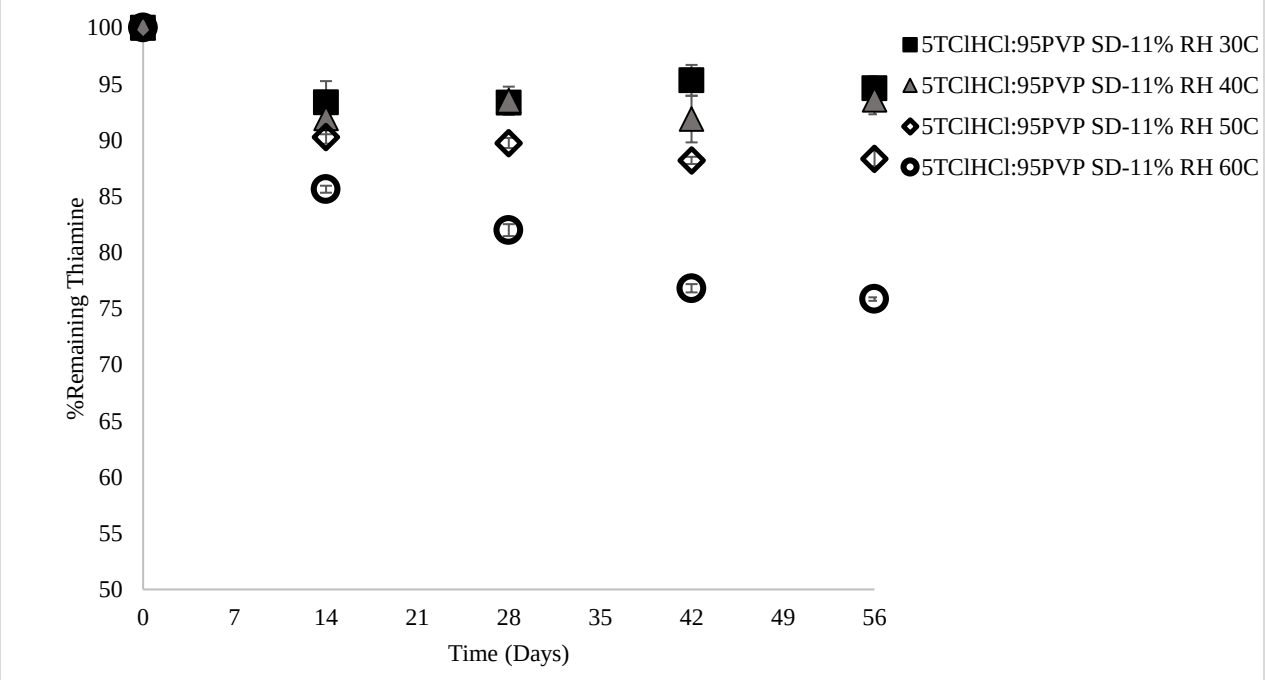
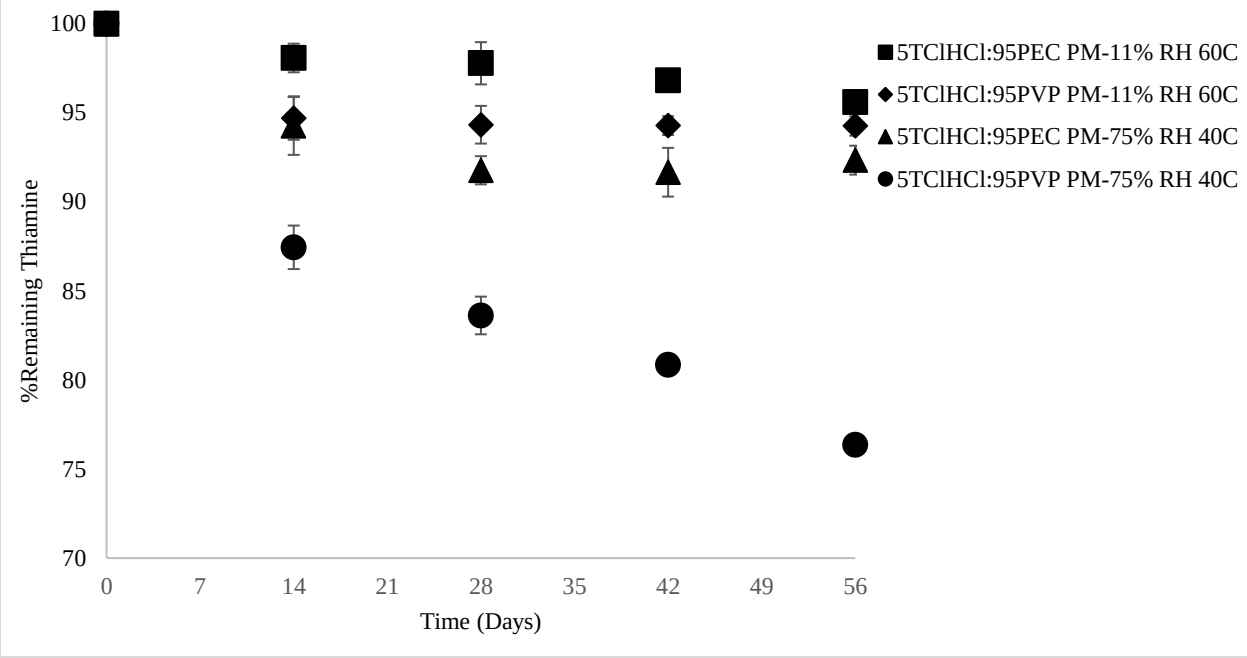


Figure 2.3 Chemical stability of thiamine. **A)** in 5TClHCl:95PEC solid dispersions (SD), **B)** in 5TClHCl:95PVP solid dispersions (SD) stored at 11% RH and 30-60^{o}C for 56 days.

A)



B)

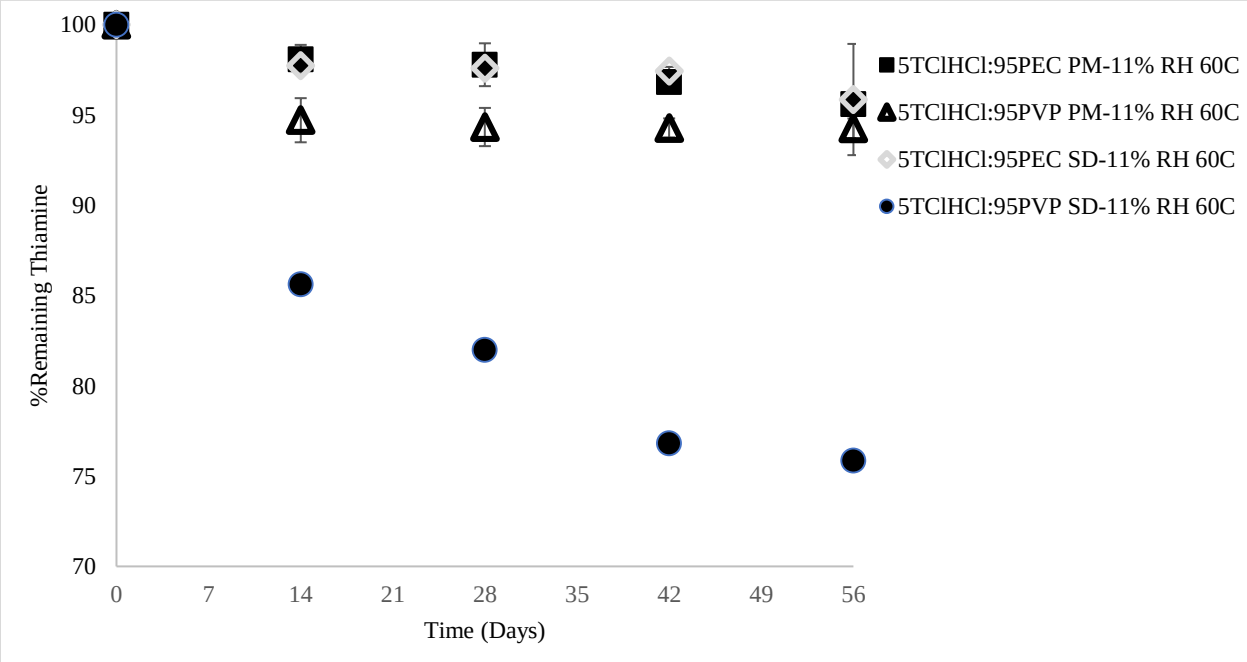
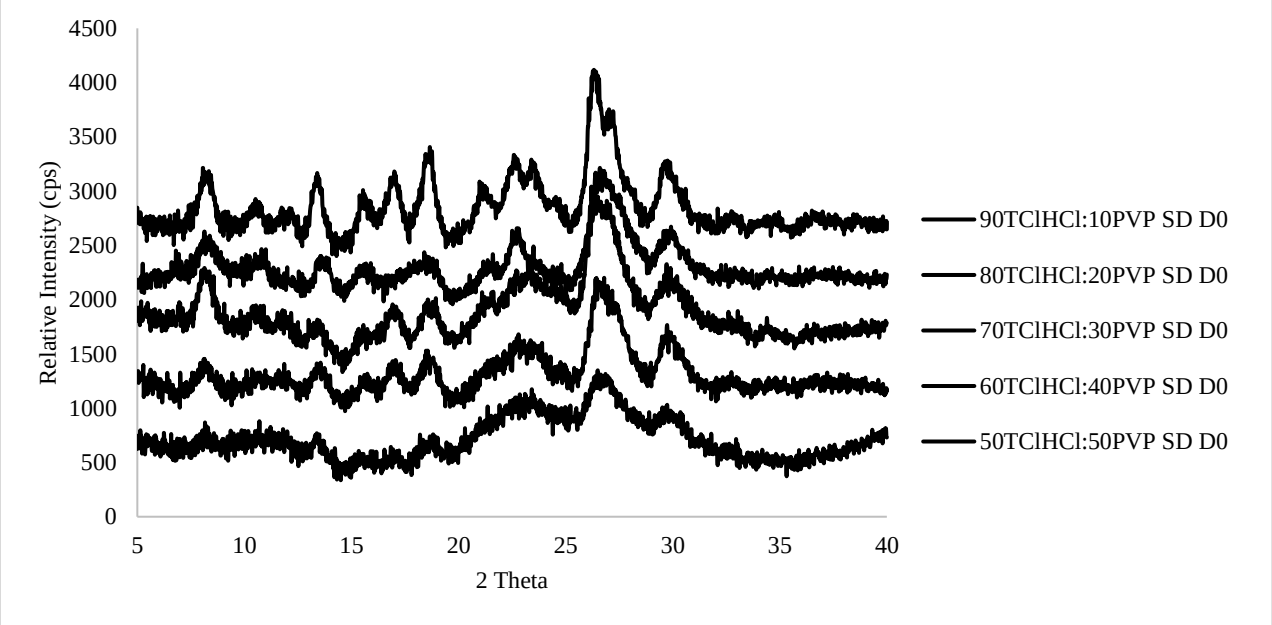


Figure 2.4 Chemical stability of thiamine **A)** in 5TClHCl:PEC-PVP physical mixtures stored at 11% RH and 60ºC and 75%RH and 40ºC for 56 days, **B)** in 5TClHCl:95PEC and PVP physical mixtures (PM) and solid dispersions (SD) stored at 11% RH and 60ºC for 56 days.

A)



B)

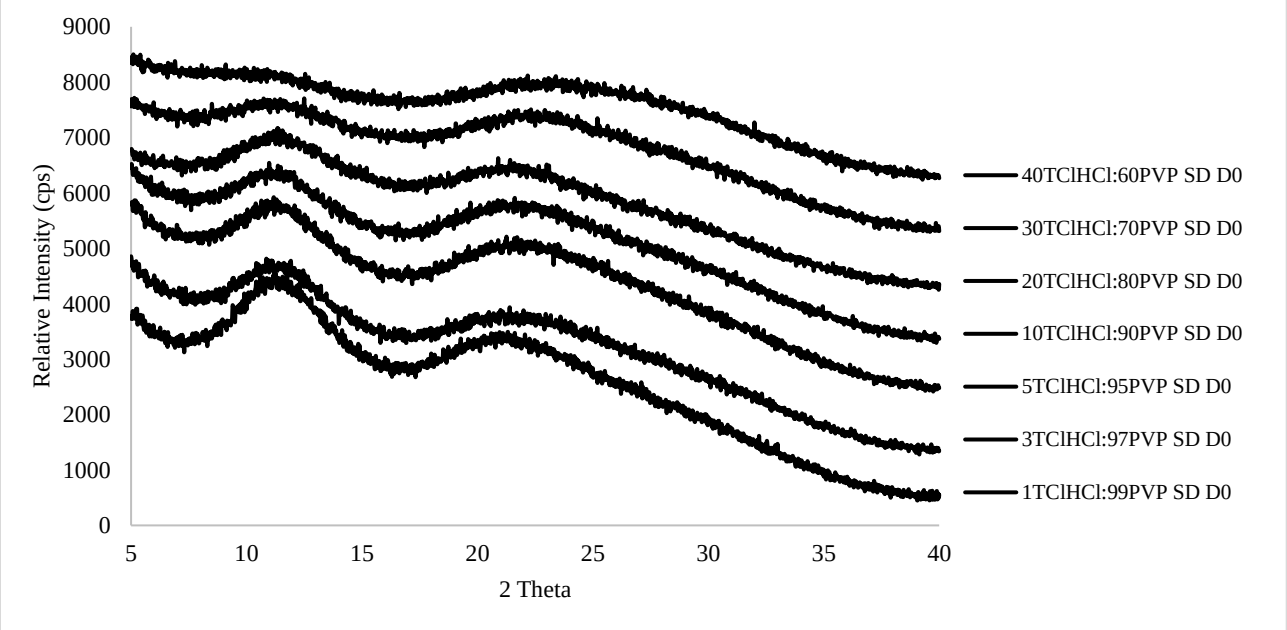


Figure 2.5 X-ray powder diffraction patterns of TClHCl:PVP solid dispersions (SD) prepared **A)** from 50TClHCl:50PVP to 90TClHCl:10PVP **B)** from 1TClHCl:99PVP to 40TClHCl:60PVP.

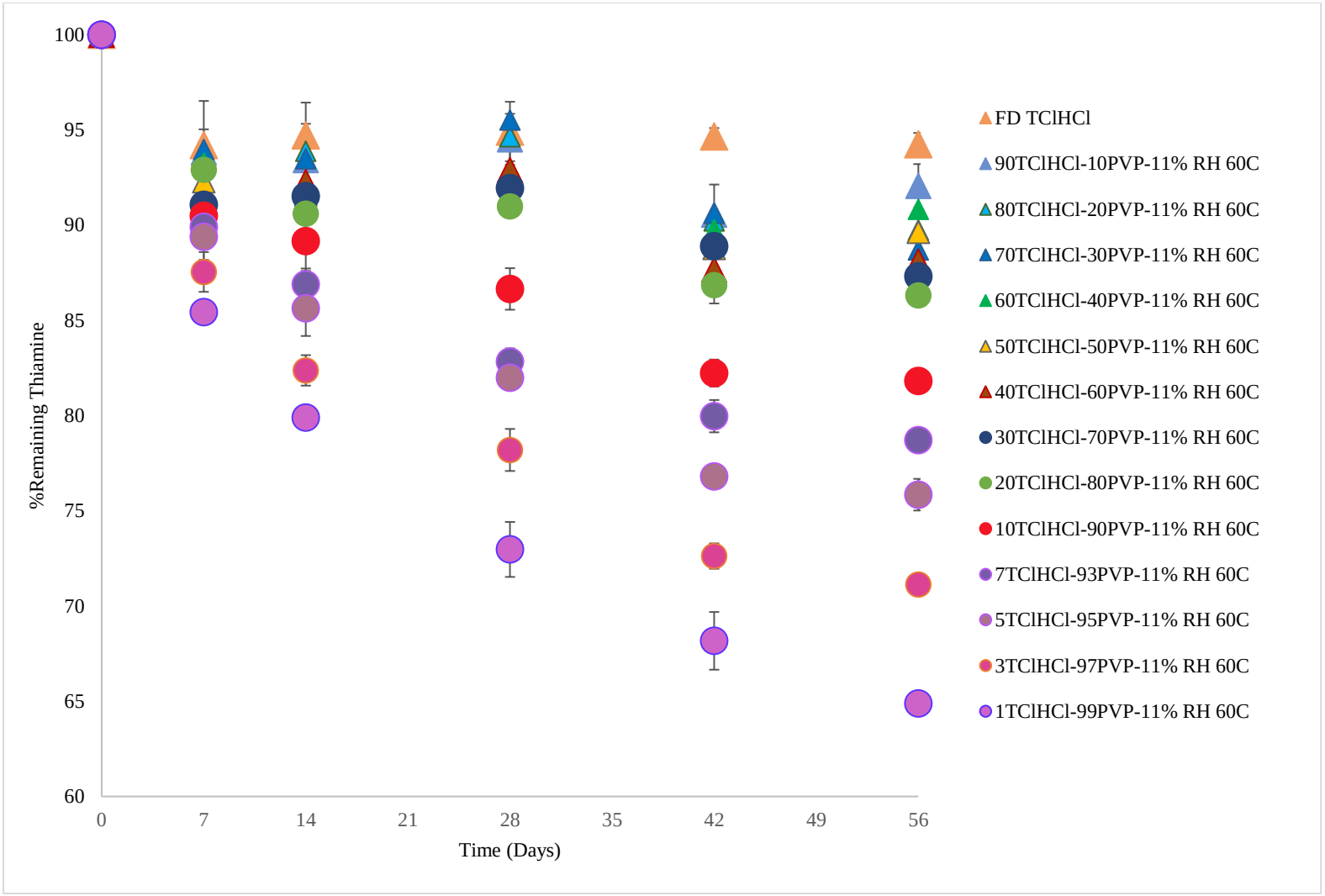


Figure 2.6 Chemical stability of thiamine in various TClHCl to PVP solid dispersions stored at 11% RH and 60°C for 56 days.

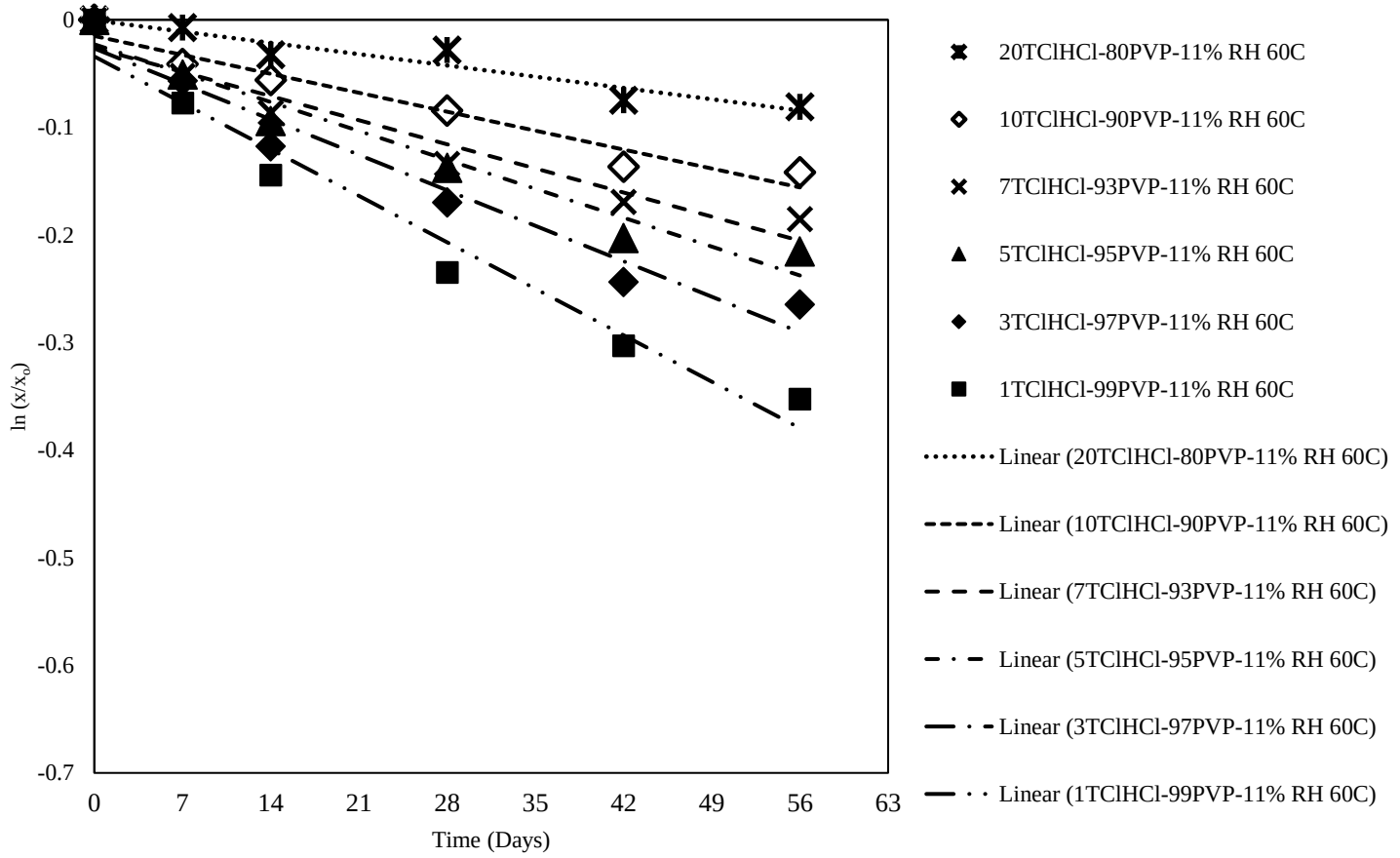


Figure 2.7 First-order degradation regression lines of thiamine in various TClHCl to PVP dispersions stored at 11% RH and 60^{o}C for 56 days.

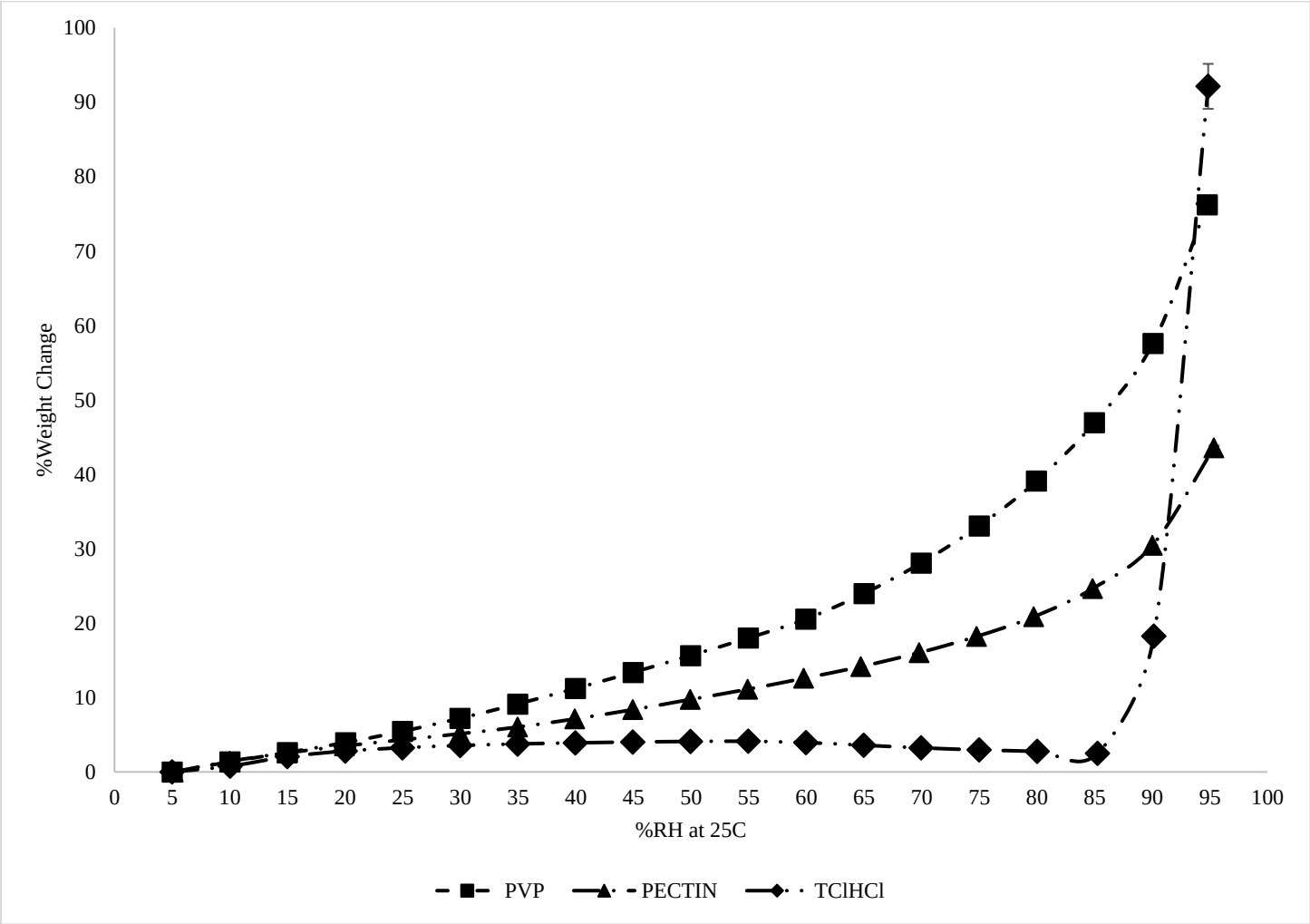


Figure 2.8 Moisture sorption profiles of PVP, PEC, and TClHCl.

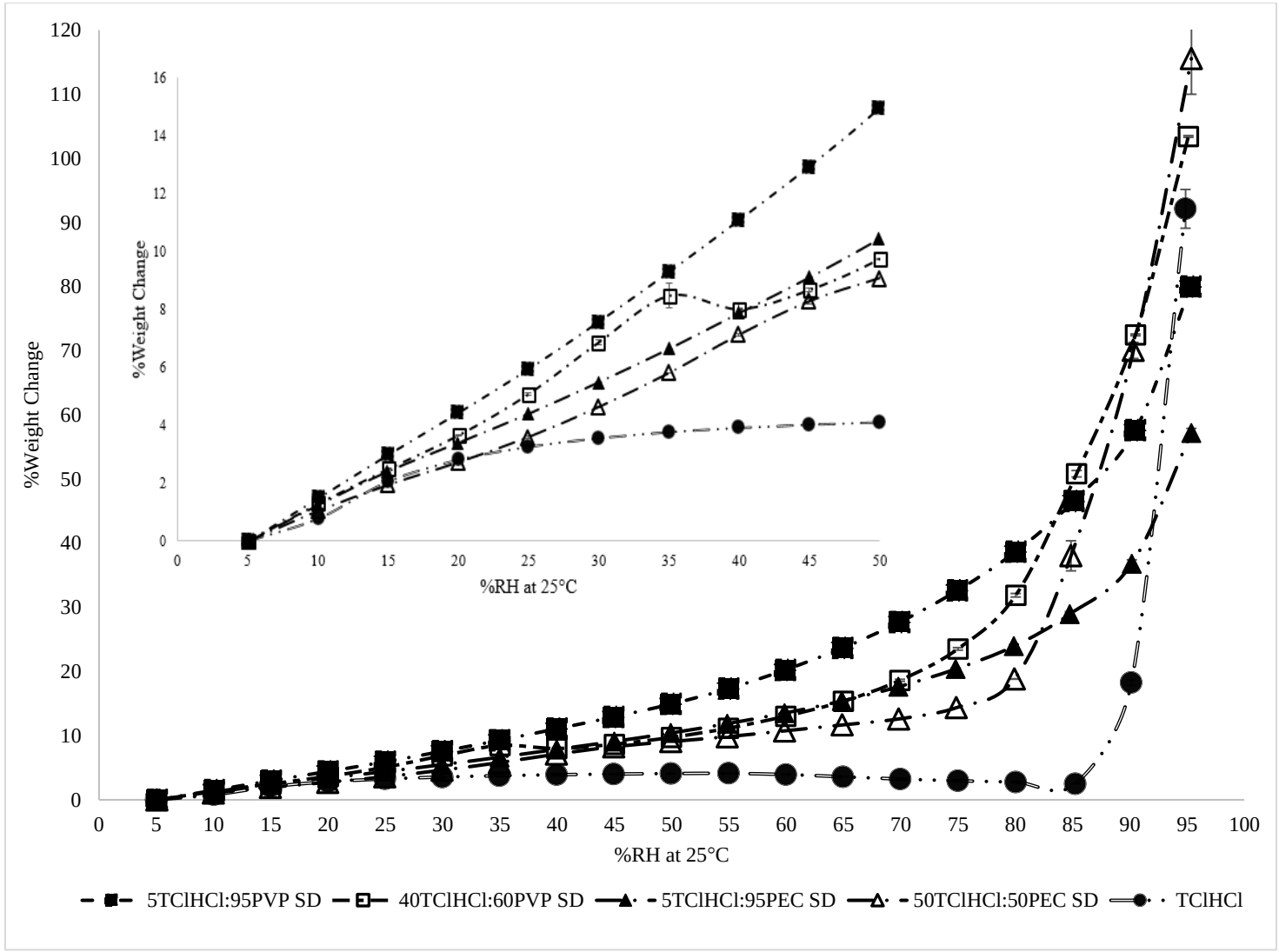
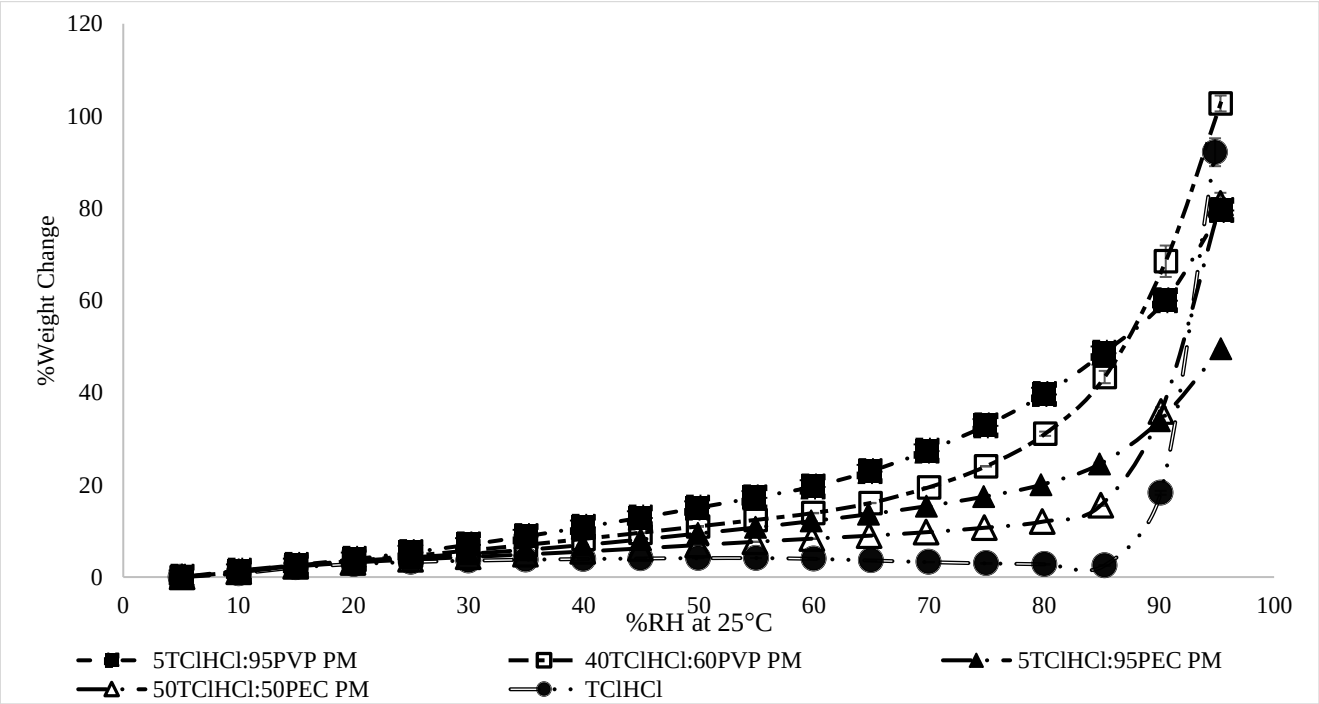


Figure 2.9 Moisture sorption profiles of TClHCl, 50TClHCl:50PVP, 40TClHCl:60PVP, 5TClHCl:95PVP, and 5TClHCl:95PEC solid dispersions (SD) at 25^{o}C.

A)



B)

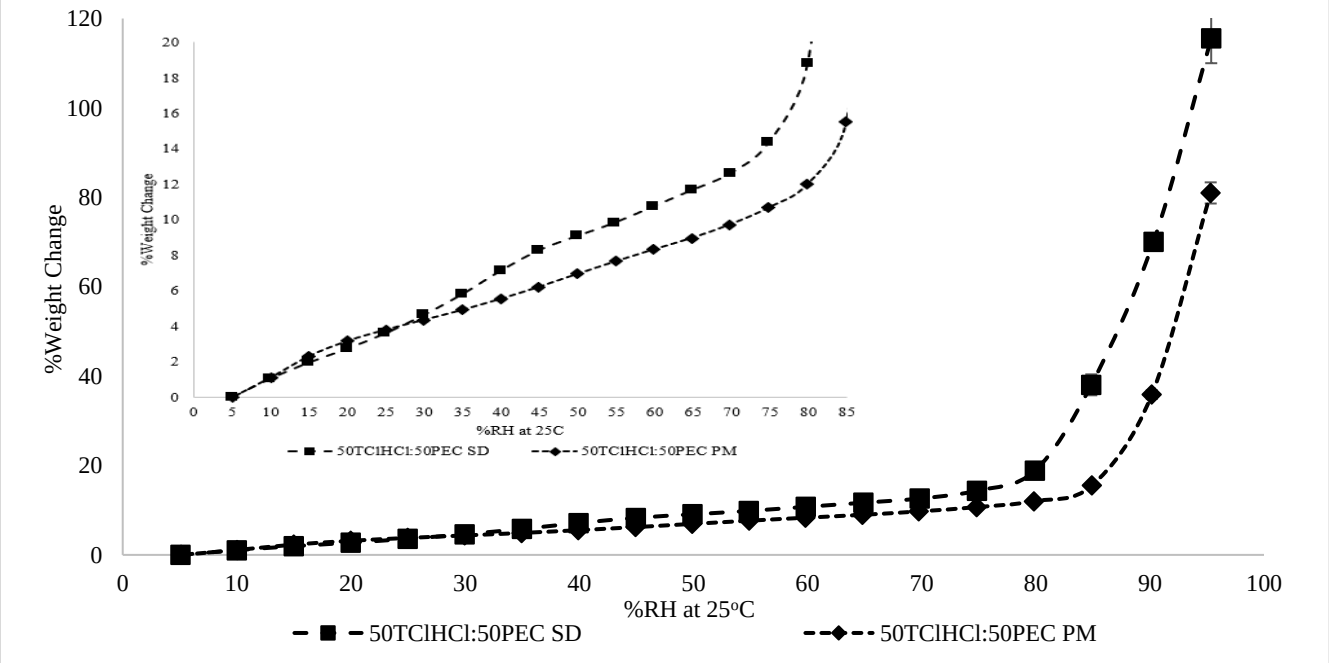
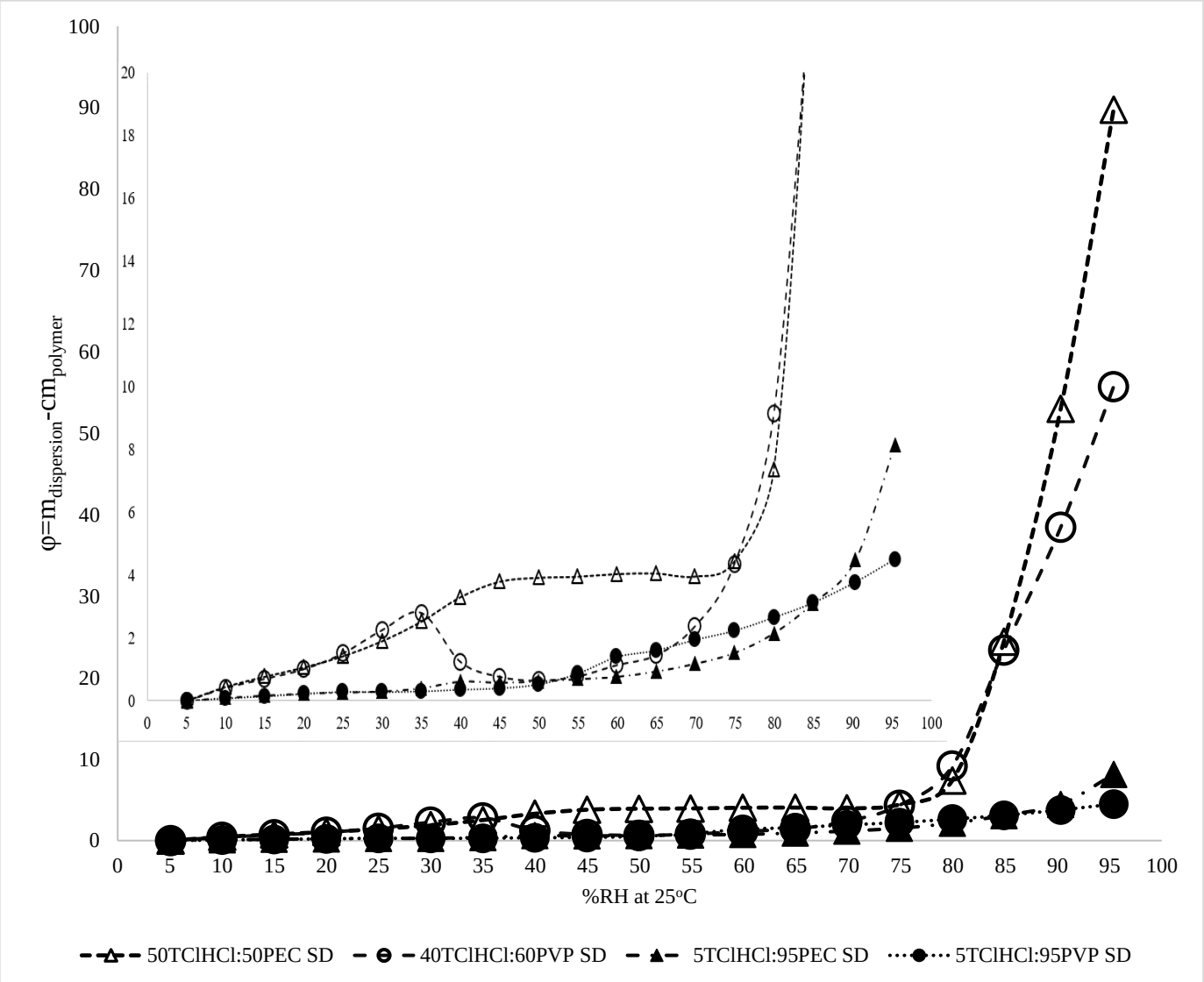


Figure 2.10 Moisture sorption profiles of TClHCl, **A)** 50TClHCl:50PVP, 40TClHCl:60PVP, 5TClHCl:95PVP, and **B)** 5TClHCl:95PEC physical mixtures (PM) at 25°C.

Figure 2.10 continued

C)



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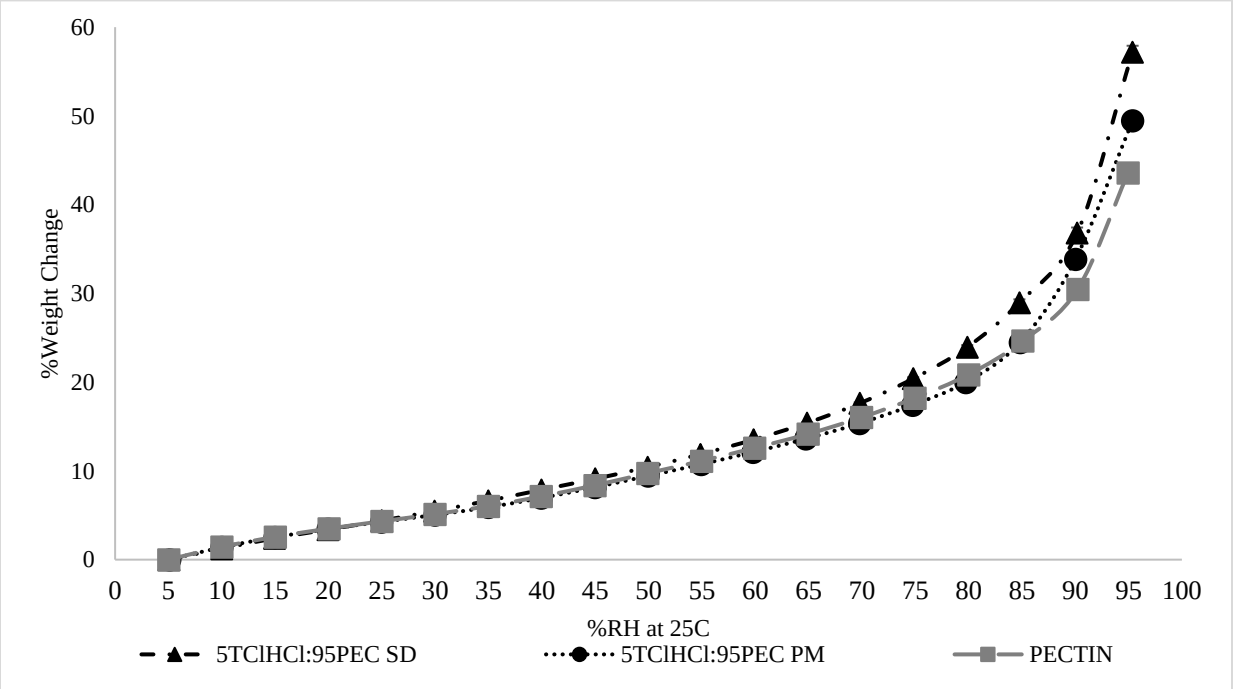

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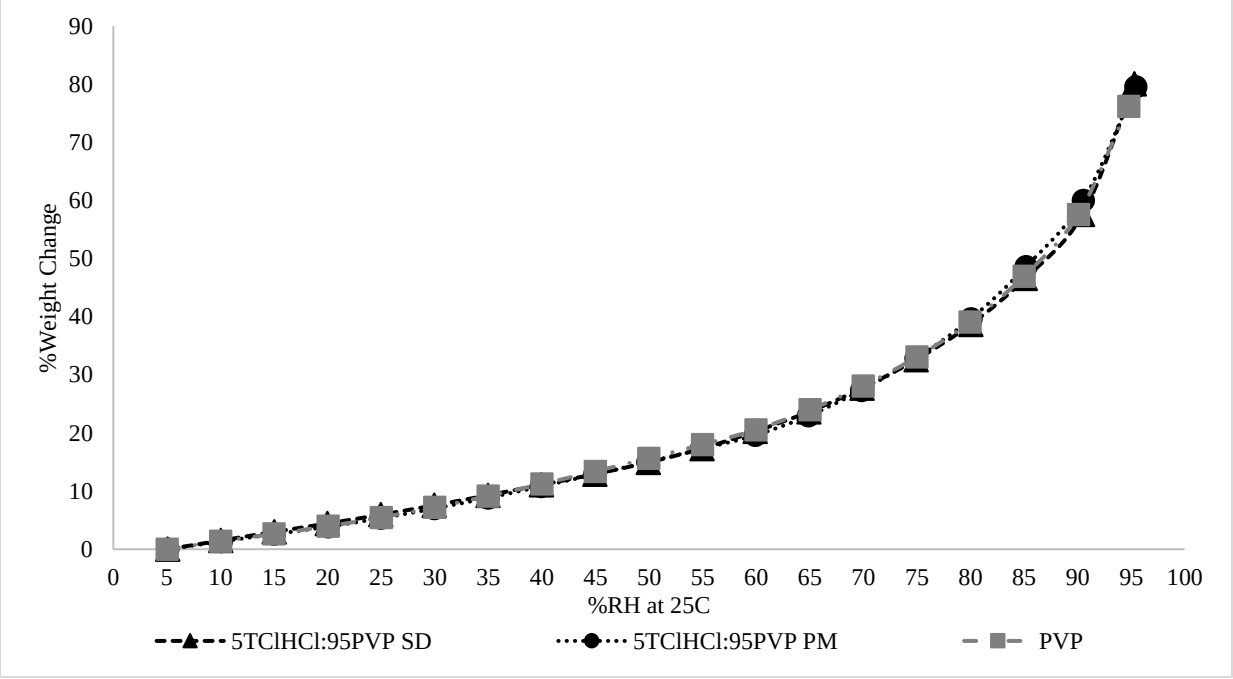


Figure 2.11 Comparison of moisture sorption profiles of **A)** PEC and solid dispersions (SD) and physical mixtures (PM) of 5TClHCl:95PEC, **B)** PVP and solid dispersions (SD) and physical mixtures (PM) of 5TClHCl:95PVP.

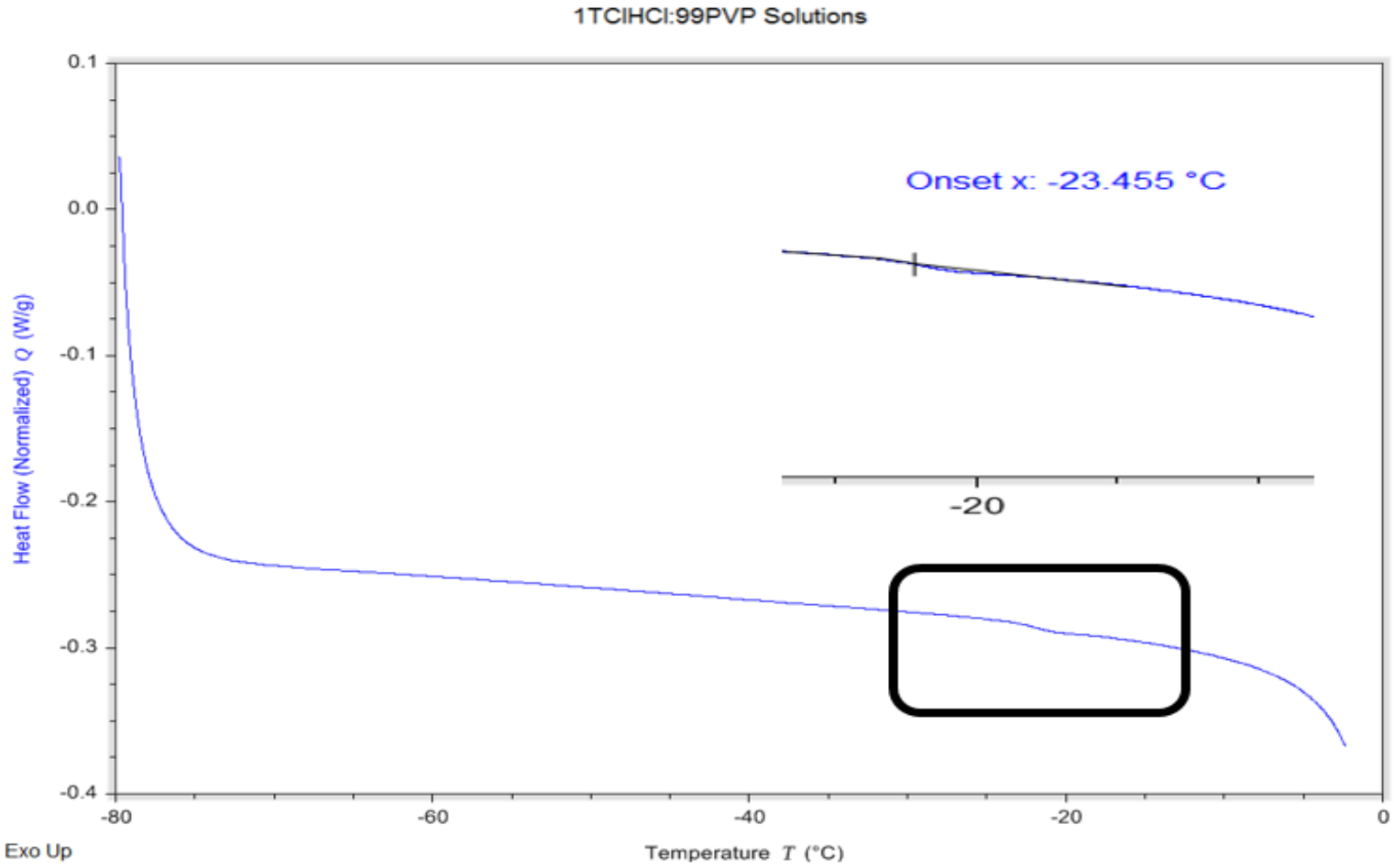
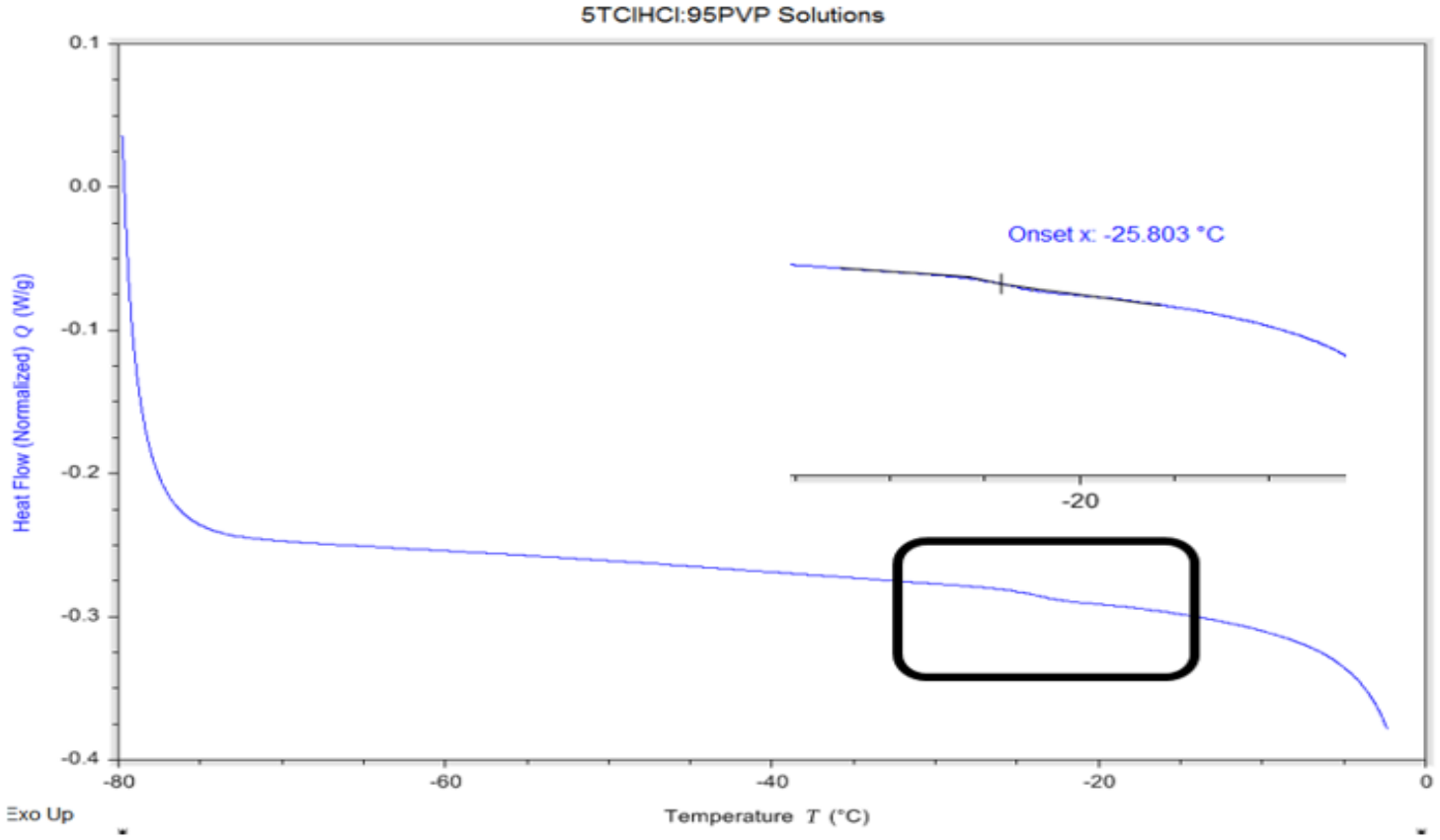


Figure 2.12 DSC graphs of TClHCl: polymer solutions showing the T_g's.

Figure 2.12 continued



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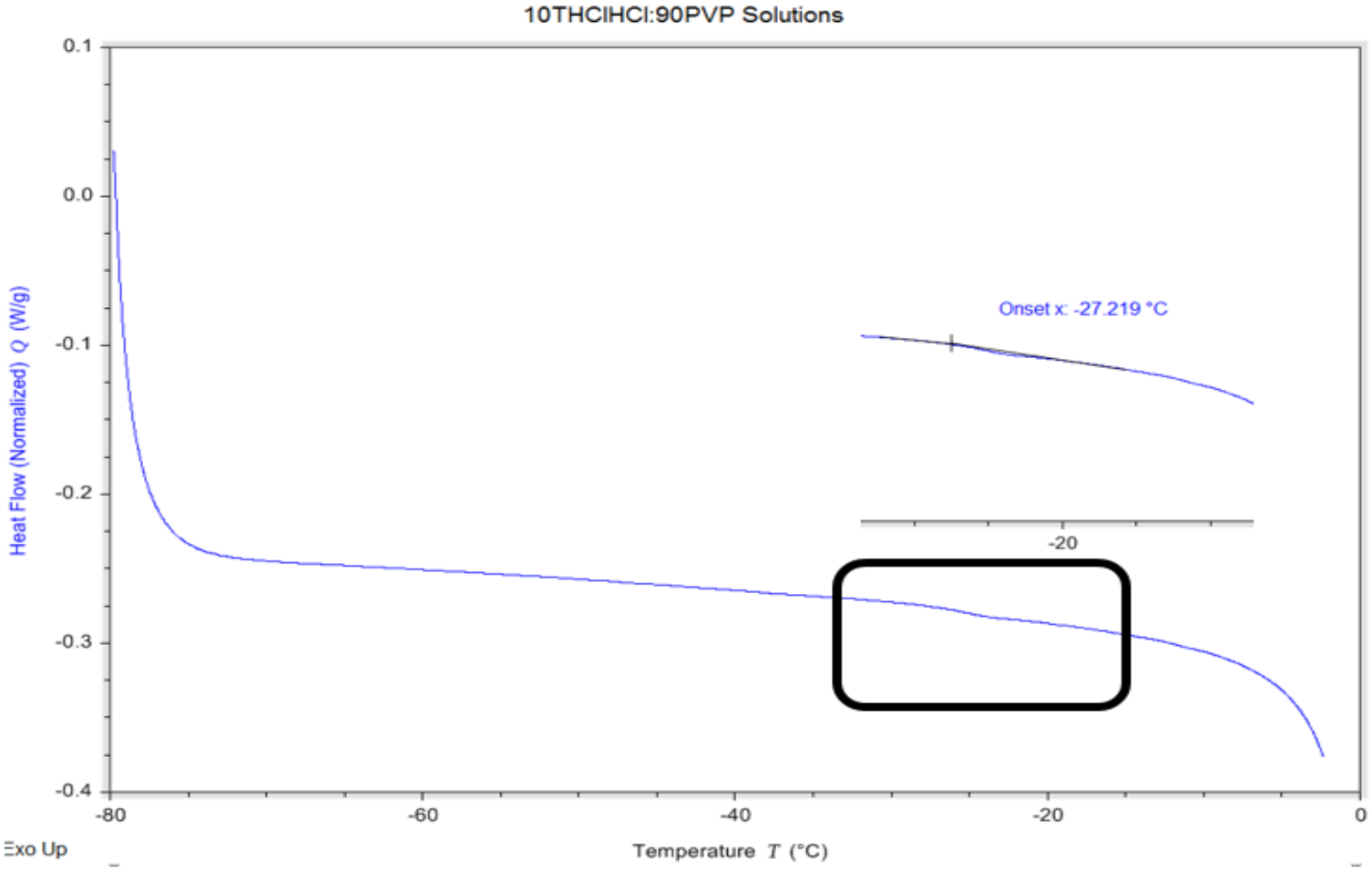

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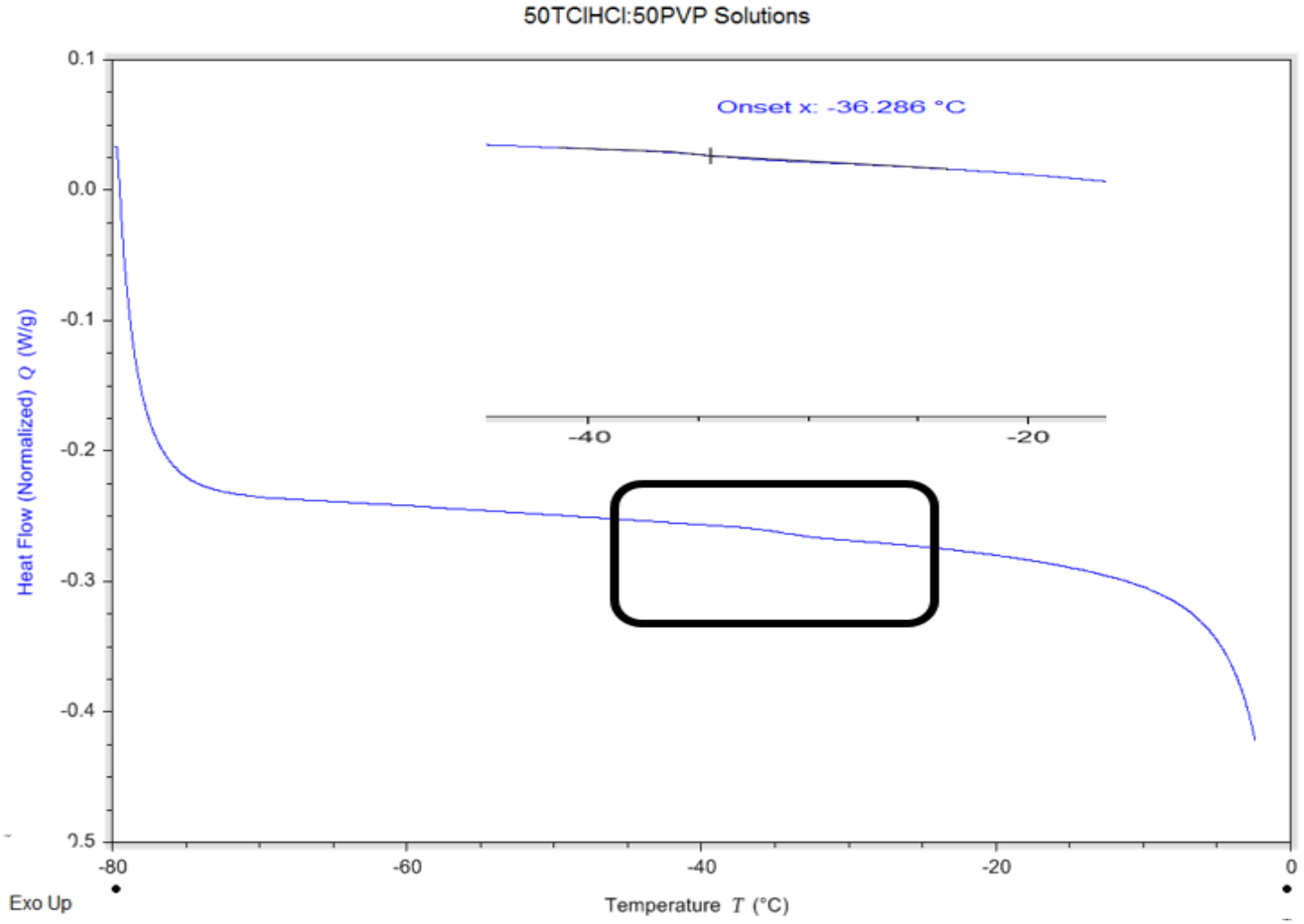

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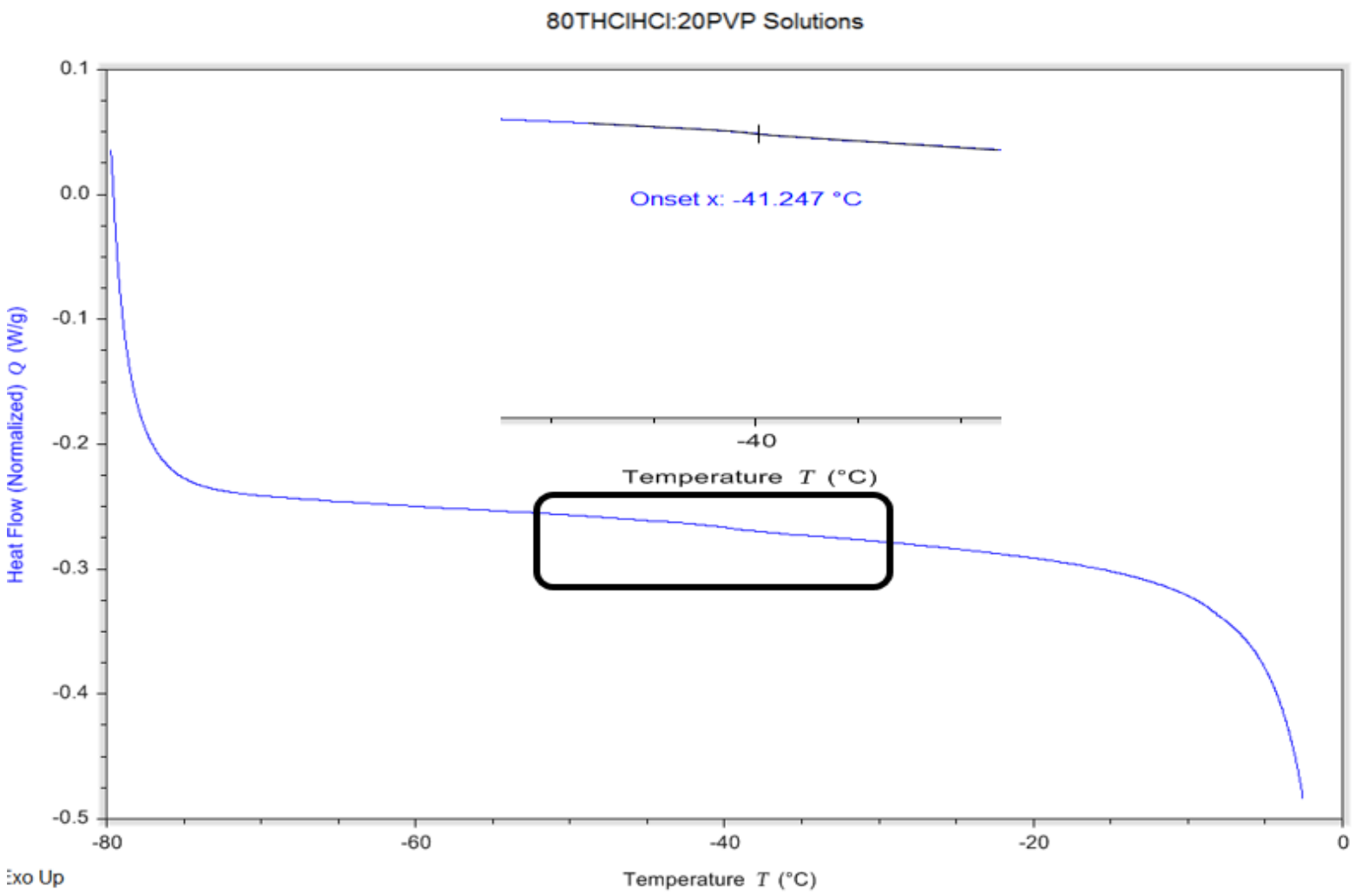

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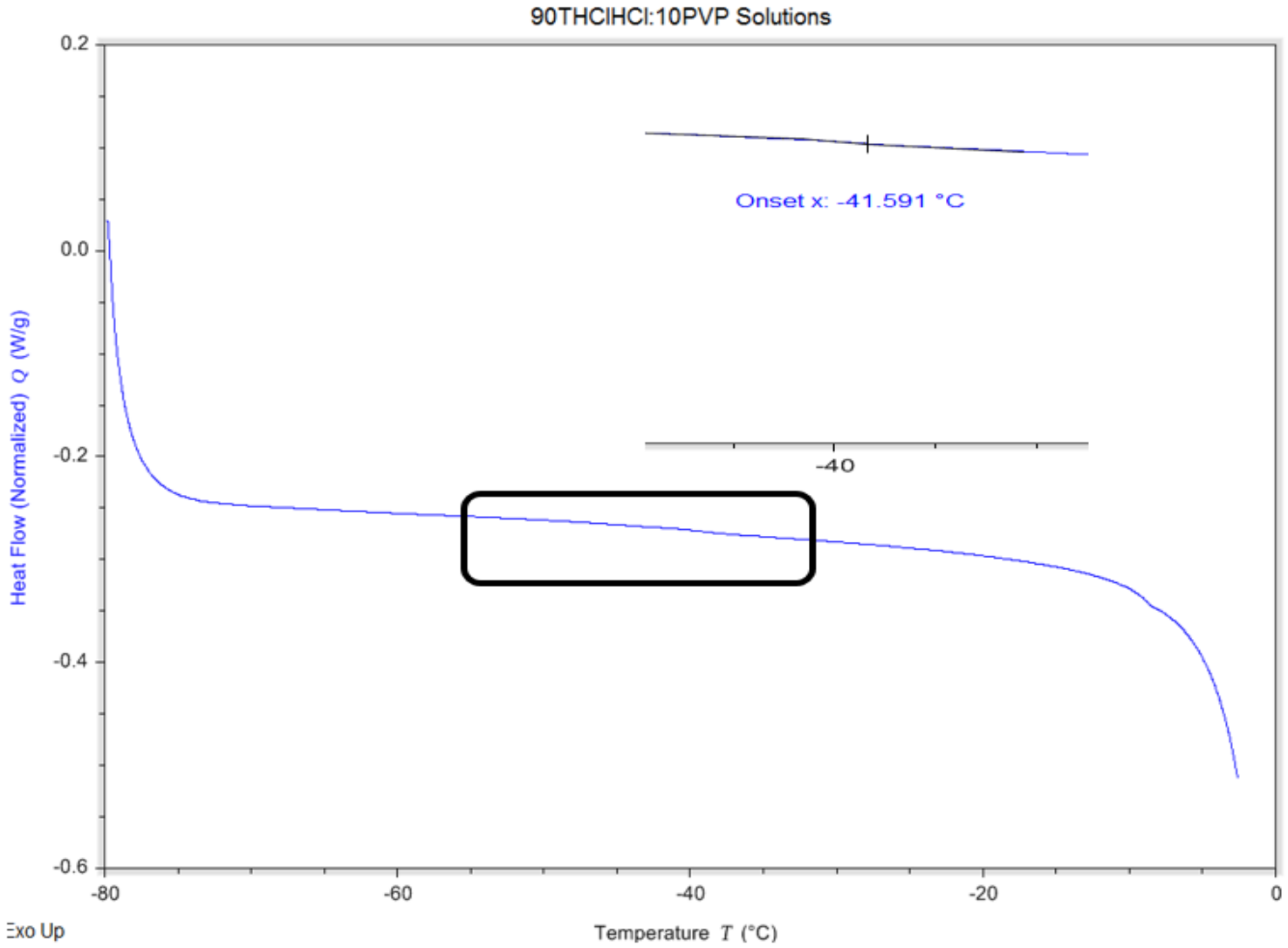

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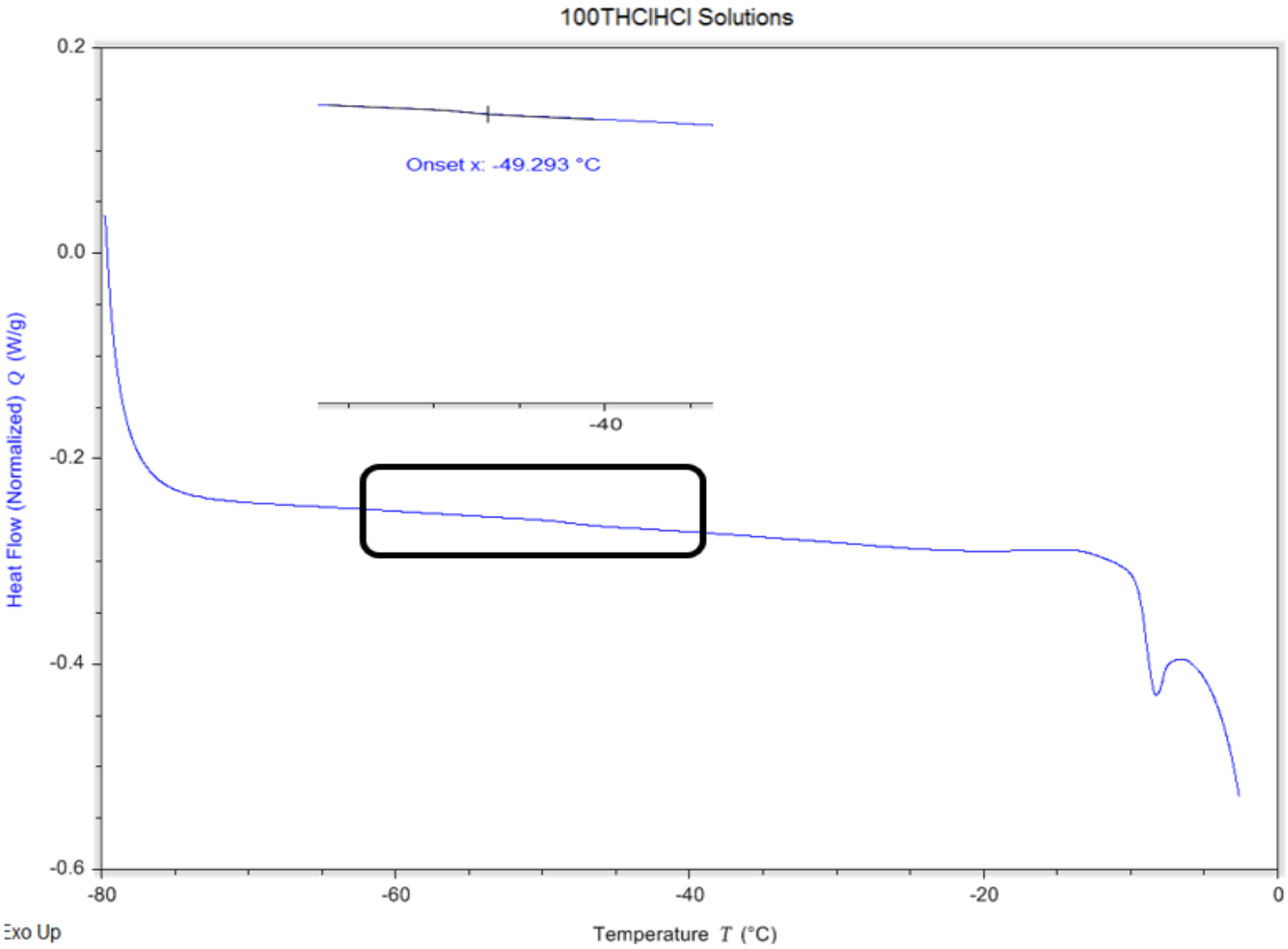

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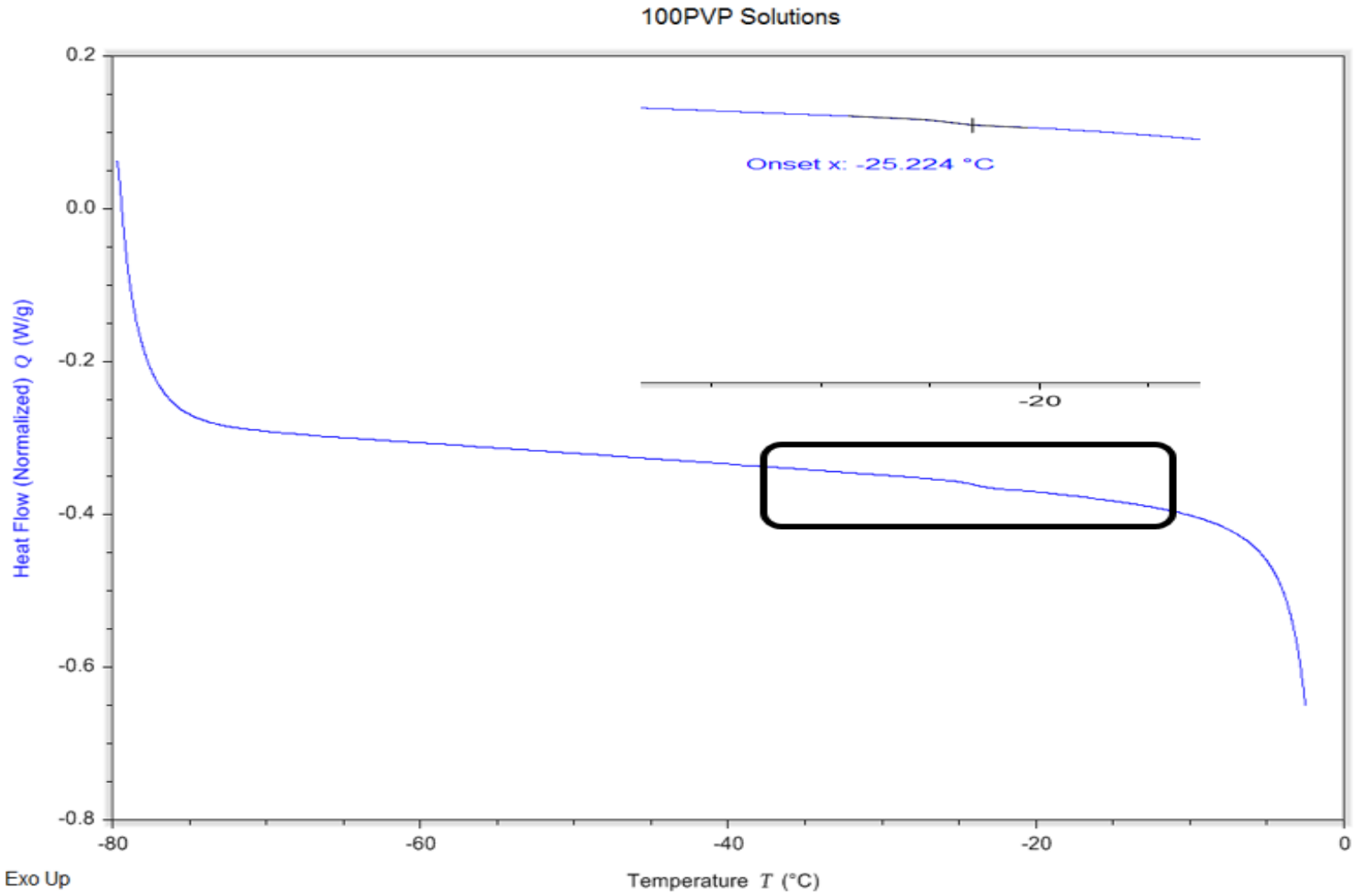

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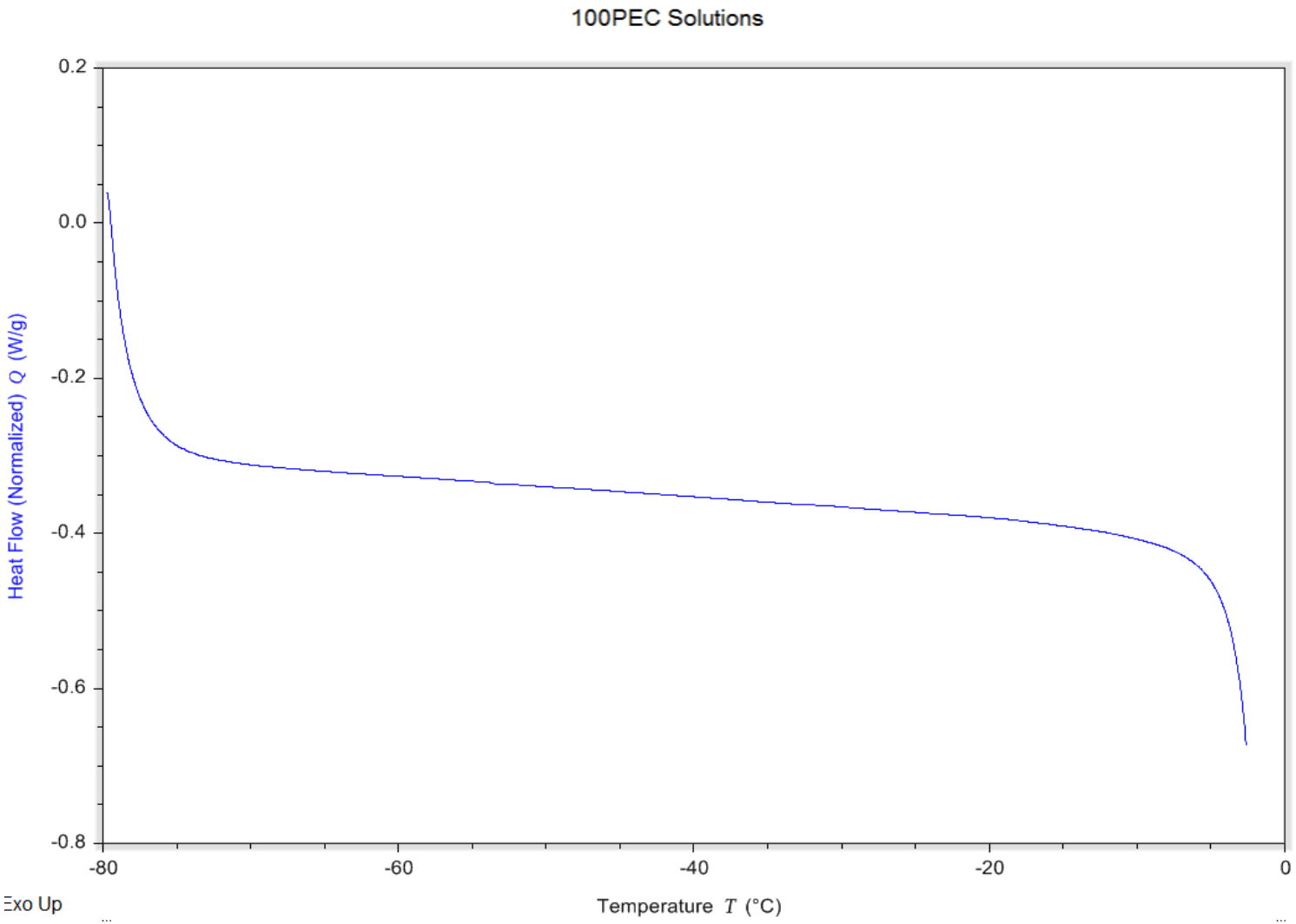

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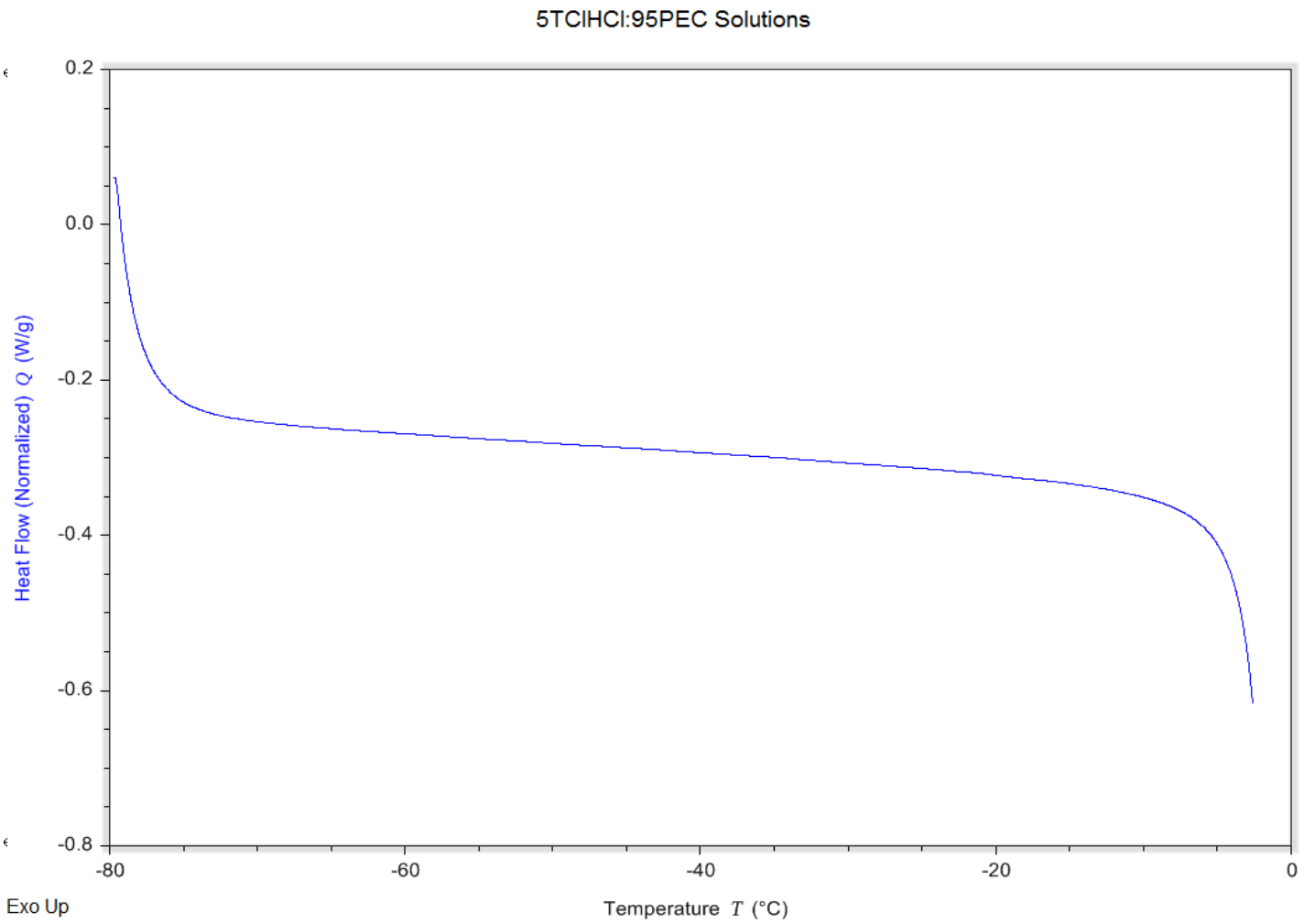


2.6 References

Al-Rashood, K. A. M., Al-Shammary, F. J., & Mian, N. A. A. (1990). Analytical Profile of Thiamine Hydrochloride. In K. Florey, A. A. Al-Badr, G. A. Forcier, H. G. Brittain, & L. T. Grady (Eds.), *Analytical Profiles of Drug Substances* (Vol. 18, pp. 413-458): Academic Press.

Arabshahi, A., & Lund, D. B. (1988). Thiamin Stability in Simulated Intermediate Moisture Food. *Journal of Food Science, 53*(1), 199-203. doi:10.1111/j.1365-2621.1988.tb10208.x

Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International, 99*, 363-374. doi:https://doi.org/10.1016/j.foodres.2017.05.021

Beaman, G. R. (1952). Relation between (apparent) second-order transition temperature and melting point. *Journal of Polymer Science, 9*(5), 470-472.

Bell, L. N., & Hageman, M. J. (1994). Differentiating between the Effects of Water Activity and Glass Transition Dependent Mobility on a Solid State Chemical Reaction: Aspartame Degradation. *Journal of Agricultural and Food Chemistry, 42*(11), 2398-2401. doi:10.1021/jf00047a007

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

Connors, K. A. (1986). *Chemical stability of pharmaceuticals : a handbook for pharmacists* (2nd ed.. ed.). New York: New York : Wiley.

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

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

Franks, F. (2007). Chapter 8 Primary Drying: The Sublimation of Ice. In *Freeze-drying of Pharmaceuticals and Biopharmaceuticals: Principles and Practice* (pp. 105-120): The Royal Society of Chemistry.

Hancock, B. C., & Zografi, G. (1997). Characteristics and Significance of the Amorphous State in Pharmaceutical Systems. *Journal of Pharmaceutical Sciences, 86*(1), 1-12. doi:https://doi.org/10.1021/js9601896

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

Lai, M. C., Hageman, M. J., Schowen, R. L., Borchardt, R. T., & Topp, E. M. (1999). Chemical stability of peptides in polymers. 1. Effect of water on peptide deamidation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) matrixes. *Journal of Pharmaceutical Sciences, 88*(10), 1073-1080. doi:https://doi.org/10.1021/js980227g

Lonsdale, D. (2006). Review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evidence-Based Complementary and Alternative Medicine, 3*(1), 49-59. doi:10.1093/ecam/nek009A

Luthra, S. A., Shalaev, E. Y., Medek, A., Hong, J., & Pikal, M. J. (2012). Chemical Stability of Amorphous Materials: Specific and General Media Effects in the Role of Water in the Degradation of Freeze-Dried Zoniporide. *Journal of Pharmaceutical Sciences, 101*(9), 3110-3123. doi:https://doi.org/10.1002/jps.23128

Mauer, L. J., & Bradley, R. L. (2017). Moisture and Total Solids Analysis. In S. S. Nielsen (Ed.), *Food Analysis* (pp. 257-286). Cham: Springer International Publishing.

McCandless, D. W. (2010). Thiamine Deficiency and Associated Clinical Disorders. *Thiamine Deficiency and Associated Clinical Disorders*, 1-192.

Nath, A., Shope, T. R., & Koch, T. R. (2017). Chapter 51 - Thiamine (Vitamin B_1) After Weight Loss Bariatric Surgery A2 - Preedy, Victor R. In R. Rajendram & C. R. Martin (Eds.), *Metabolism and Pathophysiology of Bariatric Surgery* (pp. 479-489). Boston: Academic Press.

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

Pachapurkar, D., & Bell Leonard, N. (2006). Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions. *Journal of Food Science, 70*(7), c423-c426. doi:10.1111/j.1365-2621.2005.tb11463.x

Pachapurkar, D., & Bell, L. N. (2006). Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions. *Journal of Food Science, 70*(7), c423-c426. doi:10.1111/j.1365-2621.2005.tb11463.x

Pourcel, L., Moulin, M., Fitzpatrick, T.B. (2013). Examining strategies to facilitate vitamin B1 biofortification of plants by genetic engineering. *Frontiers in Plant Science, 4*, 160.

Prinzo, Z. W. (1999). *Thiamine Deficiency and Its Prevention and Control in Major Emergencies*: Micronutrient series; World Health, Organization (WHO). Department of Nutrient for Health and Development; Office of the United Nations High Commissioner for Refugees (UNHCR).

Roos, Y. H., & Drusch, S. (2016). Chapter 8 - Reaction kinetics. In *Phase Transitions in Foods (Second Edition)* (pp. 275-313). San Diego: Academic Press.

Sanchez J., O., Ismail, Y., Christina, B., & Mauer Lisa, J. (2018). Degradation of L-Ascorbic Acid in the Amorphous Solid State. *Journal of Food Science, 83*(3), 670-681. doi:10.1111/1750-3841.13998

Slade, L., Levine, H., & Reid, D. S. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition, 30*(2-3), 115-360. doi:10.1080/10408399109527543

Voelker, A. L., Miller, J., Running, C. A., Taylor, L. S., & Mauer, L. J. (2018). Chemical stability and reaction kinetics of two thiamine salts (thiamine mononitrate and thiamine chloride hydrochloride) in solution. *Food Research International, 112*, 443-456. doi:https://doi.org/10.1016/j.foodres.2018.06.056

Waterman, K. C., Gerst, P., & Dai, Z. (2012). A Generalized Relation for Solid-State Drug Stability as a Function of Excipient Dilution: Temperature-Independent Behavior. *Journal of Pharmaceutical Sciences, 101*(11), 4170-4177. doi:https://doi.org/10.1002/jps.23268

Windheuser, J. J., & Higuchi, T. (1962). Kinetics of Thiamine Hydrolysis. *Journal of Pharmaceutical Sciences, 51*(4), 354-364. doi:https://doi.org/10.1002/jps.2600510415

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 retention*. Paper presented at the HPLC, San Francisco, CA.

CHAPTER 3. AMORPHIZATION OF THIAMINE: A STUDY OF THE PHYSICAL AND CHEMICAL STABILITY OF THIAMINE IN THIAMINE MONONITRATE AMORPHOUS SOLID DISPERSIONS

3.1 Abstract

Thiamine mononitrate (TMN) and thiamine chloride hydrochloride (TClHCl) are two salt forms of thiamine that are widely used for food fortification purposes. Both salt forms are found in crystalline form; however, due to interactions with polymeric ingredients and processing conditions, thiamine is likely to convert into the amorphous form, which is generally considered to be physically and chemically less stable. TMN is primarily used as an additive in flour and other low moisture products, many of which contain polymers. The physical and chemical stability of amorphous thiamine in TMN remain unelucidated. The objective of this study was to investigate the differences in thiamine loss between amorphous and crystalline thiamine forms by measuring its degradation in crystalline TMN, physical blends of crystalline TMN with polymers [pectin and polyvinylpyrrolidone (PVP)], and TMN amorphous solid dispersions at controlled environmental conditions [various temperature (30, 40, 50, 60^{o}C) and relative humidity (11% and 75% RH)] over the 8 weeks of storage. A freeze drier was used as an amorphization technique. The physical structure of solid dispersions was periodically verified using powder X-ray diffraction, and HPLC was performed biweekly to determine thiamine loss. Glass transition temperatures (T_g) of select samples were measured by differential scanning calorimetry, and moisture sorption isotherms were also collected. Intermolecular hydrogen bonding interactions were identified using FTIR. Our results indicate that no significant degradation was found when TMN was crystalline (alone or in physical blends with polymers). However, significant thiamine loss was observed when it was in amorphous solid dispersions at low RH and high temperature conditions. T_g was important when

the samples were stored at temperatures above their T_gs. The polymer type influenced thiamine degradation; thiamine was more stable in PEC dispersions, compared to PVP dispersions. Chemical stability of amorphous thiamine was primarily based on the hydrogen bonding formed between TMN and polymers, with PEC forming stronger hydrogen bonding compared to PVP. Thiamine was found to degrade more when it was present in lower quantities, which has implications for its stability in food systems. The findings of this study should be considered for thiamine fortification applications to ensure its delivery to populations.

3.2 Introduction

Thiamine, also knowns as Vitamin B_1, is an essential vitamin which, therefore, needs to be obtained through the diet. The physiological roles of thiamine in the human body are critical. Thiamine functions included synthesis of neurotransmitters, taking place in energy metabolism of carbohydrates as a cofactor, and being involved in in brain and organ activity (Nath, Shope, & Koch, 2017). It is recommended to take 1.2 mg of thiamine (daily) for both female and male (U.S. Food & Drug Administration, 2018).

Meat, yeast, whole grains, egg yolk, and nuts are good sources for natural thiamine. However, loss in thiamine occurs in the cereal grains due to production of polished rice and white flour. Therefore, in developing countries where more refined products (e.g. polished rice and refined wheat flour) and less meat are widely consumed, such deficiencies are seen at higher rates. In developed countries, although the rate of thiamine deficiency decreases in healthy population, alcoholics and people with Celiac's disease still suffer from thiamine deficiency (Prinzo, 1999). The moderate symptoms of thiamine deficiency starts to be observed in 10 days, which are fatigue, blurred vision, confusion, and weight loss (McCandless, 2009). However, prolonged thiamine

deficiency was reported to result in more severe disorders including Wernicke-Korsakoff syndrome, Leigh syndrome, beriberi, or death (McCandless, 2009).

Thiamine is known as one of the most sensitive vitamins: it is highly affected by heat, light, alkaline pH, presence of some other ingredients, radiation, inorganic bases, and processing treatments (Dwivedi & Arnold, 1973; Mulley, Stumbo, & Hunting, 1975). When thiamine degrades, off-flavors develop due to formation of sulfur containing degradation products (Dreher, Rouseff, & Naim, 2003; Dwivedi & Arnold, 1973; Grosch & Zeiler-Hilgart, 1992). The susceptibility of thiamine to these conditions makes its delivery in processed foods even more challenging.

In order to avoid deficiency, fortification strategies have been developed. Two synthetic crystalline salt forms of thiamine, namely thiamine mononitrate (TMN) and thiamine chloride hydrochloride (TClHCl), have been extensively used for enrichment purposes. These salt forms of thiamine possess different physicochemical features. For example, TMN is less soluble than TClHC; thus, TMN is commonly used for dry products (Labuza & Kamman, 1982).

Both TMN and TClHCl are distributed as crystalline ingredients. However, a recent study conducted in our laboratory showed that TClHCl can be solidified into amorphous form due to its interaction with a variety of polymers (Arioglu-Tuncil, Bhardwaj, Taylor, & Mauer, 2017). Polymers that could form the most extensive hydrogen bonding and/or ionic interactions with TClHCl were found to be most effective crystallization inhibitors. Among them, PEC was observed to be the best one which had ability to interact with TClHCl via hydrogen bonding and ionic interactions. In contrast, PVP acted as the poorest stabilizer for amorphous TClHCl due to lack of intermolecular interactions with TClHCl. Interesting insights into physical stability of amorphous TClHCl were developed and are extended here to TMN for its physical and chemical

stability in amorphous form. It is anticipated that co-formulation of TMN with polymers will result in amorphization of the vitamin, as a result of its interactions with polymeric ingredients in low and moderate moisture food matrices. Moreover, processing treatments (such as lyophilization) could be contributing factors for amorphous TMN formation in complex food matrices. However, the challenge for amorphous compounds is that they are less stable (both physically and chemically) than their corresponding crystalline forms (Hancock & Zografi, 1997). Although TMN is potentially found in amorphous form in the food products, there has been no study conducted to investigate its physical and chemical stability in amorphous form. Therefore, the objectives of this study were: 1) to investigate physical and chemical stability of amorphous TMN in solid dispersions made by PVP and pectin (which were selected based on their T_g, hydrogen bonding ability, molecular structures, and hygroscopicity) and documented performance in solid dispersions with TClHCl at various storage conditions; 2) to compare thiamine degradation in amorphous and crystalline state; and 3) to determine the factors causing chemical instability in amorphous form, and 4) to compare thiamine stability in dispersions made from TMN and to a previous report of those made from TClHCl (Arioglu-Tuncil et al., 2017) (Chapter 2).

3.3 Materials and Methods

3.3.1 Materials

Crystalline thiamine mononitrate (TMN) was purchased from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ). Pectin from citrus peel with a ~61% degree of esterification (PEC) and poly (vinylpyrrolidone) with a molecular weight of 40,000 (PVP) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Eleven-percent RH conditions in desiccators were created using anhydrous lithium chloride (LiCl) purchased from EMD Millipore (Billerica, MA). Sodium chloride (Sigma-Aldrich Inc., St. Louis, MO) was used to prepare 75% RH in desiccators. HPLC

grade trifluoroacetic acid (TFA) and acetonitrile were obtained from Sigma-Aldrich Inc. (St. Louis, MO), and Fisher Scientific Co., LLC (Pittsburgh, PA), respectively. Deionized and purified water was used in the study. Barnstead E-Pure ultrapure water purification system (ThermoScientific, 98 Waltham, MA) was used with a resistivity ~17.5 MΩ·cm for deionization of water.

3.3.2 Preparations of TMN solid dispersions via lyphilization

Initially, vitamin loss was tracked in the dispersions containing the 95:5 mass ratio of TMN to polymers. These dispersions were prepared by dissolving 100 mg total solid in 10 mL of deionized water (w/v). In addition, fourteen different mass ratios of TMN to PVP (1%, 3%, 5%, 7%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) were prepared by varying the TMN:PVP ratios while maintaining the total solids content constant, in order to investigate the effect of vitamin proportion and varying T_gs on the chemical stability. All the samples were prepared in triplicate. TMN: polymer solutions were mixed with a Roto-Shake Genie® SI-1100 (Scientific Industries, Inc., Bohemia, NY) for 10 minutes. The pH of the solutions was measured with an Orion Star A211 pH meter (Thermo Fisher Scientific Inc., Waltham, MA). The solutions were then frozen overnight at -20^{o}C prior to lyophilization. Frozen samples were then loaded into a VirTis Genesis 25ES shelf freeze dryer (SP Scientific, Stone Ridge, NY). Initially, samples were frozen for 6 hours at -40^{o}C and 300 mTorr. Then, the first drying step was initiated in order to remove the bulk of water via sublimation at -40^{o}C by maintaining the vacuum at 150 mTorr for 24 hours. In the secondary drying step, samples were held for 9 hours each at 10^{o}C intervals from -40^{o}C to 20^{o}C. As a last step, the samples were kept at 25^{o}C and 300mTorr for 6 hours. Immediately after the freeze drying cycle was done, samples were placed into the desiccators.

3.3.3 Storage treatments

Samples were stored in desiccators at select temperature and RH conditions. Four different temperatures at the same RH were chosen in order to be able to calculate reaction kinetics of thiamine degradation: 11%RH and 30°C, 40°C, 50°C and 60°C. In addition to these conditions, a higher storage RH (75%) at two different temperatures (25°C and 40°C) was selected to investigate the effect of high RH on thiamine degradation. Saturated salt solutions were used to control the RH in desiccators, and RH values were verified by an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, WA). Temperature conditions were controlled with water jacketed incubators (Forma Scientific, Inc., Marietta, OH) in which the desiccators were placed. Samples were analyzed biweekly for eight weeks and discarded after analysis.

3.3.4 Powder X-ray diffraction (PXRD)

A Rigaku Smartlab™ diffractometer (Rigaku Americas, Texas, USA) equipped with a Cu-Kα radiation source and D/ tex ultra-detector was used to analyze the physical structure of the starting ingredients. Additionally, TMN: polymer solid dispersions stored at select conditions were analyzed as a function of time. Scans were generated between 5 and 40 degrees as a function of 2-theta with an increment of 0.02° and a rate of 4° per minute. Samples where sharp peaks were observed were labeled as crystalline, onset of crystallization when peaks greater than two standard deviations above the baseline were present and samples that exhibited a diffuse halo were considered to be XRD amorphous.

3.3.5 Chemical stability determination with HPLC

A Waters 2690SM (Waters Corp., Milford, MA) HPLC with a Waters Xselect HSS T3 (3.5µm, 4.6x100mm) column and a Waters 2996 photodiode array detector were used for vitamin quantification in crystalline TMN, physical blends of crystalline TMN with polymers, and solid

dispersions. The amount of thiamine remaining was quantified by the standard curve prepared at a concentration range from 0.005 to 1 mg/ mL prior to analysis (r^2=0.9997-1.0000). Samples were diluted with solvent to a final thiamine concentration of 0.25-0.5 mg/mL and filtered through a 0.2 µm syringe filter. Acetonitrile (solvent A) and water with 0.1% TFA (solvent) were used as mobile phases. The following gradient method was adapted from Xia et al., (Xia et al., 2006) and performed as follows: 0/100 at 0 min (immediate), 3/97 at 4 min (linear), 10/90 at 6 min (linear), 0/100 10 min (linear), and 0/100 from 10 to 15 min (immediate). The flow rate and the injection volume used were 1 mL/min and 10 µL, respectively. Samples were scanned between 235-400 nm, and the integration was conducted at 280 nm using Masslynx V4.1 software (Waters Corp., Milford, MA).

3.3.6 Reaction kinetics calculations

Thiamine has been observed to follow first order or pseudo-first order reaction kinetics (Labuza & Kamman, 1982; Pachapurkar & Bell Leonard, 2006; Voelker, Miller, Running, Taylor, & Mauer, 2018; Windheuser & Higuchi, 1962). Experimental data were fitted to the following equation to calculate reaction rate constants:

$$\ln\frac{x}{x_0} = -kt \qquad 3.1$$

where x corresponds to thiamine concentration at t (day), x_0 is the initial thiamine concentration, and k stands for reaction rate constant (days^{-1}). The value of t_{90}, time to where 90% of the initial concentration of thiamine is left, was evaluated using the following equation:

$$t_{90} = \frac{ln\left(\frac{x_0}{0.9x_0}\right)}{k} \qquad 3.2$$

where x_0 is the initial concentration of thiamine and k is the reaction rate constant (days^{-1}).

3.3.7 Moisture sorption isotherm analysis

A SPSx-1 μ Dynamic Vapor Sorption Analyzer (Project Messtechnik, Ulm, Germany) was used to obtained moisture sorption profiles of individual ingredients, physical mixtures, and solid dispersions. Samples (100-300 mg) were loaded into aluminum pans in a 24-ring sample holder. Equilibrium criteria and maximum step time were set at a weight change of 0.001% in 30 min and 12 hours, respectively. Samples were first equilibrated at 0% RH for 12 hours and then analyzed from 5 to 95% RH at 25^{o}C with a 5% RH step size. All the samples were measured in duplicates.

3.3.8 Differential scanning calorimetry (DSC)

Thermal analyses of the samples were conducted using a Discovery DSC equipped with a refrigerated cooling accessory (TA Instruments, New Castle, DE). Nitrogen served as the purge gas at a rate of 50 mL/min. Approximately 7-12 mg of sample was weighted and sealed into Tzero pans (TA Instruments). Samples were heated from -20^{o}C to a temperature 20-30^{o}C higher than the expected T_g values at a rate of 20^{o}C/min, followed by cooling to -20^{o}C at a rate of 10^{o}C/min. Then, the second heating scan was applied to 150^{o}C at 20^{o}C/min. T_g was determined in this second heating step (where a baseline shift occurred in the endothermic direction) using TRIOS software by drawing a tangent on the straight line and also on the slope. Then, cross point was calculated by TRIOS software (TA Instruments). In addition, onset T_g' of solutions was measured to test the efficiency of the lyophilization process. For this purpose, approximately 25 μL of pre-lyophilization solutions were pipetted into Tzero pans and sealed (TA Instruments). Solutions were first cooled to -80^{o}C, held for 5 min at this temperature, followed by heating to 0^{o}C at a rate of 10^{o}C/min.

3.3.9 Fourier transform infrared spectroscopy (FTIR)

A Nexus 670 FTIR spectrometer (ThermoNicolet, Madison, WI) equipped with an MCTA detector and DRIFTS Avatar Diffuse Reflectance Smart Accessory (ThermoElectron Corp., Madison, WI) was used to collect FTIR spectra of samples, as previously described by Arioglu-Tuncil et al., (2017). The spectra of samples were analyzed using OMNIC software (ThermoElectron Corp., Madison, WI).

3.3.10 Statistical analysis

All analyses were performed in triplicate, unless otherwise noted, and data are presented as mean±SD. SAS Software Version 9.4 (SAS Institute, Cary, NC) was used to conduct statistical analyses. Analysis of variance (ANOVA) was performed at $\alpha = 0.05$ significance level to determine differences among the samples and controls. Tukey's multiple comparison test ($\alpha=0.05$) was used to test whether the samples were statistically different.

3.4 Results and Discussion

3.4.1 Physical state stability of TMN in different systems and environmental conditions

TMN was successfully amorphized in the presence of 95% of PVP and PEC (**Fig. 3.1**). The physical stabilities against crystallization of these dispersions stored at 11% RH (30^{o}C, 40^{o}C, 50^{o}C, and 60^{o}C) and 75% (25^{o}C and 40^{o}C) for eight weeks are reported in (**Figs. 3.2-4**). Regardless of the storage conditions studied, 5TMN:95PEC solid dispersions remained amorphous during the time frame of the study (**Fig. 3.2**). Similarly, 5TMN: 95PVP solid dispersions stored at 11% RH and various temperatures (30^{o}C, 40^{o}C, 50^{o}C, and 60^{o}C) maintained their amorphous structures for eight weeks (**Fig. 3.3**). However, 5TMN: 95% PVP dispersions formed solution when they were stored at 75% RH and 25^{o}C and 40^{o}C. This is mainly due to moisture absorption into the bulk

structure at this high RH condition. No evidence of TMN crystallization was found in any sample containing 5TMN:95polymer.

In addition to these dispersions, a new set of samples containing fourteen different ratios of TMN to PVP and PEC were prepared (**Figs. 3.4A-B**), and stored at 11% and 60ºC. Their physical structures were monitored overtime for eight weeks. It is worth noting that, unlike the thiamine chloride hydrochloride (TClHCl) in which minimum amount of PVP and PEC required for amorphization was 60% and 40%, respectively (Arioglu-Tuncil et al., 2017), at least 90% of PVP (**Fig. 3.4A**) and 80% of PEC were necessary to form amorphous TMN in the solid dispersions (**Fig. 3.4B**). This clearly indicates that TMN has higher crystallization tendency, compared to TClHCl. FTIR spectra showing the hydrogen bonding interactions between TClHCl and polymers were evident in crystalline lattice disruption and amorphous TClHCl formation, as further confirmed by PXRD (Arioglu-Tuncil et al., 2017). Similarly, to be able to amorphize TMN, enough energy was required to disrupt molecular interactions between crystalline TMN molecules. Since PEC was able to form stronger hydrogen bonding interaction with TMN (which was discussed in FTIR section), when it was present in lower quantity compared to PVP, less PEC was required to form amorphous TMN. The significance of hydrogen bonding interactions between compounds to create amorphous solids was also shown in an independent study (Wang, Pellerin, & Lebel, 2009). PVP successfully inhibited the crystallization of amorphous TMN in dispersions prepared using ≤ 10% of TMN at 11% RH and 60ºC for eight weeks (**Fig. 3.4A**). Note to here that, peak locations of PXRD patterns of TMN:PVP and TMN:PEC dispersions when thiamine crystallization occurred were matching. Maintaining the initial amorphous form of solid dispersions (5TMN:95polymer) at all temperature conditions applied at 11% RH and at any ratio allowed us to investigate the effect of physical structure (crystalline vs. amorphous states) on the

chemical stability of TMN successfully. The TMN was amorphous in the solid dispersions but crystalline in the physical blends.

3.4.2 Moisture sorption profiles

The moisture sorption profiles of TMN, PEC, and PVP are illustrated in **Fig. 3.5**. TMN is a deliquescent crystalline compound with a RH_0 of 98.5% (Hiatt, Ferruzzi, Taylor, & Mauer, 2008). Since this value is above the measurable RH by the equipment, the moisture uptake by TMN was limited to surface adsorption and capillary condensation (**Fig. 3.5),** since TMN is not known to form crystal hydrate. Both PVP and PEC showed sigmoidal shape isotherm, which is a characteristic of an amorphous compound (Sing et al., 1985). Based on the moisture sorption profiles, there was no difference observed between the polymers for the amount of moisture sorbed until 30% RH and PVP sorbed more moisture than PEC under the conditions where the RH > 30%. For example, PVP absorbed 15% more moisture than PEC at 75% RH, which was one of the conditions selected to store the samples for the HPLC experiment described below (give section number). Moreover, as expected, the differences in moisture uptake between the polymers increased with an increasing RH% (**Fig. 3.5**)

The moisture sorption isotherms of TMN solid dispersions (5:95) were also compared to each other in **Fig. 3.6**. Similar to polymers, TMN-polymer dispersions showed a sigmoidal shape for moisture sorption due to their amorphous characteristics. The solid dispersions followed the same trend as polymers, with 5TMN: 95PVP dispersions sorbing significantly more moisture than 5TMN: 95PEC dispersions at the RHs above 45% RH. Specifically, 5TMN: 95PVP dispersions absorbed 12% more moisture than 5TMN: 95PEC dispersions at 75% RH. In 5TMN:95PVP dispersions, increment in sorbed moisture at a specific RH compared to previous RH step was between 1-2% until 50% RH and increased over 50% RH. For example, 5% moisture was sorbed

as RH increased from 70% to 75% RH. Dissolution of compounds might be induced due to high amount of moisture gain into the bulk structure.

Since physical mixtures of crystalline TMN with the polymers were used as a second control for the HPLC analyses, the moisture sorption profiles of the physical blends were also measured (**Fig. 3.7**) and compared with the polymers and dispersions in **Figs. 3.8**. Sigmoidal moisture sorption isotherms were observed for the physical blends, although TMN was in the crystalline state. This might be attributed to the high proportion of amorphous polymers used. There was no difference found between polymer of PVP, 5TMN: 95PVP physical blends and the corresponding dispersions in terms of the moisture uptake. In other words, their moisture sorption profiles were almost identical as shown in **Fig. 3.8A.** Similarly, moisture uptakes at all the RH conditions studied were quite similar for polymer of PEC, 5TMN: 95PEC physical blends and dispersions, with the RHs of 35%-90% being exceptions, where solid dispersions absorbed 1-6% more moisture than PEC and TMN:PEC physical blends (**Fig. 3.8B)**. These results suggest that moisture sorption of the dispersions and blends tested in this study was mainly governed by the polymers, since they were used in higher quantities relative to TMN.

Overall, significantly more moisture was absorbed by PVP, its physical blends, and solid dispersions with TMN, compared to those of PEC samples, especially at high RHs. On the other hand, there were no differences for the weight gain in the physical blends and dispersions of PVP, throughout all the RHs tested, indicating that more hygroscopic nature of amorphous TMN compared to crystalline TMN was overshadowed by PVP. In contrast, the effect of hygroscopicity of amorphous TMN was more evident in TMN:PEC dispersions compared to its physical mixtures. Under the light of these findings, any significant differences, if found, in thiamine degradation between solid dispersions and physical blends of the same polymer would not be mainly due to

the difference in moisture sorbed by these samples, unless TMN goes into solution. Moreover, the effect of varying water contents between polymer types on thiamine degradation could have more apparent particularly at higher RHs (>30%).

3.4.3 Glass transition temperature

The glass transition temperatures (T_g) of 5TMN: 95PVP and 10TMN: 90PVP amorphous solid dispersions as well as 5TMN: 95PVP solid dispersions equilibrated at 11% RH (30^{o}C and 60^{o}C) are provided in **Table 3.1**. The T_gs of PVP and PEC were reported as 134^{o}C ± 0.2 and 90 ^{o}C ± 2, respectively (Arioglu-Tuncil et al., 2017). Attempts to amorphize TMN in the absence of a polymer failed using the freeze drying technique performed here. Therefore, the T_g of TMN alone could not be determined in this way. T_g' of TMN solutions might be a contributing factor for the failure of TMN amorphization alone, if it is lower than the temperature of the first drying step of freeze drying method (-40^{o}C) used in this study. Similar to T_g, attempts to measure T_g' of TMN solutions in the absence of a polymer were unsuccessful. However, it is noteworthy here that the T_g' of PVP, 1TMN:99PVP, 5TMN:95PVP, and 10TMN:90PVP dispersions were measured to be -25.3±0.1^{o}C, -23.3±0.1^{o}C, -26.7±0.3^{o}C, and -25.4±0.4^{o}C respectively (**Figs. S3.10**), suggesting that the samples were exposed to temperatures below their T_g' in the primary drying step which was -40^{o}C. However, the T_g' values of 80TMN:20PVP, 90TMN:10PVP, and 100TMN solutions could not be measured (**Figs. S3.10**). Using a melt quench method to measure the T_g of TMN could be an option, if it did not degrade with melting (T_m: 196-200^{o}C) (Macek, Feller, & Hanus, 1950). Beaman proposed a rule, called the Boyer-Beaman rule, which states that the T_g of a compound usually equals to 2/3 of its melting temperature in Kelvin (Beaman, 1952). The T_g of TMN was calculated to be 40^{o}C by applying Boyer-Beamen rule.

Since T_g of PVP is well above the estimated T_g of TMN and the amount of PVP is higher in the solid dispersion, based on the Fox and Gordon-Taylor equations (Fox, 1956; Gordon & Taylor, 1952), the T_g of solid dispersion needs to be closer to that of PVP. The low T_g values of 5TMN: 95PVP dispersions measured immediately after lyophilization was caused by the remaining moisture in the samples during lyophilization (**Table 3.1**). Since, the samples were not exposed to any additional drying step before they were placed into the storages for long term physical and chemical stability analyses (over 8 weeks), it is important to report T_g values 'as is' after lyophilization.

Strong correlation between solid state stability and T_g was proposed by several authors, due to the fact that T_g is associated with molecular mobility (Hancock & Zografi, 1997; Slade & Levine, 1991). However, this general assumption of T_g being the most important factor that dictates the chemical stability failed for small compounds in many publications (Bell & Hageman, 1994; Lai, Hageman, Schowen, Borchardt, & Topp, 1999; Luthra, Shalaev, Medek, Hong, & Pikal, 2012; Sanchez, Ismail, Christina, & Mauer, 2018). For example, an independent study conducted in our laboratory showed that the chemical stability of ascorbic acid decreased as T_g of ascorbic acid: PVP dispersions increased when samples were stored at 60^{o}C and 11% RH (Sanchez et al., 2018), indicating that chemical stability of small compounds could not be predicted by T_g. It is important to note here that the storage temperature was above the T_g of ascorbic acid on its own (40.1°C ± 3.6^{o}C) (Sanchez et al., 2018).

In the ratio study (TMN: PVP, conducted at 11% RH and 60^{o}C), only T_gs of 5TMN: 95PVP and 10TMN: 90PVP were measured and reported in **Table 3.1,** since the dispersions containing ≥20 TMN were found in crystalline form. As PVP proportion increased in the dispersion, T_g also increased (**Table 3.1**). If the T_g is the key factor for chemical stability, the samples containing

higher ratio of PVP are likely to be chemically more stable, which will be discussed in the next section. In addition, it was observed that the 5TMN: 95PVP dispersions stored at 11% RH and 60^{o}C were stored in conditions above the T_g (**Table 3.1**) and its effect on stability will be discussed in the next section.

3.4.4 Vitamin quantification with HPLC

Degradation of thiamine as a response to various environmental conditions was first studied in the crystalline form of TMN in the absence of a polymer (control) for eight weeks (**Fig. 3.9A)**. Regardless of the storage RH% and temperature, no significant degradation was found in the control samples throughout the study (8 weeks) (**Fig. 3.9A**). This could be attributed to the insufficient moisture gain (**Fig. 3.5**) to degrade thiamine, which was limited to adsorption and capillary condensation below the RH_0 of TMN (98.5% at 25^{o}C) (Hiatt et al., 2008). Thus, we concluded that the effects of RH and temperature (studied here) on vitamin loss in crystalline TMN were negligible.

In order to evaluate the influence of a polymer present in the same environment with crystalline TMN, its physical blends with 95%PEC and 95%PVP were stored at 11% RH (40^{o}C and 60^{o}C) and 75% RH (40^{o}C) for 8 weeks and remaining thiamine was measured biweekly (**Fig. 3.9B**). Thiamine loss was not significant in TMN physical blends composed of PEC and PVP throughout the study, with PVP blends stored at 75% and 40^{o}C being the exception. There was 60% thiamine loss was observed in PVP blends stored at 75% and 40^{o}C. This could be attributed to the very hygroscopic nature of PVP that caused absorption of enough amount of moisture to dissolve TMN partially at 75% RH (**Fig. 3.9B**). Due to partial dissolution of crystalline TMN, thiamine degradation proceeded faster and 60% of vitamin loss was observed after 56 days of storage (thiamine degrades more in solution).

Thiamine degradation was further monitored in 95% PEC and 95% PVP solid dispersions at 11% RH and four different temperatures (30°C, 40°C, 50°C and 60°C) (**Figs. 10A-B**) in which thiamine remained in amorphous form during the time frame of the study (**Figs. 2-3**). No significant degradation was found among TMN amorphous dispersions made by PEC (**Fig. 10A**). Similarly, thiamine was also quite stable in PVP dispersions at 11% RH and lower temperature conditions (30°C and 40°C), where only 5-8% degradation was observed on day 56 (**Fig. 10B**). On the other hand, as temperature increased, the proportion of thiamine degraded increased; for example, nearly 12% of thiamine degraded at 11% RH at 50°C after 56 days of storage. Even more severe thiamine degradation (23%) was observed when the storage temperature was further increased to 60°C. This dramatic increase in thiamine loss at 60°C, compared to other storage temperatures (≤50°C) was attributed to T_g. The 5TMN: 95PVP amorphous solid dispersions were observed to be present above the T_g at 11%, but no crystallization found above T_g. RH and 60°C, but below the T_g when stored at 11%RH and 50°C **(Table 3.1**), because amorphous compounds are known to be less chemically stable in the storage conditions below their T_gs.

Thiamine stability was also investigated at higher RH conditions (75% RH and 25°C and 40°C) for eight weeks (**Fig. 3.10C**). Here again, thiamine was found to be more stable in amorphous dispersions made by 95% PEC, compared to PVP. For example, only 10% of thiamine degraded in PEC dispersions stored at 75% RH and 40°C for eight weeks; however, thiamine degradation proceeded more rapidly in 5TMN: 95PVP samples at 75% RH and both temperatures studied: within only two weeks, 87% and 92% of thiamine degradations were observed at 25°C and 40°C, respectively. After this two week period, thiamine continued to be degraded in a relatively small quantity. At the end of eight weeks, remaining thiamine was 10% and 6% at 25°C and 40°C, respectively, at 75% RH. These dramatic increases in thiamine degradations were associated with

full dissolution of TMN in the 95% PVP dispersions due to significantly high amount of moisture gained (**Fig. 3.6**).

In addition to dispersions explained above, PVP dispersions composed of various ratios of TMN were prepared and stored at 11% RH and 60ºC for 56 days (**Fig. 3.11**) in order to investigate the effects of thiamine ratio present in the dispersions on the stability of thiamine. PVP was chosen for this part of the study, since thiamine degraded more in PVP dispersions than in PEC dispersions. Throughout the storage, the samples were analyzed weekly for physical state stability using PXRD and biweekly for chemical stability using HPLC. Our results revealed that the minimum amount of PVP needed for amorphization of TMN was 90% (**Fig. 3.3**). Thus, studying the samples containing 10% or less TMN could provide detailed information for thiamine degradation in amorphous state, but the rest for thiamine degradation in crystalline state. Moreover, such samples would allow us to compare the effects of physical state (amorphous vs crystalline) on chemical stability of thiamine. Regardless of the PVP ratio, thiamine present in the crystalline solid dispersions was not significantly degraded over storage ($p < 0.05$) (**Fig. 3.11**); specifically, only 2-5% of thiamine degradation was observed in these the dispersions in which thiamine had crystallized after 56 days of storage, with 20TMN:80PVP dispersions being an exception, wherein 8% of thiamine degraded and it was statistically significant ($p <0.05$) compared to other crystalline dispersions prepared $\geq$ 40% TMN. This could be due to having some proportions of thiamine present still amorphous. In contrast to crystalline TMN:PVP samples, in amorphous dispersions that are composed of at least 90% PVP, more thiamine degradation was found (**Fig. 3.11**). Specifically, 11% of thiamine degraded in the 10TMN: 90PVP amorphous dispersions on day 14, which further increased to 17% on day 56. Interestingly, our result revealed that more thiamine was likely to degrade when the proportion of thiamine in the amorphous

dispersions decreased; for instance, 23-26% of thiamine degraded in the samples containing 3-7% of TMN after 56 days of storage (**Fig. 3.11**). Moreover, vitamin loss occurred even more rapidly in amorphous solid dispersions containing only 1% TMN; for example, on day 14, 24% of thiamine was lost in this sample, while the degraded amount of thiamine reached to 34% at the end of 56 days (**Fig. 3.11**).

Thiamine is known to degrade in a first-order or pseudo-first order reaction rate (Labuza & Kamman, 1982; Pachapurkar & Bell Leonard, 2006). Based on the Equation 2, reaction rate constants were calculated for the samples with different TMN and PVP ratios **(Table 3.2)**. Experimental data obtained from the samples containing 20% or less TMN fitted the first order kinetic model with high R^2 values. Since the other samples degraded very slowly, the model could not be applied for them. It was found that reaction rate constants slightly increased when PVP proportion increased from 80% to 99% in the dispersion **(Fig. 3.12)**. In addition, as the PVP ratio decreased to 80% where TMN was in the crystalline form, the t_{90} (time where 90% of the initial concentration of thiamine left) value dramatically increased (**Table 3.2)**, indicating that the samples with crystalline TMN were more stable than samples containing amorphous TMN. The reason of having more degradation as the amount of PVP increased in amorphous samples cannot be explained by T_g. It is important to note here again that (as explained before), 5TMN:PVP dispersions were found to be less chemically stable when stored at 11% RH and 60^{o}C than the samples (5TMN:95PVP) stored at lower temperatures (11% RH and 30-50^{o}C). It was attributed to their T_g values (51.5^{o}C) which were below the highest storage temperature (60^{o}C). Although T_g had an impact on chemical stability in these samples, general assumption of its effect on stability ('samples with higher T_gs are prone to be more stable') could be misleading, particularly if the samples compared to each other are not identical. In other words, this assumption is more valid for

identical samples, prepared by same polymer: small compound and proportions, when they are stored at different temperatures (50^{o}C vs. 60^{o}C). However, if the parameters are different (TMN: PEC dispersions vs. TMN: PVP dispersions or 5TMN:PVP dispersions vs. 1TMN:99PVP dispersions), it needs to be always remembered that another factors could be more determinant than T_g only (for example, intermolecular interactions) on stability. As PVP proportion increased, the T_g of dispersions increased; however, it did not result in greater stability of TMN against degradation. Similar results were obtained by Sanchez et al., (Sanchez et al., 2018) for degradation of amorphous ascorbic acid in different ratios of PVP dispersions. Ascorbic acid was found to be more labile when a higher amount of PVP was used in the dispersions. This could not be attributed to T_g phenomena, since the T_g of dispersions was higher in the presence of high proportion of PVP; instead, this observation was explained by a kinetic model proposed by Waterman et al. (Waterman, Gerst, & Dai, 2012). It is proposed in the model that the degradation rate of a drug is affected by the amount of excipient used, only if the drug is found in an excess number of excipients particles. If this happens, the degradation rate of the drug is significantly increased. On the other hand, its degradation rate is not dependent on the number of excipient particles, when the number of drug particles are comparable to that of excipient (Waterman et al., 2012). The reason behind this phenomena is that greater surface area of contact between drug and the excipient increases the degradation rate of the drug (Waterman et al., 2012). The observations made by Sanchez et al. (2018), and the results obtained in this study are also in line with another study which was the interest of this dissertation (**Chapter 2**).

The findings of this study clearly support the hypothesis that thiamine degrades more when it was in the amorphous state and chemical stability of thiamine was highly dependent on polymer type. T_g had an impact on thiamine degradation when stored at temperature conditions above the

T_g, however it was not a determinant factor dictating the stability. Hygroscopicity of polymers was important only at higher RH conditions (75% RH), however it failed to explain stability differences between PEC and PVP dispersions at low RHs.

3.4.5 Spectroscopic investigation of hydrogen bonding interactions between TMN and polymers in solid dispersions

In many studies, hydrogen bonding occurred between a small compound and polymer has been hypothesized to be the most important stabilizing factor for physical stability of solid dispersions against recrystallization (Arioglu-Tuncil et al., 2017; Christina, Taylor, & Mauer, 2015; Kestur, Van Eerdenbrugh, & Taylor, 2011; Miyazaki, Yoshioka, Aso, & Kojima, 2004; Wegiel, Zhao, Mauer, Edgar, & Taylor, 2014). The hydrogen bonding ability of a compound was known to be highly dependent on its hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) groups. In our previous study, the physical stability of amorphous TClHCl dispersions prepared with several different polymers (including PEC and PVP) was reported to be governed by hydrogen bonding and/or ionic interactions formed between TClHCl and polymers, and the potential HBD and HBA groups of compounds which could involve in hydrogen bonding were provided (Arioglu-Tuncil et al., 2017). More specifically, the largest peak shifts to a lower wavenumber in the OH/NH and carbonyl region of FTIR spectra, which is the indication of intermolecular hydrogen bonding between compounds, were observed between TClHCl and PEC in the amorphous solid dispersions. In contrast, there was no evidence found for hydrogen bonding interaction between PVP and TClHCl. Thus, PEC was found to be one of the best crystallization inhibitors due to strong hydrogen bonding interaction formed between TClHCl and PEC, whereas PVP was found to be the worst one due to the lack of hydrogen bonding between TClHCl and PVP (Arioglu-Tuncil et al., 2017). These findings led us to believe that possible hydrogen bonding might occur between TMN and PEC, but not between TMN and PVP, and this interaction could

physically and chemically stabilize thiamine against degradation when TMN is dispersed into matrices of PEC.

In order to test our hypothesis, spectroscopic investigations of hydrogen bonding interactions between TMN and polymers in solid dispersions were performed using FTIR (**Figs. 3.13)**. The crystalline TMN possesses three distinct peaks in the OH/NH region which were located at 3329 cm^{-1}, 3141 cm^{-1}, and 3047 cm^{-1}, and several other peaks were seen in the carbonyl region of the TMN spectrum (**Fig. 3.13B**). Since TMN does not carry a carbonyl group in its structure, those peaks are not associated with any carbonyl group. Peak shifts towards lower wavenumbers relative to FTIR spectrum of polymer in the OH/NH and/or carbonyl region are indicators of hydrogen bonding formation between TMN and polymers in the dispersions. The maximum peak in the hydroxyl region of PEC occurred at 3419 cm^{-1} and this peak shifted to 3363 cm^{-1} once amorphous TMN was formed in the solid dispersions (20TMN:80PEC) **(Fig. 3.13A)**. Moreover, distinct peaks located in the OH/NH region of the TMN spectrum disappeared upon amorphous TMN formation, and, instead, a broad peak was formed. Similarly, crystalline TMN: PEC dispersions showed similar peaks as TMN itself in the carbonyl region of FTIR spectra of TMN:PEC dispersions (**Fig. 3.13B**). When amorphization of TMN was achieved in 20TMN:80PEC dispersions, these distinct peaks disappeared. PEC showed two peaks in the carbonyl region which were located at 1743 cm^{-1} and 1622 cm^{-1} (**Fig. 3.13B**). The first peak (1743 cm^{-1}) stayed in the same location, indicating that it was not involved in hydrogen bonding formation with TMN. However, the second peak (1622 cm^{-1}) shifted to 1606 cm^{-1} and 1610 cm^{-1} in the amorphous 20TMN:80PEC and 5TMN:95PEC dispersions, respectively. The peak shifts occurred in the carbonyl region of TMN:PEC dispersions can be attributed to hydrogen bonding formation between TMN and PEC in amorphous dispersions. Unlike PEC, the only functional

group of PVP that can participate in hydrogen bonding is the amide carbonyl group, which gives absorption in the carbonyl region of FTIR spectrum (1669 cm^{-1}) **(Fig. 3.13C)**. However, the peak in the carbonyl region of PVP either shifted to a higher wavenumber (which indicates less interaction between TMN and PVP, and more interaction between PVP molecules) or remained in the same location relative to FTIR spectrum of PVP, meaning that there was no strong intermolecular hydrogen bonding occurred between PVP and TMN.

Based on the FTIR results, stronger hydrogen bonding interaction formation between TMN and PEC was found to be the key factor to ensure physical and chemical stability of amorphous thiamine due to the reduced molecular movement.

3.4.6 Comparison of thiamine stability in TMN and TClHCl solid dispersions

The minimum PEC and PVP amount needed to amorphize TMN and TClHCl was compared in **Fig. S3.1**. TMN required more polymer than TClHCl for amorphization regardless of the polymer type used. For example, 40% and 60% of PEC and PVP were enough to amorphize TClHCl, respectively, whereas at least 80% PEC and 90% PVP were required to create amorphous TMN (**Fig. S3.1**). The nature of counterion leads to a different crystal lattice packing, thus physicochemical properties of different salt forms of the same compound vary (David, Timmins, & Conway, 2012). The reason of TMN to require more polymer than TClHCl for amorphization might be due to having a different counterion which could lead to a stronger crystal lattice. When the crystal lattice is stronger, it is more difficult to break. For this reason, more polymer might be required to disrupt hydrogen bonding between TMN molecules and form new hydrogen bonding with polymers which results in amorphization of TMN. Compared to PVP, PEC acted as a better stabilizer meaning that PEC was likely to show a better performance for maintaining the physical and chemical stability of amorphous thiamine over the storage. When the less polymer was needed

for amorphization, that polymer was likely to show a better performance for physical and chemical stability. A similar observation was made by Wegiel et al., for naringenin and quercetin (Wegiel, Mauer, Edgar, & Taylor, 2013), where E100 was observed to be a better crystallization inhibitor than PAA for amorphous naringenin and quercetin. Amorphization of naringenin and quercetin were achieved when 15% and 30% E100 were used, respectively, while at least 70% and 80% PAA were needed (Wegiel et al., 2013). In addition, the performance of PEC and PVP on 5TMN:95polymer and 5TClHCl:95polymer solid dispersions as crystallization inhibitors were same (all the samples remained amorphous) when the samples were stored at 11% RH and 30^{o}C-60^{o}C for 8 weeks. Similarly, both TMN and TClHCl solid dispersions prepared by various ratios of PVP maintained their initial physical status by eight weeks. Based on the observation made in this part of the study, the general take home message is that the type and the amount of the polymer have a significant impact on amorphization and amorphous stability against crystallization. Moreover, different salt forms of a compound could show quite different responses to presence of different types of polymers, mainly due to differences in extent of hydrogen bonding between the compound and polymer.

As seen from the moisture sorption profiles (**Fig. S3.2)**, TClHCl is more hygroscopic than TMN, which is the main reason of more widespread use of TMN in dry products. The deliquescence points (RH_0) of TClHCl and TMN are 88% RH (**Fig. S3.2**) and 98% RH (Hiatt et al., 2008), respectively. On the other hand, the moisture sorption behavior of TMN and TClHCl solid dispersions were similar to each other across all the RHs studied since both TMN and TClHCl were initially amorphous and the polymer amount used in both dispersions were quite high **(Fig. S3.3A**). The contribution of amorphous TClHCl and TMN on moisture gain in 95% polymer solid dispersions were compared in **Fig. S3.3B**. Weight gain due to amorphous vitamin did not exceed

1% up to 70% RH, regardless of the polymer type, with 5TClHCl:95PVP dispersions being exception wherein phi value was calculated to be 1.4% at 60% RH. As RH increased above 70%, it was observed that amorphous TClHCl contributed moisture sorption more than amorphous TClHCl. Thus, amorphous TClHCl was found more hygroscopic than amorphous TMN, as they were in their crystalline form. In general, due to high proportion of polymer present in the dispersions (95%), phi values were not higher than 8%. Similar to solid dispersions, the weight gain of crystalline TMN:polymer and crystalline TClHCl:polymer physical mixtures were same up to deliquescence point of TClHCl and after that point TClHCl:polymer mixtures gained 5-7% more moisture than TMN:polymer physical blends. Hygroscopicity is a very important characteristic of an amorphous solid for both physical and chemical stability. However, based on the moisture sorption data provided here, it can be stated that the difference between chemical stability of TMN and TClHCl could not be directly related to hygroscopicity, unless solution formation occurs.

There was no significant difference found between crystalline TMN (control) and TClHCl (control) for thiamine degradation at 75% RH and 40^{o}C throughout 56 days of storage (**Fig. S3.5A**). Similarly, thiamine loss was not significant in the physical blends of TMN and TClHCl with the polymers stored at 11% RH and 60^{o}C on day 56, regardless of the polymer type. On the other hand, significantly ($p<0.05$) more thiamine degraded in the physical blends of PVP with crystalline TMN than that of with crystalline TClHCl stored at 75% RH and 40^{o}C during eight weeks of storage (**Fig. S3.5A**). It is important to note here again that partial dissolution of TMN and TClHCl were observed in their physical blends with PVP at 75% RH and 40^{o}C due to moisture absorption; and thiamine degradation was highly affected by this circumstance. For example, 19% of thiamine degraded in the 5TClHCl:95PVP physical blends on day 42, while thiamine loss was

32% in the 5TMN:95PVP physical blends. The difference in thiamine loss between TMN and TClHCl could be attributed to difference in pH of the samples, which were measured as 3.8± 0.04 and 5.22± 0.02 prior to lyophilization for 5TClHCl:95PVP and 5TMN:95PVP solutions, respectively (in 10 mL of H_2O at 25^oC). Voelker et al., (2018) investigated TMN and TClHCl degradation in solution state as a function of temperature and concentration. No significant thiamine degradation in TClHCl was found at 40^oC during 174 days of storage at any concentration studied. On the other hand, remaining thiamine amount in TMN solutions were between 44-87% at 40^oC on day 63 and was highly dependent on concentration of the solutions. When comparing the data obtained in this dissertation with the study conducted by Voelker et al., (2018), it can be stated that thiamine degradation was not affected only by pH in the solutions, but also existence of another compound in solution had a prominent impact. The HPLC chromatograms of 5TMN:95PVP and 5TClHCl:95PVP physical mixtures were presented in **Fig. S3.8**. The retention time of thiamine in 5TClHCl:95PVP physical blends were 4.51 min, and there was no any other compounds detected (**Fig. S3.8A**). In contrast, in addition to thiamine peak (retention time of 4.45 min), 5TMN:95PVP physical mixtures were shown to have some degradation compounds which were detected at the retention times of 3.08, 3.85, and 5.47 min **(Fig. S3.8B**). A similar observation was made in the solid dispersions of 5TMN:95PVP and 5TClHCl:95PVP stored at 75% RH and 40^oC (**Fig. S3.5B)** where thiamine degradation was significantly more in 5TMN:95PVP dispersions than 5TClHCl:95PVP dispersions. For instance, thiamine remaining was 68% in 5TClHCl:95PVP dispersions at the end of 28 days of storage, whereas only 7% thiamine remained in 5TMN:95PVP dispersions on day 28 (**Fig. S3.5B**). The hygroscopic nature of PVP and initial amorphous form of TMN and TClHCl led to absorpsion of considerable amounts of moisture in which fully dissolution of TClHCl and TMN were occurred. Similar to physical blends, the

difference for thiamine degradation in dispersions made by PVP was also associated with difference in pH. HPLC chromatogram of 5TClHCl:95PVP dispersions showed some degradation products with very small quantity at the retention time of 3.45, 4.27, and 6.01 (**Fig. S3.9A**). On the other hand, the quantity and number of degradation compounds occurred in 5TMN:95PVP dispersions were higher and they appeared at 2.84, 3.33, 4.12, 5.81, 7.26, and 8.34 min (**Fig. S3.9B**). The difference in HPLC chromatograms of 5TMN:95PVP and 5TClHCl:95PVP dispersions and physical blends confirmed that thiamine degradation was highly dependent on the pH. TMN samples possessed higher pH than TClHCl samples due to nature of the counterion, which further induced thiamine degradation (thiamine is known to be less stable in higher pHs). Although the difference in pH values between 5TMN:95PVP and 5TClHCl:95PVP samples mentioned here were obvious, it is also important to note here that the reported pH values belong to TMN and TClHCl solutions with PVP prior to lyophilization at 25^{o}C. Thus, the samples mentioned here could have slightly different pHs than the reported values, since the concentration and the temperature were not the same. The effect of pH on thiamine degradation in TMN and TClHCl solutions were reported by Voelker et al. (Voelker et al., 2018). It was found that thiamine stability depended on the salt form of thiamine, being more stable in the solutions of TClHCl than that of TMN in all the temperature conditions studied, which was attributed to more acidic pHs of TClHCl solutions (Voelker et al., 2018). Nature of counterion is known to influence several properties of a compound (David et al., 2012). Thus, different salt forms of the same compound are likely to have different properties (such as melting point, solubility, hygroscopicity, and pH) mainly due to having different counterion in the crystal lattice. There are some studies showing the influence of counterion on the degradation kinetics by accounting for the pH (which differs due to nature of counterion) (Badawy, 2001; Guerrieri, Jarring, & Taylor, 2010). The significant

difference between 5TMN:95PVP and 5TClHCl:95PVP samples for thiamine degradation at 75% RH and 40^{o}C (where solution formation occurred) was attributed to different environmental pH, created by counterion. Chemical stability of thiamine was also compared in TMN and TClHCl solid dispersions made by PEC and PVP at 11% RH and 30-60^{o}C (**Figs. S3.6A-B**) and thiamine degradation was not significantly different between any TMN and TClHCl dispersions treated with the same condition (**Figs. S3.6A-B**). When the dispersions of TMN prepared with various proportions of PVP were compared to TClHCl:PVP dispersions at 11% RH and 60^{o}C, it was observed that the difference in thiamine degradation was between 1-3% during 56 days of storage in the samples that thiamine was found in amorphous structure in both salt form of thiamine (from 90% PVP to 99% PVP) (**Figs. S3.7**). When PVP proportions were between 60-80% where thiamine was found in crystalline form in TMN dispersions, but in amorphous form in TClHCl dispersion, 6-7% of more thiamine was degraded in TClHCl dispersions on day 56 compared to crystalline TMN dispersions.

In general, both salt forms of thiamine showed a similar degradation behavior in the solid form and polymer type had the same impact on thiamine stability in both salt forms. In addition, hygroscopicity was more important when the samples were stored at higher RH conditions; the effect of different salt forms on thiamine degradation was more noticeable when thiamine dissolved in the system partially or fully, which was primarily affected by pH.

3.5 Conclusion

Amorphization of TMN was achieved when at least 80% of PEC and 90% of PVP were used and both polymers were effective at inhibiting TMN crystallization in solid dispersions at 11% RH (30-60^{o}C). In addition, PEC inhibited the crystallization of TMN in solid dispersions, when stored at even higher RH and temperature conditions (75% RH and 25^{o}C and 40^{o}C). No

significant thiamine loss was found in crystalline TMN and physical blends of crystalline TMN with the polymers. In contrast to crystalline samples, thiamine degradation was accelerated when it was in the amorphous form. Polymer type highly affected the stability of amorphous thiamine where it was chemically more stable in PEC dispersions than PVP dispersions at low RH conditions. This could not be attributed to hygroscopicity of polymers, since no significant difference was found between amount of moisture absorption by PEC and PVP dispersions at 11% RH. Similarly, chemical stability of amorphous thiamine in PEC dispersions could not be correlated with T_g of polymers (PVP possess higher T_g than PEC). On the other hand, stronger hydrogen bonding interaction between TMN and PEC was proved by FTIR, indicating that intermolecular interaction formation between TMN and polymers was the main and most important stabilizing factor against degradation of amorphous thiamine. In addition, significantly more amorphous thiamine degraded in the dispersions containing higher amount of PVP, which was attributed to greater surface area of contact between TMN and PVP. The findings of this study will improve the mechanistic understanding of a variety of factors, from least effective to most effective one (including polymer type, polymer proportion, storage conditions, intermolecular interaction ability of polymers, hygroscopicity, and T_g), on physical and chemical stability of thiamine in complex food matrices. Thus, these parameters need to be taken into the account to design food formulations to improve the quality.

Table 3.1 Onset glass transition temperatures of solid dispersions after lyophilization and equilibrated at 11% RH-30ºC and 60ºC.

SAMPLE	**Onset T_g (ºC)**
5TMN SD: 95PVP ('As is' after lyophilization)	65.0 ± 6.9^{A}
10TMN SD:90 PVP ('As is' after lyophilization	60.0 ± 4.2^{A}
5TMN SD: 95 PVP (11%RH and 30ºC)	49.4 ± 1.3^{A}
5TMN SD:95 PVP (11%RH and 60ºC)	51.5 ± 3.2^{A}

Table 3.2 Rate constants and t_{90} values for thiamine in solid dispersions prepared by different proportions of PVP, upon storage at 11%RH and 60ºC.

k	**1TMN:99PVP**	**3TMN:97PVP**	**5TMN:95PVP**	**7TMN:93PVP**	**10TMN:90PVP**	**20TMN:80PVP**
k_{obs} (day^{-1})	0.0055	0.0054	0.005	0.0049	0.0038	0.0018
R^2	0.7981	0.9217	0.8637	0.9371	0.9155	0.9464
t_{90} (days)	19.15646	19.51121	21.0721	21.50215	27.72645	58.53362

***t_{90}**: Time where 90% of the initial concentration of thiamine is left.

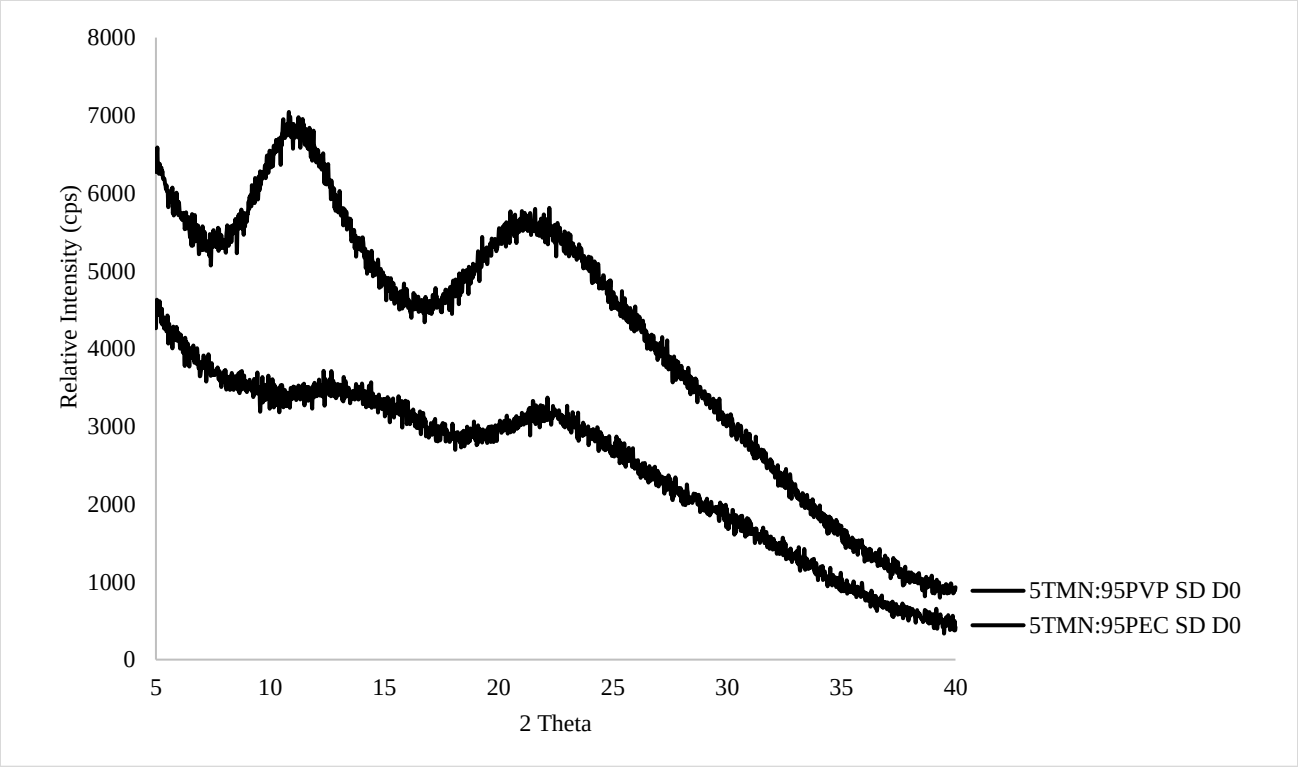


Figure 3.1 X-ray powder diffraction patterns of 5TMN:95PEC and 5TMN:95PVP solid dispersions (SD) on day 0.

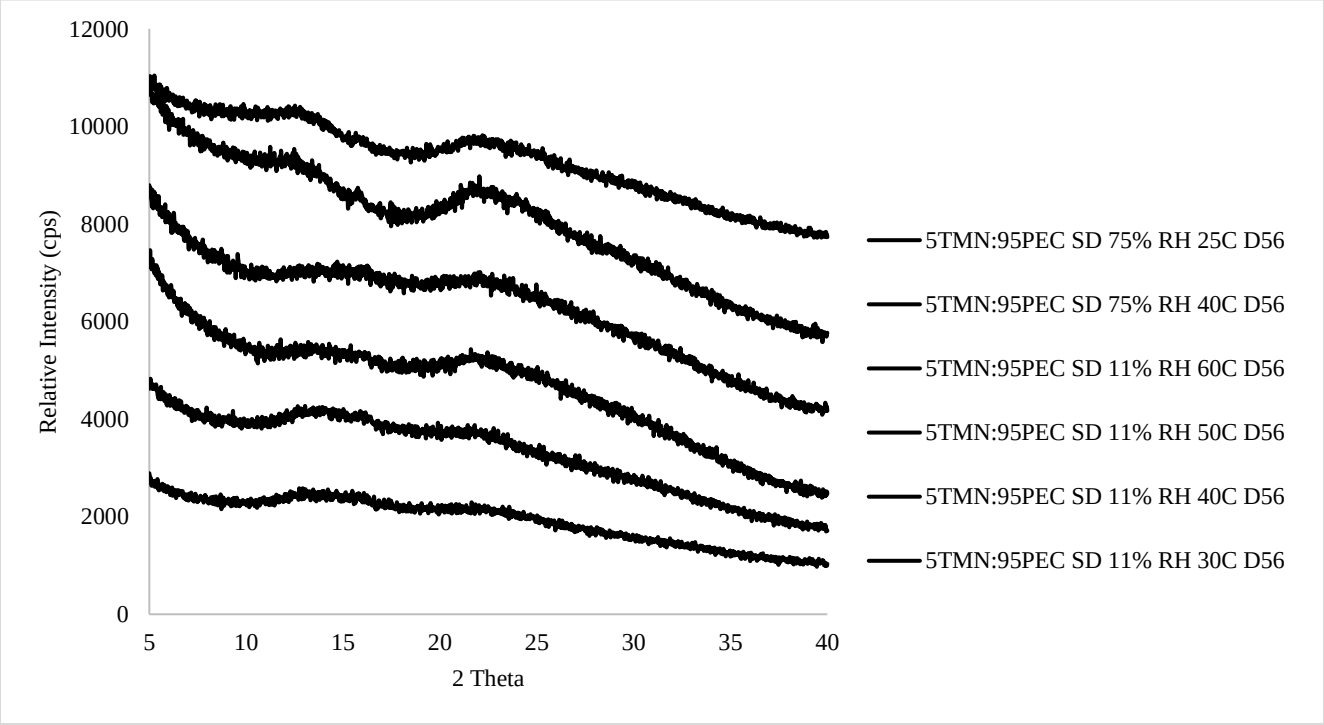


Figure 3.2 X-ray powder diffraction patterns of 5TMN:95PEC solid dispersions (SD) stored at 11% RH and 30ºC-60ºC and 75% RH (25ºC and 40ºC) on day 56.

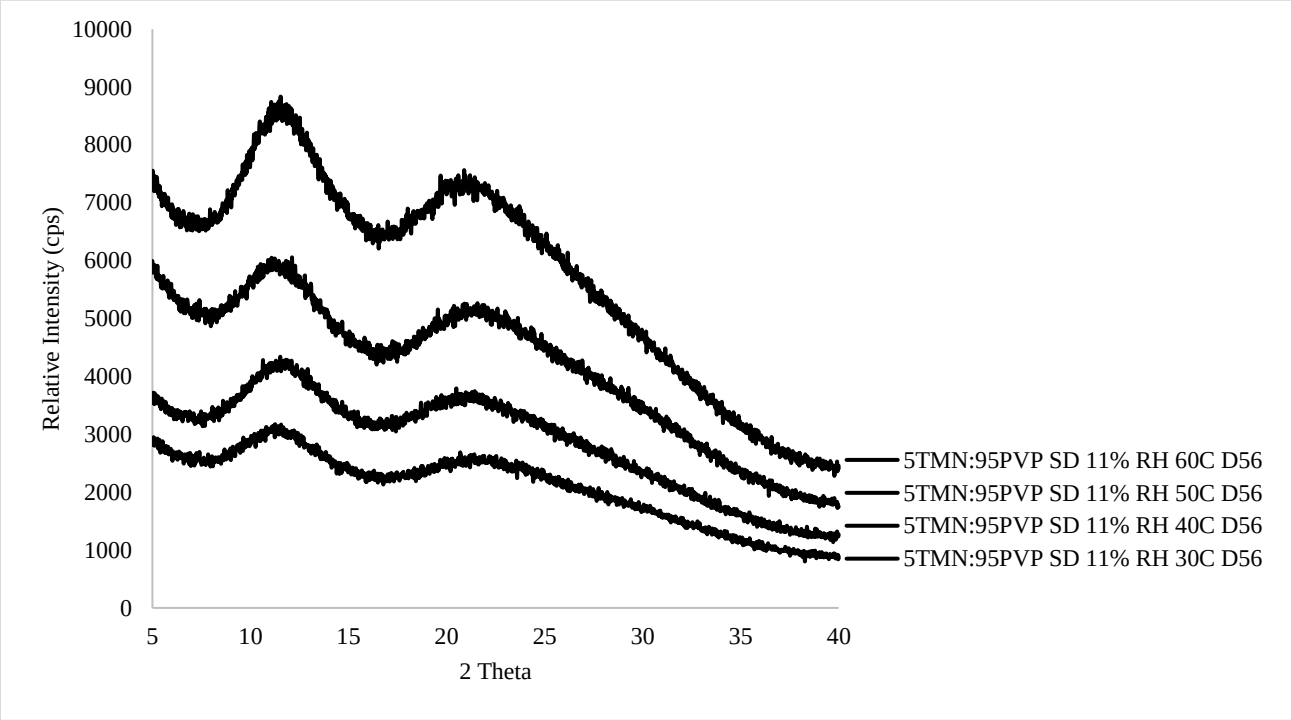


Figure 3.3 X-ray powder diffraction patterns of 5TMN:95PVP solid dispersions (SD) stored at 11% RH and 30ºC-60ºC on day 56.

A)

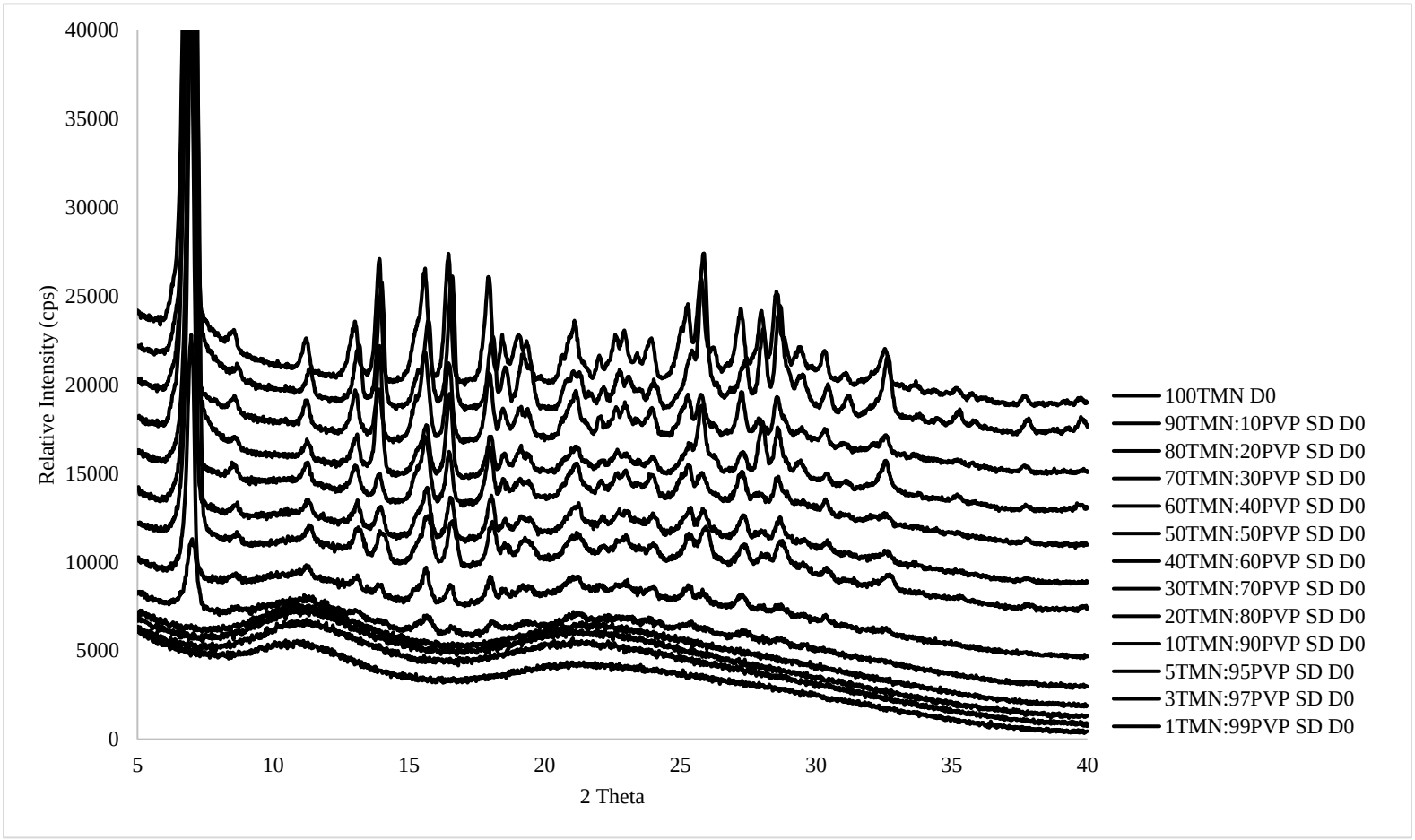
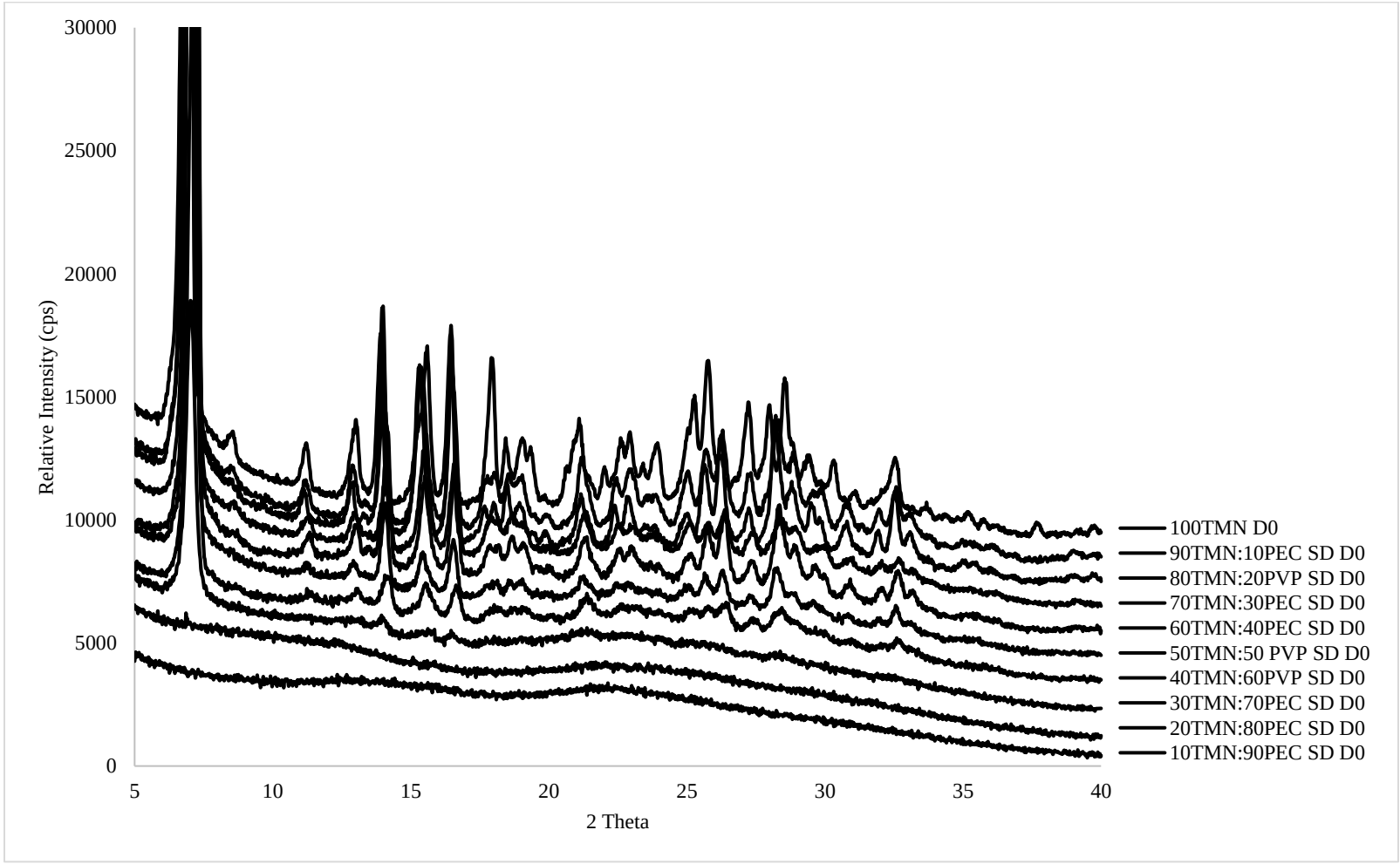


Figure 3.4 X-ray powder diffraction patterns of **A)** TMN:PVP solid dispersions (SD) prepared varying ratios of TMN to PVP on day 0, **B)** TMN:PEC solid dispersions (SD) prepared varying ratios of TMN to PVP on day 0.

Figure 3.4 continued

B)



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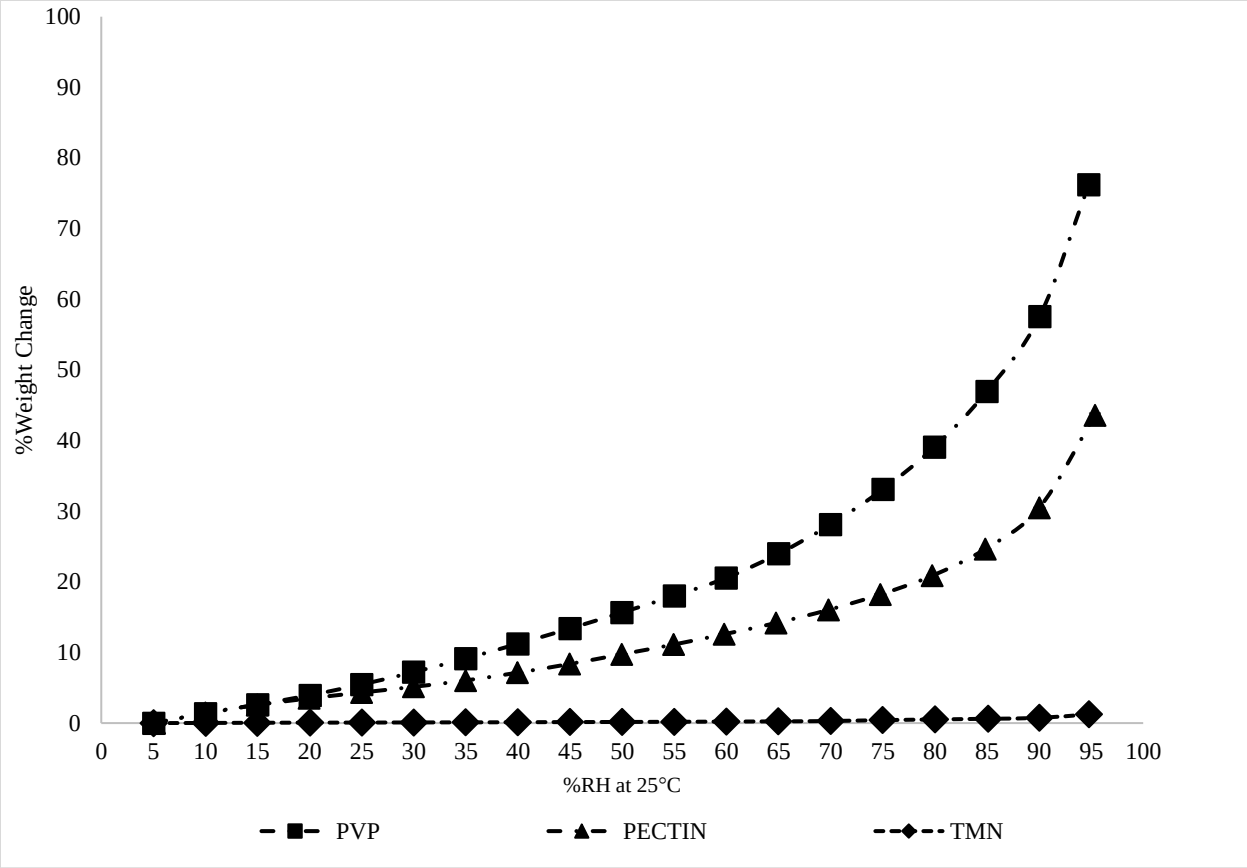


Figure 3.5 Moisture sorption profiles of PVP, PEC, and TMN.

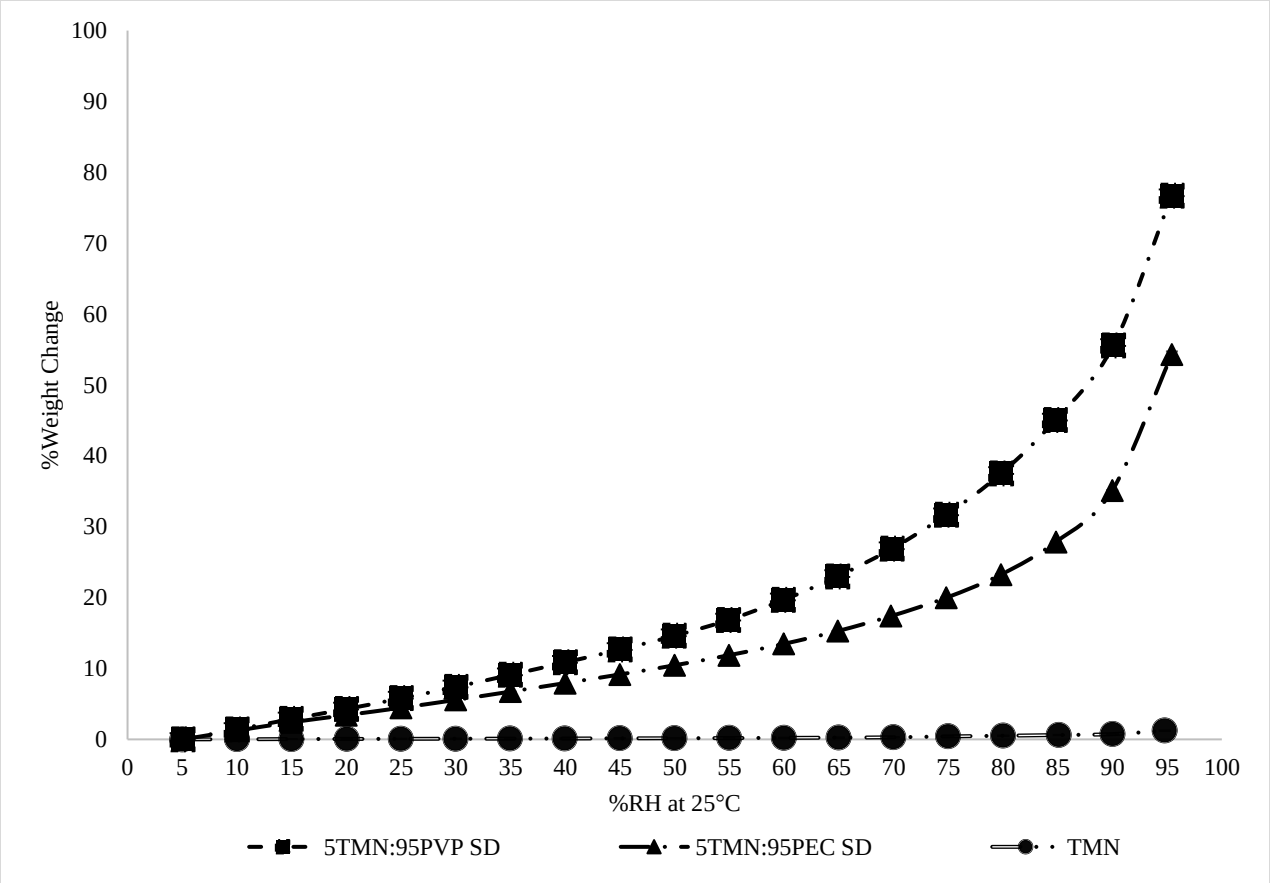


Figure 3.6 Moisture sorption profiles of TMN, 5TMN:95PVP, and 5TMN:95PEC solid dispersions (SD) at 25ºC.

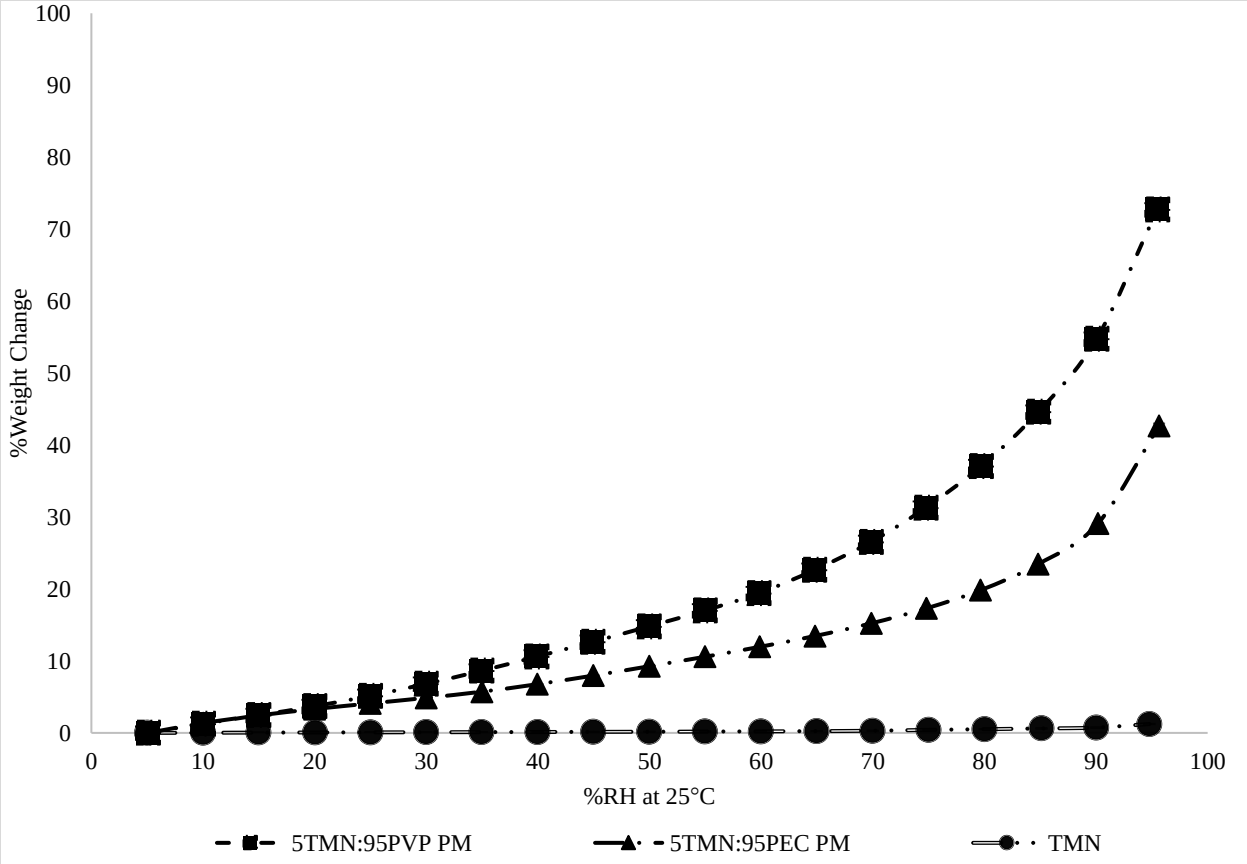
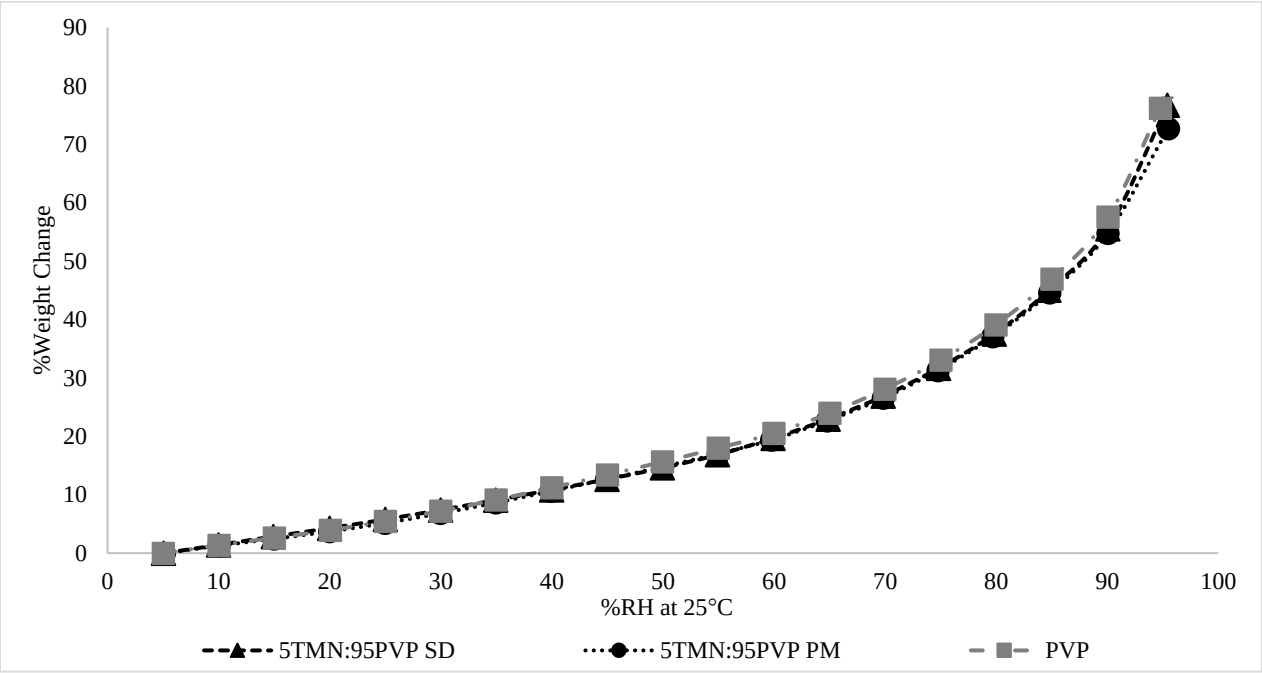


Figure 3.7 Moisture sorption profiles of TMN, 5TMN:95PVP, and 5TMN:95PEC physical mixtures (PM) at 25ºC.

A)



B)

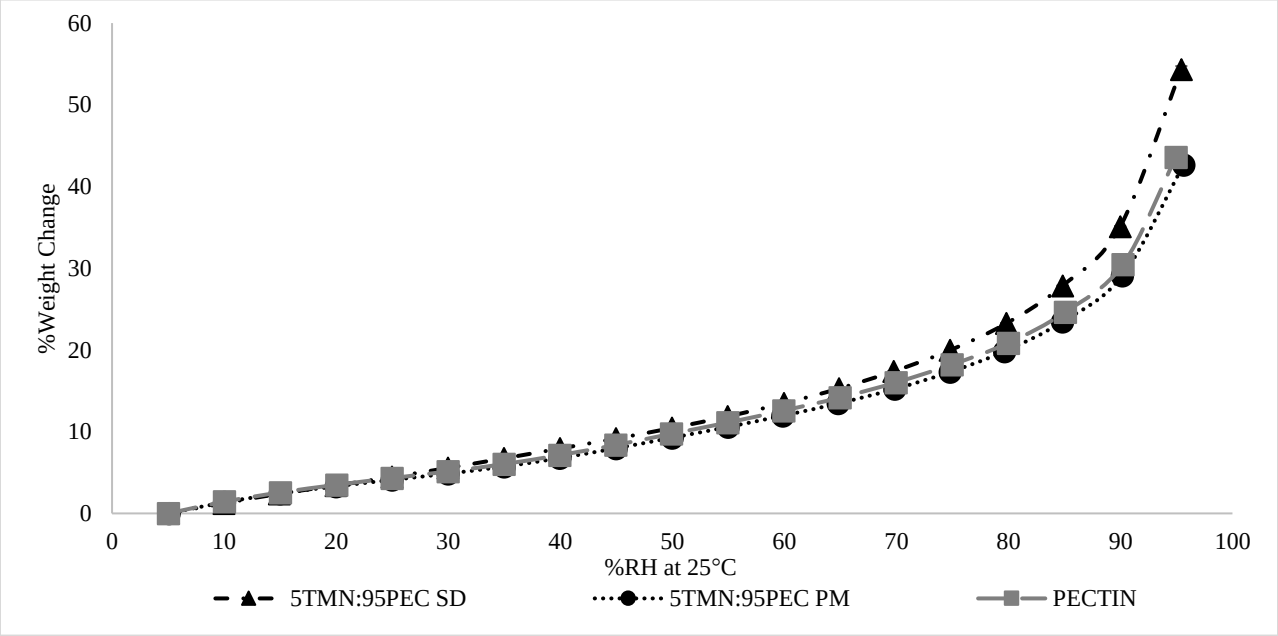
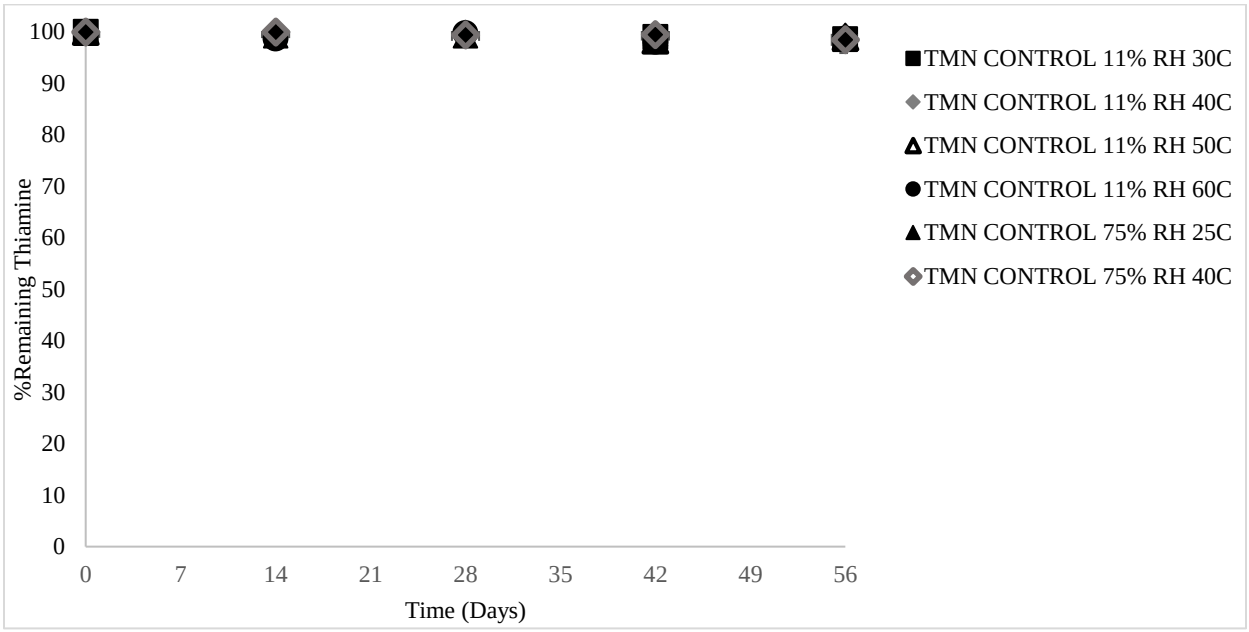


Figure 3.8 Comparison of moisture sorption profiles of **A)** PVP and solid dispersions (SD) and physical mixtures (PM) of 5TMN:95PVP, **B)** PEC and solid dispersions (SD) and physical mixtures (PM) of 5TMN:95PEC.

A)



B)

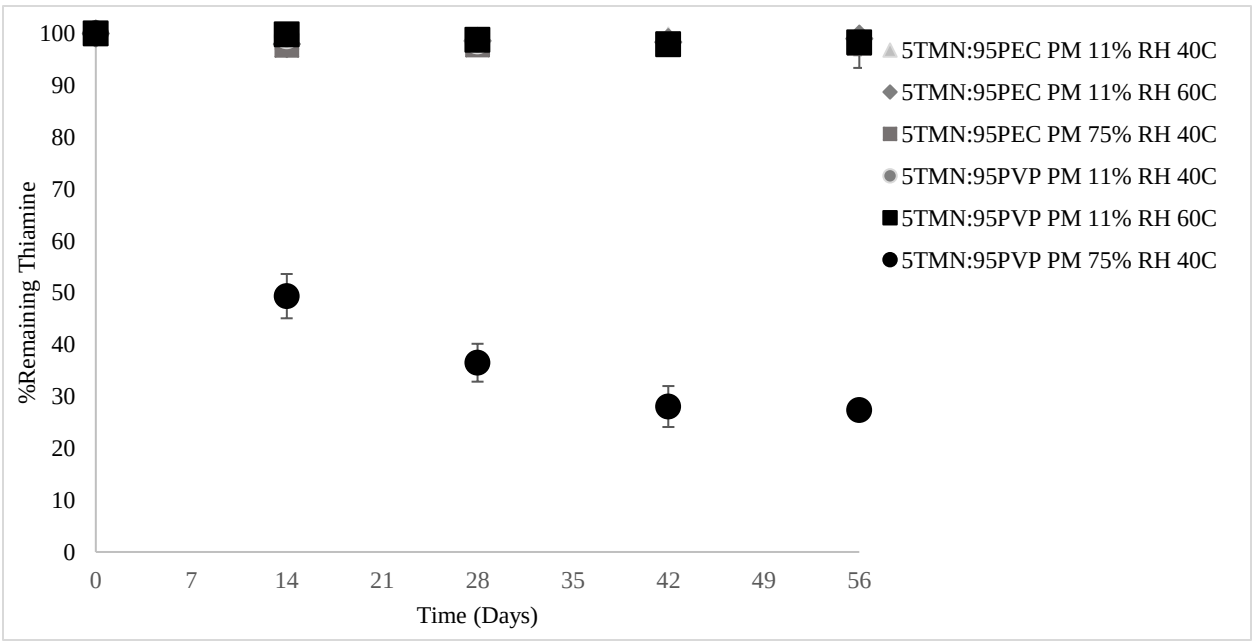
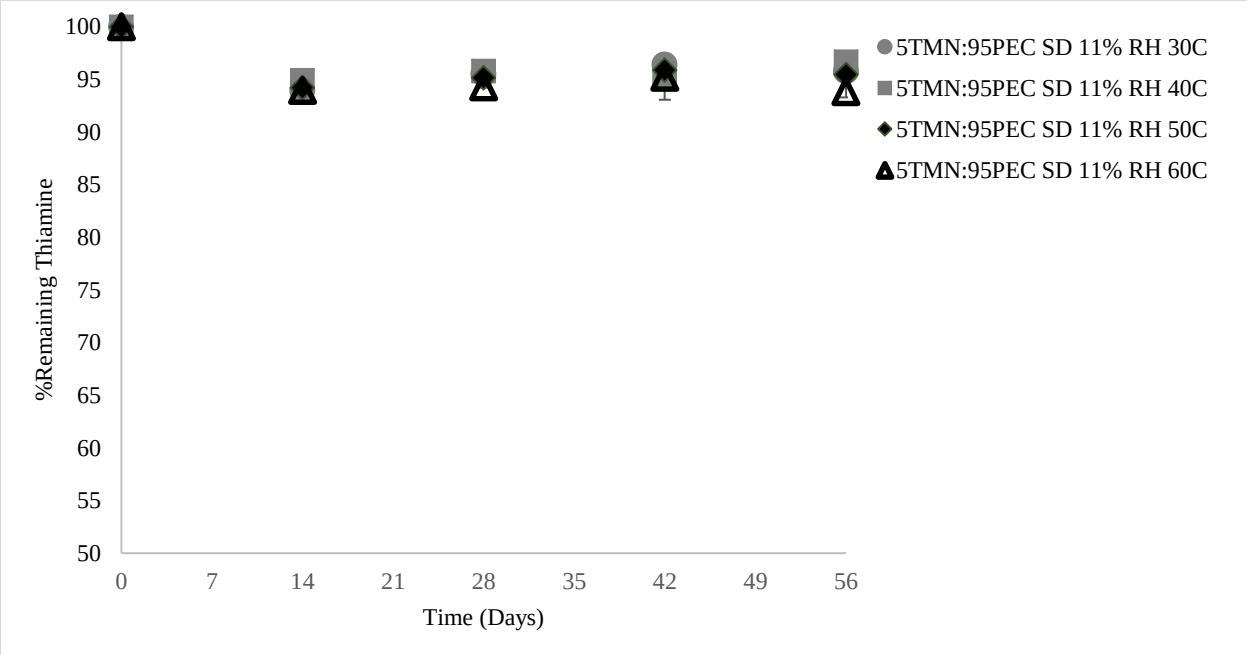


Figure 3.9 Chemical stability of thiamine **A)** in crystalline TMN form, **B)** in 5TMN:95PEC and 5TMN:95PVP physical mixtures (PM).

A)



B)

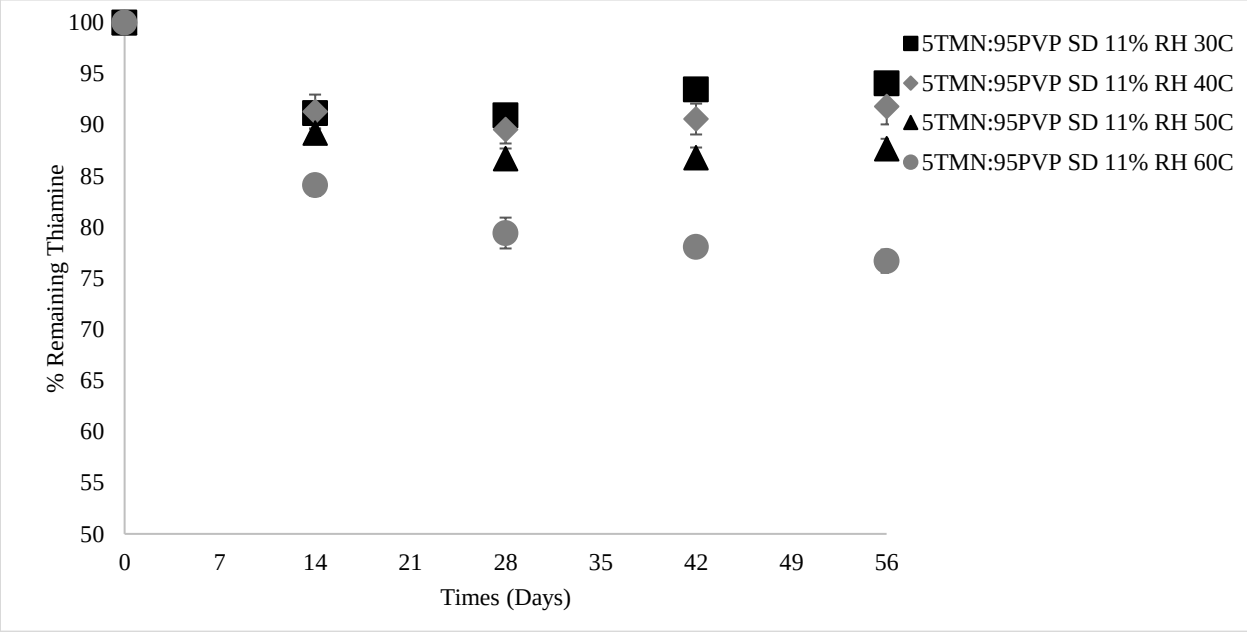
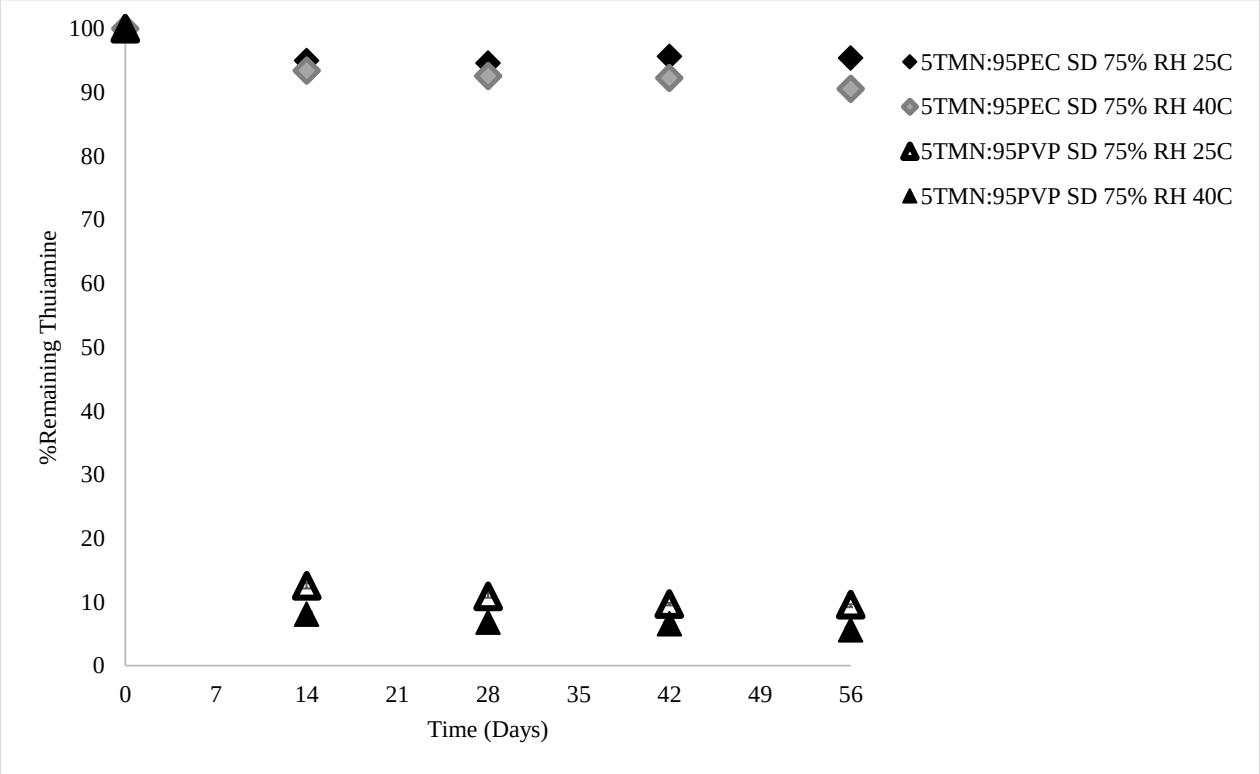


Figure 3.10 Chemical stability of thiamine **A)** in 5TMN:95PEC solid dispersions (SD), **B)** in 5TMN:95PVP dispersions (SD) stored at 11% RH and 30-60^{o}C for 56 days, **C)** in 5TMN:95PEC solid dispersions (SD) and 5TMN:95PVP dispersions stored at 75% RH (25^{o}C and 40^{o}C) for 56 days.

Figure 3.10 continued

C)



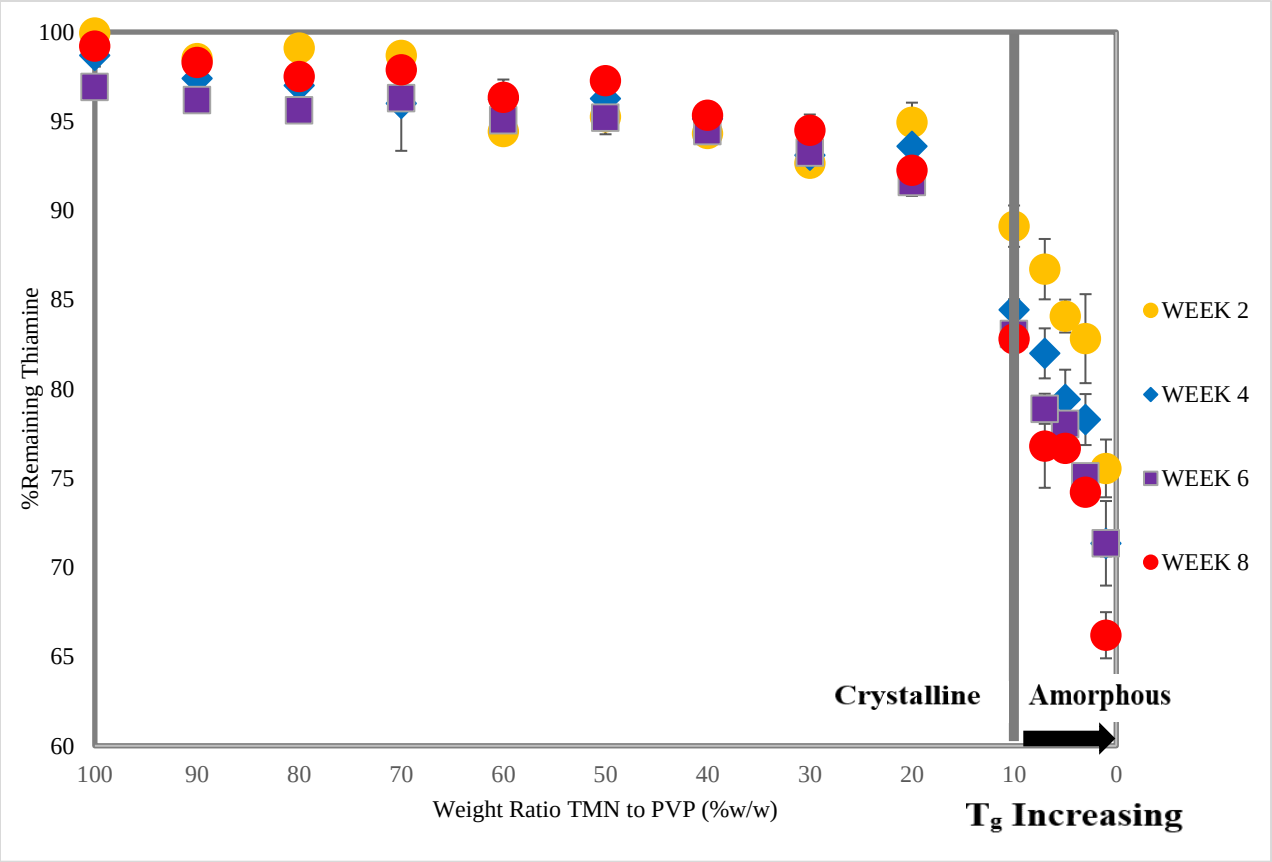


Figure 3.11 Chemical stability of thiamine in various ratios of TMN to PVP solid dispersions stored at 11% RH and 60ºC for 56 days.

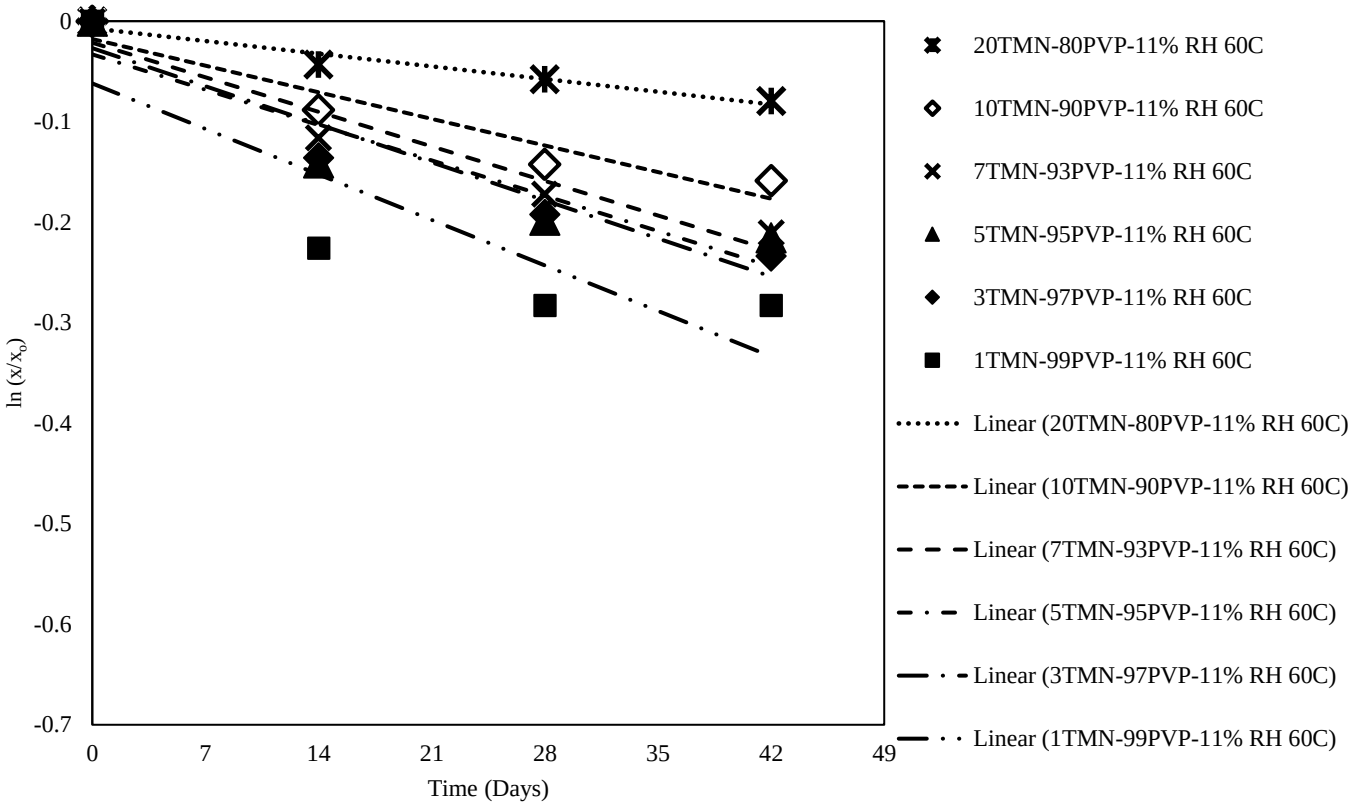
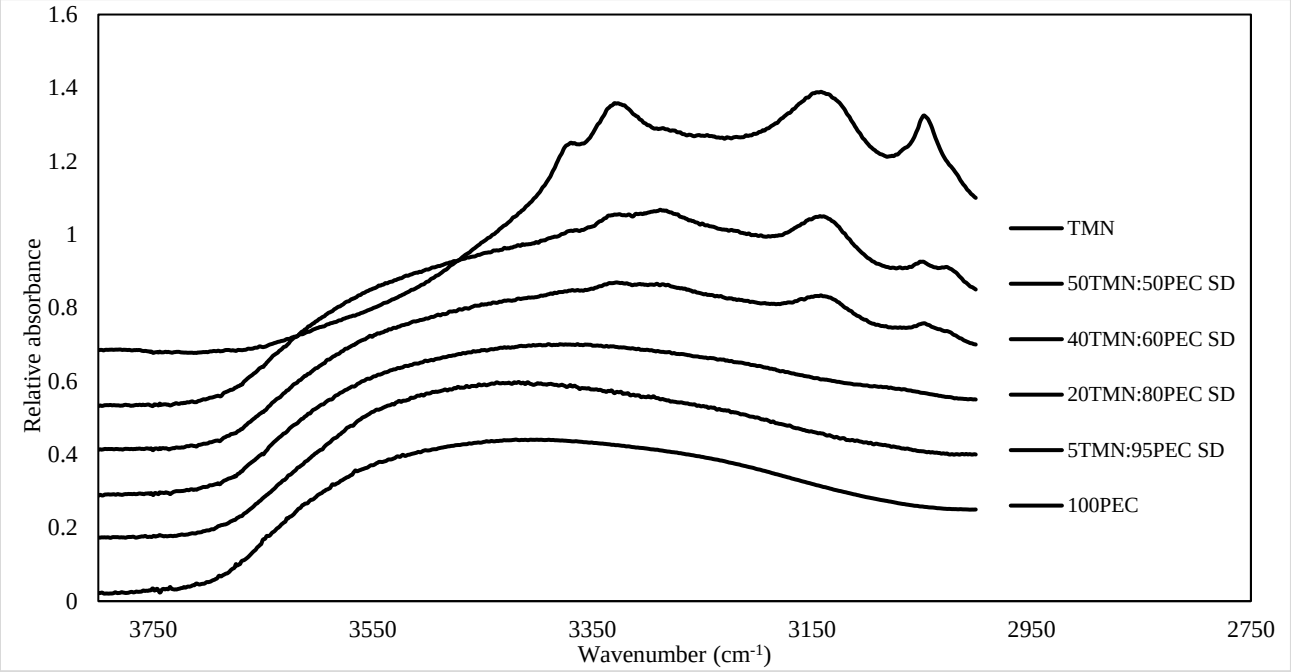


Figure 3.12 First-order degradation regression lines of thiamine in various TMN to PVP dispersions stored at 11% RH and 60ºC for 56 days.

A)



B)

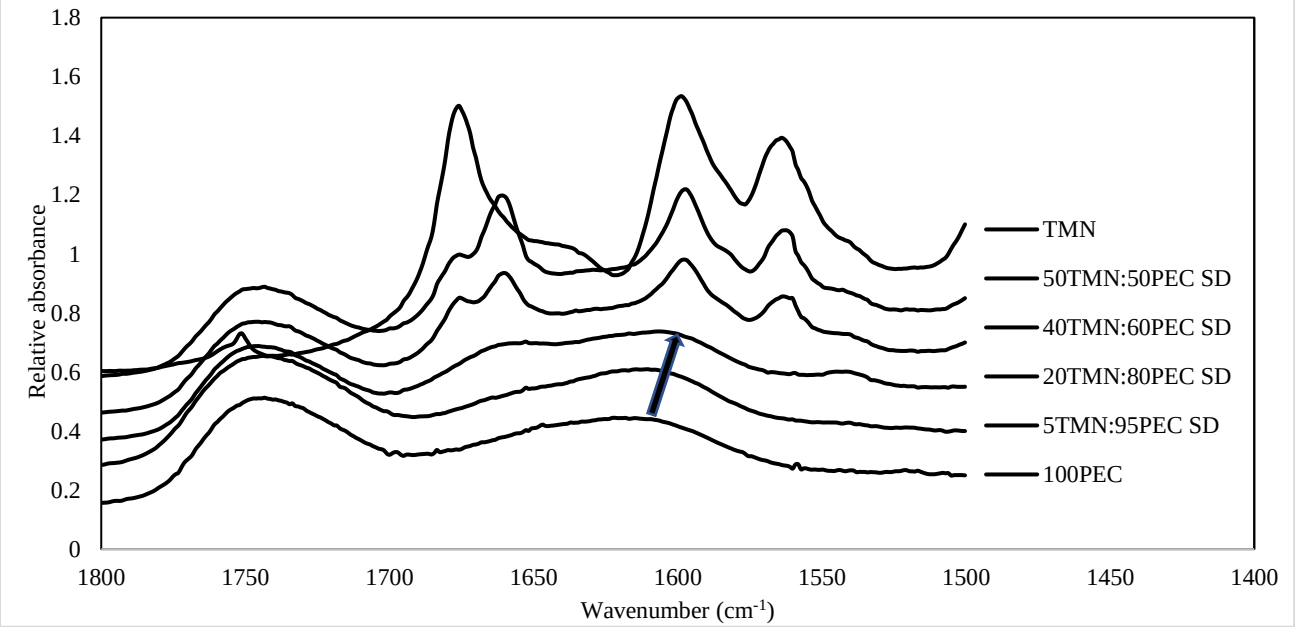
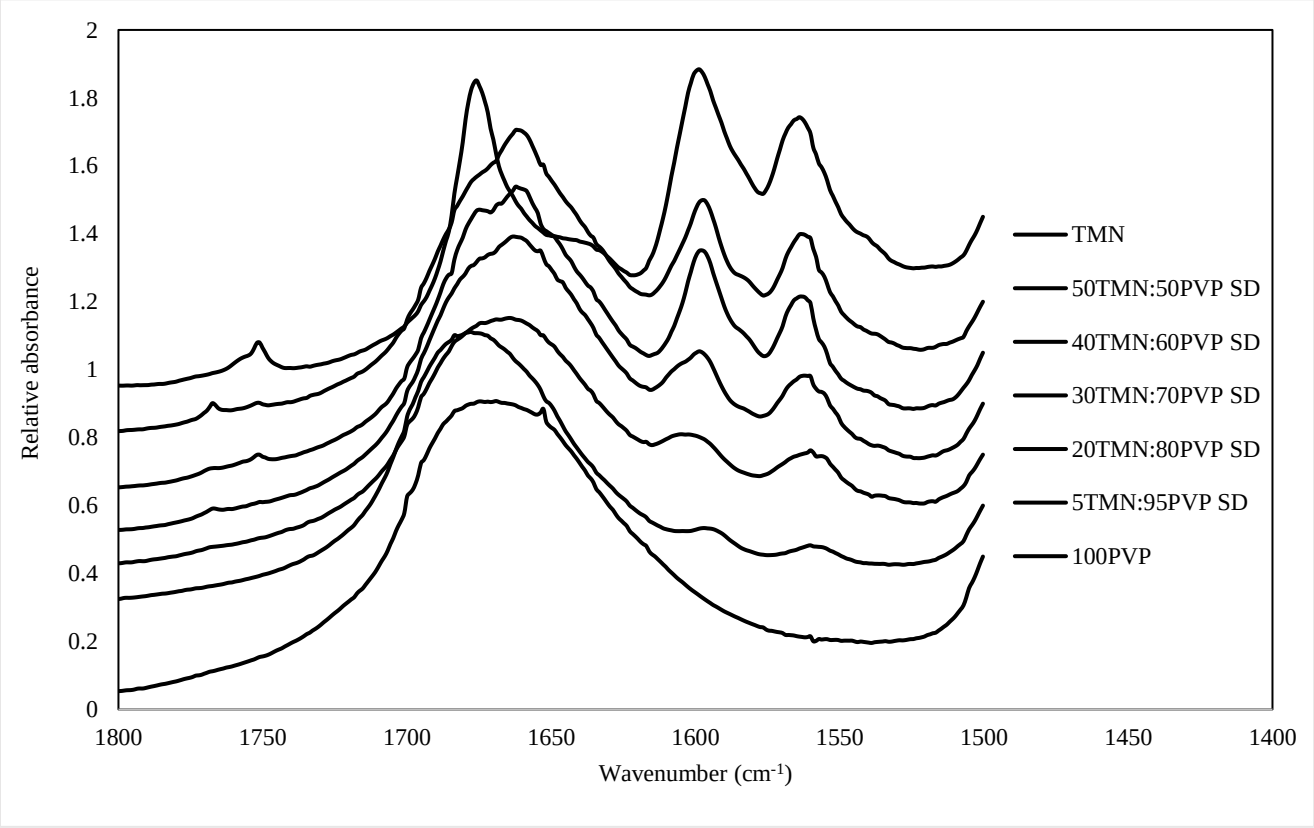
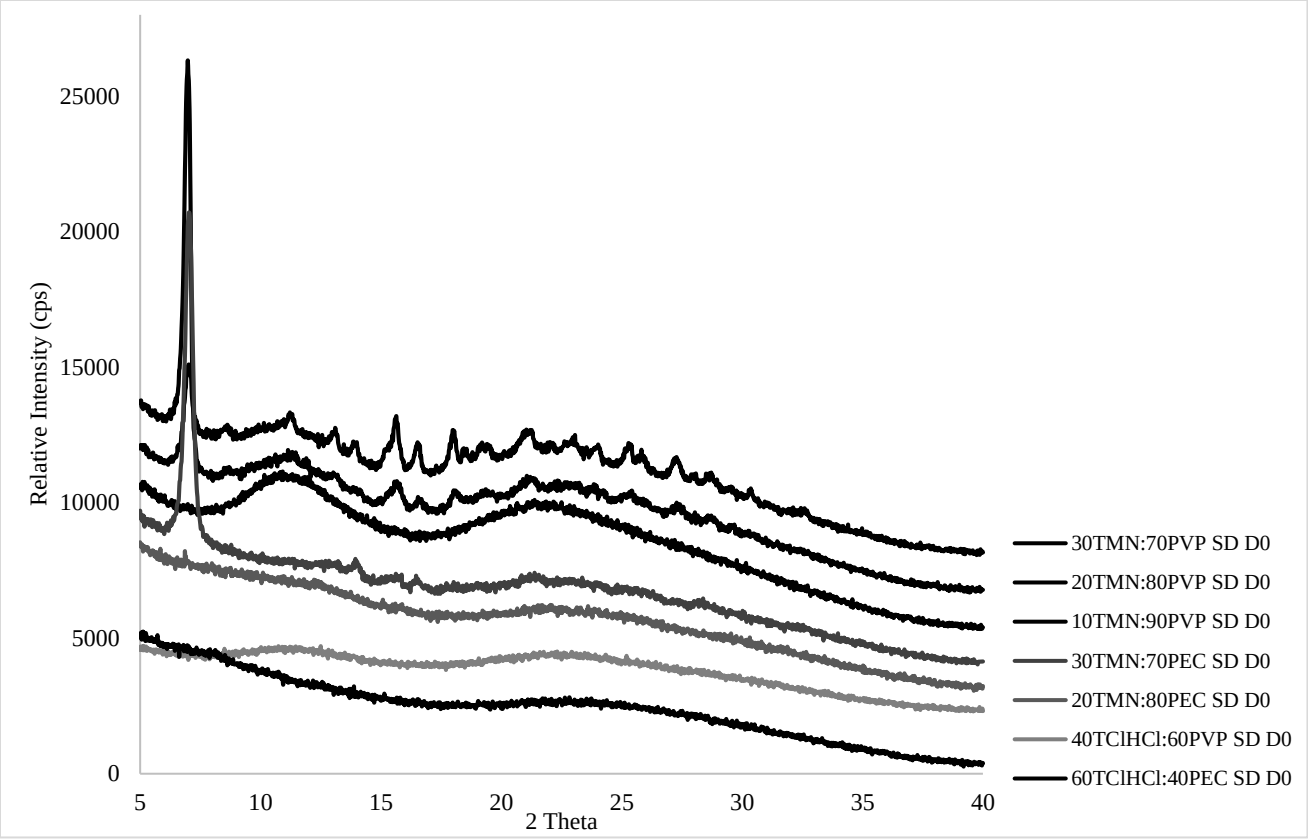


Figure 3.13 FTIR spectra of the **A)** hydroxyl region of TMN:PEC solid dispersions, **B)** carbonyl region of TMN:PEC solid dispersions, and **C)** carbonyl region of TMN:PVP solid dispersions.

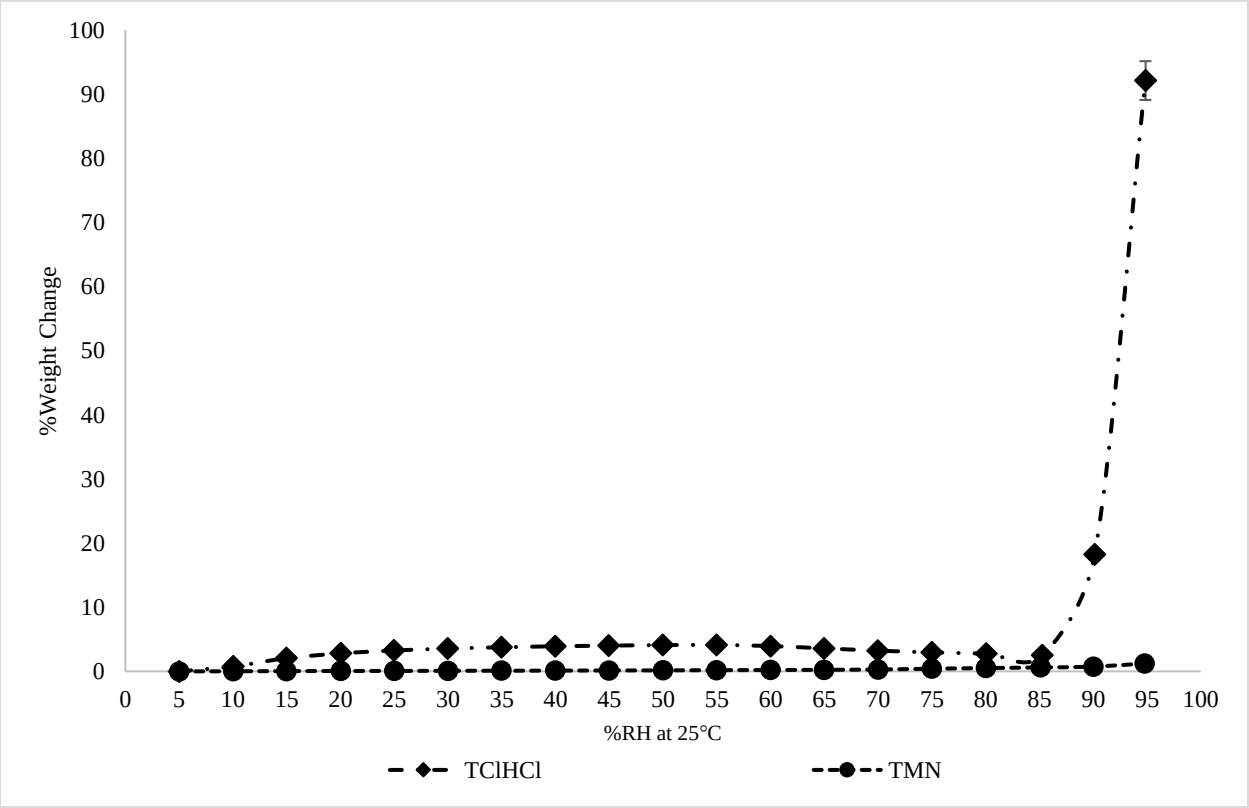
Figure 3.13 continued

C)



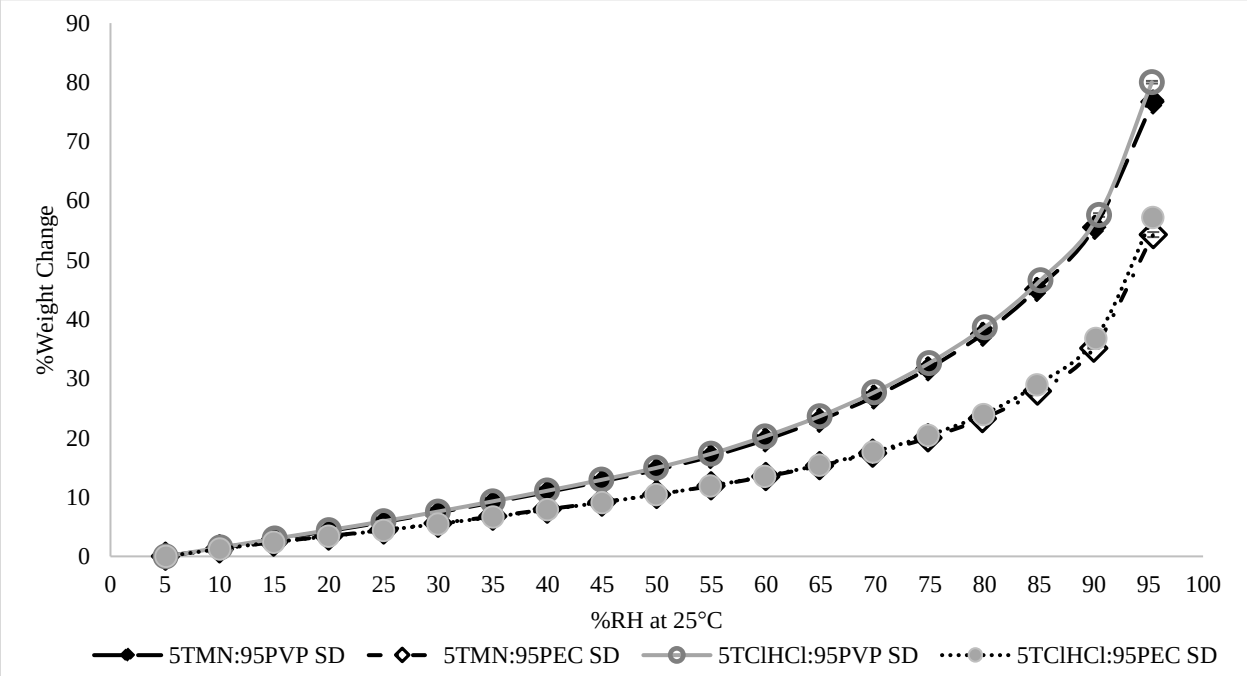


Supplementary Figure 3.1 Comparison of the minimum amount of PVP and PEC needed to amorphize TMN and TClHCl.

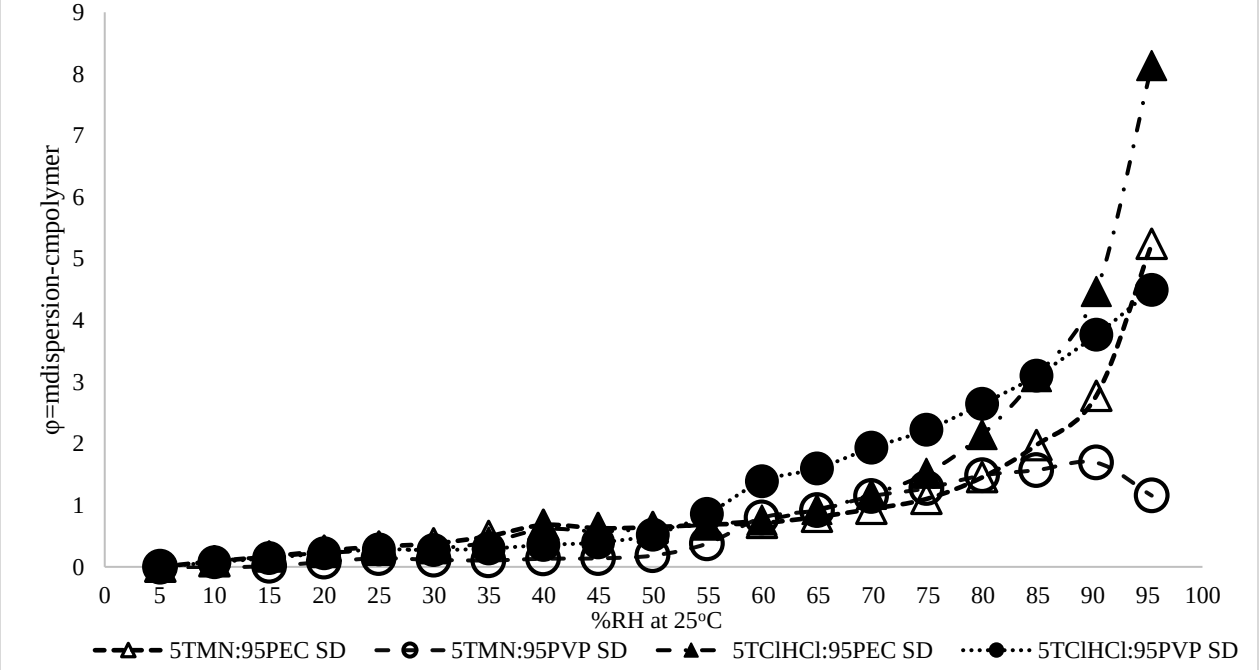


Supplementary Figure 3.2 Comparison of moisture sorption isotherms of TMN and TClHCl.

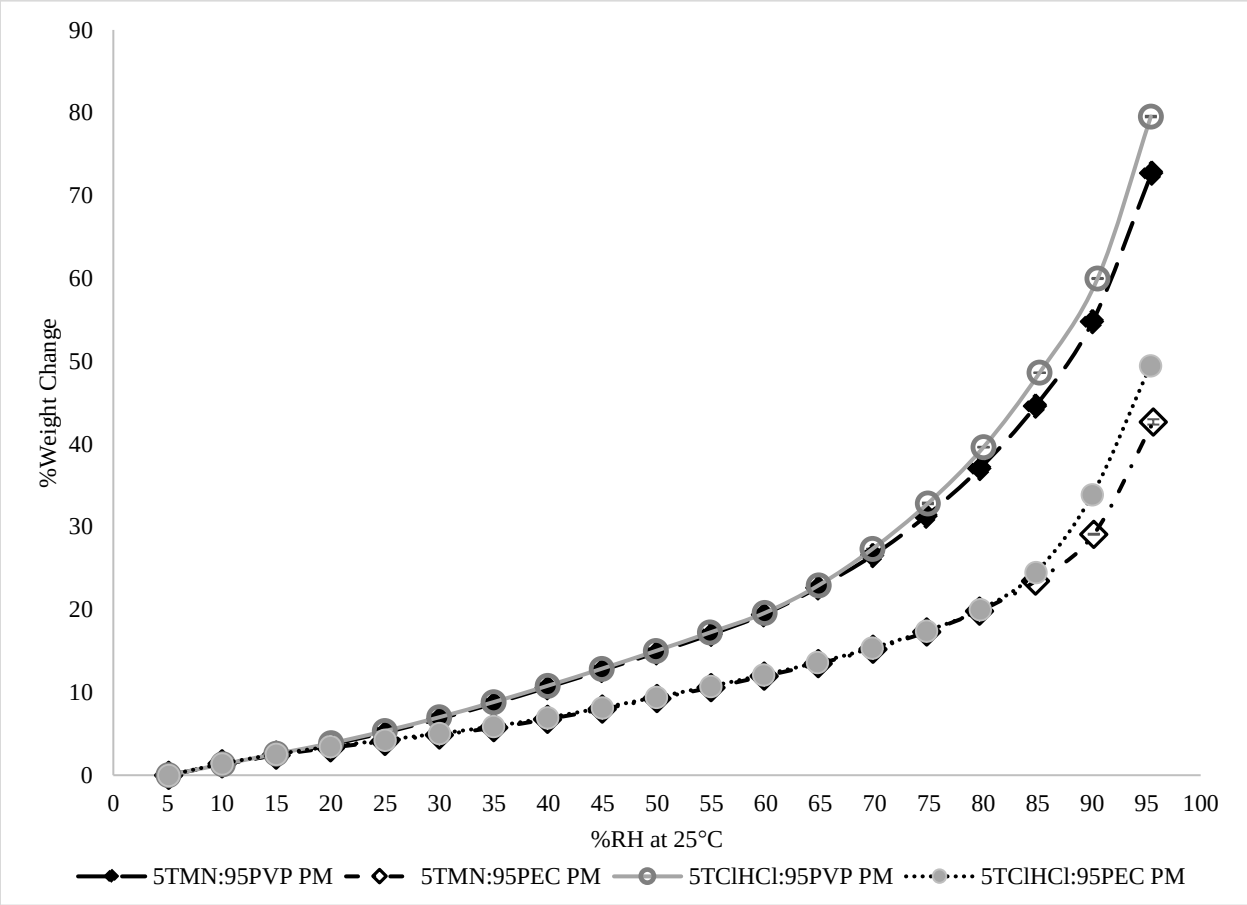
A)



B)

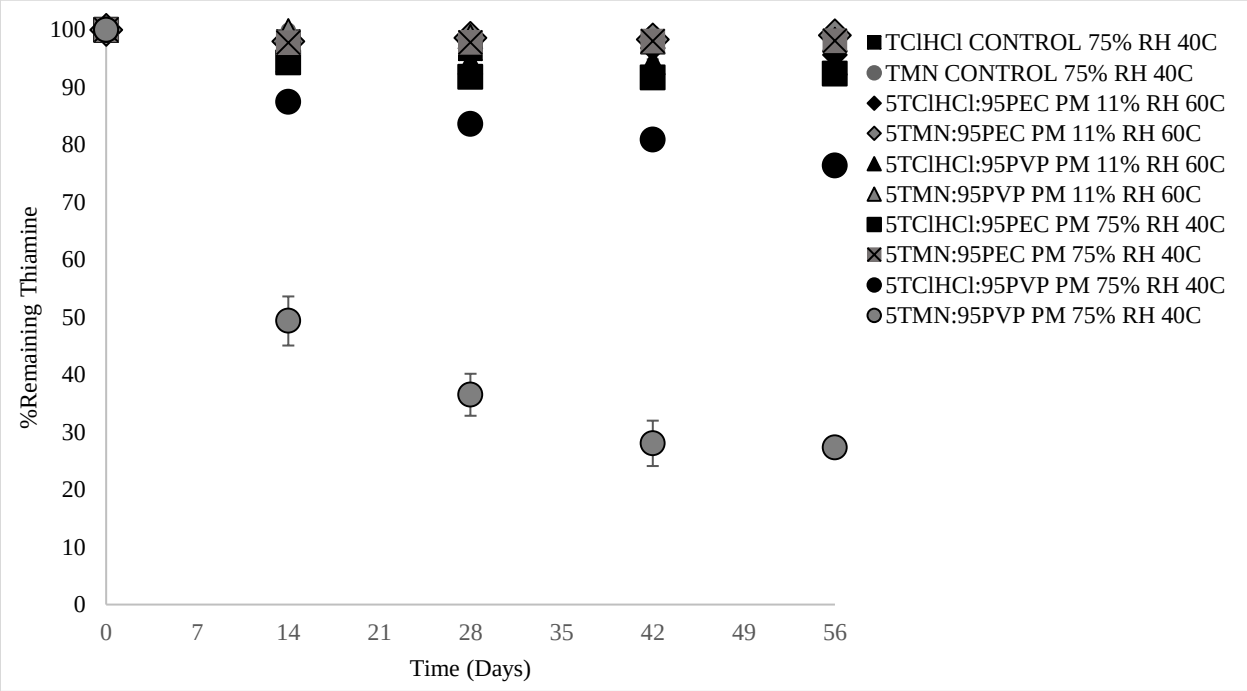


Supplementary Figure 3.3 **A)** Comparison of moisture sorption isotherms of 5TMN:95polymer solid dispersions (SD) and 5TClHCl:95polymer solid dispersions (SD), **B)** Difference between polymer and TMN, and TClHCl dispersions moisture sorption.

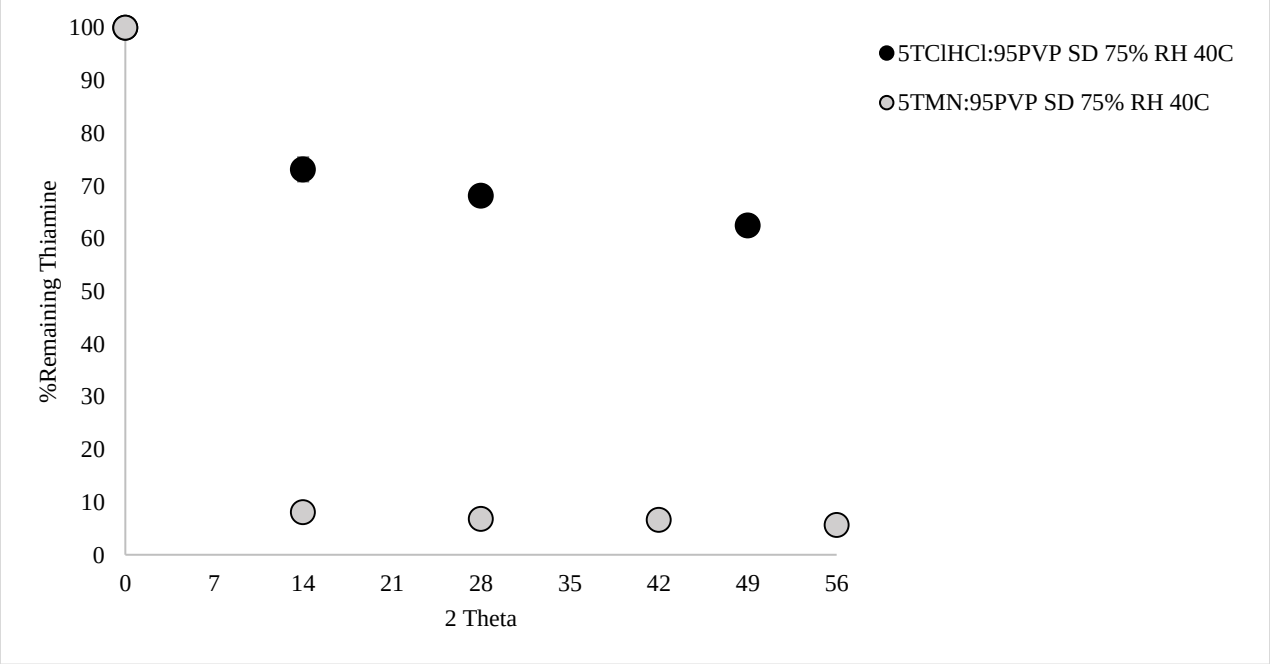


Supplementary Figure 3.4 Comparison of moisture sorption isotherms of 5TMN:95polymer physical mixtures (PM) and 5TClHCl:95polymer physical mixtures (PM).

A)

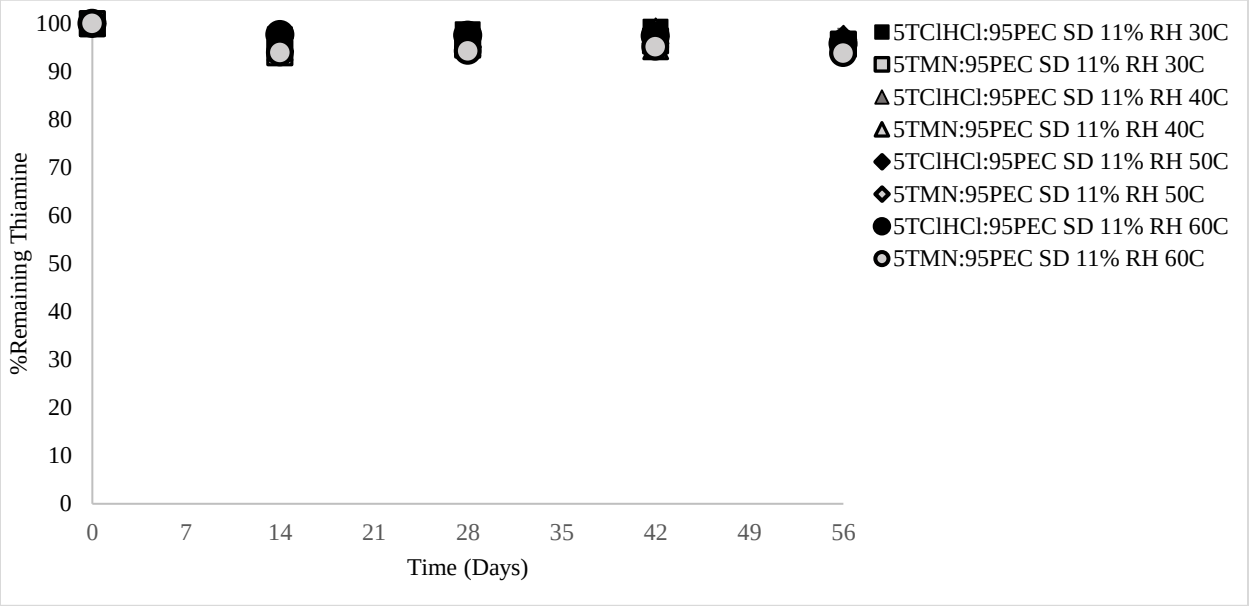


B)

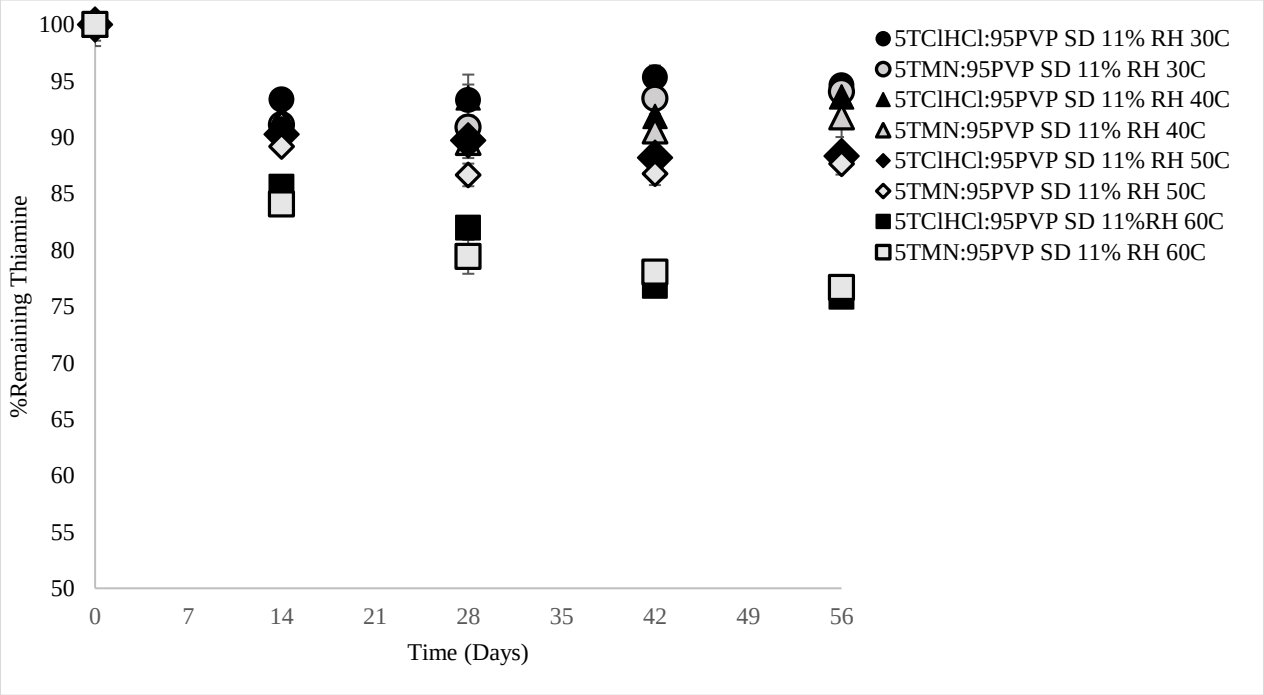


Supplementary Figure 3.5 Comparison of chemical stability of thiamine, **A)** in crystalline TMN, TClHCl, 5TMN:95polymer physical mixtures (PM) and 5TClHCl:95polymer physical mixtures (PM), **B)** in 5TMN:95PVP and 5TClHCl:95PVP dispersions at 75% RH and 40^{o}C.

A)

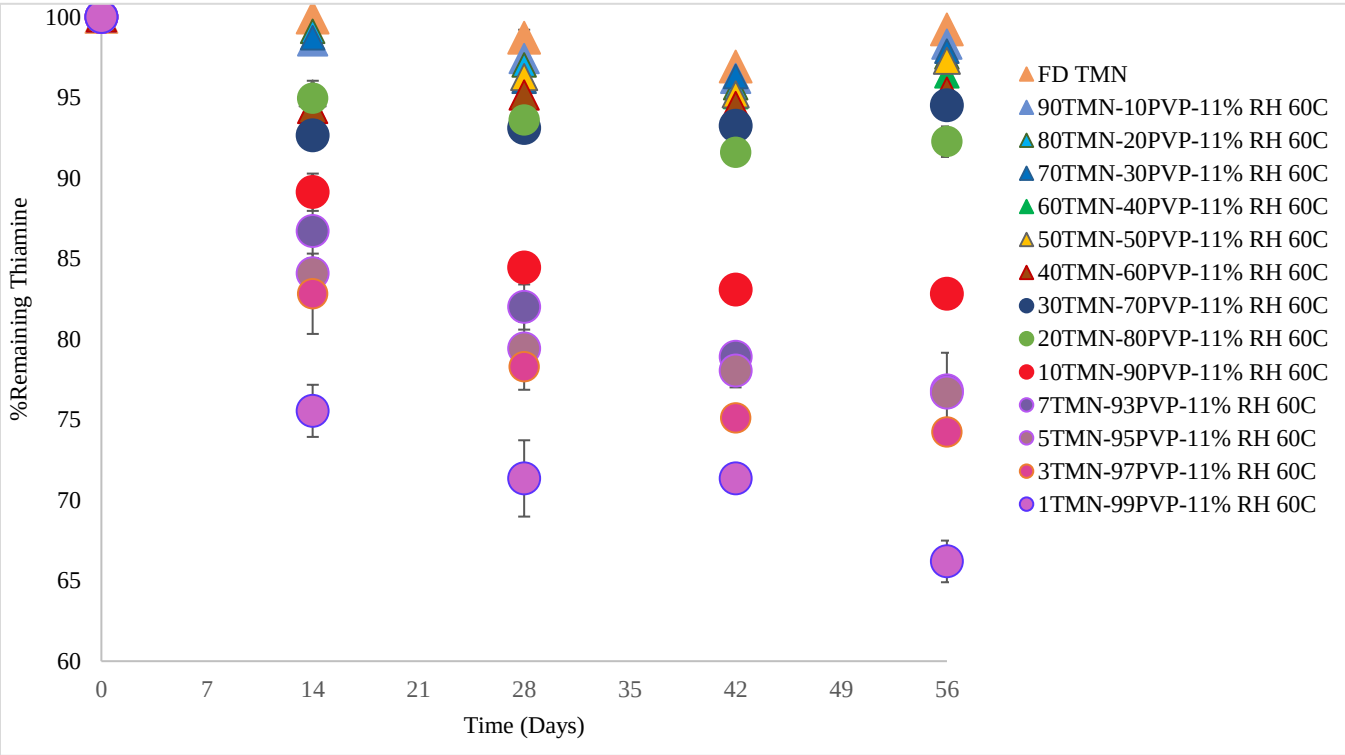


B)

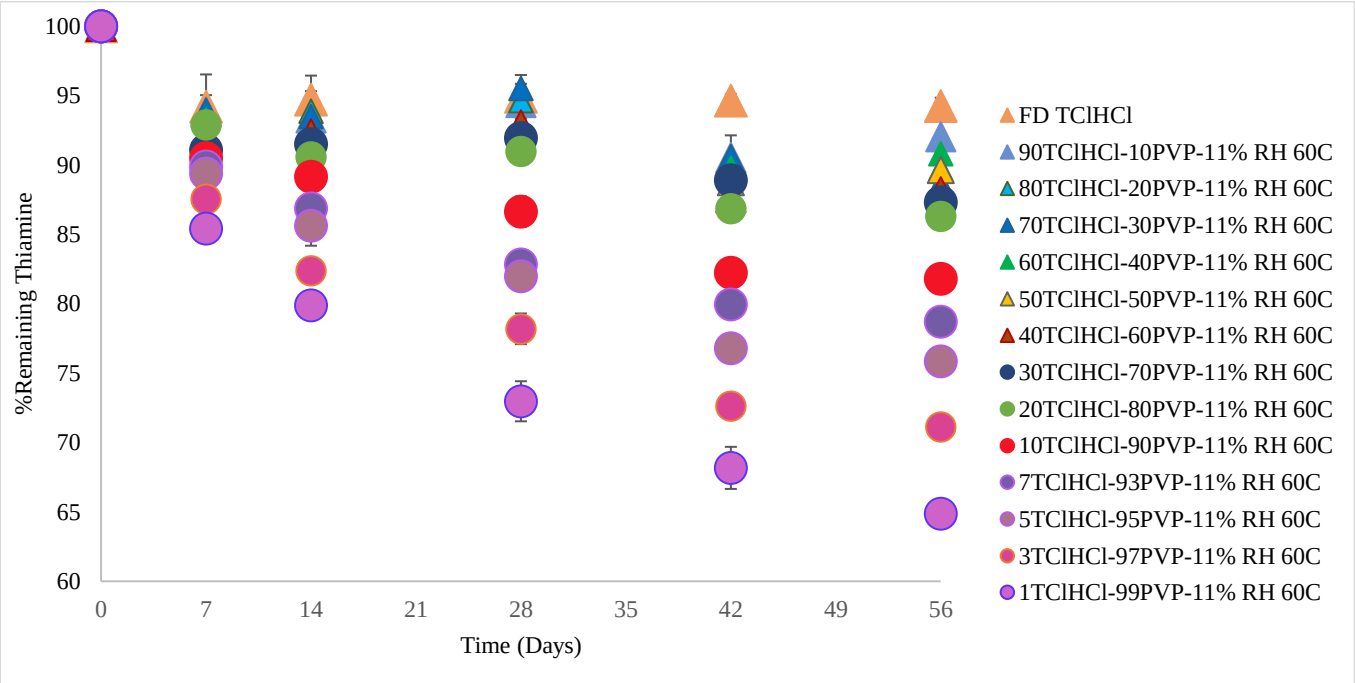


Supplementary Figure 3.6 Comparison of chemical stability of thiamine, **A)** in 5TMN:95PEC and 5TClHCl:95PEC solid dispersions (SD) at 11% RH and 30-60ºC, **B)** in 5TMN:95PVP and 5TClHCl:95PVP solid dispersions (SD) at 11% RH and 30-60ºC.

A)

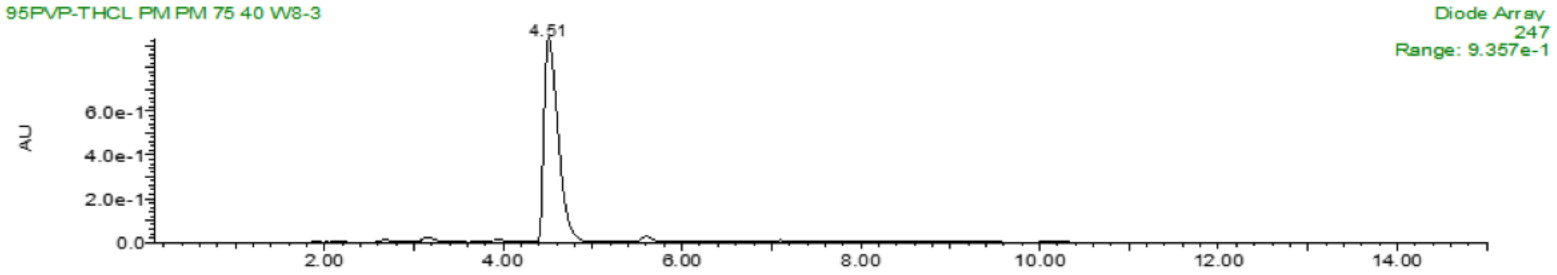


B)

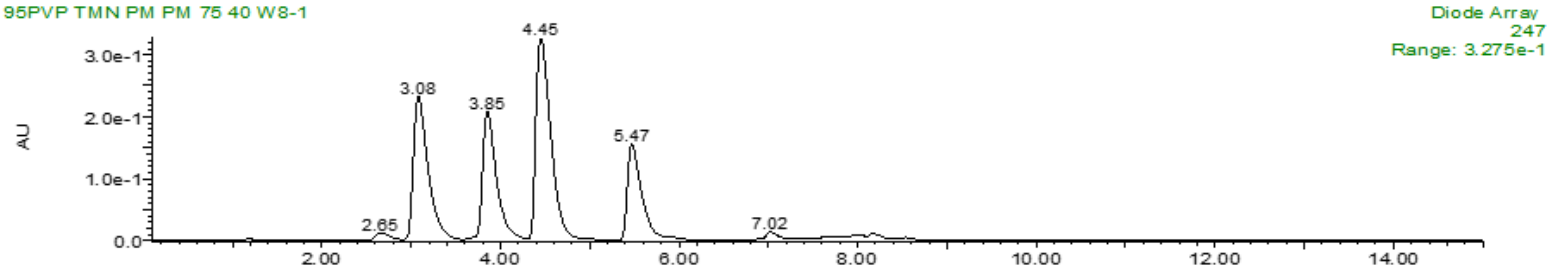


Supplementary Figure 3.7 Chemical stability of thiamine in various ratios of **A)** TMN and **B)** TClHCl to PVP solid dispersions stored at 11% RH and 60°C for 56 days.

A)

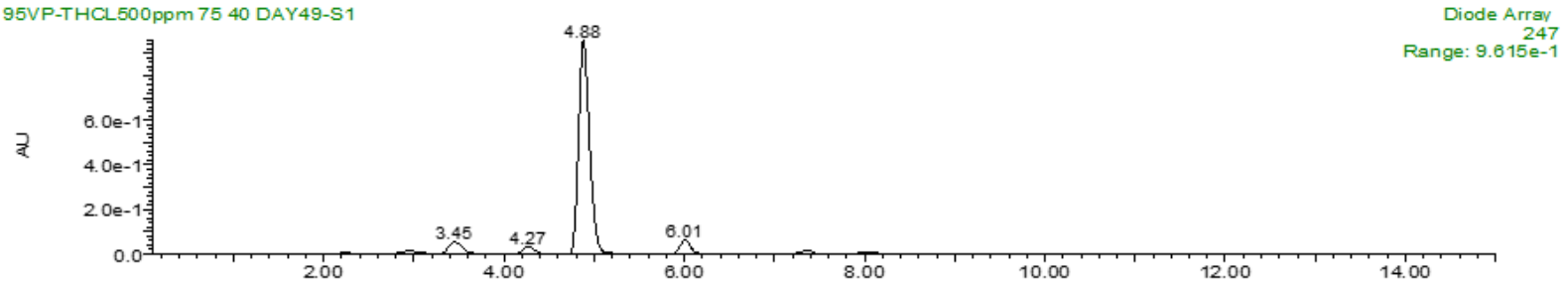


B)



Supplementary Figure 3.8 HPLC chromatograms of **A)** 5TClHCl:95PVP physical mixtures (PM) at 75% RH and 40^{o}C, and **B)** 5TMN:95PVP physical mixtures (PM) at 75% RH and 40^{o}C on day 56.

A)



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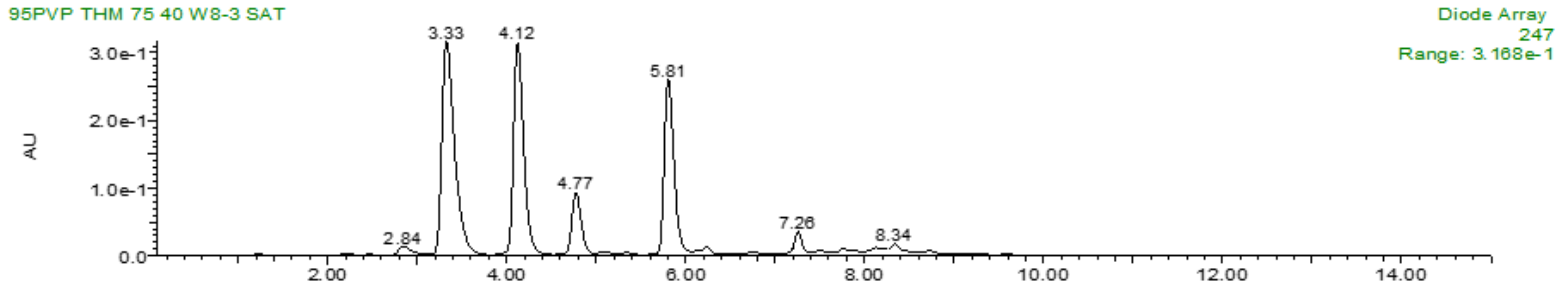
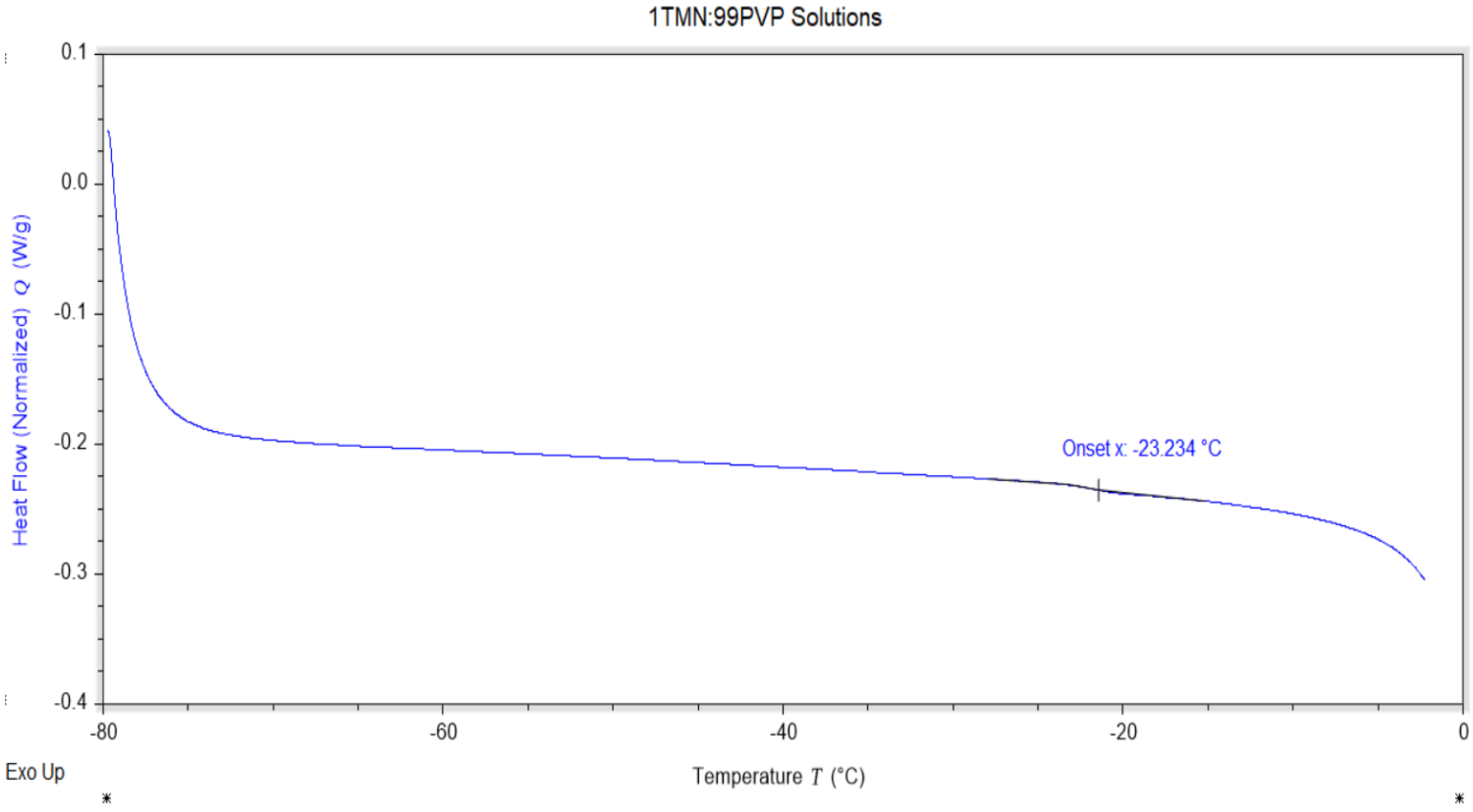
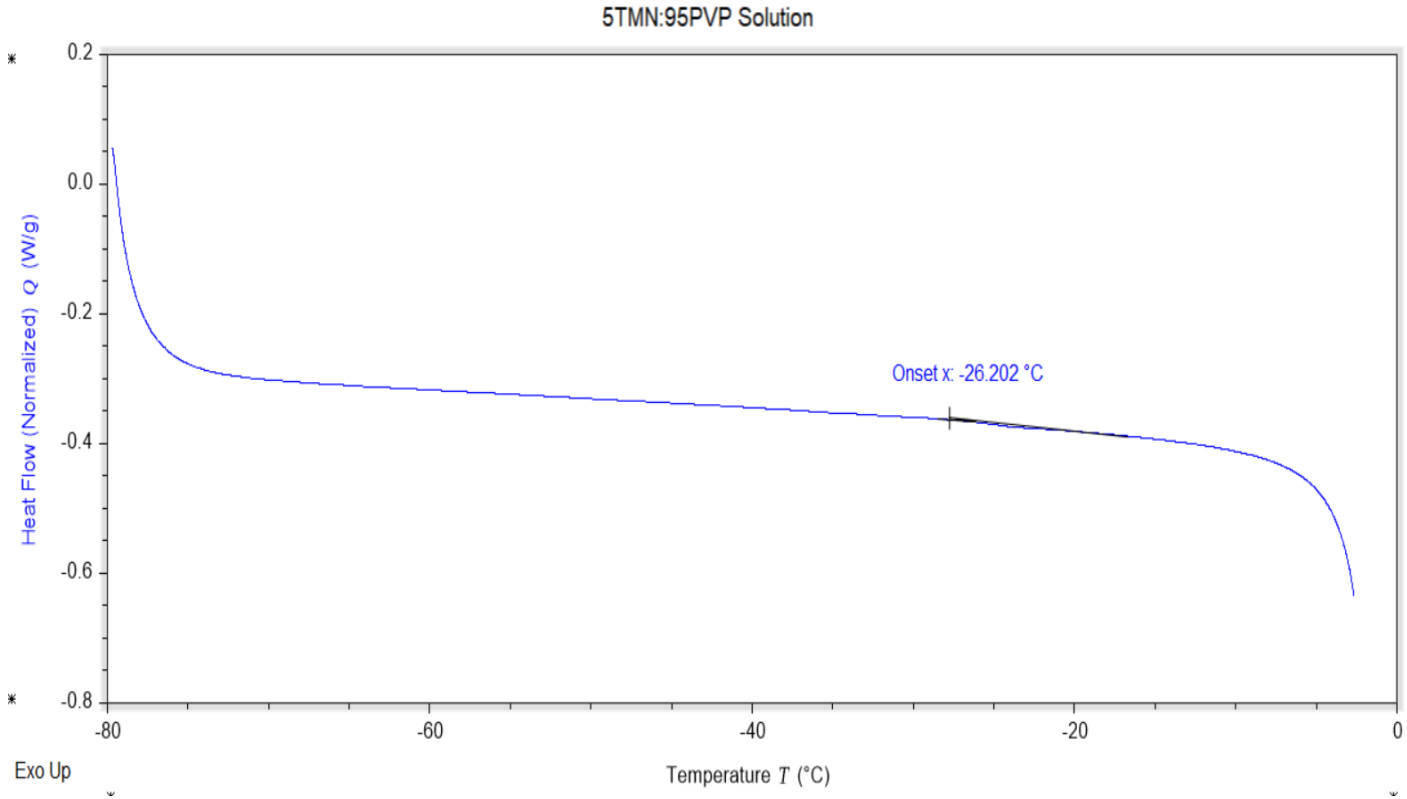


Supplementary Figure 3.9 HPLC chromatograms of **A)** 5TClHCl:95PVP dispersions at 75% RH and 40^{o}C, and **B)** 5TMN:95PVP dispersions at 75% RH and 40^{o}C.



Supplementary Figure 3.10 DSC graphs of solutions of TMN, polymers, and TMN:polymer for T_g' measurement.

Supplementary Figure 3.10 continued



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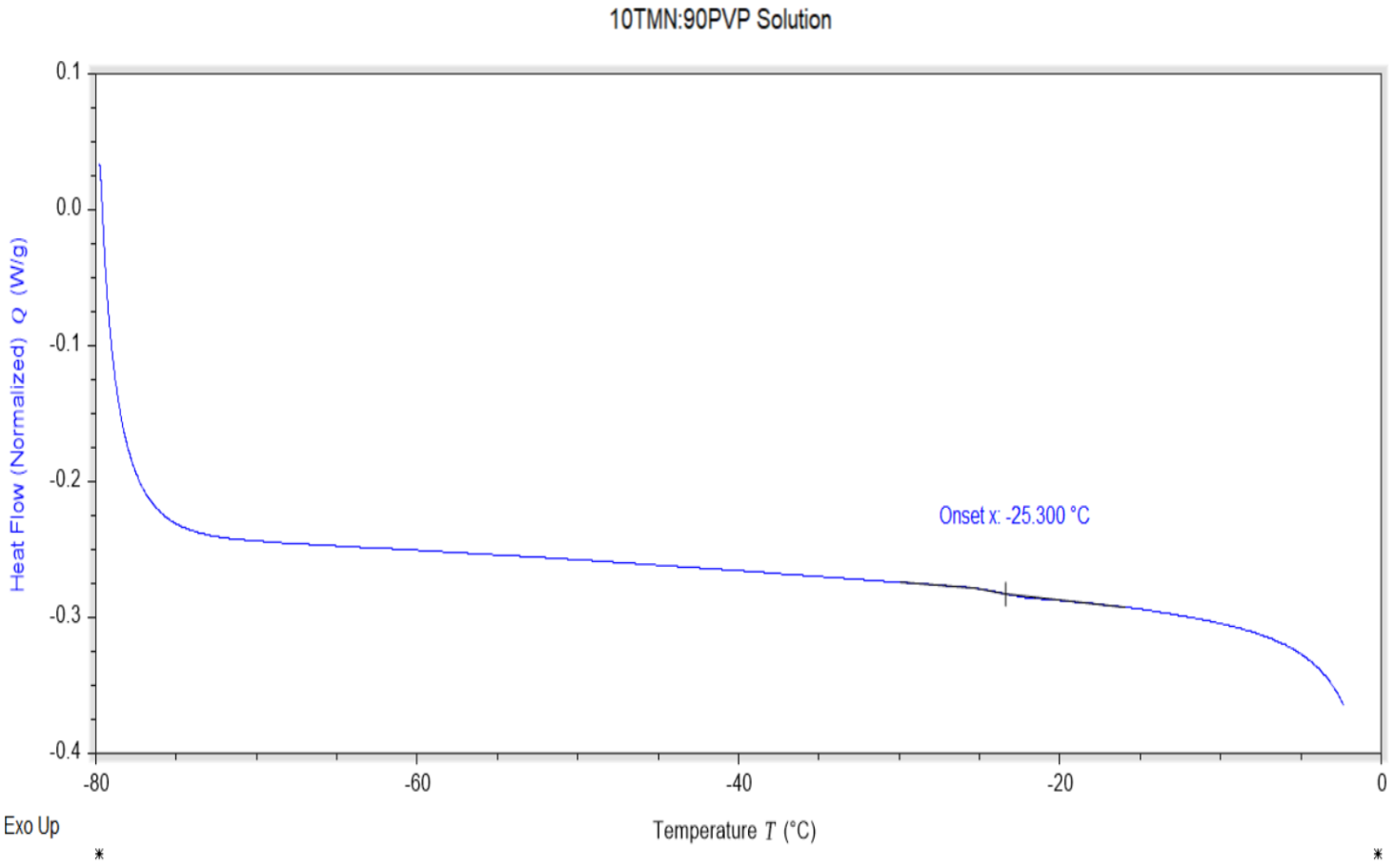

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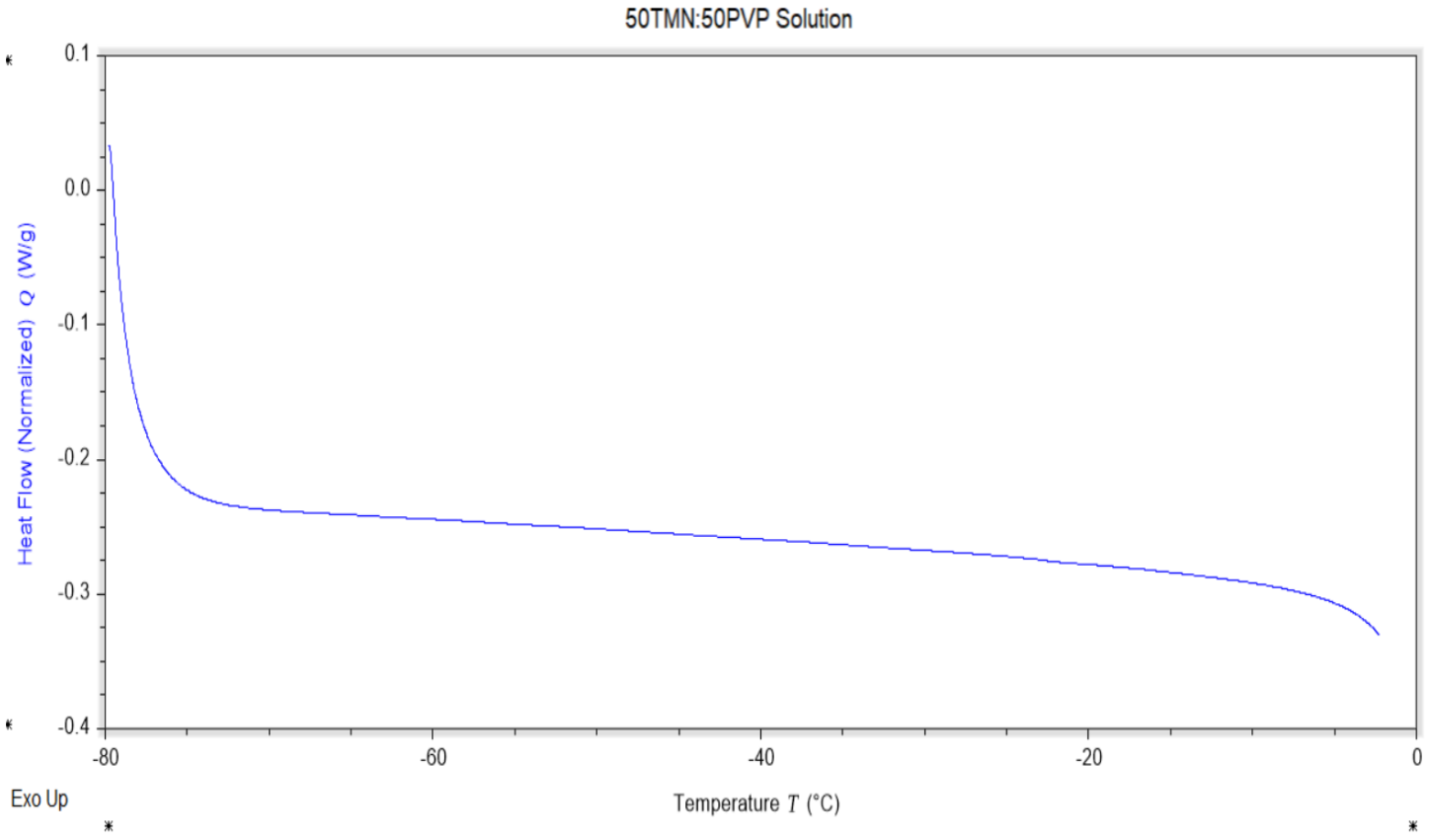

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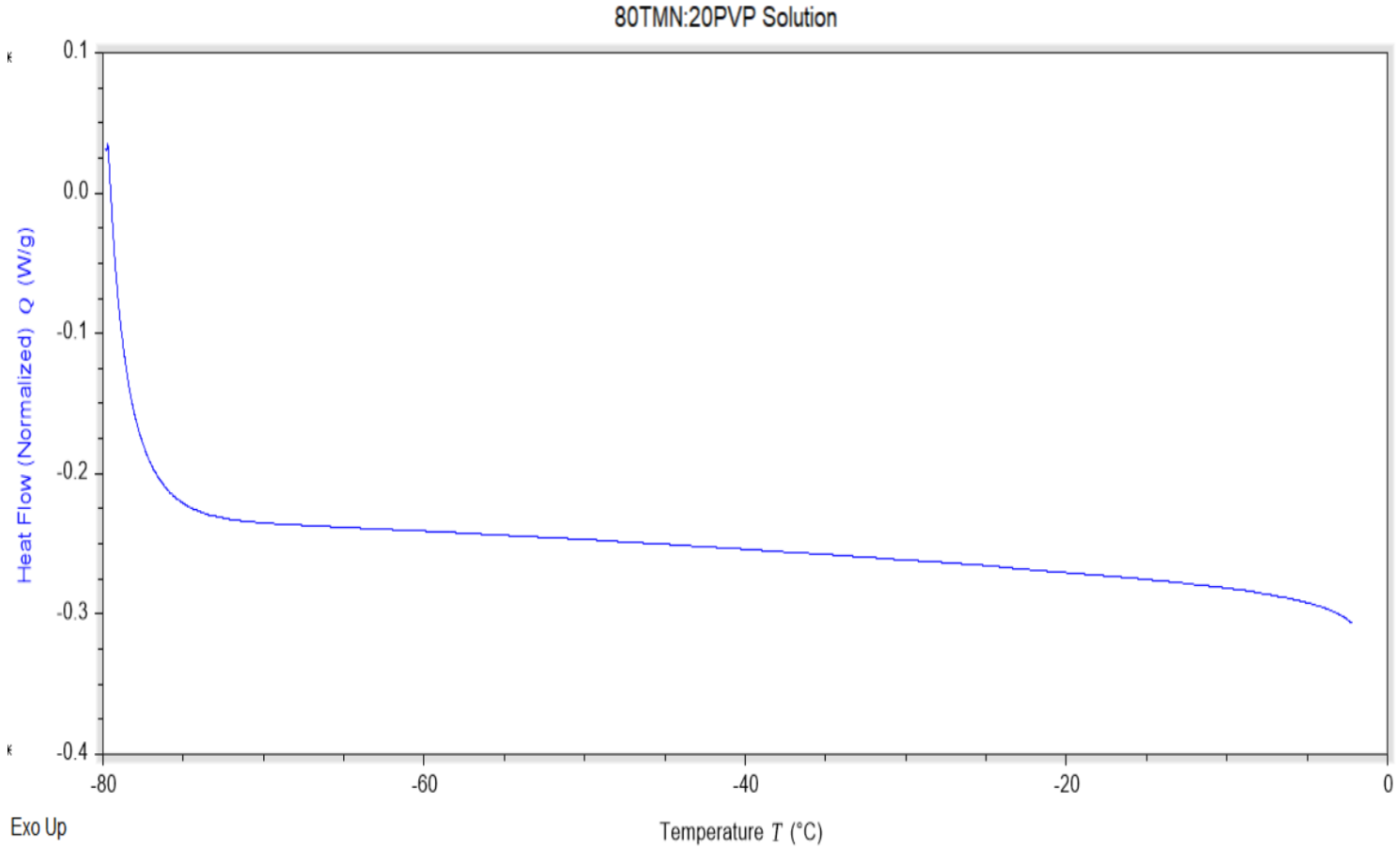

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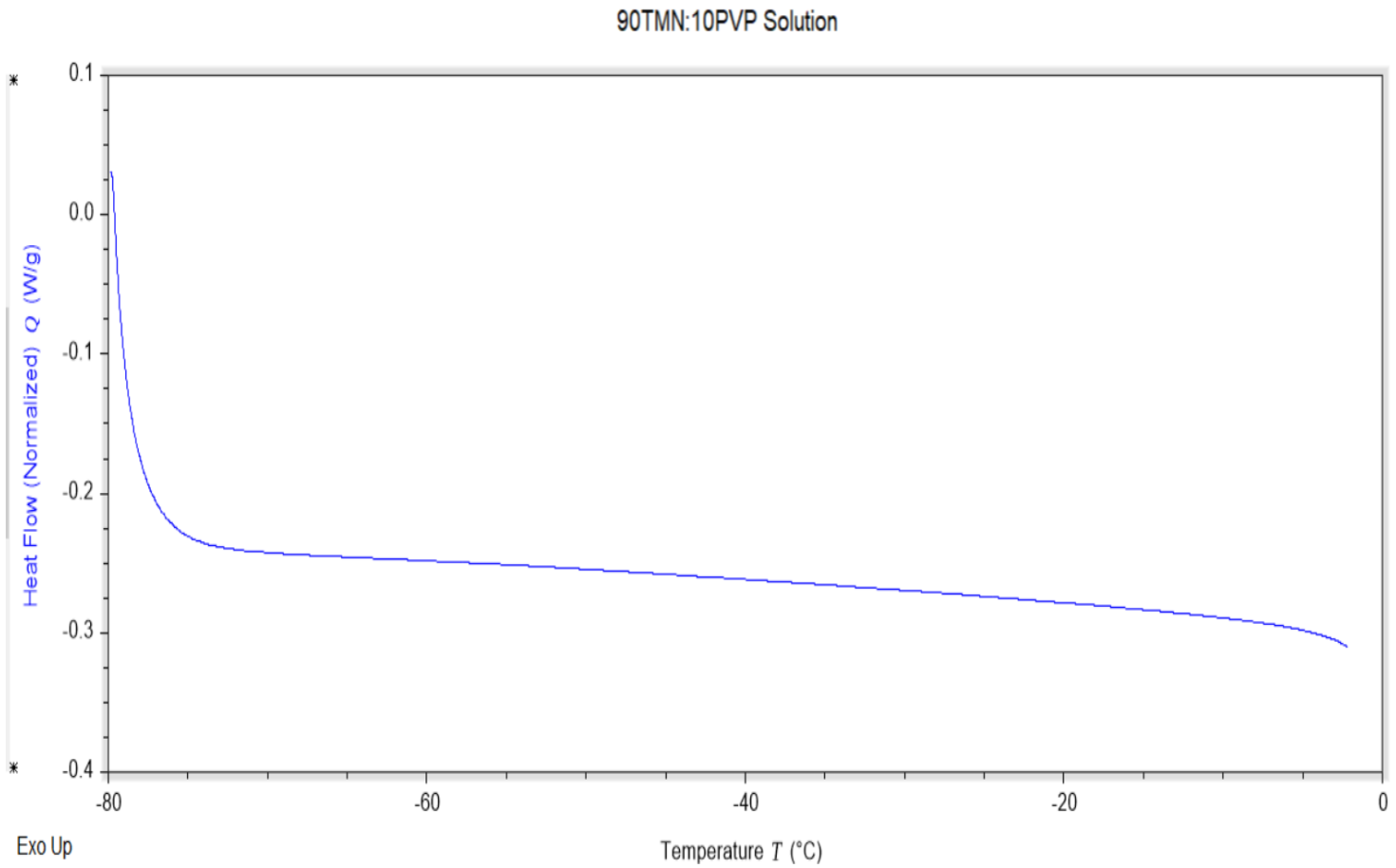

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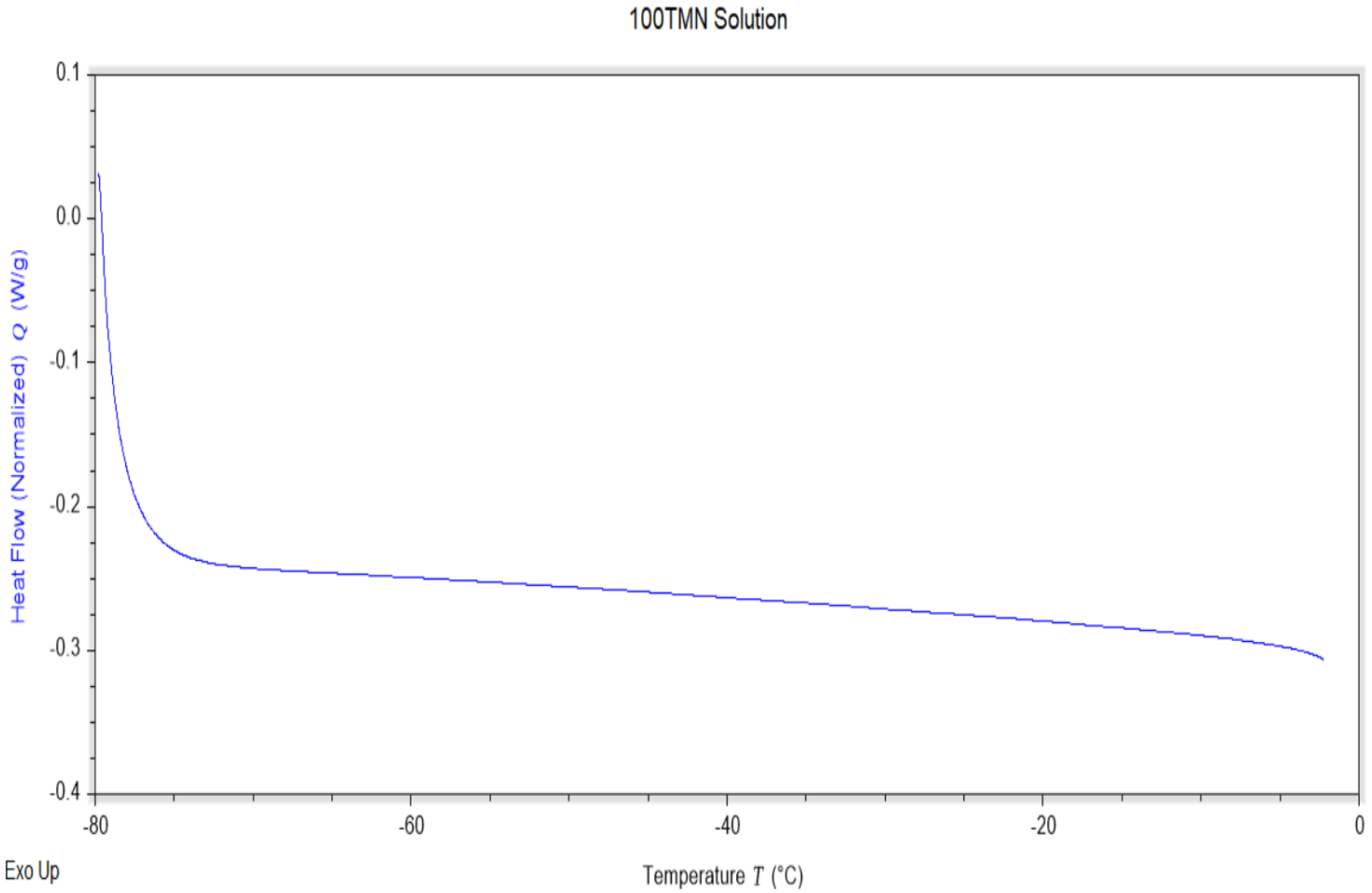

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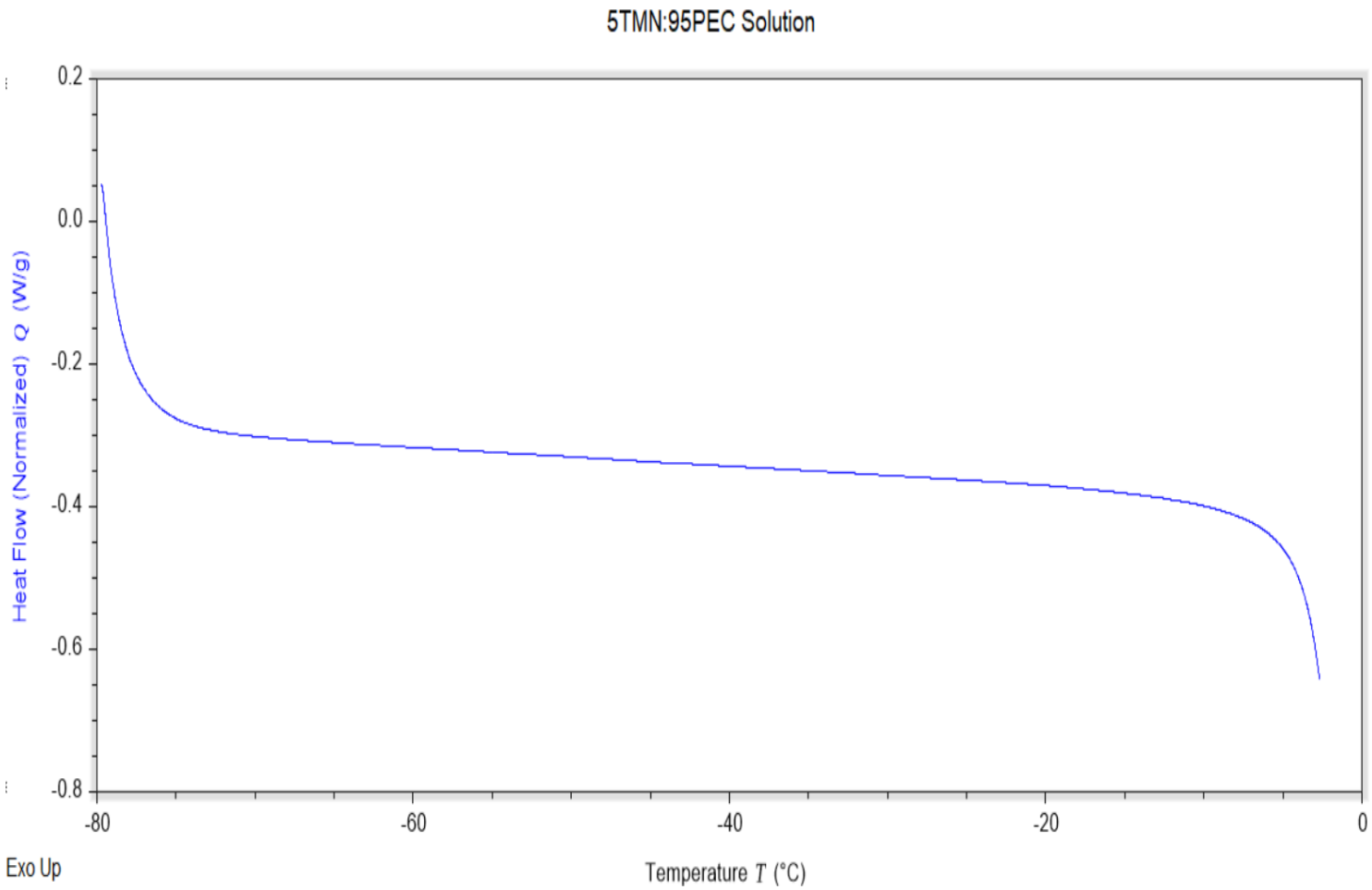

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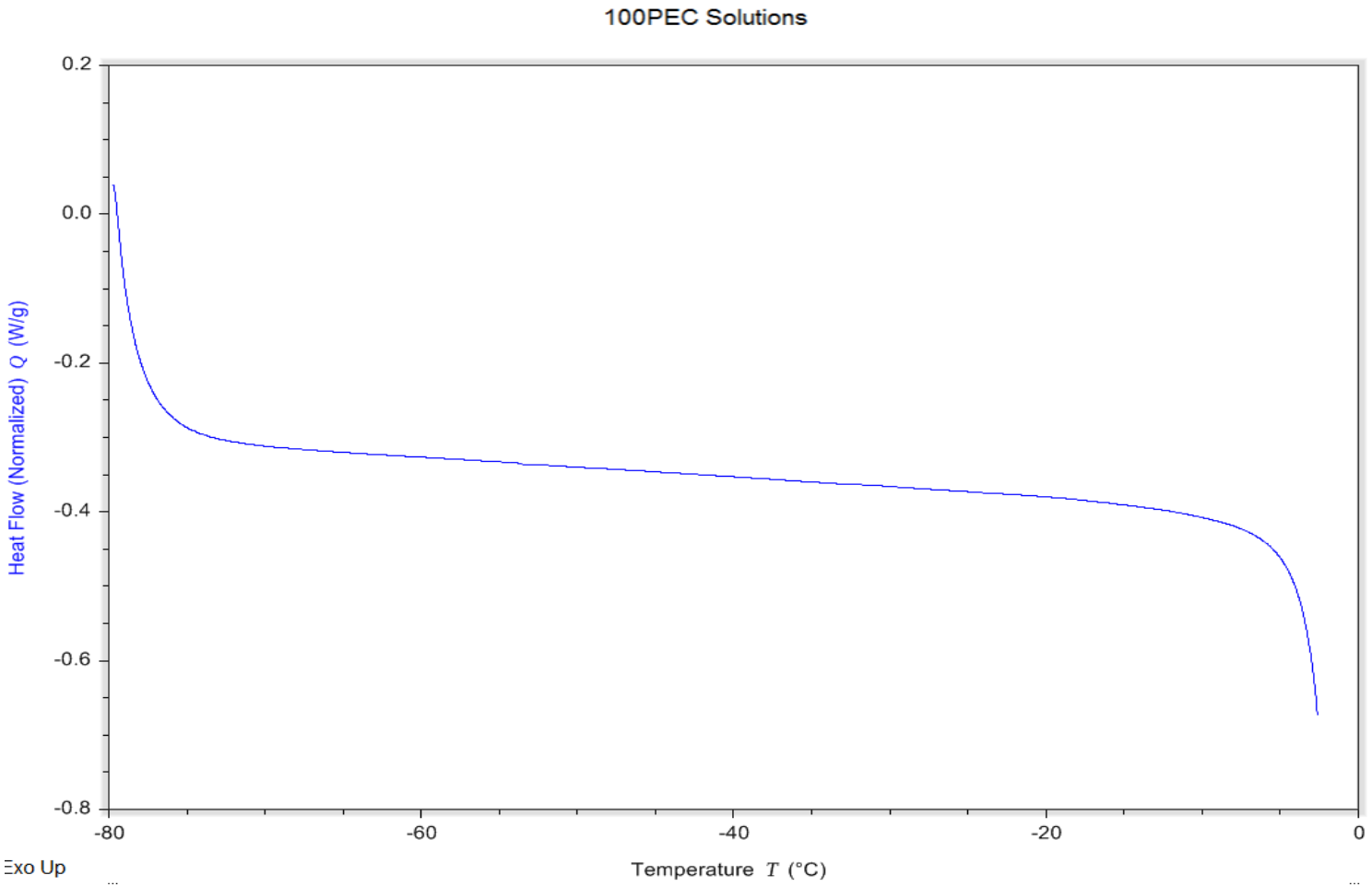


3.6 References

Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International, 99*, 363-374. doi:https://doi.org/10.1016/j.foodres.2017.05.021

Badawy, S. I. (2001). Effect of salt form on chemical stability of an ester prodrug of a glycoprotein IIb/IIIa receptor antagonist in solid dosage forms. *International Journal of Pharmaceutics, 223*(1), 81-87. doi:https://doi.org/10.1016/S0378-5173(01)00726-8

Beaman, G. R. (1952). Relation between (apparent) second-order transition temperature and melting point. *Journal of Polymer Science, 9*(5), 470-472.

Bell, L. N., & Hageman, M. J. (1994). Differentiating between the Effects of Water Activity and Glass Transition Dependent Mobility on a Solid State Chemical Reaction: Aspartame Degradation. *Journal of Agricultural and Food Chemistry, 42*(11), 2398-2401. doi:10.1021/jf00047a007

Christina, B., Taylor, L. S., & Mauer, L. J. (2015). Physical stability of l-ascorbic acid amorphous solid dispersions in different polymers: A study of polymer crystallization inhibitor properties. *Food Research International, 76*, 867-877. doi:https://doi.org/10.1016/j.foodres.2015.08.009

David, S. E., Timmins, P., & Conway, B. R. (2012). Impact of the counterion on the solubility and physicochemical properties of salts of carboxylic acid drugs. *Drug Development and Industrial Pharmacy, 38*(1), 93-103. doi:10.3109/03639045.2011.592530

Dreher, J. G., Rouseff, R. L., & Naim, M. (2003). GC−Olfactometric Characterization of Aroma Volatiles from the Thermal Degradation of Thiamin in Model Orange Juice. *Journal of Agricultural and Food Chemistry, 51*(10), 3097-3102. doi:10.1021/jf034023j

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

Fox, T. G. (1956). Influence of diluent and of copolymer composition on the glass temperature of a polymer system. *Bulletin of the American Physical Society, 1*(2), 123-135.

Gordon, M., & Taylor, J., S. (1952). Ideal copolymers and the second-order transitions of synthetic rubbers. i. non-crystalline copolymers. *Journal of Applied Chemistry, 2*(9), 493-500. doi:10.1002/jctb.5010020901

Grosch, W., & Zeiler-Hilgart, G. (1992). Formation of Meatlike Flavor Compounds. In *Flavor Precursors* (Vol. 490, pp. 183-192): American Chemical Society.

Guerrieri, P., Jarring, K., & Taylor, L. S. (2010). Impact of Counterion on the Chemical Stability of Crystalline Salts of Procaine. *Journal of Pharmaceutical Sciences, 99*(9), 3719-3730. doi:https://doi.org/10.1002/jps.22009

Hancock, B. C., & Zografi, G. (1997). Characteristics and Significance of the Amorphous State in Pharmaceutical Systems. *Journal of Pharmaceutical Sciences, 86*(1), 1-12. doi:https://doi.org/10.1021/js9601896

Hiatt, A. N., Ferruzzi, M. G., Taylor, L. S., & Mauer, L. J. (2008). Impact of Deliquescence on the Chemical Stability of Vitamins B1, B6, and C in Powder Blends. *Journal of Agricultural and Food Chemistry, 56*(15), 6471-6479. doi:10.1021/jf800709f

Kestur, U. S., Van Eerdenbrugh, B., & Taylor, L. S. (2011). Influence of polymer chemistry on crystal growth inhibition of two chemically diverse organic molecules. *Crystengcomm, 13*(22), 6712-6718. doi:10.1039/c1ce05822c

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

Lai, M. C., Hageman, M. J., Schowen, R. L., Borchardt, R. T., & Topp, E. M. (1999). Chemical stability of peptides in polymers. 1. Effect of water on peptide deamidation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) matrixes. *Journal of Pharmaceutical Sciences, 88*(10), 1073-1080. doi:https://doi.org/10.1021/js980227g

Luthra, S. A., Shalaev, E. Y., Medek, A., Hong, J., & Pikal, M. J. (2012). Chemical Stability of Amorphous Materials: Specific and General Media Effects in the Role of Water in the Degradation of Freeze-Dried Zoniporide. *Journal of Pharmaceutical Sciences, 101*(9), 3110-3123. doi:https://doi.org/10.1002/jps.23128

Macek, T. J., Feller, B. A., & Hanus, E. J. (1950). Pharmaceutical Studies with Thiamine Mononitrate. *Journal of the American Pharmaceutical Association (Scientific ed.), 39*(7), 365-369. doi:https://doi.org/10.1002/jps.3030390702

McCandless, D. W. (2009). Early Thiamine Deficiency. In D. W. McCandless (Ed.), *Thiamine Deficiency and Associated Clinical Disorders* (pp. 9-16). Totowa, NJ: Humana Press.

Miyazaki, T., Yoshioka, S., Aso, Y., & Kojima, S. (2004). Ability of Polyvinylpyrrolidone and Polyacrylic Acid to Inhibit the Crystallization of Amorphous Acetaminophen. *Journal of Pharmaceutical Sciences, 93*(11), 2710-2717. doi:https://doi.org/10.1002/jps.20182

Mulley, E. A., Stumbo, C. R., & Hunting, W. M. (1975). Kinetics of thiamine degradation by heat. Effect of pH and form of the vitamin on its rate of destruction. *Journal of food science, Sept*(5), 989-992.

Nath, A., Shope, T. R., & Koch, T. R. (2017). Chapter 51 - Thiamine (Vitamin B1) After Weight Loss Bariatric Surgery A2 - Preedy, Victor R. In R. Rajendram & C. R. Martin (Eds.), *Metabolism and Pathophysiology of Bariatric Surgery* (pp. 479-489). Boston: Academic Press.

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

Pachapurkar, D., & Bell Leonard, N. (2006). Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions. *Journal of Food Science, 70*(7), c423-c426. doi:10.1111/j.1365-2621.2005.tb11463.x

Prinzo, Z. W. (1999). *Thiamine Deficiency and Its Prevention and Control in Major Emergencies*: Micronutrient series; World Health, Organization (WHO). Department of Nutrient for Health and Development; Office of the United Nations High Commissioner for Refugees (UNHCR).

Sanchez, J., Ismail, Y., Christina, B., & Mauer, J. L. (2018). Degradation of L-Ascorbic Acid in the Amorphous Solid State. *Journal of Food Science, 83*(3), 670-681. doi:10.1111/1750-3841.13998

Sing, K. S. W., Everett, D. H., Haul, R. A. W., Moscou, L., Pierotti, R. A., Rouquerol, J., & Siemieniewska, T. (1985). Reporting Physisorption Data for Gas Solid Systems With Special Reference to the Determination of Surface-Area and Porosity (Rrecommendations 1984). *Pure and Applied Chemistry, 57*(4), 603-619. doi:10.1351/pac198557040603

Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition, 30*(2-3), 115-360. doi:10.1080/10408399109527543

Voelker, A. L., Miller, J., Running, C. A., Taylor, L. S., & Mauer, L. J. (2018). Chemical stability and reaction kinetics of two thiamine salts (thiamine mononitrate and thiamine chloride hydrochloride) in solution. *Food Research International, 112*, 443-456. doi:https://doi.org/10.1016/j.foodres.2018.06.056

Wang, R. Y., Pellerin, C., & Lebel, O. (2009). Role of hydrogen bonding in the formation of glasses by small molecules: a triazine case study. *Journal of Materials Chemistry, 19*(18), 2747-2753. doi:10.1039/b820294j

Waterman, K. C., Gerst, P., & Dai, Z. (2012). A Generalized Relation for Solid-State Drug Stability as a Function of Excipient Dilution: Temperature-Independent Behavior. *Journal of Pharmaceutical Sciences, 101*(11), 4170-4177. doi:https://doi.org/10.1002/jps.23268

Wegiel, L. A., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2013). Mid-infrared spectroscopy as a polymer selection tool for formulating amorphous solid dispersions. *Journal of Pharmacy and Pharmacology, 66*(2), 244-255. doi:10.1111/jphp.12079

Wegiel, L. A., Zhao, Y., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2014). Curcumin amorphous solid dispersions: the influence of intra and intermolecular bonding on physical stability. *Pharmaceutical Development and Technology, 19*(8), 976-986. doi:10.3109/10837450.2013.846374

Windheuser, J. J., & Higuchi, T. (1962). Kinetics of Thiamine Hydrolysis. *Journal of Pharmaceutical Sciences, 51*(4), 354-364. doi:https://doi.org/10.1002/jps.2600510415

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 retention*. Paper presented at the HPLC, San Francisco, CA.

CHAPTER 4. A PRELIMINARY STUDY ON THE EFFECT OF PH OF PRE-LYOPHILIZED SOLUTIONS ON THE CHEMICAL DEGRADATION OF THIAMINE IN THIAMINE CHLORIDE HYDROCHLORIDE SOLID DISPERSIONS PREPARED BY POLYVINYLPYRROLIDONE

4.1 Abstract

Thiamine has been known to be highly susceptible to pH changes in solutions, being less stable as pH increases. The studies in this regard are conducted in solution systems, but not in a solid matrix. When considering thiamine as a widely used ingredient for solid food products, it is important to assess its stability at different pH conditions in a solid matrix. Here, the effects of pH on the chemical stability of thiamine stored at different environmental conditions (11% RH and 30^{o}C; 11% RH and 60^{o}C; 75% RH and 40^{o}C) were investigated using solid dispersions, prepared by lyophilization, containing 5% thiamine chloride hydrochloride (TClHCl) and 95% polyvinylpyrrolidone (PVP). The solutions were adjusted to pHs of 4, 5, and 6 prior to lyophilization using citrate buffer. Thiamine degradation in the solid dispersions was quantified by measuring the remaining vitamin biweekly for eight weeks using high performance liquid chromatography (HPLC). Thiamine loss was minimal in the samples stored at low RH and temperature conditions (11% RH and 30^{o}C), regardless of initial pH. However, thiamine stability significantly decreased with increasing the temperature at low RH in all pH conditions. Thiamine dissolved in the samples stored at 75% RH and 40^{o}C, which further increased the degradation, especially at higher pH conditions. For instance, there was 80% thiamine loss in the dispersion stored at 75% RH and 40^{o}C at pH 6 for 8 weeks, whereas only 4% thiamine degradation was

*The term of pH used in this preliminary report corresponds to pre-lyophilization pH of solutions.

observed in its counterpart stored at 11% RH and 30^{o}C. In general, these results indicate that pre-lyophilization solution pH is a determinant factor for chemical degradation of thiamine in solid products and thus, it needs to be considered as an important variable for solid state food formulations to ensure food safety, quality and nutrient delivery.

4.2 Introduction

The pH is a measurement of the hydrogen ions that is generally used to specify the acidity and basicity of an aqueous solutions. In food and pharmaceutical applications, pH is an important determinant factor for the quality of many products, as it dictates the stability of the majority of the constituent ingredients. Although the term of pH is usually used to refer solutions, it is thought to play important role in the stability of compounds in the solid state. For example, if a compound is pH sensitive in solutions, it is reasonable to expect that its stability is likely to be affected also by pH in the solid state. Several methods have been tried to be established to measure the pH of solids (Costantino et al. 1997, Brunner, Mäder and Göpferich 1999, Fu et al. 2000); however, there is no generally accepted standard method to accurately determine pH of a solid. In order to define the pH of solid state, several studies have used the term 'effective pH', which, more specifically, refers to the pH of pre-lyophilized solutions or pH of reconstituted lyophiles (Strickley 1996, Costantino, Langer and Klibanov 1994, Oliyai et al. 1994). It was shown previously that the pH of a solution and its corresponding lyophilized form are quite similar (only 0.3 unit differences) (Costantino et al. 1997). This suggests that pH is also an important factor that can influence the chemical stability of compounds found in the solid state, as it does in solutions.

The lyophilization technique has been widely applied to food and pharmaceutical products to obtain high quality dehydrated products, ensure safety, prolong shelf-life, and control drug release (Marin 2003, Franks 2007). Since it is a widely used technique, the lyophiles need to be

subjected to physical and chemical stability studies, including pH stability which is the main focus of this preliminary study. The pH dependent-solid state stability against degradation has been studied by several authors, who showed that pH has a pronounced effect on the stability of constituents (Song et al. 2001, Strickley 1996, Costantino et al. 1994, Oliyai et al. 1994, Hailu and Bogner 2010, Li, Guo and Zografi 2002). For example, it was shown by Pikal et al. that aggregate formation of lyophilized human growth hormone was dependent on the solid state pH (Pikal et al. 1991). Moreover, a study on dextran-sucrose-citrate and polyvinyl pyrrolidone (PVP)-sucrose-citrate lyophiles revealed that chemical reactivity was significantly affected by solid state pH (Chatterjee et al. 2008).

Thiamine is one of the most unstable vitamins, and its chemical stability is known to be highly influenced by pH in solutions (Windheuser and Higuchi 1962). Chemical stability of thiamine has been investigated by several researchers in different buffer systems to document pH effect on its degradation in solutions (Pachapurkar and Bell 2006, Windheuser and Higuchi 1962, Mulley, Stumbo and Hunting 1975). However, no study has been conducted to evaluate effect of pH on thiamine stability in solid-state lyophiles wherein thiamine may solidify in the amorphous state (Arioglu-Tuncil et al. 2017). When considering the pH sensitivity of thiamine as well as the previous publications which revealed that pH of pre-lyophilized solutions dictates chemical stability, a mechanistic understanding of the effect of pH on thiamine degradation is needed to ensure thiamine retention in a solid matrix. Therefore, the objective of this preliminary study was to investigate influence of pre-lyophilized solution pH on thiamine retention in a PVP matrix at select environmental conditions. PVP and pectin were used in previous works presented in this dissertation as polymers to study chemical degradation of amorphous thiamine in solid dispersions prepared by lyophilization. Since thiamine loss was observed to be greater in PVP dispersions,

PVP was selected as a polymer in this preliminary study. Given the pH susceptibility of thiamine and previous publications on importance of pH of pre-lyophilized solutions on chemical stability, it was hypothesized that thiamine stability will decrease in the lyophiles with increasing the pH of the pre-lyophilized solutions, especially at high temperature and RH conditions. The data obtained from this preliminary study could be used to improve shelf life predictions and to optimize thiamine stability in the solid state.

4.3 Materials and methods

4.3.1 Materials

Thiamine chloride hydrochloride (TClHCl) and poly (vinylpyrrolidone) (PVP, MW 40,000) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). Anhydrous citric acid and sodium citrate dihydrate were obtained from VWR (VWR International LLC., Bristol, CT) and Mallinckrodt Pharmaceuticals (Dublin, Ireland), respectively. Specific relative humidity (RH) conditions (reported here at 25°C) were created using saturated salt solutions of sodium chloride (NaCl, 75% RH) (Sigma-Aldrich Inc., St. Louis, MO) and lithium chloride (LiCl, 11% RH) (EMD Millipore, Billerica, MA). HPLC grade trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich Inc. (St. Louis, MO), and acetonitrile was purchased from Fisher Scientific Co., LLC (Pittsburgh, PA).

4.3.2 Preparation of TClHCl:PVP solid dispersions via lyophilization and pH adjustment

TClHCl solid dispersions were prepared in triplicate using 5% TClHCl and 95% PVP (w/w). Citrate buffer was prepared based on the Henderson-Hasselbalch equation provided below to adjust the pH of the solutions to pH of 4, 5, and 6.

$$pH = pKa + \log\left(\frac{[A^-]}{[HA]}\right) \quad 4.1$$

Briefly, the calculated amounts of anhydrous citric acid and sodium citrate dihydrate for each pH were weighed and dissolved in water. The PVP and TClHCl were then weighted and dissolved in the buffers, separately. After that, buffer solutions containing PVP and TClHCl were added to 20 mL amber glass vials and mixed with a Roto-Shake Genie® SI-1100 (Scientific Industries, Inc., Bohemia, NY) for 20 minutes. An Orion Star A211 pH meter (Thermo Fisher Scientific Inc., Waltham, MA) was used to measure the pH of the buffers, the solutions prior to lyophilization, and the reconstituted lyophiles right after freeze drying to confirm the pHs. The solutions were then frozen overnight at -20^{o}C prior to lyophilization. After removal from the freezer, samples were placed into a VirTis Genesis 25ES shelf freeze dryer (SP Scientific, Stone Ridge, NY) and frozen for 6 hours at -40^{o}C and 300 mTorr. For the primary drying step, high vacuum was applied (150 mTorr) at -40^{o}C for 24 hours to remove the bulk of water via sublimation. Secondary drying was then achieved by holding the samples for 9 hours each at 10^{o}C intervals from -40^{o}C to 20^{o}C. Lastly, the samples were held for 6 hours at 25^{o}C at 300mTorr. The pH of the samples were within 0.1 pH units prior to and after lyophilization. The lyophilized samples were immediately placed into RH-controlled desiccators.

4.3.3 Storage treatments

The following temperature and RH conditions were chosen as storage treatments: 11%RH and 30^{o}C and 60^{o}C, and 75%RH and 40^{o}C. The condition of 11% RH and 30^{o}C was chosen to determine if pH has an effect at low RH and temperature conditions. At higher temperature (60^{o}C) at the same RH was chosen to document the impact of the temperature along with pH on degradation. Lastly, 75% RH and 40^{o}C was studied to better understand the impact of high RH on thiamine stability along with pH. This condition is also the recommendation from the ICH guidelines for monitoring the shelf life of solid products. RH was controlled by using saturated

salt solutions in desiccators (with water activity verified by measurement using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, WA), and the desiccators were placed into water jacketed incubators (Forma Scientific, Inc., Marietta, OH) to control the temperature. Samples were stored for eight weeks, removed biweekly for HPLC analysis, and discarded after analysis.

4.3.4 Thiamine quantification by HPLC

Thiamine was quantified biweekly for eight weeks using a Waters 2690SM (Waters Corp., Milford, MA) HPLC with a Waters Xselect HSS T3 (3.5µm, 4.6x100mm) column and a Waters 2996 photodiode array detector. Prior to each analysis, standard curves of thiamine from TClHCl at a concentration range from 0.005 to 1 mg/mL were prepared (r^2=0.9997-1.0000). Samples were diluted with solvent to a final estimated vitamin concentration 0.5 mg/mL, and filtered through a 0.2 µm syringe filter. The mobile phase containing solvent A (acetonitrile) and solvent B (water and 0.1% TFA) was used with the following gradient procedure adapted from (Xia et al. 2006): 0/100 at 0 min (immediate), 3/97 at 4 min (linear), 10/90 at 6 min (linear), 0/100 at 10 min (linear) and 0/100 from 10 to 15 min (immediate), for a total chromatographic run time of 15 min. The flow rate was 1 mL/min, and the samples were scanned between 235-400 nm. Integration was conducted at 247 nm using Masslynx V4.1 software (Waters Corp., Milford, MA).

4.3.5 Statistical analysis

All analyses were performed in triplicate and data are presented as mean±SD. The samples possessing the same pre-lyophilization solution pH but stored at different conditions as well as the samples with different pHs and stored in the same conditions were statistically compared to each other. SAS Software Version 9.4 (SAS Institute, Cary, NC) was used to conduct statistical analyses. Analysis of variance (ANOVA) was performed at $\alpha = 0.05$ significance level to

determine differences among the samples. Tukey's multiple comparison test (α=0.05) was used to test whether the results were statistically different.

4.4 Results and Discussion

4.4.1 Preliminary results of vitamin quantification in different 'pH's and storage conditions

Preliminary results of remaining thiamine at different pre-lyophilization solution pH and storage conditions are presented in **Figs. 4.1-3**. When the sample pH was 4 but the storage conditions were changed increasing temperature at low RH (11%) and increasing the storage RH both significantly increased thiamine degradation. No significant thiamine degradation was observed at pH 4 11% RH and 30^{o}C (**Fig. 4.1A**). As storage temperature increased to 60^{o}C at the same RH, the stability of thiamine dramatically decreased, and thiamine loss reached 22% after 56 days of storage (**Fig. 4.1A**). As expected, increasing the storage RH to 75% further induced thiamine degradation (41% of loss at week 8), which was attributed to dissolution of thiamine due to significantly high amount of moisture absorption. The greatest amount and rate of vitamin loss at 11% RH and 60^{o}C as well as at 75% RH and 40^{o}C occurred in the PVP matrix in the first two weeks of the storage, which was 12%, and 20%, respectively (**Fig. 4.1A1).** Thiamine degradation at pH 4 were also compared to un-buffered systems (which had a pH of 3.8) in 5TClHCl:95PVP dispersions at 11% RH-30^{o}C and 60^{o}C, and 75% RH and 40^{o}C (**Fig. 4.1A2**). No significant difference were found for thiamine degradation at 11% RH-30^{o}C and 60^{o}C. Since the data points selected for thiamine detection were different for 5TClHCl:95PVP dispersions in buffered and un-buffered system, the samples could not be compared to each other on day 42 and 56. However, the trends for thiamine loss in these two systems were likely to be same.

Similar findings were observed for the samples in which pH was adjusted to 5 prior to lyophilization (**Fig. 4.1B)**. Thiamine loss was negligible in the samples stored at low RH and

temperature. Thiamine loss increased with increasing the storage temperature at the same RH and pH. Similar to samples stored at pH 4, thiamine degradation was significantly affected at 75% RH and 40^{o}C, and 45% of vitamin loss was detected after 56 days of storage, which was again due to solution formation.

At pH 6, the highest pH condition studied in this preliminary research, thiamine loss was minimal at 11% RH and 30^{o}C which was in line with the previous samples at the same storage condition (**Fig. 4.1C**). However, a drastic increase in vitamin loss was found with increasing the temperature at low RH condition (11% RH and 60^{o}C). The amount of thiamine degraded in this condition was found to be 24% even after two weeks of storage. After a fast-initial loss of thiamine in the first two weeks, vitamin loss occurred at a relatively slower rate, and it reached to 31% on day 56. Dissolution of TClHCl at 75% RH and 40^{o}C significantly affected chemical stability of thiamine at pH 6 (**Fig. 4.1C**). Thiamine loss was 80% at the end of 42 days of storage.

When different pH conditions in the same storage treatment were compared to each other, no significant difference ($p < 0.05$) in thiamine loss was found between any given pH conditions at low RH and temperature (11% RH and 30^{o}C) (**Fig. 4.2A**). It was also revealed that samples stored at pH 4 and 5 showed quite similar pH stability behavior at 11% RH and 60^{o}C. (**Figs. 4.2A-B**). However, slight differences (4-7% difference depending on the time points) in degradation were found between the samples stored at 75% RH and 40^{o}C at pH 4 and 5 (**Fig. 4.2C**). At any given pH and time point, thiamine loss was significantly highest ($p<0.05$) in all the samples stored at 75% RH and 40^{o}C, followed by 11% RH and 60^{o}C. Although thiamine was expected to degrade more at 75% RH due to the formation of solution, compared to low RH environment (11% RH), interestingly, at pH 6, remaining thiamine after 56 days of storage at 11% RH and 60^{o}C (where thiamine was in solid form) was only 3% higher than the samples with pH 5 stored at 75% RH and

40^{o}C (where thiamine was in solution) (**Fig. 4.3A**). As seen from the speciation plot of thiamine (**Fig. 4.3B**), thiamine stability is highly dependent on the pH. As the pH increases, the fraction of unprotonated species which are less stable increases. For example, the fraction of species in unprotonated form are calculated to be 0.14 and 0.61 at pH 4 and pH 5, respectively. As pH raises to 6, a dramatic increase in the fraction of unprotonated species is seen and the value reaches up to 0. 94. Since the percentage of unprotonated thiamine species increases, the stability of thiamine decreases, especially at pHs near and above 6. These data collectively indicate that pH plays an important role on determining the stability of thiamine in a solid matrix.

HPLC chromatograms of samples with pHs of 4, 5, and 6 were compared in **Figs. 4.4** after 42 days of storage at 75% RH and 40^{o}C. The chromatogram of pH 6 showed many degradation products with different quantities (**Fig. 4.4C**). Although the HPLC chromatograms revealed that the samples at pH 4 and 5 released the same degradation products as the sample at pH 6 did (based on retention time $\leq$7), the quantity of the products observed in samples at pH 4 and 5 were relatively small (**Figs. 4.4**). Several more degradation products were found between retention times of 7 and 9 minutes in samples with pH 6 (**Fig. 4.4C**), which might indicate that thiamine degradation at these conditions followed different pathways or caused formation of different (more) degradation products. However, it may also be due to a difference in the amount of thiamine loss, thereby difference in the amount of new products formed. In other words, if thiamine degradation was further induced in the conditions of pH 4 and 5, the products formed in pH 6 (with the retention time between 7-9 min) (**Fig. 4.4C**), could also be yielded.

To better understand this phenomenon, the HPLC chromatogram of pH 6 samples stored at 11% and 60^{o}C and that of pH 5 stored at 75% RH and 40^{o}C were compared (**Figs. 4.5**). These samples were chosen, because they showed very similar loss in thiamine amount, although the pH

and storage conditions were different. The degradation products formed in these samples were same, with an exception that pH 5 stored at 75% RH and 40^{o}C revealed one more degradation product having a retention time of 7.80 (**Fig. 4.5B**). However, the quantity of this product was very small to come to a conclusion. It was previously reported that thiamine degradation in solutions follows different pathways, depending on pH under buffer-free conditions. However, these differences in degradation pathways were classified as: pH below 1, pH between 1-6, and pH above 6.5 (Windheuser and Higuchi 1962). Based on this classification system, all the pH conditions studied here belongs to same category. In addition, the system used here was a buffered systems and all the samples were found in solid form (at least initially for the samples stored at 75% RH and 40^{o}C). It was previously stated that thiazole and pyrimidine fractions are formed a result of water cleavage of thiamine (Dwivedi and Arnold 1973, Windheuser and Higuchi 1962), which was, most probably, the case for the samples stored at 75% RH and 40^{o}C.

HPLC chromatograms of pH 6 samples stored at 11% RH and 60^{o}C and 75% RH and 40^{o}C for 42 days are also provided in **Figs. 4.6**. Although the pH was the same, different degradation products with retention time of ≥ 7 were formed at different storage conditions (**Fig. 4.6B**). This furher confirms the importance of storage conditions selected for thiamine degradation.

4.5 Conclusion

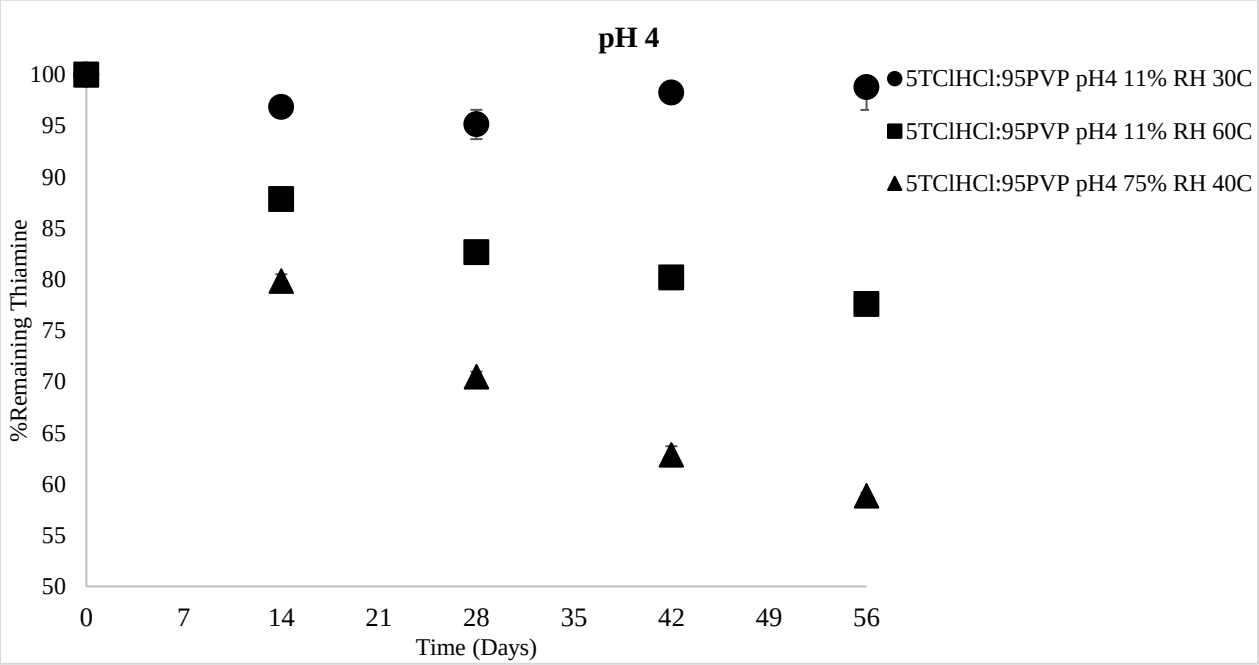
Preliminary results revealed that the pH of pre-lyophilized solutions have a significant impact on thiamine degradation, particularly at higher temperatures and/or RH conditions. In addition, vitamin loss at high RH where thiamine dissolves resulted in greater loss of thiamine. In general, thiamine degraded more as pH increased and its stability was highly dependent on storage treatments along with pH. Thus, pH is an important parameter that needs to be carefully considered for ingredient selection to ensure thiamine delivery and pH adjustment to maintain thiamine in the

protonated form may significantly improve thiamine retention in solid state. As future studies, the thiamine degradation pathway can be documented in a wider range of pH conditions. In addition, the effect of different temperatures at the same pH or vice versa can be investigated to understand the influence of pH by accounting the temperature. Thiamine chemical stability can also be tracked in the presence of different polymers to see how excipient type and properties have impact on stability at different pHs. Lastly, thiamine degradation can be tracked in a longer period of time to be able to obtain and differentiate degradation products better.

Table 4.1 Percent remaining thiamine stored at different storage conditions and pHs.

5TClHCl:95PVP Dispersions	**Time (Days)**			
pH 4	**14**	**28**	**42**	**56**
11%RH and 30ºC	96.8±0.8^{A}	95.1±1.4^{A}	98 ±1^{A}	98.8±2.2^{A}
11%RH and 60ºC	87.8±0.9^{B}	82.6±0.4^{B}	80.2±0.6^{B}	77.6±0.5^{B}
75%RH and 40ºC	79.8±0.7^{C}	70.5±0.5^{C}	62.8±0.9^{C}	58.8±0.3^{C}
pH 5	**14**	**28**	**42**	**56**
11%RH and 30ºC	96 ±2^{A}	96. 7±0.5^{A}	98.6±0.6^{A}	99.72±1.01^{A}
11%RH and 60ºC	87.2±0.2^{B}	81.1±0.8^{B}	77.6±1.1^{B}	77.4±0.4^{B}
75%RH and 40ºC	73.7±0.8^{C}	63.4±0.8^{C}	57.8±0.2^{C}	55.12±1.04^{C}
pH 6	**14**	**28**	**42**	**56**
11%RH and 30ºC	94.1±1.2^{A}	94.5±0.2^{A}	95.6±0.8^{A}	95.6±0.6^{A}
11%RH and 60ºC	76.9±0.5^{B}	67.7±0.2^{B}	61.6±0.6^{B}	58.8±1.7^{B}
75%RH and 40ºC	49±1^{C}	20.9±4.6^{C}	19.8±2.9^{C}	N/A
5TClHCl:95PVP Dispersions	**Time (Days)**			
11%RH and 30ºC	**14**	**28**	**42**	**56**
pH 4	96.8±0.8^{a}	95.1±1.4^{ab}	98 ±1^{a}	98.8±2.2^{a}
pH 5	96 ±2^{a}	96. 7±0.5^{a}	98.6±0.6^{a}	99.72±1.01^{a}
pH 6	94.1±1.2^{a}	94.5±0.2^{b}	95.6±0.8^{b}	95.6±0.6^{b}
11%RH and 60ºC	**14**	**28**	**42**	**56**
pH 4	87.8±0.9^{a}	82.6±0.4^{a}	80.2±0.6^{a}	77.6±0.5^{a}
pH 5	87.2±0.2^{a}	81.1±0.8^{b}	77.6±1.1^{b}	77.4±0.4^{a}
pH 6	76.9±0.5^{b}	67.7±0.2^{c}	61.6±0.6^{c}	58.8±1.7^{b}
75%RH and 40ºC	**14**	**28**	**42**	**56**
pH 4	79.8±0.7^{a}	70.5±0.5^{a}	62.8±0.9^{a}	58.8±0.3^{a}
pH 5	73.7±0.8^{b}	63.4±0.8^{b}	57.8±0.2^{b}	55.12±1.04
pH 6	49±1^{c}	20.9±4.6^{c}	19.8±2.9^{c}	N/A

A1)



A2)

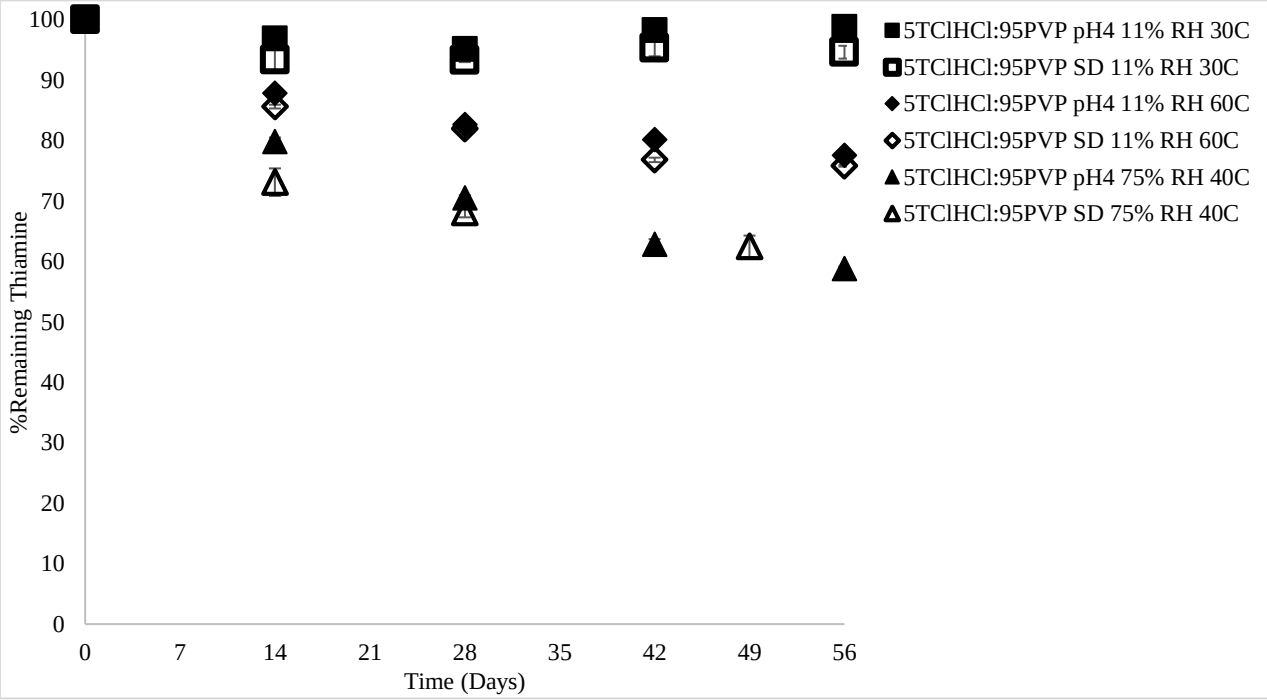
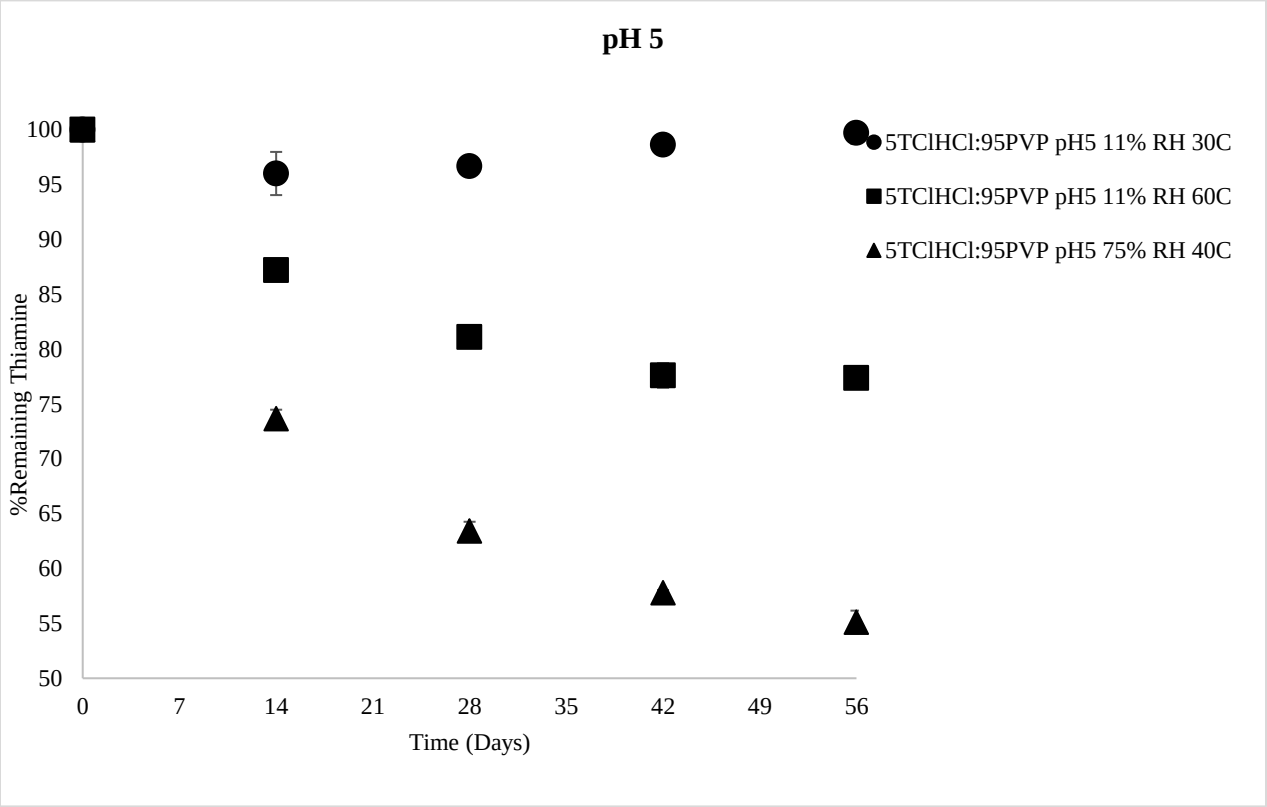


Figure 4.1 Chemical stability of thiamine in 5TClHCl:95PVP dispersions stored at 11% RH and 30ºC, 60ºC, and 75% RH and 40ºC **A1)** at pH 4, **A2)** in buffered samples (pH 4) vs. un-buffered samples (pH 3.8), **B)** at pH 5, **C)** at pH 6.

Figure 4.1 continued.

B)



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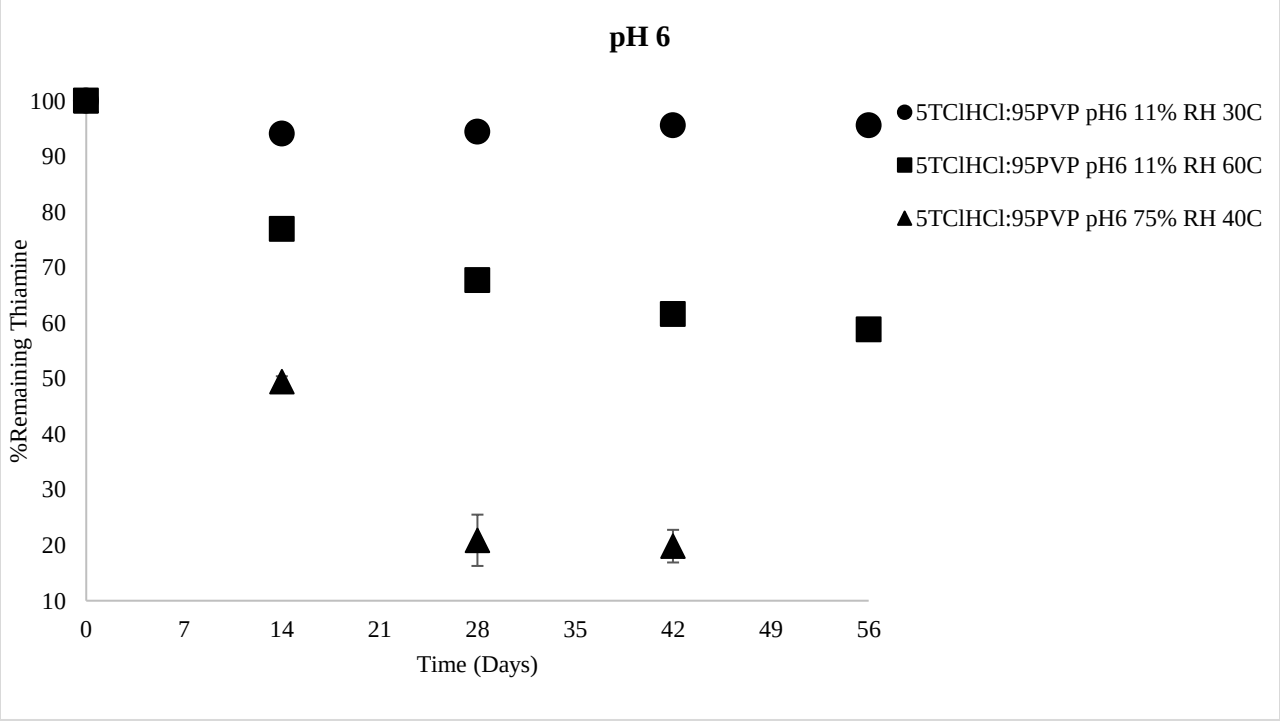

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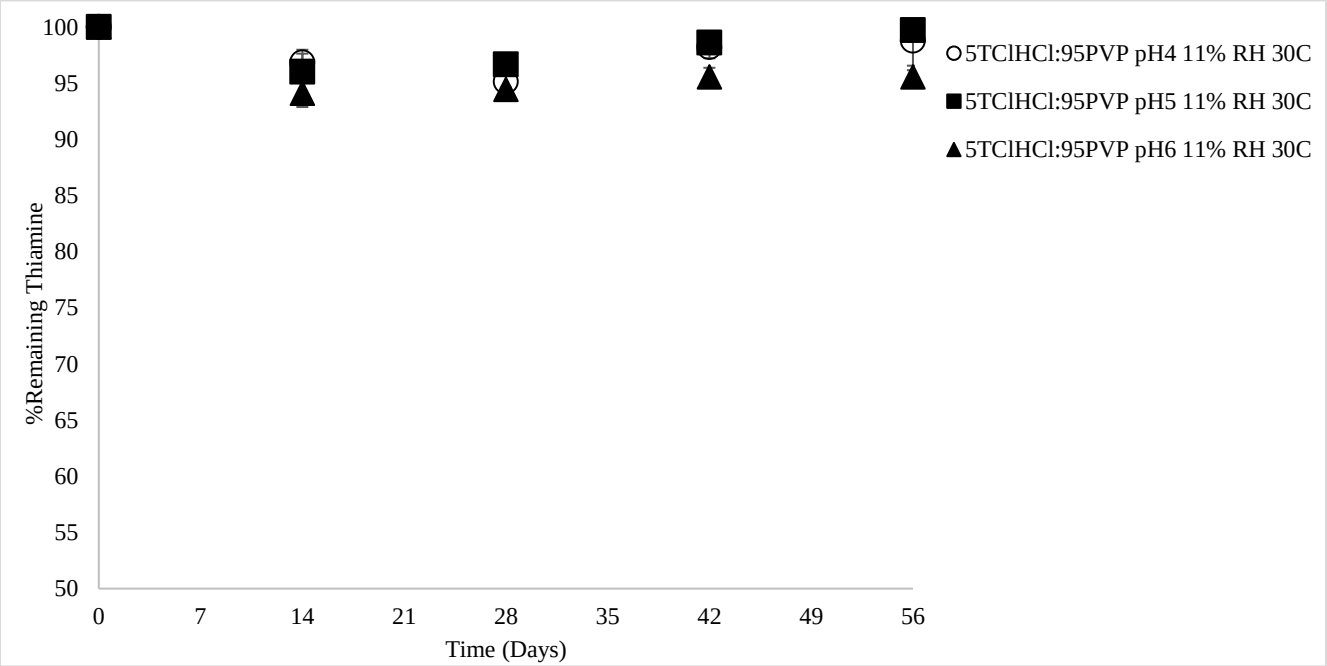

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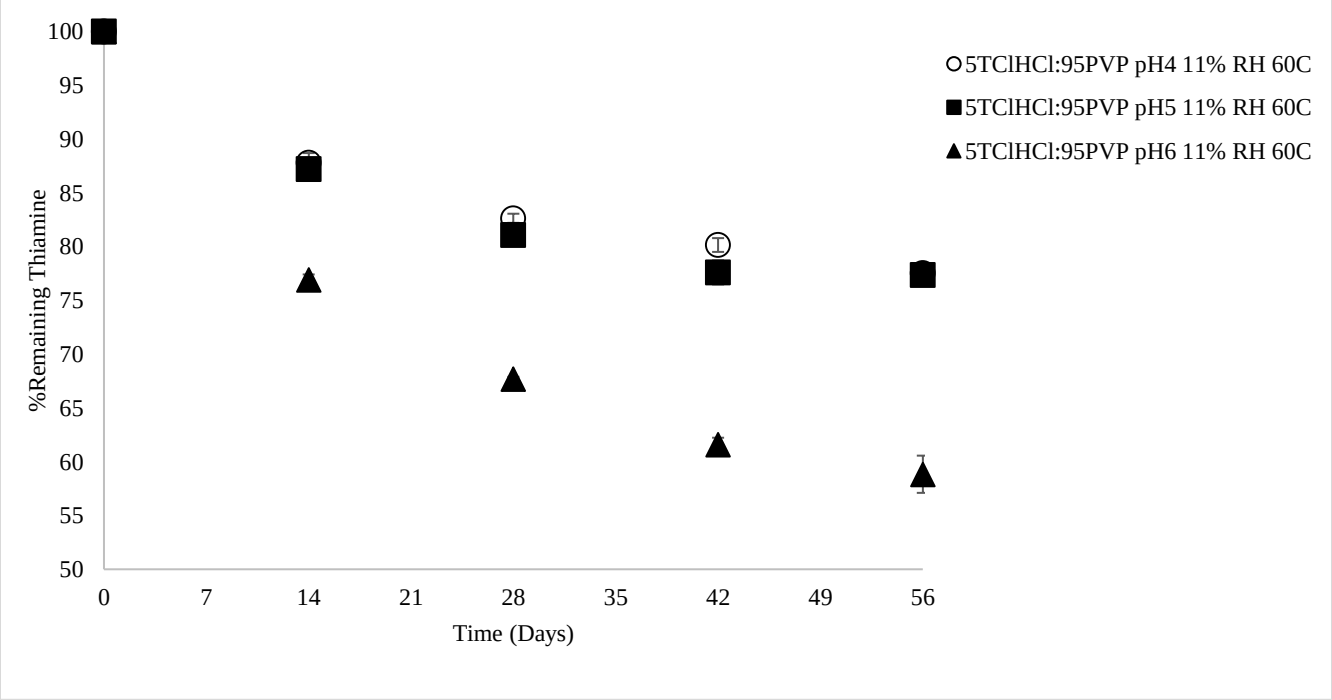
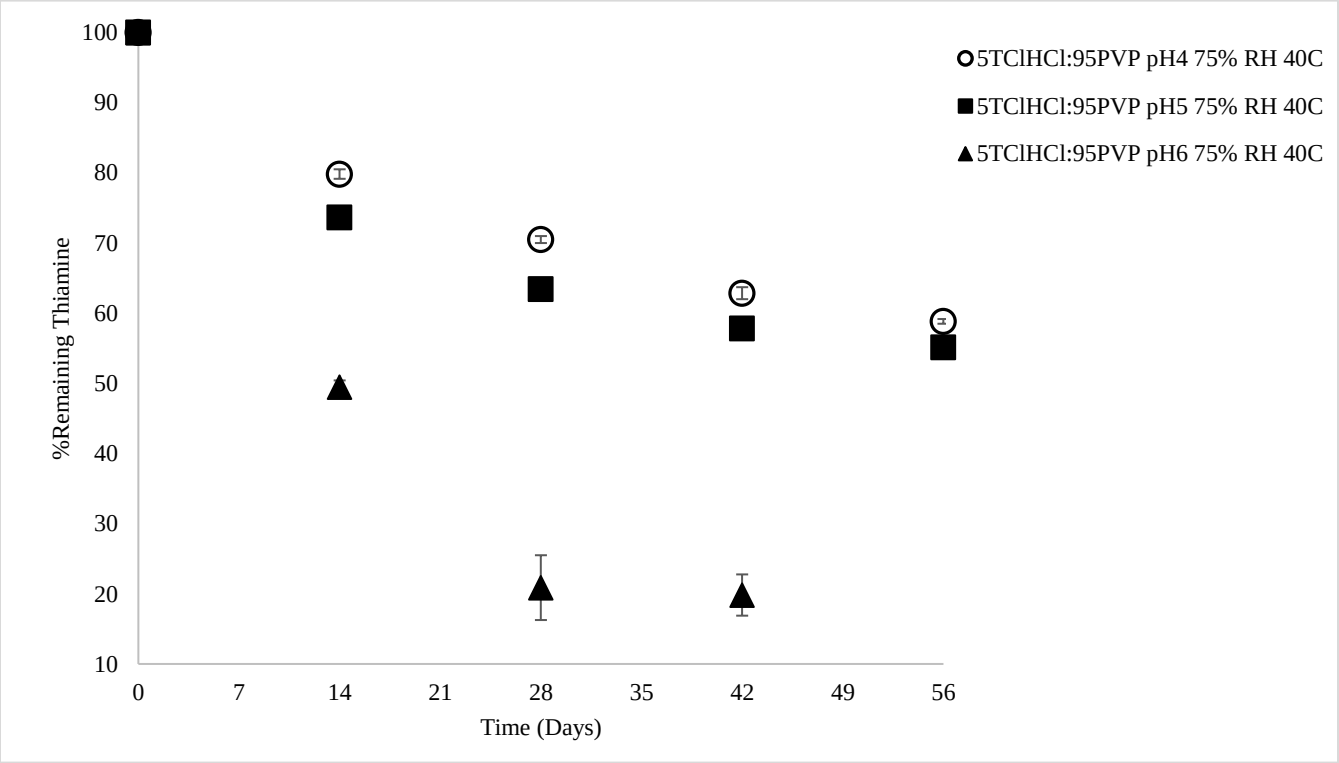


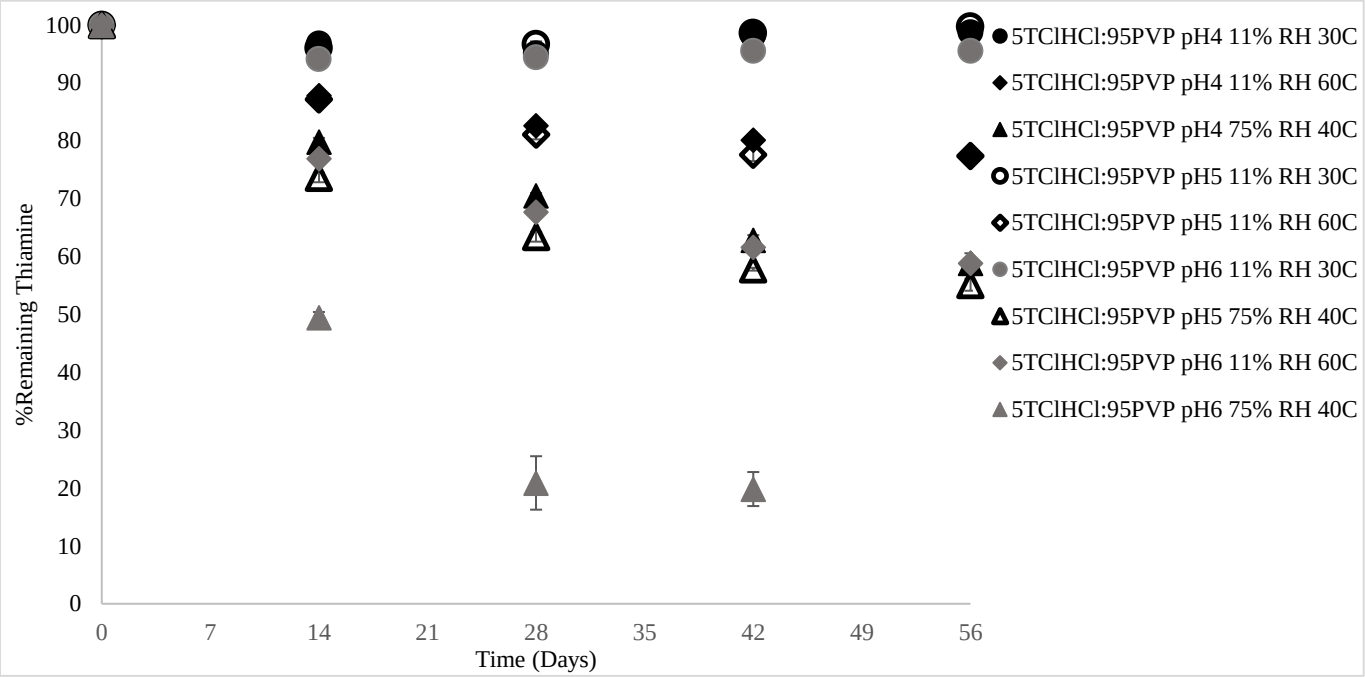
Figure 4.2 Chemical stability of thiamine in 5TClHCl:95PVP dispersions at pH 4, 5, and 6, **A)** stored at 11% RH and 30ºC, **B)** stored at 11% RH and 60ºC, and **C)** stored at 75% RH and 40ºC.

Figure 4.2 continued

C)



A)



B)

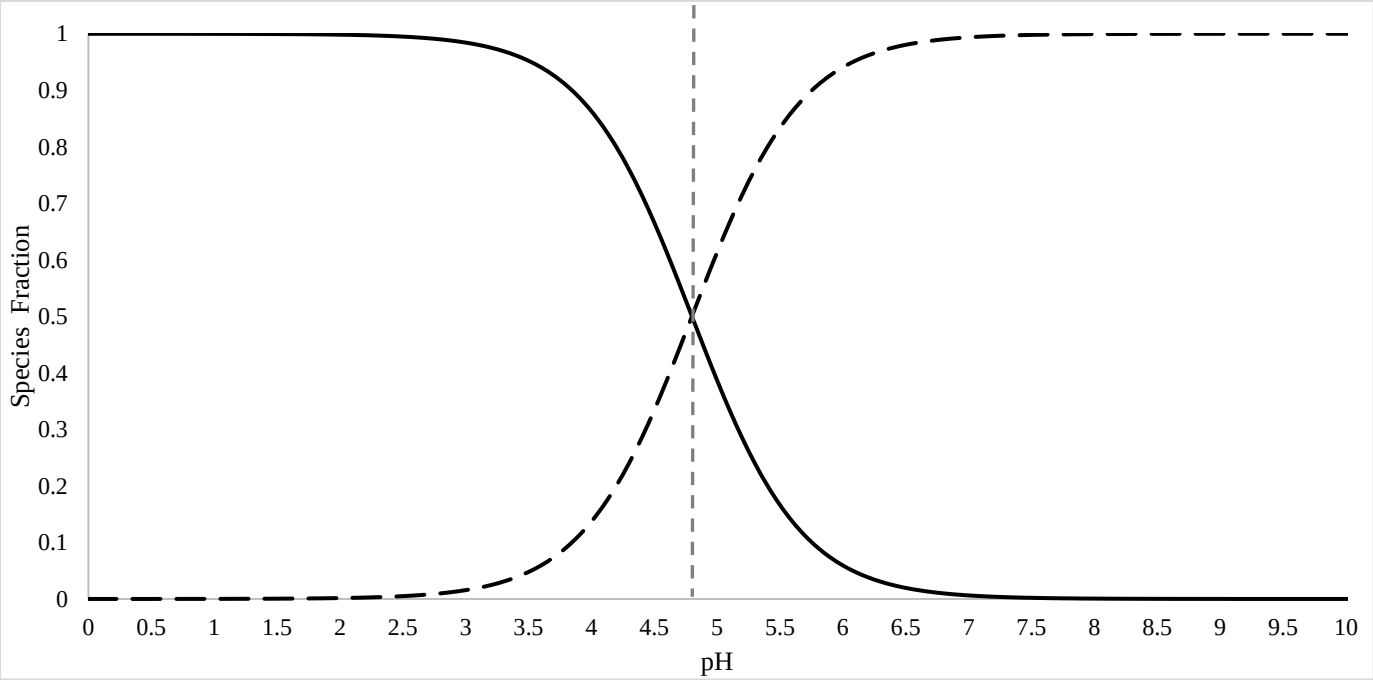
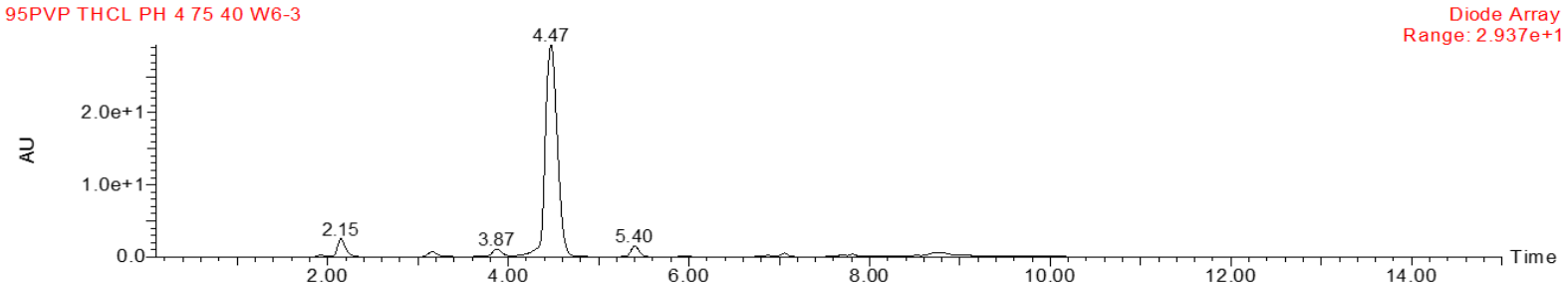


Figure 4.3 **A)** Chemical stability of thiamine in 5TClHCl:95PVP dispersions at pH 4, 5, and 6, **B)** distribution of species for thiamine.

A)



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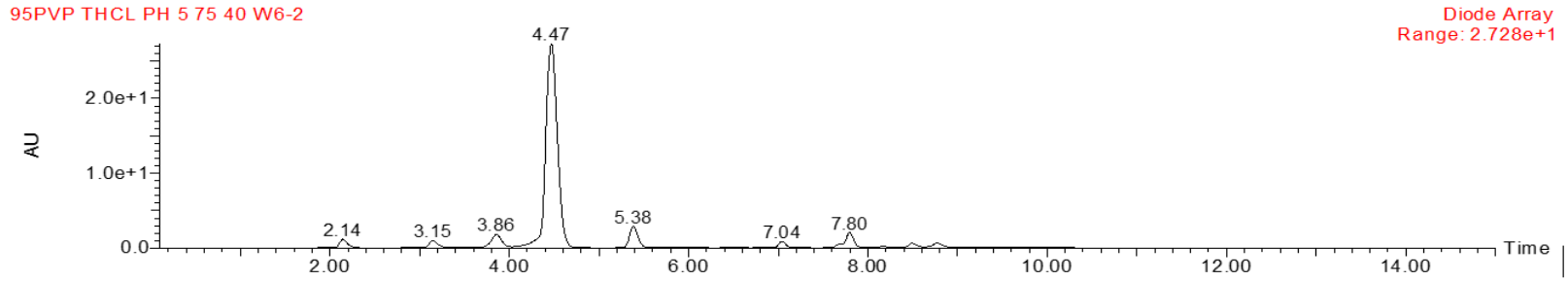


C)

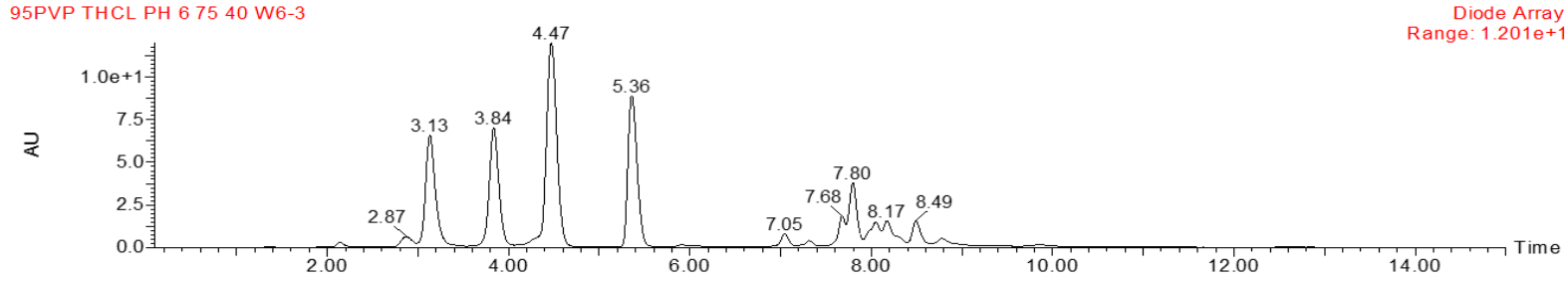
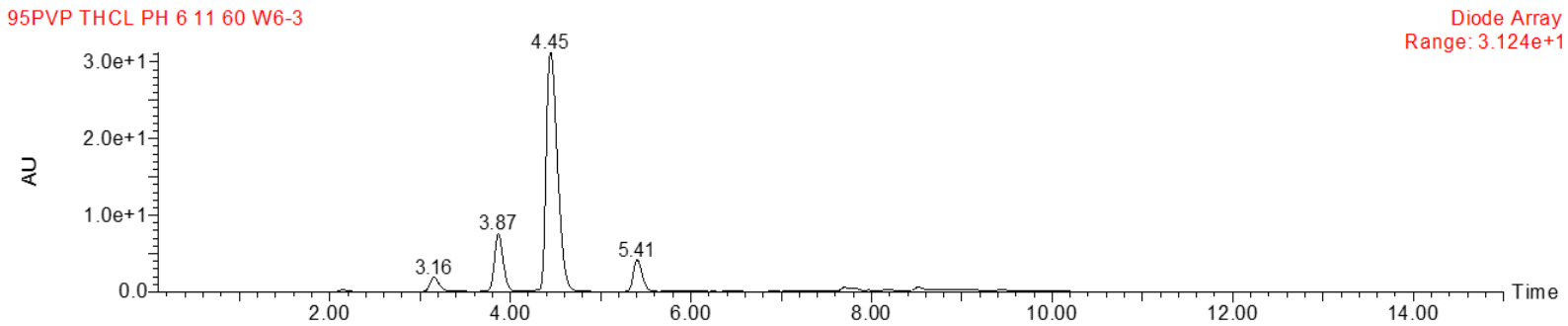


Figure 4.4 HPLC chromatograms of 5TClHCl:95PVP dispersions on day 42 stored at 75% RH and 40ºC, **A)** at pH 4, **B)** at pH 5, **C)** at pH 6.

A)



B)

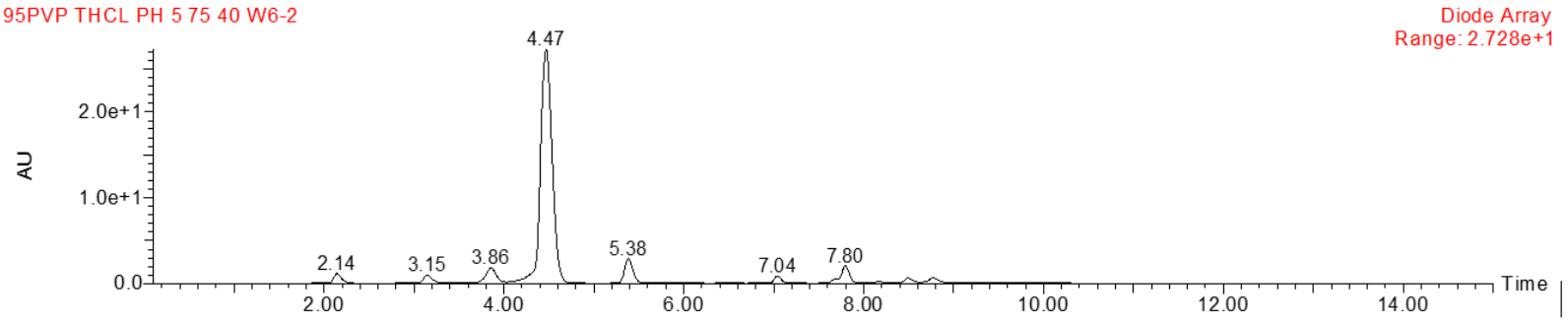
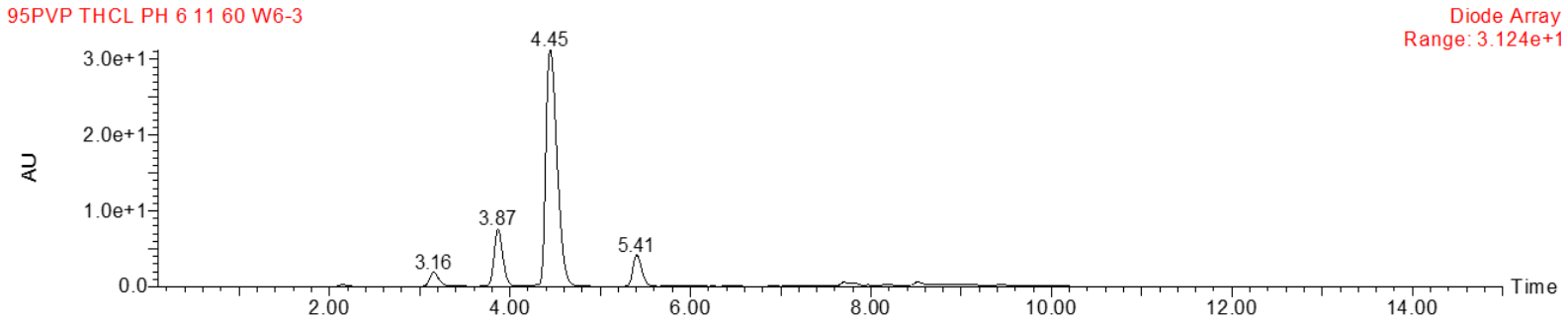


Figure 4.5 HPLC chromatograms of 5TClHCl:95PVP dispersions on day 42, **A)** at pH 6 stored at 11% RH and 60ºC, **B)** at pH 5 stored at 75% RH and 40ºC.

A)



B)

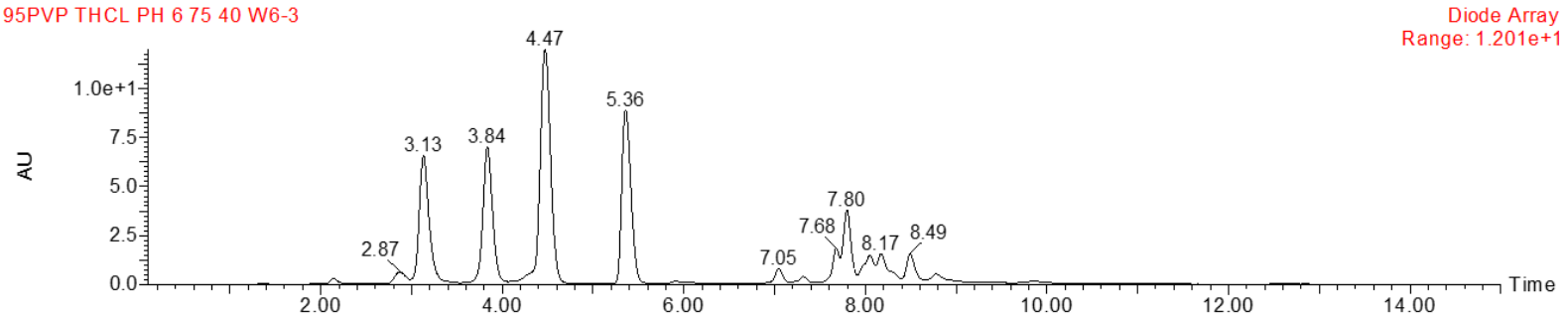


Figure 4.6 HPLC chromatograms of 5TClHCl:95PVP dispersions on day 42 at pH 6, **A)** stored at 11% RH and 60ºC, **B)** stored at 75% RH and 40ºC.

4.6 References

Arioglu-Tuncil, S., V. Bhardwaj, L. S. Taylor & L. J. Mauer (2017) Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International,* 99**,** 363-374.

Brunner, A., K. Mäder & A. Göpferich (1999) pH and Osmotic Pressure Inside Biodegradable Microspheres During Erosion1. *Pharmaceutical Research,* 16**,** 847-853.

Chatterjee, K., E. Y. Shalaev, R. Suryanarayanan & R. Govindarajan (2008) Correlation between Chemical Reactivity and the Hammett Acidity Function in Amorphous Solids Using Inversion of Sucrose as a Model Reaction. *Journal of Pharmaceutical Sciences,* 97**,** 274-286.

Costantino, H., R. Langer & A. Klibanov (1994) Moisture-Induced Aggregation of Lyophilized Insulin. *Pharmaceutical Research,* 11**,** 21-29.

Costantino, H. R., R. Langer, K. Griebenow & A. M. Klibanov (1997) On the pH memory of lyophilized compounds containing protein functional groups. *Biotechnology and Bioengineering,* 53**,** 345-348.

Dwivedi, B. K. & R. G. Arnold (1973) Chemistry of thiamine degradation on food products and model systems. Review. *Journal of Agricultural and Food Chemistry,* 21**,** 54-60.

Franks, F. 2007. 1.1 History of Drying as a Preservation Method. In *Freeze-drying of Pharmaceuticals and Biopharmaceuticals*. Royal Society of Chemistry.

Fu, K., D. Pack, A. Klibanov & R. Langer (2000) Visual Evidence of Acidic Environment Within Degrading Poly(lactic-co-glycolic acid) (PLGA) Microspheres. *Pharmaceutical Research,* 17**,** 100-106.

Hailu, S. A. & R. H. Bogner (2010) Solid-State Surface Acidity and pH-Stability Profiles of Amorphous Quinapril Hydrochloride and Silicate Formulations. *Journal of Pharmaceutical Sciences,* 99**,** 2786-2799.

Li, J., Y. Guo & G. Zografi (2002) Effects of a Citrate Buffer System on the Solid-State Chemical Stability of Lyophilized Quinapril Preparations. *Pharmaceutical Research,* 19**,** 20-26.

Marin, M. 2003. FREEZE-DRYING | Structural and Flavor (Flavour) Changes. In *Encyclopedia of Food Sciences and Nutrition (Second Edition),* ed. B. Caballero, 2701-2705. Oxford: Academic Press.

Mulley, E. A., C. R. Stumbo & W. M. Hunting (1975) Kinetics of thiamine degradation by heat. Effect of pH and form of the vitamin on its rate of destruction. *Journal of food science,* Sept**,** 989-992.

Oliyai, C., J. Patel, L. Carr & R. Borchardt (1994) Chemical Pathways of Peptide Degradation. VII. Solid State Chemical Instability of an Aspartyl Residue in a Model Hexapeptide. *Pharmaceutical Research,* 11**,** 901-908.

Pachapurkar, D. & L. N. Bell (2006) Kinetics of Thiamin Degradation in Solutions under Ambient Storage Conditions. *Journal of Food Science,* 70**,** c423-c426.

Pikal, M. J., K. M. Dellerman, M. L. Roy & R. M. Riggin (1991) THE EFFECTS OF FORMULATION VARIABLES ON THE STABILITY OF FREEZE-DRIED HUMAN GROWTH-HORMONE. *Pharmaceutical Research,* 8**,** 427-436.

Song, Y., R. L. Schowen, R. T. Borchardt & E. M. Topp (2001) Effect of 'pH' on the rate of asparagine deamidation in polymeric formulations: 'pH'–rate profile. *Journal of Pharmaceutical Sciences,* 90**,** 141-156.

Strickley, R. G. (1996) Solid-state stability of human insulin. I. Mechanism and the effect of water on the kinetics of degradation in lyophiles from pH 2-5 solutions. *Pharmaceutical Research,* 13**,** 1142.

Windheuser, J. J. & T. Higuchi (1962) Kinetics of Thiamine Hydrolysis. *Journal of Pharmaceutical Sciences,* 51**,** 354-364.

Xia, F., P. Hong, B. Alden, C. Boissel, D. Swanson, E. Chambers, D. Diehl, P. Iraneta, J. Mazzeo, K. Wyndham, N. Lawrence & T. Walter. 2006. Improvements in reversed-phase HPLC columns designed for polar compound retention. In *HPLC*. San Francisco, CA.

CHAPTER 5. IMPACT OF POLYMER PHYSICOCHEMICAL FEATURES ON THE AMORPHIZATION AND CRYSTALLIZATION OF CITRIC ACID IN SOLID DISPERSIONS

5.1 Abstract

The amorphization and crystallization of citric acid in the presence of a variety of polymers were investigated. Polymers were chosen for their different physicochemical features, including hygroscopicity, glass transition temperature (T_g), and functional groups capable of forming hydrogen bonds and/or ionic interactions with citric acid. Citric acid solutions with varying amounts of pectin (PEC), guar gum (GG), κ-carrageenan (KG), gelatin (GEL), (hydroxypropyl) methyl cellulose (HPMC), and carboxymethylcellulose sodium (CMC-Na) were lyophilized. The resulting solid dispersions were stored for up to 6 months in controlled temperature and relative humidity environments and periodically monitored using powder X-ray diffraction, differential scanning calorimetry, and Fourier transform infrared spectroscopy. Moisture sorption isotherms were generated, and moisture contents were determined. Amorphous solid dispersions of citric acid were successfully formed in the presence of ≥20% w/w CMC-Na and pectin or ≥30% w/w of the other polymers except KG which required a minimum of 40% polymer. All samples remained amorphous at 0% RH (25°C and 40°C), but increasing the RH to 32% RH resulted in citric acid crystallization in the κ-carrageenan dispersions, and further increasing the RH to 54% RH resulted in crystallization in all samples at both temperatures. The polymers ranked from most to least effective for inhibiting citric acid crystallization are: CMC-Na>PEC≥GEL>HPMC>GG>KG. To create and maintain amorphous citric acid, it is recommended to select polymers with greater propensity for intermolecular noncovalent interactions with the citric acid rather than higher T_g or lower hygroscopicity.

5.2 Introduction

Citric acid is an organic acid which is naturally present in a variety of fruits and vegetables and is distributed as a crystalline ingredient in anhydrous and monohydrate forms. Citric acid has GRAS (Generally Recognized as Safe) status and is used as a flavor enhancer, acidulant, chelating agent, and antioxidant in various products in the food and pharmaceutical industries (Igoe, 2011). When food or pharmaceutical products are processed, and particularly with the addition and removal of water and/or heat such as in freeze drying and spray drying (Hancock & Zografi, 1997), initially crystalline ingredients can be solidified in the amorphous state, as can compounds naturally found in fruits and vegetables.

Different physical forms of an ingredient (anhydrate crystal, hydrate crystal, amorphous form) exhibit different properties, including temperature sensitivity, solubilities, dissolution kinetics, (Hancock & Zografi, 1997) and sensory perceptions (Ribeiro et al., 2015). Crystalline solids melt at a certain temperature, whereas amorphous solids are softened at a temperature lower than the melting temperature known as the 'glass transition temperature' (T_g). Amorphous forms have faster dissolution rates and higher solubilities, and often higher hygrocapacities at low and intermediate relative humidities (RHs), than their crystalline counterparts (Ambike, Mahadik, & Paradkar, 2005; Kim et al., 2008; Law et al., 2004). This is attributed to the lack of long range atomic order and crystal lattice energy, as well as the higher free volume found in amorphous structures (Roy, Lipert, & Rodriguez-Hornedo, 2012).

When an ingredient can adopt different physical states, phase transformations can become problematic for product quality and shelf-life. An RH-temperature phase diagram has been generated for citric acid to document the environmental boundaries at which citric acid crystals will hydrate, dehydrate, or deliquesce (Allan & Mauer, 2017). Regarding amorphous citric acid, there are number of studies that have investigated the glass forming ability of citric acid in solid

dispersions prepared by solvent evaporation and melt-quenching methods (Chiou & Riegelman, 1969; Hoppu, Hietala, Schantz, & Juppo, 2009; Hoppu, Jouppila, Rantanen, Schantz, & Juppo, 2007; Lu & Zografi, 1997, 1998; Summers, 1978; Summers & Enever, 1976; Timko & Lordi, 1979). Amorphous solids have a tendency to crystallize over time (Yoshioka, Hancock, & Zografi, 1994). Citric acid has a T_g below ambient temperature (11^oC) and a high crystallization tendency on its own (Lu & Zografi, 1997). However, the presence of various polymers in solid dispersions has been shown to delay crystallization of small molecules (Chiou & Riegelman, 1971). There are a few studies in which citric acid was used as a carrier and the physical stability of amorphous dispersions was monitored overtime. For example, the solid dispersions of citric acid with loratadine and paracetamol were reported to be remained amorphous for three months at 0% and 60%RH at 25^oC, and 27 weeks in dry conditions, respectively (Hoppu et al., 2007; Wang et al., 2017). Amorphous stability of the dispersions was attributed to the intermolecular hydrogen bonding (Hoppu et al., 2007; Wang et al., 2017) and elevated T_g (Wang et al., 2017).

Due to the widespread use of citric acid in products that contain a variety of ingredients (including polymers) and the potential for citric acid to solidify in the amorphous state, it was of interest to explore citric acid in solid dispersions with polymers with different physicochemical features (hygroscopicity, hydrogen bonding ability, and T_g) to determine if the trends in polymer crystallization inhibition for different classes of small molecules (including thiamine chloride hydrochloride, ascorbic acid, resveratrol and curcumin) (Arioglu-Tuncil, Bhardwaj, Taylor, & Mauer, 2017; Christina, Taylor, & Mauer, 2015; Wegiel, Mauer, Edgar, & Taylor, 2013; Wegiel, Zhao, Mauer, Edgar, & Taylor, 2014) hold true for citric acid.

5.3 Materials and methods

5.3.1 Materials

Citric acid anhydrous and κ-carrageenan (KG) were purchased from VWR Scientific (Radnor, PA, USA). Guar gum (GG), (hydroxypropyl) methyl cellulose (HPMC), carboxymethylcellulose sodium (CMC-Na), and pectin (PEC) (from citrus peel with a ~61% degree of esterification) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Gelatin (GEL) was obtained from Ward's Science (Rochester, NY). Drierite™, used to create 0% RH storage conditions, was obtained from W.A. Hammond Drierite Company, LTD (Xenia, OH). Magnesium chloride ($MgCl_2$, 32% RH at 25°C) and magnesium nitrate ($Mg(NO_3)_2$, 54% RH at 25°C) that were used to create specific RH conditions in desiccators were purchased from Sigma-Aldrich Inc. (St. Louis, MO).

5.3.2 Formation of solid dispersions via lyophilization

Citric acid solid dispersions in polymers were initially prepared using a 1:1 weight ratio of anhydrous citric acid and polymer. Polymers (0.25g) were weighted and added to 30 mL of deionized water. Then, heat was applied using a digital heat block (VWR International LLC., Bristol, CT) at 60°C for 30 minutes, to solubilize the polymers properly. The solutions were cooled to the room temperature, and 0.25g of citric acid was then added. Citric acid:polymer solutions were then mixed with a Roto-Shake Genie® SI-1100 (Scientific Industries, Inc., Bohemia, NY) until uniform one-phase solutions were visually confirmed. Further studies used samples containing different ratios of citric acid and polymer, from 0% to 100%, in 10% increments, keeping the total solids at 0.5 g added to 30 mL water. For the controls, lyophilized citric acid and polymers were prepared by dissolving 0.5g of the individual ingredients in 30 mL of water. Then, the solutions were kept at -20°C for at least 40 hours prior to lyophilization. The lyophilization

technique was conducted using a VirTis Benchtop K (VirTis, Gardiner, NY) for at least 96 hours at ambient temperature and 20 mT. After lyophilization process completed, solid dispersions were transferred into 20 mL glass vials and placed into desiccators with select %RH conditions. In addition, physical blends of crystalline citric acid with the polymers were prepared by weighting each ingredient separately, followed by simply mixing in 20 mL glass vials.

5.3.2.1 Storage treatments

Solid dispersions were kept in select %RH conditions (0, 32 and 54% RHs) at two temperatures (22±3^{o}C and 40^{o}C). A water jacketed incubator (Forma Scientific, Inc., Marietta, OH) was used to maintain the 40^{o}C. Solid dispersions showing the amorphous halo were analyzed weekly up to 189 days.

5.3.3 Powder X-ray Diffraction (PXRD)

Solid state characterization of the samples was done immediately after lyophilization and at set time points during the study with a Rigaku Smartlab^{TM} diffractometer (Rigaku Americas, Texas, USA) Cu-Kα radiation source and D/ tex ultra-detector. Samples were scanned between 5-40° 2θ at 4^{o}/min with a 0.02° step size. Solid dispersions with a diffuse halo in the diffractograms were characterized as XRD amorphous, and samples containing sharp peaks at least two standard deviations above baseline were labeled as crystalline.

5.3.4 Fourier transform infrared spectroscopy (FTIR)

The interactions between citric acid and polymers were investigated using a ThermoNicolet Nexus 670 FT-IR spectrometer (ThermoNicolet, Madison, WI) equipped with an MCTA detector and DRIFTS Avatar Diffuse Reflectance Smart Accessory (ThermoElectron Corp., Madison, WI). An IR spectrum of KBr was collected before each series of analyses as a background. The scan

range was set from 4000 to 650 cm^{-1} and 128 scans were recorded with a resolution of 2 cm^{-1}. Prior to placement in the DRIFTS accessory; polymers, citric acid, freeze dried citric acid, citric acid: polymer dispersions, and citric acid: polymer physical mixtures were weighted (5% w/w of KBr) and then samples were milled for 30 seconds in a screw type capsule with a stainless-steel ball pestle, using a Crescent Digital Wig-L-Bug C020200 Mixer (Dentsply Rinn Inc., Elgin, IL). The spectra were analyzed using OMNIC software (ThermoElectron Corp., Madison, WI).

5.3.5 Moisture sorption isotherm analysis

Moisture sorption isotherms were collected using a SPSx-1µ Dynamic Vapor Sorption Analyzer (Projekt Messtechnik, Ulm, Germany), with the equilibrium criteria set at a weight change of 0.01% in 15 minutes. Five hours was used as a maximum step time. Duplicate samples (100-300 mg) were loaded into the aluminum pans and were first equilibrated at 5% RH in the instrument prior to the analysis. Samples then were analyzed from 5-95% RH and 5-80% RH at 25°C and 40°C, respectively, using a step size of 5% RH. An additive model, basically the weighted average of moisture sorption of each individual ingredient, was used to compare the experimental moisture sorption isotherms of solid dispersions with the predicted values. In addition, the differences between the moisture sorption of the polymers and the solid dispersions (φ) were calculated as using the equation provided below:

$$\varphi = m_{dispersion} - cm_{polymer} \quad 5.1$$

where m is the % moisture content (w/w) and c is the polymer fraction in the solid dispersion. This approach has been used when the moisture sorption profile of the compound if interest, cannot be in the amorphous form collected (Arioglu-Tuncil et al., 2017; Sanchez, Ismail, Christina, & Mauer, 2018).

5.3.6 Differential scanning calorimetry (DSC)

Glass transition temperatures (T_g) of the samples were measured using a Discovery DSC (TA Instruments, New Castle, DE) equipped with a refrigerated cooling accessory. Nitrogen at 50 mL/min served as the purge gas. A sample size of 7-12 mg was weighted in duplicate and sealed hermetically into the Tzero pans (TA Instruments). Samples were equilibrated at -80^{o}C, then were heated at least 20-30^{o}C higher than T_g at a heating rate of 20^{o}C/min, then quickly cooled to -80^{o}C at a cooling rate of 10^{o}C/min. A second heating step was applied by heating the samples to 140^{o}C at a rate of 20^{o}C/min. The T_g of citric acid was determined using a melt quench method. Samples (weighed into the pans with pin hole) were equilibrated at -25^{o}C, then were heated to 170^{o}C (above its melting point) at a heating rate of 10^{o}C/min, then cooled to -25^{o}C at a cooling rate of 10^{o}C/min. A second heating step was applied by heating to 170^{o}C at a rate of 10^{o}C. The onset glass transition temperature of the second heating was recorded as T_g using TRIOS software (Universal Analysis), unless otherwise stated. A tangent was drawn on the straight line of the second heating step prior to where the baseline shift occurred to the endothermic direction. Then, the second tangent was drawn on the slope, followed by calculation of the cross point using TRIOS software.

5.3.7 Statistical Analysis

SAS Software Version 9.4 (SAS Institute, Cary, NC) was used for statistical analysis. The significant differences in T_g between solid dispersions were using Tukey's multiple comparison test at alpha 0.05.

5.4 Results and Discussion

5.4.1 Long term physical stability of citric acid amorphous solid dispersions towards crystallization measured by PXRD

The physical forms of CA and FD CA were determined based on their PXRD patterns. As expected, unprocessed CA was found to be in a crystalline state (**Fig. 5.1A**). Lyophilization of CA alone in solution did not end up with amorphous form, which agrees with an earlier report where the crystallization of CA was attributed to low T_g value of CA (Lu & Zografi, 1997). Moreover, T_g' (T_g of the maximally freeze concentrated solution) is the most important parameter to consider for lyophilization procedures, since the compounds dried at temperatures above T_g' are prone to collapse (Franks, 2007). The T_g' of citric acid was reported to be -55.1^{o}C (Kadoya et al., 2008) which was lower than the temperature of freeze drying protocol used in this study. Thus, amorphization of citric acid was not successful because of exposing the samples to temperature higher than T_g' during the primary drying step.

Lyophilized CA retained most of the crystalline peaks at the same location as CA does (but with different intensities), indicating that CA mostly maintained its crystalline structure upon lyophilization alone (**Fig. 5.1A**). PXRD pattern of citric acid monohydrate (CA MH) was also compared with that of FD CA. However, it was observed that FD CA had a diffractogram that was more like anhydrous CA than CA monohydrate. The small difference in PXRD patterns of CA and lyophilized CA was ascribed to water present in the crystalline lattice of FD CA due to lyophilization.

The presence of amorphous ingredients such as hydrocolloids in the food matrix may cause amorphization of crystalline ingredients by interacting with them (Arioglu-Tuncil et al., 2017; Christina et al., 2015). This phenomenon occurred only under certain processing conditions such as lyophilization, but cannot be achieved spontaneously by just blending the amorphous polymers

with crystalline ingredients. The amount of polymer needed to produce amorphous solid dispersion varied among the polymers studied. For example, 20% w/w PEC and CMC-Na were required to amorphize CA, whereas a minimum of 30% w/w GEL, GG, and HPMC were needed (**Figs. S5.1A-F**). KG was the polymer that required the highest amount (40%) to be able to yield amorphous CA in the solid dispersion (**Fig. S5.1F**). The PXRD peaks of all the crystalline CA: polymer dispersions were observed to be at the same location as anhydrous CA does.

Amorphous solid dispersions of CA with polymers at a ratio of 1:1 were successfully prepared, as shown in **Fig. 5.1B**, and the physical stability of amorphized CA in the solid dispersion at select RH (0%, 32% and 54% RH) and temperatures (25^{o}C and 40^{o}C) were periodically determined using PXRD over the course of 189 days. The polymers showed different crystallization inhibitor ability for CA solid dispersions, as shown in **Table 5.1**. All the dispersions maintained their amorphous characteristics for more than 189 days in both temperatures at 0% RH (**Figs. S5.2A-B**). However, as %RH increased up to 32%, the abilities of the polymers to inhibit recrystallization of CA from the amorphous matrix varied. For instance, KG:CA solid dispersions crystallized within three weeks at 32%RH, and no difference was observed between the temperatures studied (25^{o}C and 40^{o}C) **(Table 5.1)**. On the other hand, CMC-Na:CA and PEC:CA were the only dispersions that retained their amorphous structures for more than 189 days at 32%RH at both temperatures tested. The temperature had more pronounced effect for the HPMC:CA, GG:CA, and GEL:CA dispersions stored at 32%RH than those stored at 0%RH. For example, at 32% RH, onset of CA crystallization occurred in 28 days for GG:CA and HPMC:CA dispersions, and in 63 days for GEL:CA at 40^{o}C **(Table 5.1)**, whereas those samples maintained their amorphous structure for more than 189 days at 25^{o}C. Onset crystallization started to be observed in these samples stored at 40^{o}C after 28 days of storage, but, the intensity of the peaks in

the PXRD did not change over longer storage periods **(Fig. S5.2C)**. At higher RH condition (54%), solid dispersions crystallized faster (**Table 5.1**). CA solid dispersions made by KG and GG crystallized within 14 days at 54% RH 25^{o}C-40^{o}C. Moreover, crystallization in PEC:CA and HPMC:CA solid dispersions was observed in 21 days at the same conditions. CMC:CA solid dispersions showed onset of crystallization in 63 days at 54% RH and 25^{o}C, while same sample crystallized at 49 days at 54%RH and 40^{o}C. Based on both minimum amount of polymer needed, as well as stability in 1:1 dispersions, the rank ordering of polymer performance from most effective to least effective was CMC-Na>PEC>GEL>HPMC>GG>KG.

5.4.2 Spectroscopic investigation of interactions between citric acid and polymers by FTIR

CA is a tricarboxylic acid meaning that it contains three carboxylic functional groups in its chemical structure and two of these carboxyl groups are equivalent (**Fig. 5.2A)**. In addition to three carboxylic groups, CA carries one hydroxyl group which is located at the center of the molecule. These functional groups of CA can be involved in hydrogen bonding. Specifically, -$OH_{(alc)}$ and -$OH_{(ac)}$ are both hydrogen bond donors (HBD) and acceptors (HBA). Moreover, the carbonyl carboxylic acids of CA play a role in hydrogen bonding as a HBA. Thus, CA is composed of four potential HBD and seven potential HBA groups. As a result of strong intermolecular interaction via hydrogen bonding, carboxylic acids generally exist as dimers (Socrates, 2001). Dimerization of CA through hydrogen bonding between the carboxylic groups was reported previously (Bichara, Lanús, Ferrer, Gramajo, & Brandán, 2011; Glusker, Minkin, & Patterson, 1969; Nordman, Weldon, & Patterson, 1960). The strong intermolecular interaction between two monomers of CA needs to be overcome to be able to form intermolecular hydrogen bonding between CA and any other compound. The study made by Bichara and others revealed the six intense bands in IR spectrum of CA, that correspond to the CA dimer structure are: at 3498 cm^{-1}, 3291 cm^{-1}, 1756 cm^{-}

^{1}, 1708 cm^{-1}, 1174 cm^{-1}, 1140 cm^{-1}, corresponding to CA dimer (Bichara et al., 2011). If these peaks disappear in IR spectra of CA:polymer solid dispersions, it could be attributed to breaking of hydrogen bonding between two CA monomers and thus a new interaction formation of CA with the polymers. Intermolecular hydrogen bonding formation between compounds are highly dependent on available functional groups for hydrogen bonding as well as their potential strengths as HBA and HBD. Therefore, the relative HBA and HBD strengths of the functional groups in CA and polymers were determined based on the pK_{BHX} scale (Laurence, Brameld, Graton, Le Questel, & Renault, 2009), as shown in **Table 5.2.**

The interaction between CA and polymers via hydrogen bonding were investigated using FTIR wherein alterations in peak shape and/or location were observed in hydroxyl and carbonyl regions when the interactions occur. As a control, the FTIR spectra of CA and FD CA are compared in **Fig. 5.2B**. Both unprocessed CA and FD CA revealed almost identical peak shapes and locations in the IR spectra. This observation is also in line with PXRD results of anhydrous CA and FD CA wherein the PXRD diffractogram of FD CA was essentially same with CA (**Fig. 5.1A**). Strong bands were observed at 3495 cm^{-1}, 3449 cm^{-1} and 3292 cm^{-1} in the hydroxyl region of the IR spectrum of CA (**Fig. 5.2B**). These bands correspond to alcohol and carboxylic OH groups of CA. Moreover, the IR spectra of CA had three bands in the 1800-1600 cm^{-1}, at 1756 cm^{-1}, 1744 cm^{-1} and 1716 cm^{-1} (**Fig. 5.2B**). The IR bands in the 1800-1600 cm^{-1} region could be ascribed to the stretching of the C=O bond of the carboxyl groups in CA.

When polymers were simply blended with CA at a 1:1 ratio, the IR spectra of the physical mixtures resembled that of CA in terms of peak shapes and positions, as shown in **Fig. 5.3**. This proved the lack of interaction between CA and the polymers. Secondly, the similarity of peak positions and shapes in IR spectrum of CA with those of CA:polymer physical mixtures

demonstrates that vibration of the functional groups, which CA consists of, are more predominant than those of polymers.

Changes in peak locations and/or shapes in IR spectra of the solid dispersions were found and were highly associated with the polymer to CA ratio. A broad single band, which non-crystalline polymers tend to contain (Socrates, 2001), was observed in the hydroxyl regions of IR spectra of the polymers. In the hydroxyl region of the IR spectra, a maximum absorbance was detected at 3400 cm^{-1}, 3419 cm^{-1}, 3322 cm^{-1}, 3461 cm^{-1}, 3388 cm^{-1}, 3422 cm^{-1} for CMC-Na, PEC, GEL, HPMC, GG, and KG, respectively (**Figs. S5.3A-C** and **Fig. 5.4A**). In addition, peaks in carbonyl region occurred at 1600 cm^{-1} for CMC-Na, 1653 cm^{-1} and 1558 cm^{-1} for GEL and 1743 cm^{-1} and 1622 cm^{-1} for PEC (**Figs. 5.4B-D**). It should be noted that HPMC, KG and GG do not carry any carbonyl groups. Therefore, a peak shift, if occurs, relative to non-hydrogen bonded carbonyl of the polymer cannot be attributed to hydrogen bonding interaction with CA (**Fig. S5.4A-C**). However, if peak alteration occurs relative to non-hydrogen bonded carbonyl of CA in that region, it can be interpreted as a formation or disruption of hydrogen bonding between CA and polymer depending on the peak alteration.

Upon formation of CA: PEC (1:1) solid dispersion, peak shift to the lower wavenumber (3400 cm^{-1}) relative to the maximum absorbance of PEC in the hydroxyl region (3419 cm^{-1}) was observed. The shift of 19 cm^{-1} relative to the non-hydrogen bonded hydroxyl in PEC (**Fig. 5.4A** and **Table 5.3**) was due to the hydrogen bonding between CA and PEC. Similarly, peak shifts to the lower wavenumber in hydroxyl region relative to the polymers were found when CA: HPMC and CA:KG solid dispersions were formed (**Figs. S5.3A-B** and **Table 5.3**). Specifically, maximum hydroxyl absorbance of HPMC (3461cm^{-1}) and KG (3422 cm^{-1}) were shifted to 3421cm^{-1} and to 3384 cm^{-1}, respectively, upon formation of CA solid dispersions with these polymers (1:1),

indicating the hydrogen bonding interaction with CA. Peak shifts relative to non-hydrogen bonded CMC-Na, GEL, and GG were not found in the hydroxyl region of CA solid dispersions (1:1) containing these polymers (**Fig. S5.3C** and **Table 5.3**). However, when IR spectra of solid dispersions were interpreted relative to the spectrum of CA itself, hydrogen bonding interaction of CA with all the polymers were proved.

As mentioned above, CA contains three main peaks (3495 cm^{-1}, 3449 cm^{-1}, and 3292 cm^{-1}) in the hydroxyl region of IR. These peaks were observed to disappear and form a broad peak, when amorphization of CA in the solid dispersions were achieved. Hydroxyl stretching region of CA: CMC-Na is shown as a function of the polymer concentration in **Fig. S5.3C**. In the presence of 10% of CMC-Na in the solid dispersion, where CA was crystalline confirmed by PXRD, FTIR peak shapes and positions were shown to be similar to IR spectrum of CA. However, a broad shoulder was formed when more than 10% CMC-Na was used, which corresponds to formation of amorphous CA in the solid dispersion. As polymer amount was increased enough to form amorphous CA, peak shape alteration represented reduction of interaction between CA molecules which indicates the formation of a new interaction between CMC-Na and CA. Similar trend was observed in the hydroxyl region of the CA solid dispersions made by the other polymers.

Furthermore, peak shift to the lower wavenumber relative to non-hydrogen bonded CMC-Na, PEC and GEL was found in the carbonyl region of the CA solid dispersions indicating the presence of hydrogen bonded carbonyl **(Table 5.3 and Figs. 5.4B-D).** Specifically, carbonyl peak of the CMC-Na was located at 1600 cm^{-1} and it shifted to 1575 cm^{-1} upon CMC-Na: CA (1:1) solid dispersion formation and it proved the interaction between CA and CMC-Na through hydrogen bonding. Similarly, carbonyl peaks of GEL that were located at 1653 cm^{-1} and 1558 cm^{-1}, shifted to 1634 cm^{-1} and 1546 cm^{-1}, respectively (**Table 5.3 and Fig. 5.4C**). Lastly, one of two

carbonyl peaks of PEC (1743cm^{-1}) shifted to 1731 cm^{-1} (**Table 5.3 and Fig. 5.4D**), which could be attributed to more favorable hydrogen binding interaction between PEC and CA, whereas the other carbonyl peak located at 1622 cm^{-1} shifted to a higher wavenumber due to hydrogen bonding among PEC molecules. In addition to these observations, it is important to note that three distinct peaks of CA in the carbonyl region of IR spectrum merged into a single peak depending on the ratio of polymer to CA. In other words, three distinct peaks in the carbonyl region of CA disappeared and merged into a single peak when amorphous CA was formed in solid dispersions, independent of the polymer type (**Figs. 5.4B-D and Figs. S5.4A-C**). Peak shape alteration in this region was the strong evidence of hydrogen bonding between CA and the polymers.

5.4.3 Potential ionic interaction between citric acid and some polymers

In addition to hydrogen bonding formation, presence of ionic interactions between CA and some of the polymers in solid dispersions have been hypothesized. CA composed of three carboxylic acid has p*K*as of 3.1, 4.8 and 6.4 (Apelblat, 2014). The pH of polymers and CA in the solution were measured as 7.21 for CMC-Na, 4.91 for GEL, 3.54 PEC, 8.18 for KG, 5.79 for HPMC, 6.24 for GG and 2.33 for CA. Upon mixing CA with the polymers, the pH of solutions changed to 3.01 for CMC-Na:CA, 2.42 for GEL:CA, 2.39 for PEC:CA, 2.37 for KG:CA, 2.32 for HPMC:CA, and 2.33 for GG solid dispersions, showing that pH of CA:polymer solutions were below the p*K*a of CA before lyophilization. CA exits mostly in the ionized form at pHs below its p*K*a, which could potentially be interacting with some polymers through ionic interactions. The speciation curve of citric acid (prepared based on the first p*K*a of 3.1) is shown in **Fig. S5.4D**. The fraction of protonated species at pHs 2.3, 2.4, and 3.0 were calculated to be 0.87, 0.85, and 0.58, respectively.

The supporting evidence is that carboxylic acids found in the structure of CA can reacts with metals, which may result in a formation of carboxylate salt of the metal. Specifically, in this study, sodium portion (metal) of CMC-Na was thought to be attracting the carboxylate anion of CA and leading to the formation of ionic network.

Similarly, chemical structure of GEL needs to be considered while interpreting its crystallization inhibitor property. GEL is composed of amino acids which carry both negative and positive charges. Therefore, GEL is capable of interacting with CA via ionic interaction. Ionic interaction may take place between amine group of GEL and ionized carboxylic acid group of CA. Further stabilization of amorphous CA solid dispersions made by CMC-Na and GEL could be maintained by potential ionic interaction, which may be stronger than hydrogen bonding (Etter, 1990).

5.4.4 Moisture sorption isotherm profiles by dynamic vapor sorption analyzer

Water is ubiquitous; therefore, exposure of dry ingredients to moisture is unavoidable during handling, processing, and storage, which highly affects the chemical and physical stability of dry or intermediate moisture blends. The relationships between RH and moisture content at a constant temperature were studied by means of moisture sorption isotherms to investigate the role of water in the physical stability of amorphous citric acid solid dispersions.

The moisture sorption profiles of CA and lyophilized CA at 25^{o}C are shown in **Fig. S5.5A.** A small amount of moisture (<0.09%) gain below the deliquescence point (RH_0), which has been reported as 75% for anhydrous CA at 25^{o}C (Salameh, Mauer, & Taylor, 2006), were observed. This is attributed to adsorption and condensation of water at contact points of citric acid crystal particles (known as capillary condensation) (Mauer & Taylor, 2010). In general, lyophilized CA gained more moisture than CA as RH increased above the RH_0 at both temperatures, even though

both of them had crystalline structures. This is because, freeze drying process caused reduction in the particle size and having some disorder, and hence increase in surface area which made lyophilized CA more susceptible to moisture.

Moisture sorption profiles of individual ingredients, physical mixtures, and solid dispersions were examined at 25^{o}C (**Fig. 5.5A-C)**. Polymers showed a Type II (sigmoidal) moisture sorption isotherm (**Fig. 5.5A**), where water uptake increased as RH increased due to the amorphous structure of the polymers. Moisture gain of the polymers up to 80% RH were more than moisture gain of CA. Moreover, CMC-Na absorbed more moisture than any other polymers throughout all the RHs studied, meaning that it was the most hygroscopic polymer in this study (**Fig. 5.5A**). On the other hand, HPMC was found to be the least hygroscopic polymer since it absorbed the least amount of water at all the RHs studied. Moisture sorption profiles of the physical mixtures were compared to the solid dispersions (**Fig. 5.5B-C**). Presence of CMC-Na, which was the most hygroscopic polymer, in the physical blends brought more moisture to the system than other polymers specifically at RHs below 80%, with the exception of KG:CA blend (**Fig. 5.5B**). The trend of CMC-Na: CA physical mixture for moisture sorption was quite similar to that of KG:CA until the RHs reached up to 60%. Above the 60% RH, weight gain by CMC-Na: CA physical blends were significantly higher than KG:CA blend. For example, the weight changes due to moisture sorption in the KG:CA physical blends were 9% w/w at 65% RH, whereas CMC-Na: CA physical blends sorbed 15% w/w at the same condition. On the other hand, all the other physical blends sorbed less moisture than CA blends made by CMC-Na and KG. In essence, CMC-Na:CA blends sorbed more moisture than any other mixtures between 60-80% RH. Solid dispersions gained more moisture than physical blends at low RHs because of the contribution of amorphous CA to the system. The magnitude of the difference between absorbed moisture by solid

dispersions and physical blends was the lowest in CMC-Na:CA and the highest in HPMC:CA than other samples. For instance, HMPC:CA solid dispersions sorbed 10% (w/w) more moisture than its physical blends at 60% RH, whereas CMC-Na:CA solid dispersion sorbed only %3 (w/w) more moisture than its physical blends at that RH (**Fig. 5.5C**).

Predicted moisture sorption of solid dispersions were calculated based on an additive model in which the contribution of individual lyophilized ingredients (lyophilized crystalline CA and lyophilized amorphous polymer) for the moisture sorption is taken into account. Moisture sorption was considered to be synergistic when actual samples brought more moisture to the system than calculated values based on the additive model. Experimental and predicted moisture sorption profiles of the solid dispersions and the difference between additive model and experimental values were showed in **Figs. S5.5B-F and Fig.5.6A**. Differences were found between the predicted and experimental moisture sorption profiles of the solid dispersions at all RHs studied but mostly at RHs higher than 30%. Differences between experimental and predicted values increased as RH% increased from 0 to 70%RH: the presence of amorphous CA was likely to have more influence for moisture sorption than crystalline CA until crystalline CA deliquesced. Moreover, KG:CA and HPMC:CA solid dispersions exhibited the highest Δ% EMC until 70% RH while CMC-Na:CA solid dispersions showed the least Δ% EMC in the same conditions (**Fig. 5.6A**). All the experimental isotherms excluding the one made by GEL and CMC-Na intersected with the additive model around 80% RH (**Figs. S5.5B-G**), meaning that the difference between additive model and experimental values were minimal near 80% RH. Above 80% RH, it was found that moisture sorption by predicted model for all the samples were higher than solid dispersions which was due to the complete dissolution of crystalline CA in the additive model. Impact of amorphous CA in the solid dispersions for moisture sorption was investigated by calculating the

φ value, which eliminates the contribution of polymer to the amount of moisture absorbed, in order to reveal the effect of CA only (**Fig. 5.6B)**. Positive φ values were found for the solid dispersions, meaning that hygrocapacity of solid dispersions was higher than the polymers as RH increases due to amorphous form of CA.

There was no direct correlation found between physical stability of the amorphous solid dispersions and the hygroscopicity of the individual ingredients or solid dispersions. Based on the PXRD results, CMC-Na was the most effective polymer for inhibiting the recrystallization of amorphous CA, even though it was the most hygroscopic polymer used in this study. The crystallization inhibitor ability of the polymers varied at the higher RH% conditions and it was not related to amount of moisture sorbed at these conditions. For example, the amount of moisture sorbed by KG:CA (6.8% w/w) was quite similar to amount of moisture that GG:CA solid dispersion sorbed (6.1% w/w) at 35% RH; however, KG:CA dispersion crystallized in 21 days at 32%RH and 25^{o}C where GG:CA dispersion was amorphous for more than 189 days in this environment.

5.4.5 Glass Transition Temperatures by DSC

There are controversial reports in the literature regarding the effects of T_g on amorphous stability. For example, Van den Mooter and colleagues found that increasing the T_g of amorphous solids decreased the molecular mobility, which resulted in stabilization of amorphous solid dispersions against recrystallization at temperatures below T_g (Van den Mooter et al., 2001). In contrast, several others reported that T_g was not the key element for maintaining the physical stability in the amorphous phase; instead, formation of hydrogen bonding and/or ionic interactions among the compounds of the dispersion was the most important contributor (Christina et al., 2015; Matsumoto & Zografi, 1999; Taylor & Zografi, 1997; Wegiel et al., 2013). Due to these

controversial findings in the literature, there is a need for further examination of T_g and its effect on amorphization stability of compounds found in solid dispersions

Due to failure of lyophilization technique to form amorphous CA in the absence of a polymer, the T_g of CA could not be determined using lyophilized CA in this study. However, T_g of CA was measured to be 11^oC by means of melt quench technique in DSC using a pin hole which is in a good agreement with an early report (Lu & Zografi, 1997). The experimental T_g of amorphous CA was in line with its predicted T_g based on Boyer-Beaman rule, which states that the T_m and T_g of a compound in Kelvin are related and the ratio of T_g to T_m is usually around 0.6-0.7 (Beaman, 1952). Since the T_m of CA is 153^oC, the estimated T_g of CA was calculated to be 11^oC. The onset T_gs of polymers were also measured**.** The T_gs of GEL and PEC were determined to be 151±3^oC, and 90±2^oC, respectively. However, T_gs of HPMC, GG, CMC-Na and KG could not be determined using the method described here. This could be due to impurities coming from the synthesis of polymers, which make transitions hard to detectable. To overcome this problem, using modulated DSC could be an option. In the literature, T_gs of HPMC, GG, CMC-NA and KG were reported as 145^oC (Wegiel et al., 2014), 108^oC (Kumar, De, & Mozumdar, 2015), 191^oC (Bochek et al., 2012), and 161^oC (Mahmood, Khan, & Yee, 2014), respectively. Based on the assumption that the polymer with the highest T_g was the best crystallization inhibitor, the expected rank order for the stability of CA amorphous solid dispersions would follow: CMC-Na>KG>GEL>HPMC>GG>PEC.

DSC measurements were conducted both in the absence and in the presence of pinhole for CA amorphous solid dispersions on the day when the samples were withdrawn from the freeze dryer (**Table 5.4)**. The samples were observed to possess the lower T_gs when the measurement was conducted without a pin hole, likely due to remaining water in the samples that acted as a

plasticizer by penetrating the internal structure of amorphous samples. Experimental T_gs of CA solid dispersions were determined using a pin hole, to remove water, as 49.5ºC, 44.4ºC, 21.8ºC, 16.6ºC, 24.7ºC, 33.5ºC for CMC-Na:CA, GEL:CA, GG:CA, HPMC:CA, KG:CA, and PEC:CA, respectively. T_gs of solid dispersions can be ranked in the order of highest to lowest as follows: CMC-Na:CA>GEL:CA>PEC:CA>KG:CA>GG:CA>HPMC:CA. T_g of solid dispersions did not follow the same order with T_g of polymers. There might be several factors causing this difference; 1) although a pin hole was used during the T_g measurement, all the moisture that solid dispersions had after lyophilization could not be possibly removed from the samples, thus decreasing the T_g. 2) the presence or lack of intermolecular interaction as well as magnitude and nature of the interactions between compounds in the solid dispersion might result in positive or negative deviation from the predicted T_g, and 3) T_g of CMC-Na, HPMC, KG and GG reported here were based on the other studies from the literature. Polymerization technique, impurity of the polymers and experimental conditions used to determine T_g may cause differences in measured T_g of the polymers. Therefore, the T_g of the polymers used in this study is not necessarily to be same as reported values from the other studies.

Although CA solid dispersions were stored in temperature conditions above their T_gs measured after lyophilization, the samples maintained their amorphous structures at 0% and 32% RHs at 25ºC (except KG:CA dispersions at 32%RH) within the time scale of the experiment (189 days). Similar observation was reported by Hoppu et al., (2006), where amorphous dispersions of citric acid with paracetamol which present in rubbery state remained amorphous for at least 27 weeks in dry conditions (Hoppu, Jouppila, Rantanen, Schantz, & Juppo Anne, 2010) Consequently, this study is in agreement with early reports where T_g was shown not to be the most

significant factor that governs the amorphous structure stability (Christina et al., 2015; Matsumoto & Zografi, 1999; Taylor & Zografi, 1997; Wegiel et al., 2013).

5.5 Conclusion

Amorphization of CA was succeeded in the presence of polymers. CA solid dispersions maintained their amorphous characteristics for more than 189 days of storage at 0% RH, 25^{o}C and 40^{o}C, regardless of the polymer type used. As storage RH increased to 32% and 54% RHs, the performance of the polymers as crystallization inhibitors varied, suggesting the following rank order: CMC-Na>PEC≥GEL>HPMC>GG>KG. The T_gs of CA amorphous solid dispersions were measured to be well below the estimated values, indicating that all the storage conditions used in this study were at the temperatures above the T_g of dispersions. Thus, all the polymers were able to inhibit recrystallization of citric acid even from the rubbery state at low RH conditions (0% RH). The performances of CMC-Na and PEC to prevent recrystallization from the rubbery state were also good at higher RH% conditions. Based on the T_g data, it can be stated that T_g did not appear to be a significant factor for ensuring of amorphous citric acid physical stability. Hygroscopicity of the polymers, on the other hand, showed pronounced impact on the physical stability of amorphous solid dispersions only at high RH conditions by promoting the recrystallization to a much greater extent than lower RHs. The polymers that were able to form the most extensive hydrogen bonding and/or ionic interaction with citric acid were found to be the best stabilizers, confirming that intermolecular interaction formation ability was the most important feature correlating to crystallization inhibition features of polymers. The findings of this study will contribute to better understanding to stabilize small molecules in the complex food matrices in order to avoid any quality defects.

Table 5.1 Interpretation of PXRD patterns of 1:1 CA: Polymer solid dispersions (SD) after 189 days at select storage treatments. PXRD patterns that contained sharp peaks were interpreted as having CA crystalline structures (C), those that have halo patterns were interpreted as being amorphous (A), and partially crystalline samples, in which onset crystallization of CA was started but peaks were present in few locations (2 Theta) with low intensity, were labeled as (PC).

RH (%) and T (°C)	CMC-Na SD	PEC SD	GEL SD	HPMC SD	GG SD	KG SD
0% RH- 25°C	>189-A	>189-A	>189-A	>189-A	>189-A	>189-A
0% RH- 40°C	>189-A	>189-A	>189-A	>189-A	>189-A	>189-A
32% RH- 25°C	>189-A	>189-A	>189-A	>189-A	>189-A	<21-C
32% RH- 40°C	>189-A	>189-A	<63-PC	<28-PC	<28-PC	<21-C
54% RH- 25°C	<63-PC	<21-C	<49-C	<21-C	<14-C	<14-C
54% RH- 40°C	<49-C	<21-C	<49-C	<21-C	<14-C	<14-C

Table 5.2 Characterization of hydrogen bond donor and acceptor group of CA and polymers based on the pK_{BHX} scale published by Laurence et al. (2009)

Compound	Hydrogen Bond Donor		Hydrogen Bond Acceptor	
	Group	Strength	Group	Strength [a]
CA	Hydroxyl	Strong	Hydroxyl	Medium
	Carboxylic	Very Strong	Carboxylic	Medium [b]
PEC	Hydroxyl	Strong	Hydroxyl	Medium
	Carboxylic	Very Strong	Carboxylic	Medium [b]
			Ether	Medium
			Ester	Medium
GG	Hydroxyl	Strong	Hydroxyl	Medium
			Ether	Medium
KG	Hydroxyl	Strong	Hydroxyl	Medium
			Ether	Medium
			S=O of sulfate ester	Medium
HPMC	Hydroxyl	Strong	Hydroxyl	Medium
			Ether	Medium
CMC-Na	Hydroxyl	Strong	Hydroxyl	Medium
			Ether	Medium

[a]Acceptor strength was determined by pK_{BHX} scale. The classification of the acceptor strength was weak < 0.5 < medium < 1.8 < strong < 3.0 < very strong (Laurence et al., 2009).

[b]No values were found for carboxylic acid acceptor group (Laurence et al., 2009), but the acceptor strength of the carboxylic acid carbonyl group was regarded as being comparable to the ester carbonyl group.

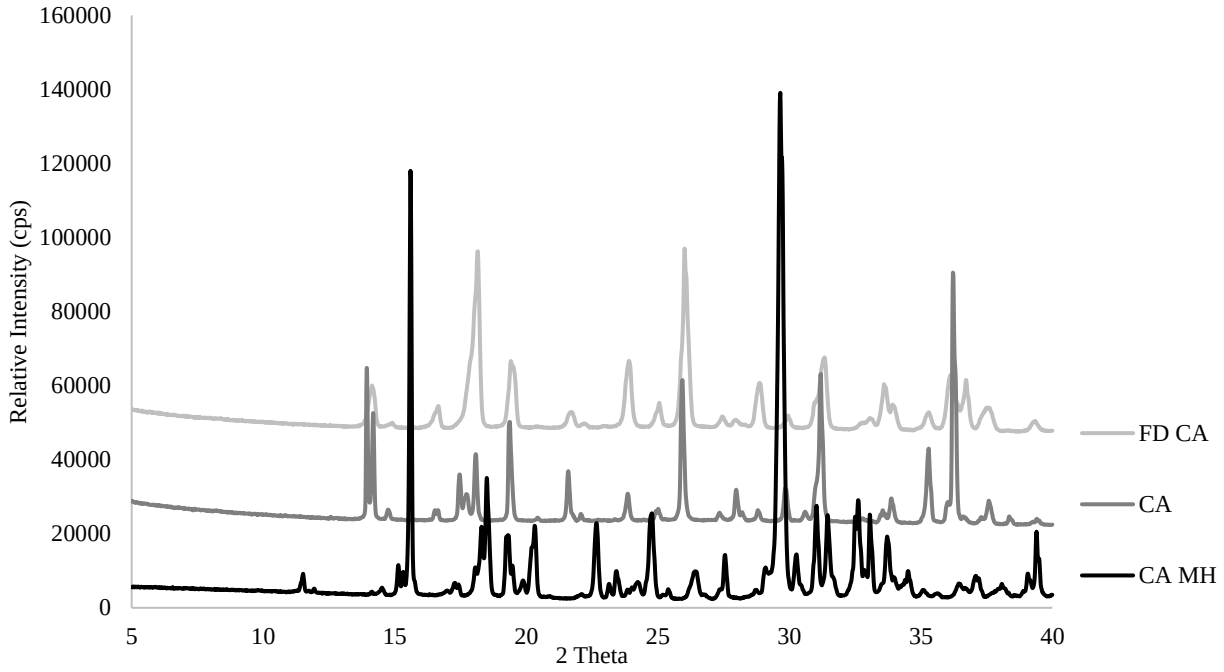
Table 5.3 FTIR peak shifts observed in the amorphous CA: polymer solid dispersions compared to the polymer alone.

CA: Polymer Dispersion	%CA	Hydroxyl Region (3600-3000 cm^{-1})	Total Shift	Carbonyl Region (1800-1500 cm^{-1})		Total Shift	
CA:PEC	0→50	3419→3400	19	1743→1734	1622→1647	9	-25
CA:KG	0→50	3422→3384	38	N/A			
CA:GG	0→50	3388→3402	-14	N/A			
CA:GEL	0→50	3322→3335	-12	1653→1634	1558→1546	19	12
CA:HPMC	0→50	3461→3421	40	N/A			
CA:CMC-Na	0→50	3400→3400	0	1600→1575		25	

Table 5.4 Onset glass transition temperatures (T_g) of citric acid and solid dispersions in the absence and presence of pinhole.

Sample (Pin hole)	**Onset $T_g$at Day 0 (Pin Hole)**	**Onset T_g at Day 0 (No Pin Hole)**
Citric Acid	11.2±0.1	
CMC-Na: CA SD	49.5 ± 1.1^{A}	14.3^{a}
GEL: CA SD	44.4 ± 1.3^{A}	13.1 ± 2.5^{a}
GG: CA SD	21.8 ± 2.4^{DC}	-9.5 ± 1.8^{b}
HPMC: CA SD	16.6 ± 1.8^{D}	-7.8^{b}
KG: CA SD	24.7 ± 0.2^{C}	-10.4 ± 0.1^{b}
PEC: CA SD	33.5 ± 0.8^{B}	-5.7 ± 0.2^{b}

A)



B)

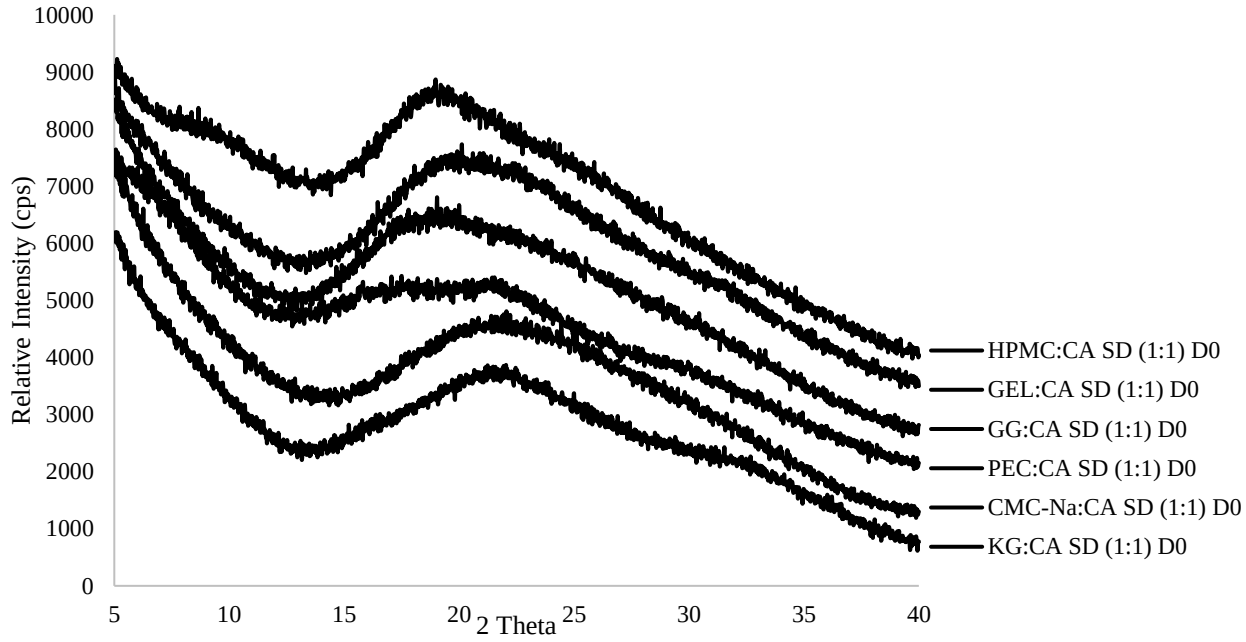
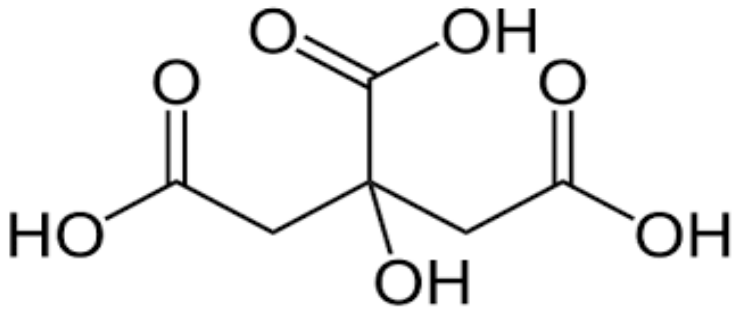


Figure 5.1 PXRD patterns of crystalline citric acids: **A)** Anhydrous citric acid (CA), citric acid monohydrate (CA MH) and lyophilized citric acid (FD CA), **B)** 1:1 CA: polymer solid dispersions (SD) at day 0.

A)



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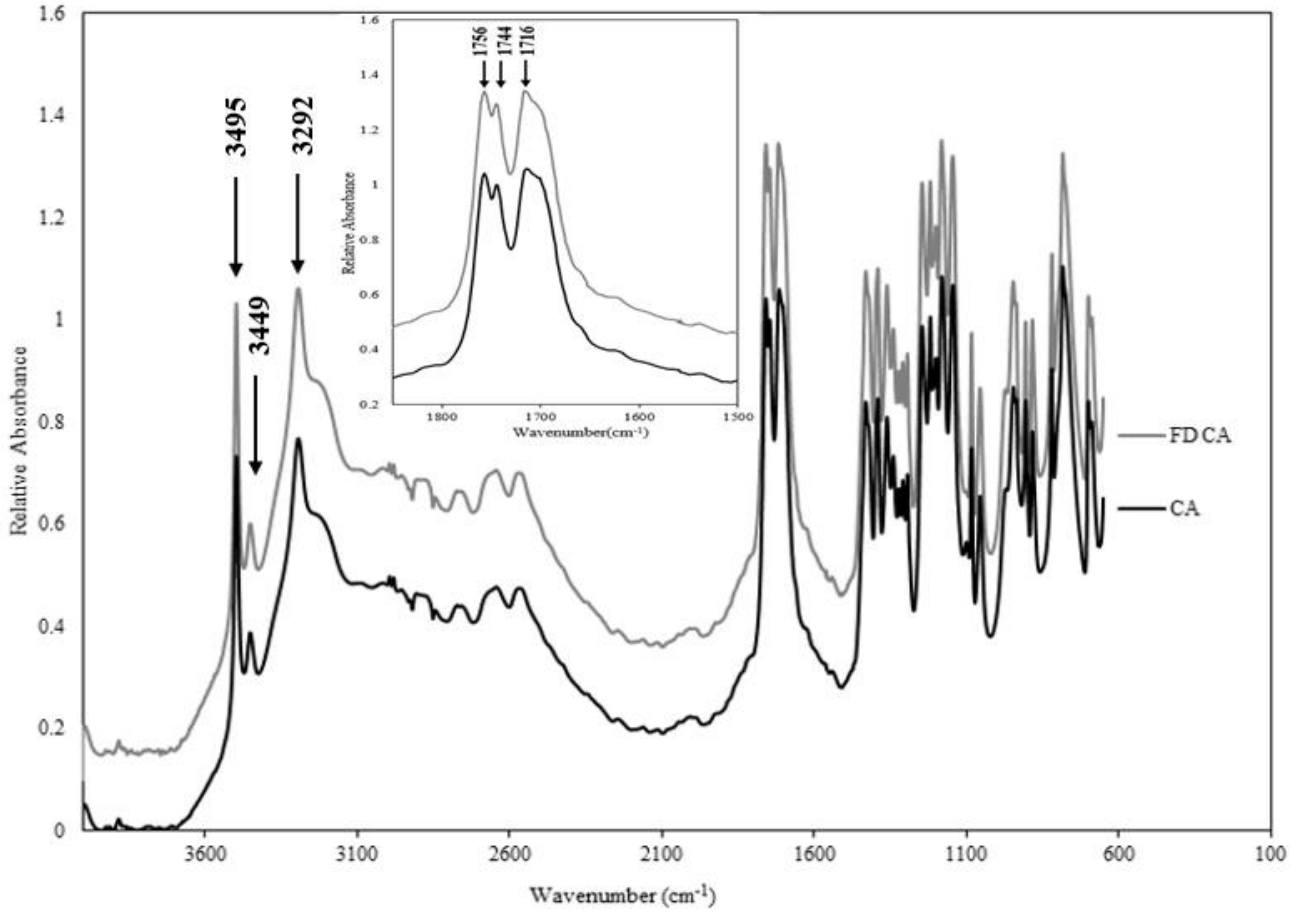


Figure 5.2 **A)** Chemical structure of CA, **B)** mid infrared spectra of crystalline citric acid samples, before (CA) and after lyophilization (FD CA). NH/OH and carbonyl stretching region are shown.

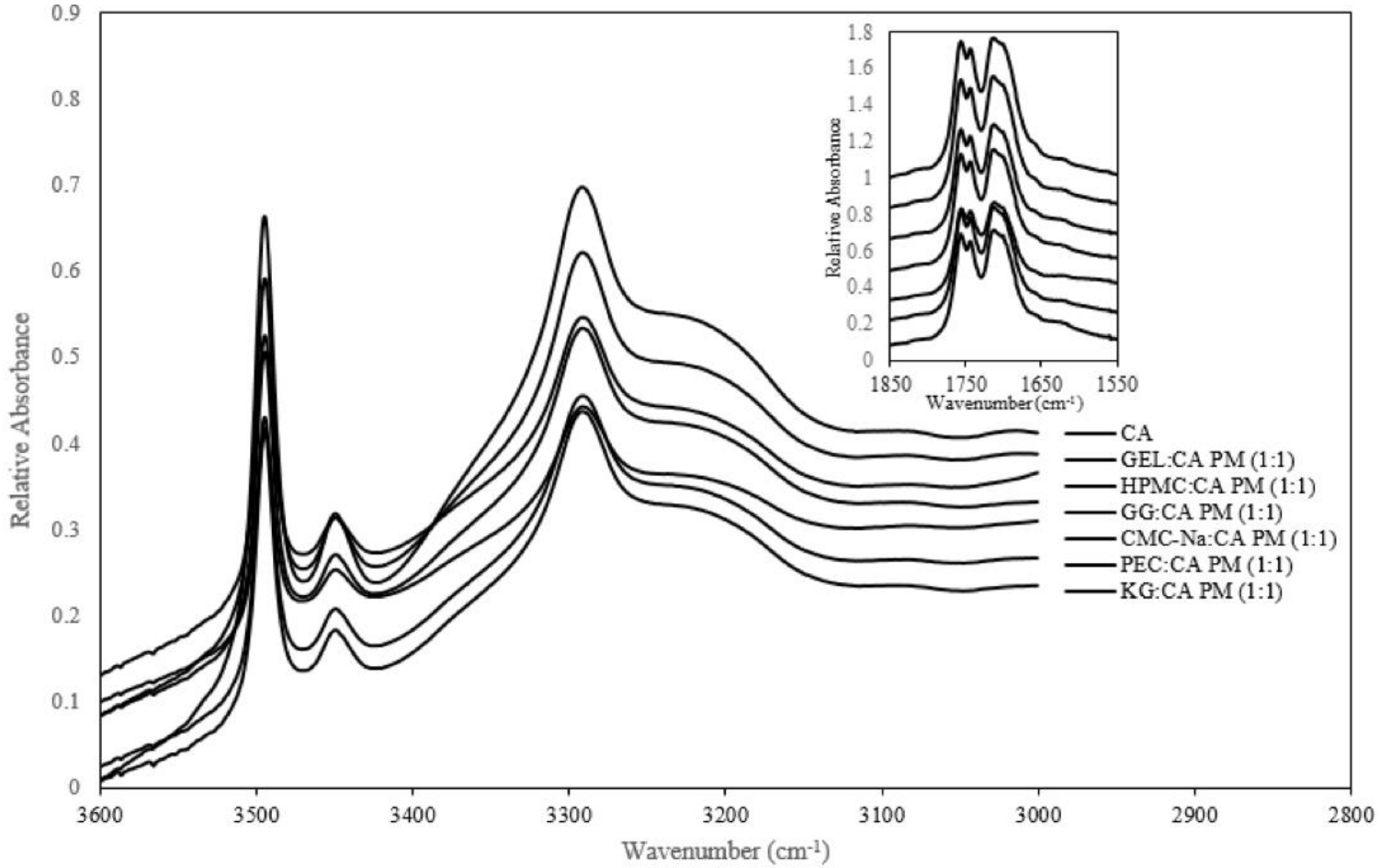


Figure 5.3 Mid infrared spectra of 1:1 CA:Polymer physical mixtures (PM).

A)

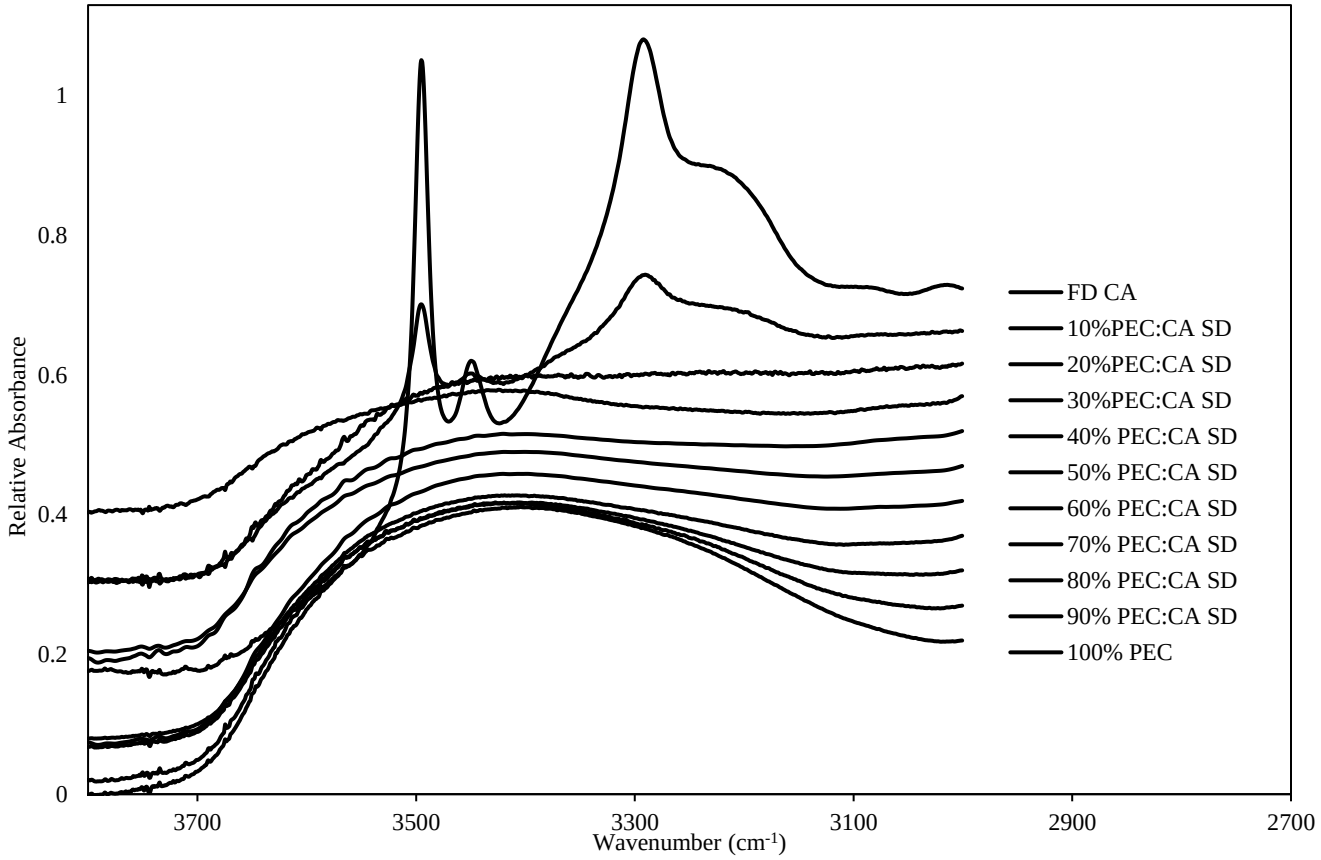


Figure 5.4 Mid infrared spectra of: **A)** CA: PEC solid dispersions (SD) made with various ratios, (NH/OH region is shown), **B)** CA:CMC-Na, **C)** CA: GEL, **D)** CA: PEC solid dispersions (SD) made with various ratios, showing the carbonyl stretching region.

Figure 5.4 continued

B)

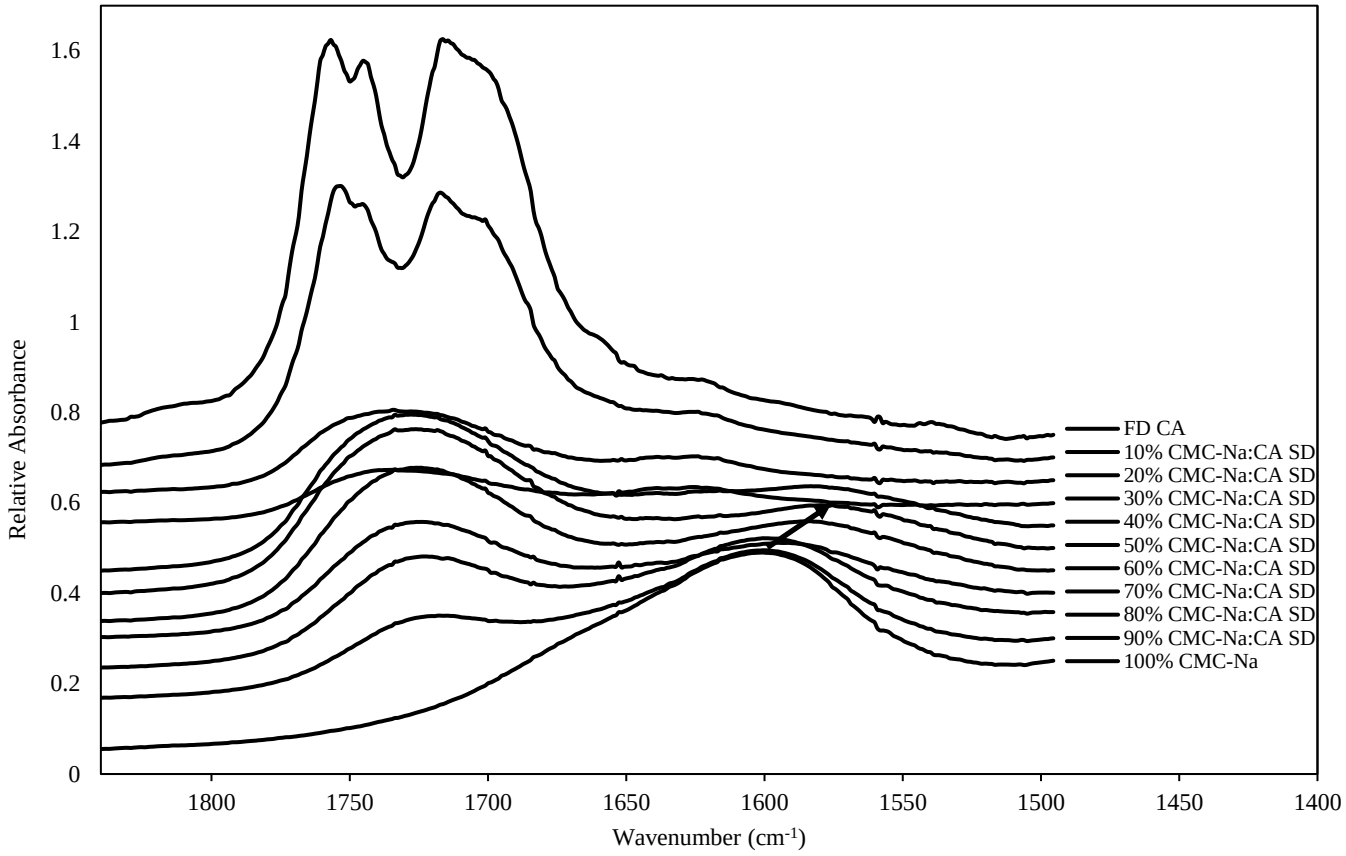


Figure 5.4 continued

C)

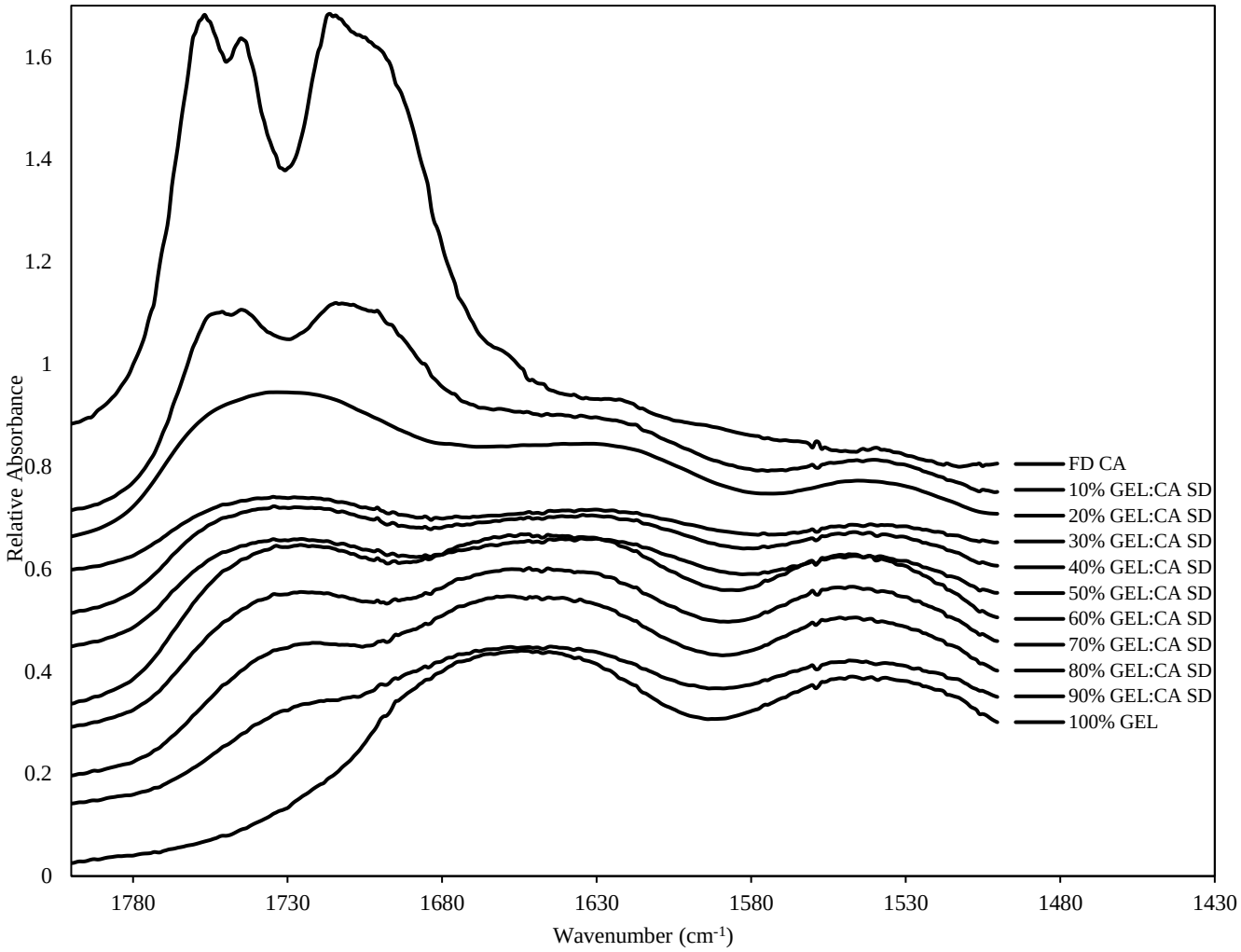
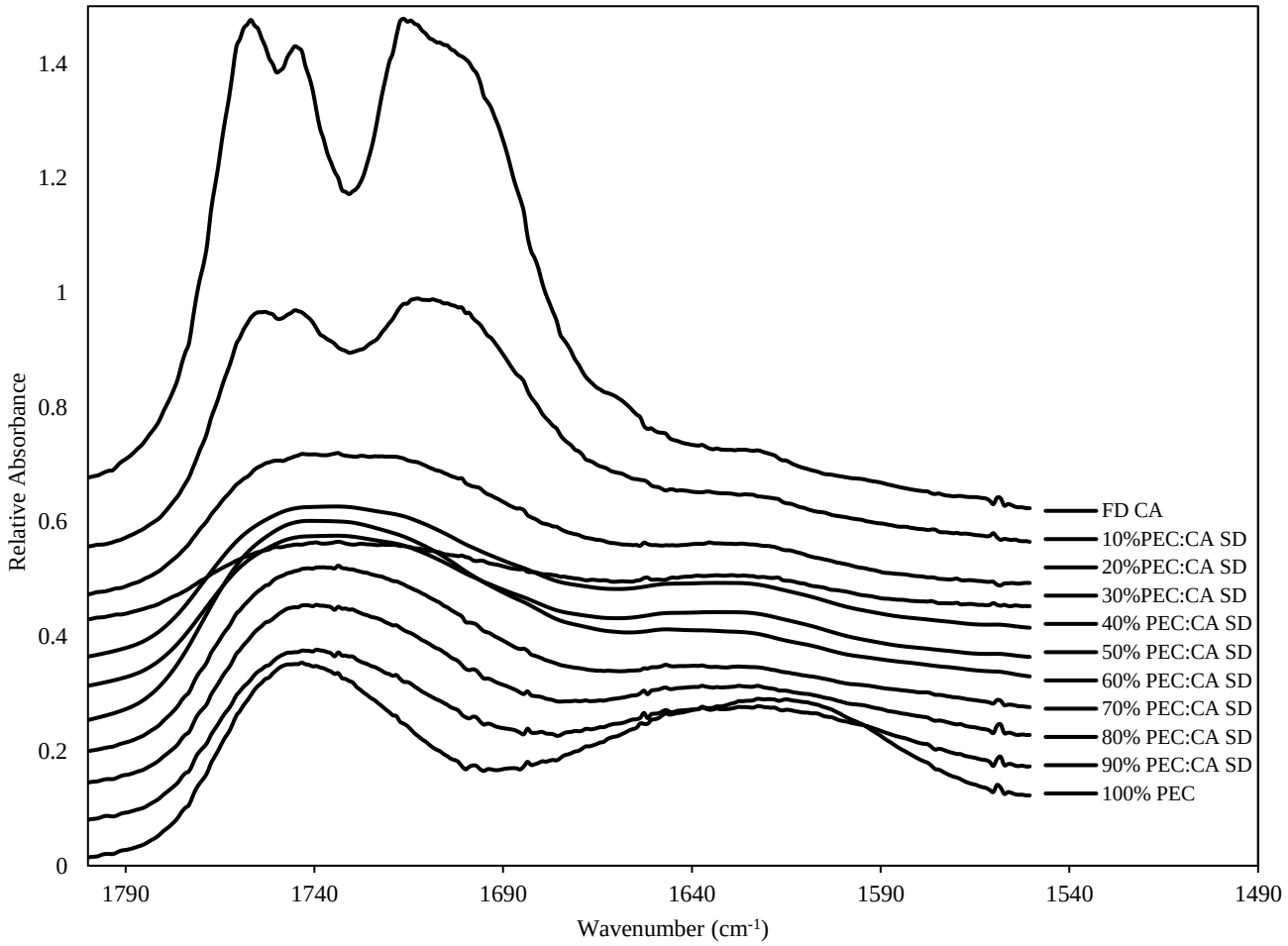


Figure 5.4 continued

D)



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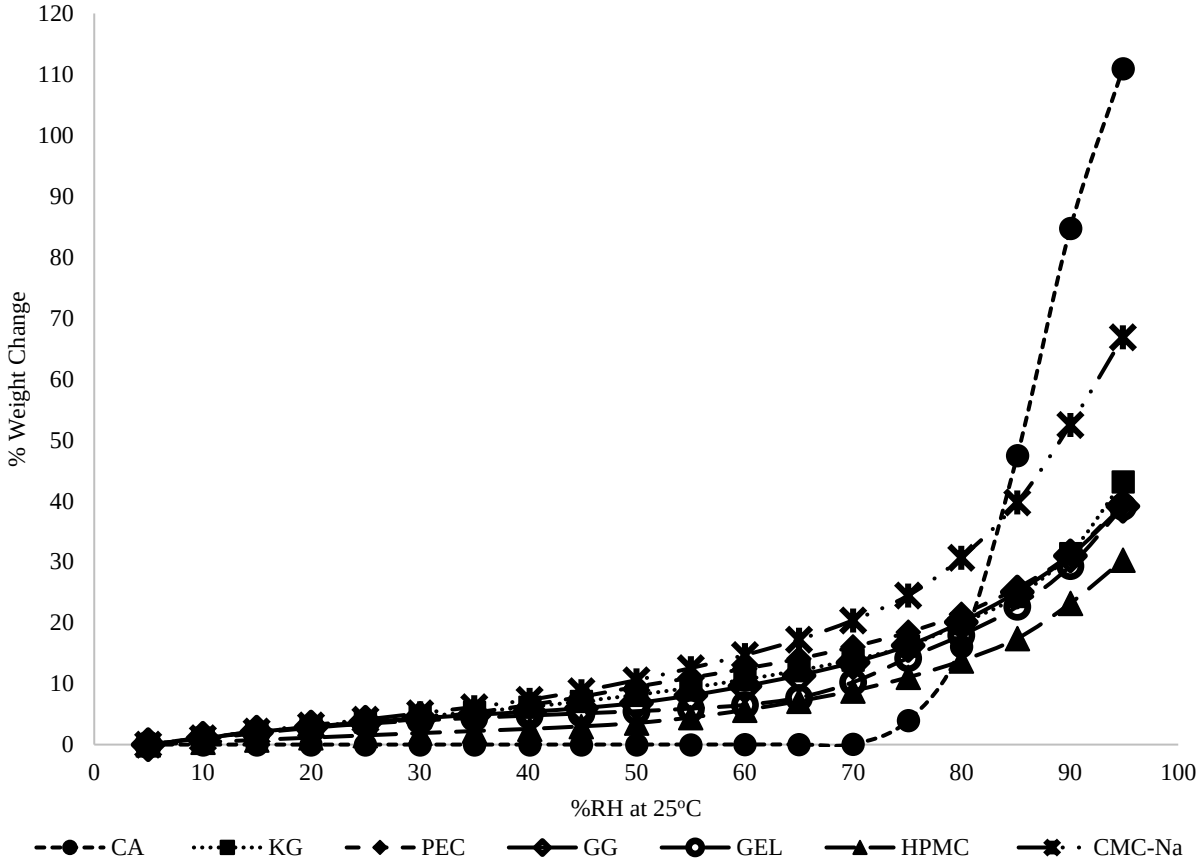


Figure 5.5 Moisture sorption profiles of samples at 25ºC: **A)** CA and polymers, **B)** 1:1 CA: polymer physical mixtures (PM), **C)** 1:1 CA: polymer solid dispersions (SD).

Figure 5.5 continued

B)

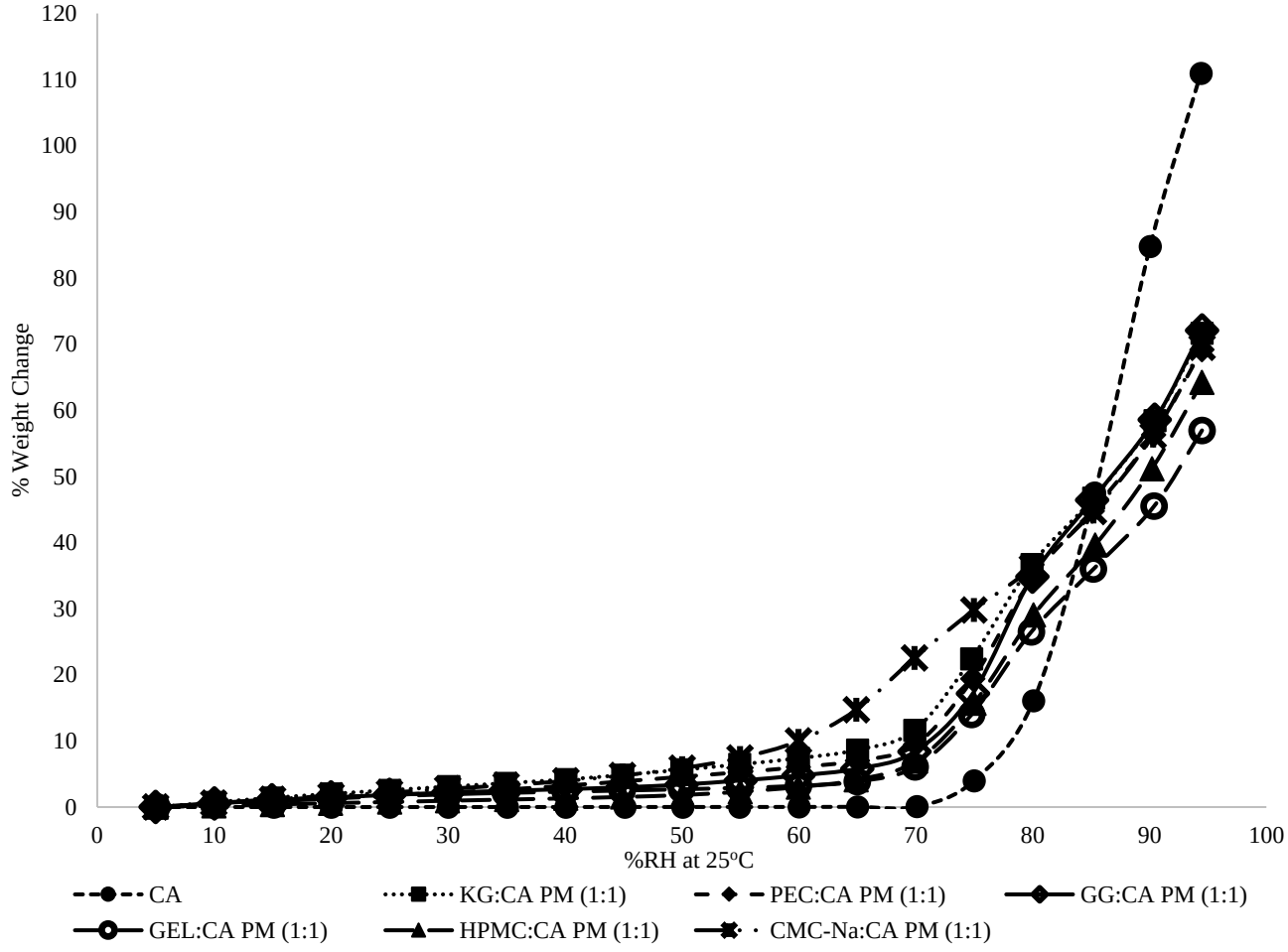
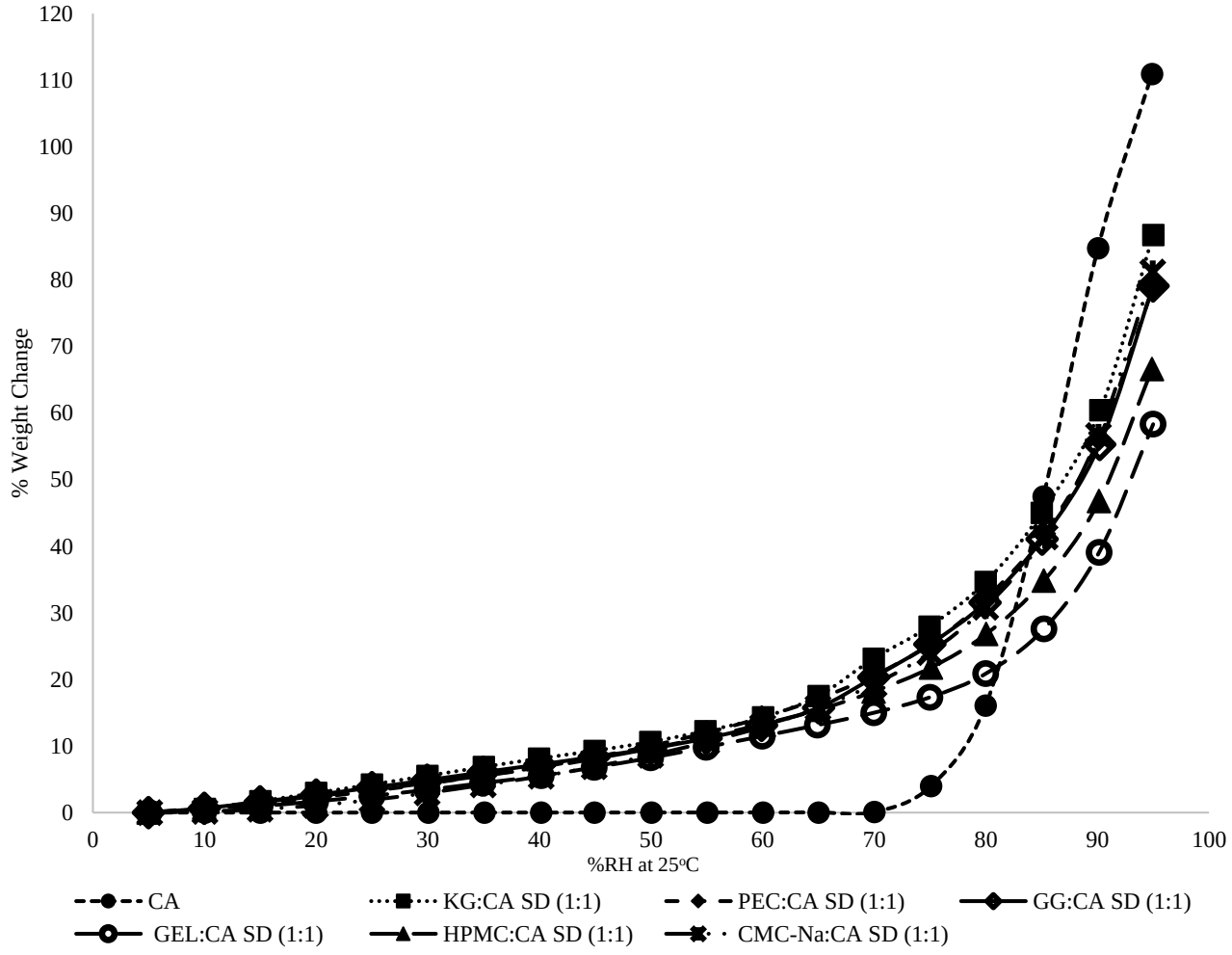


Figure 5.5 continued

C)



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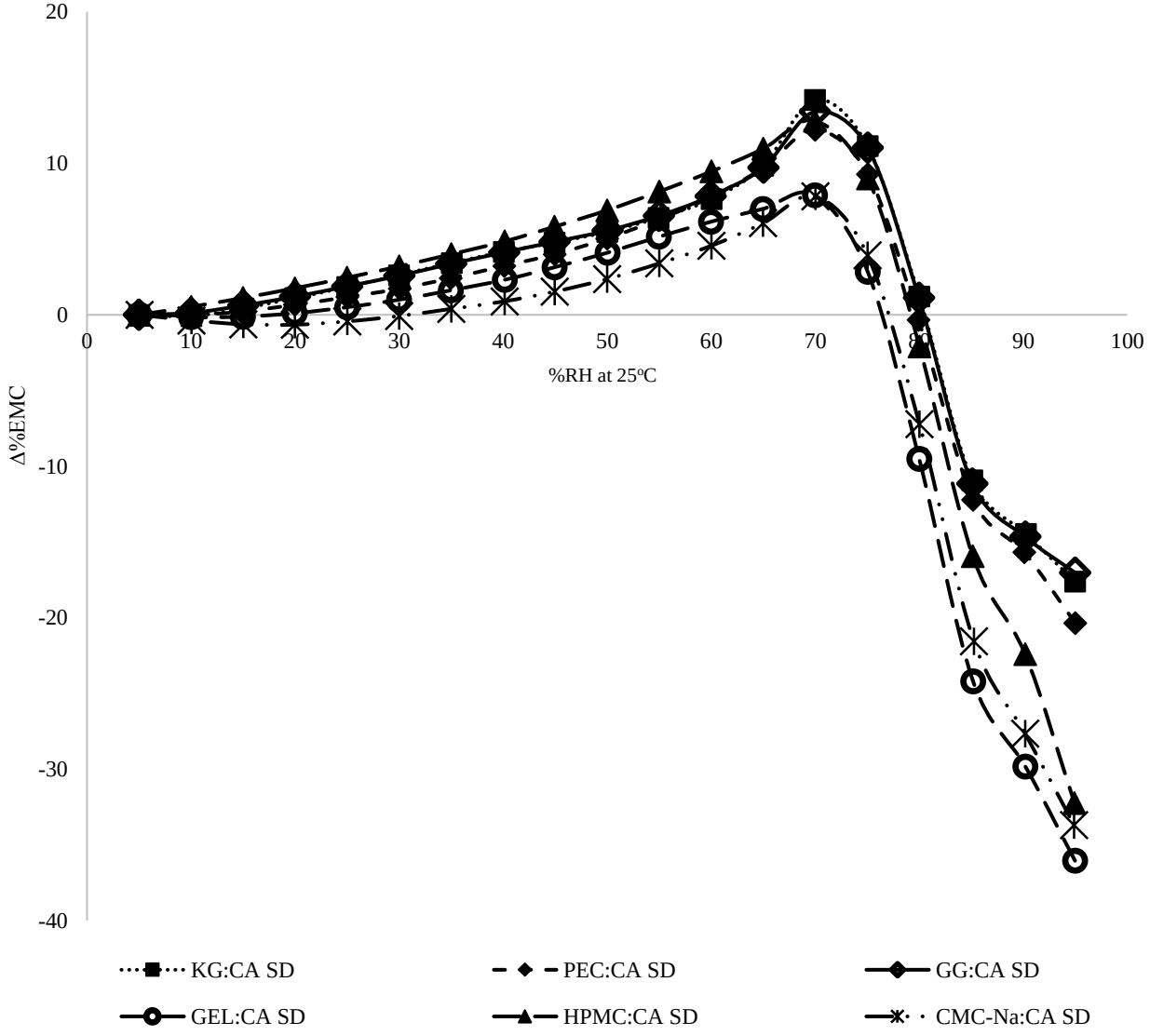
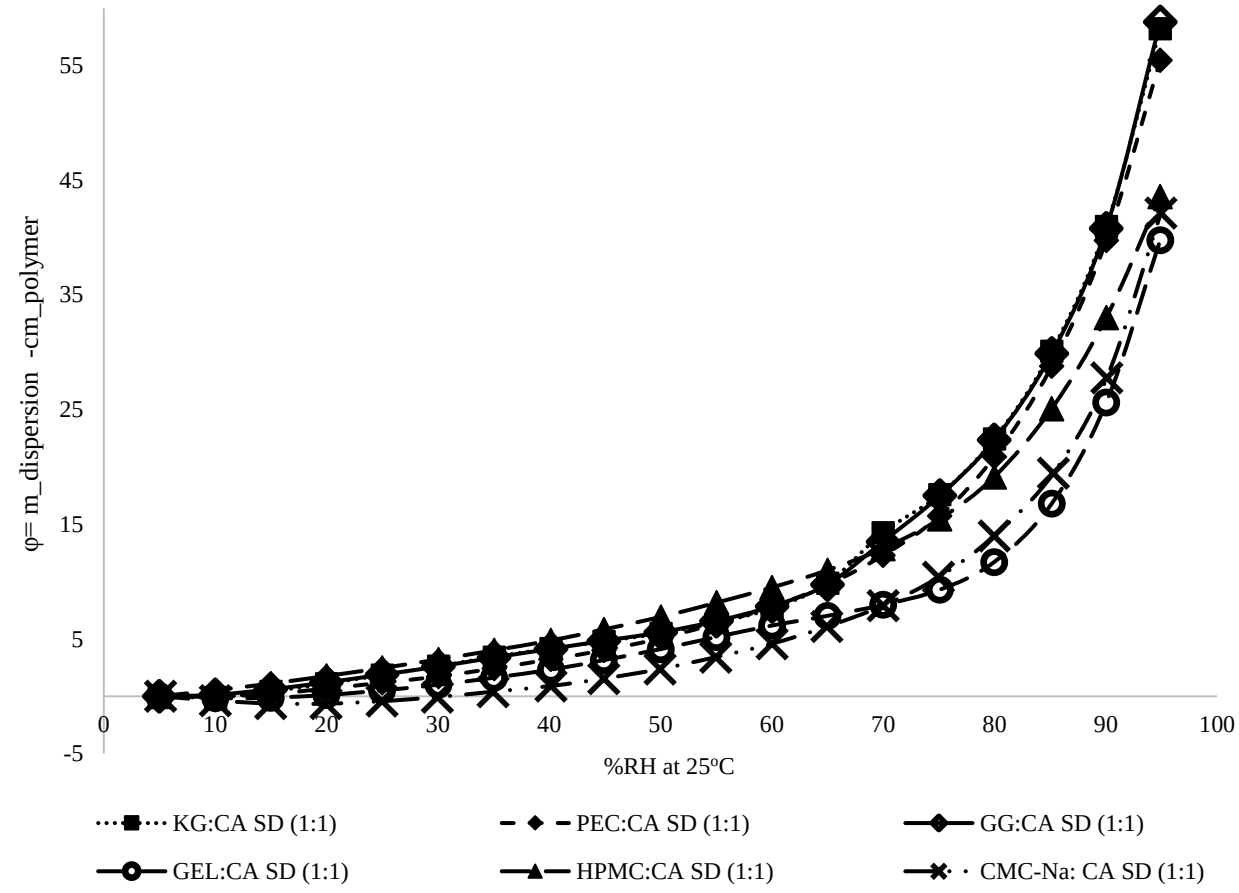


Figure 5.6 **A)** Percent equilibrium moisture content (Δ% EMC) versus %RH of all the solid dispersions (SD), **B)** Difference between polymer and dispersion moisture sorption.

Figure 5.6 continued

B)



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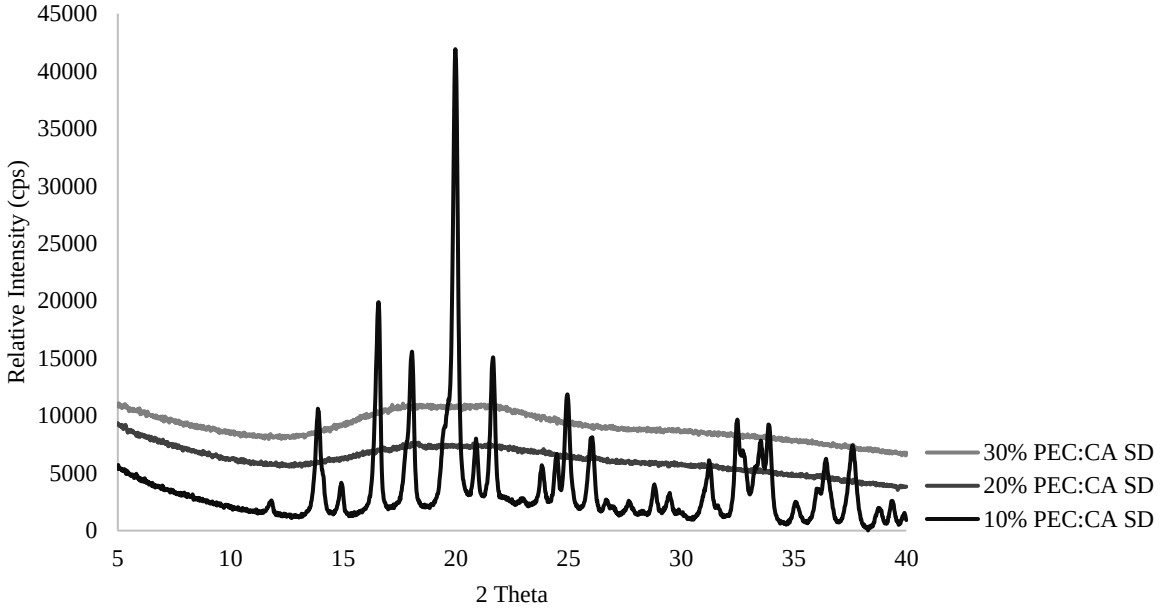
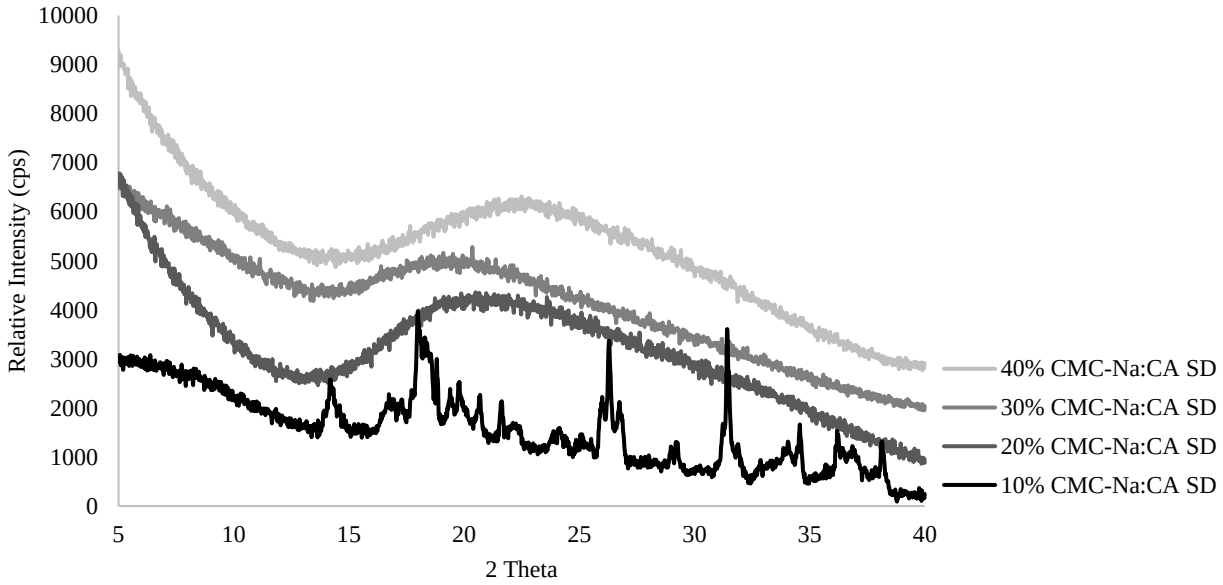


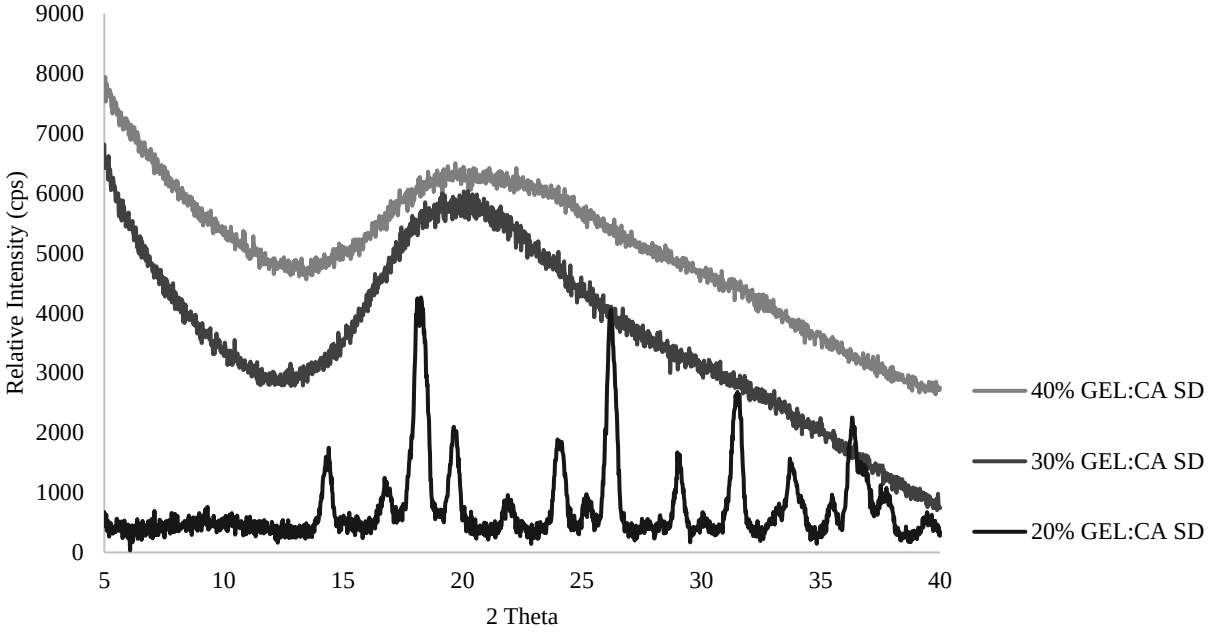
B)



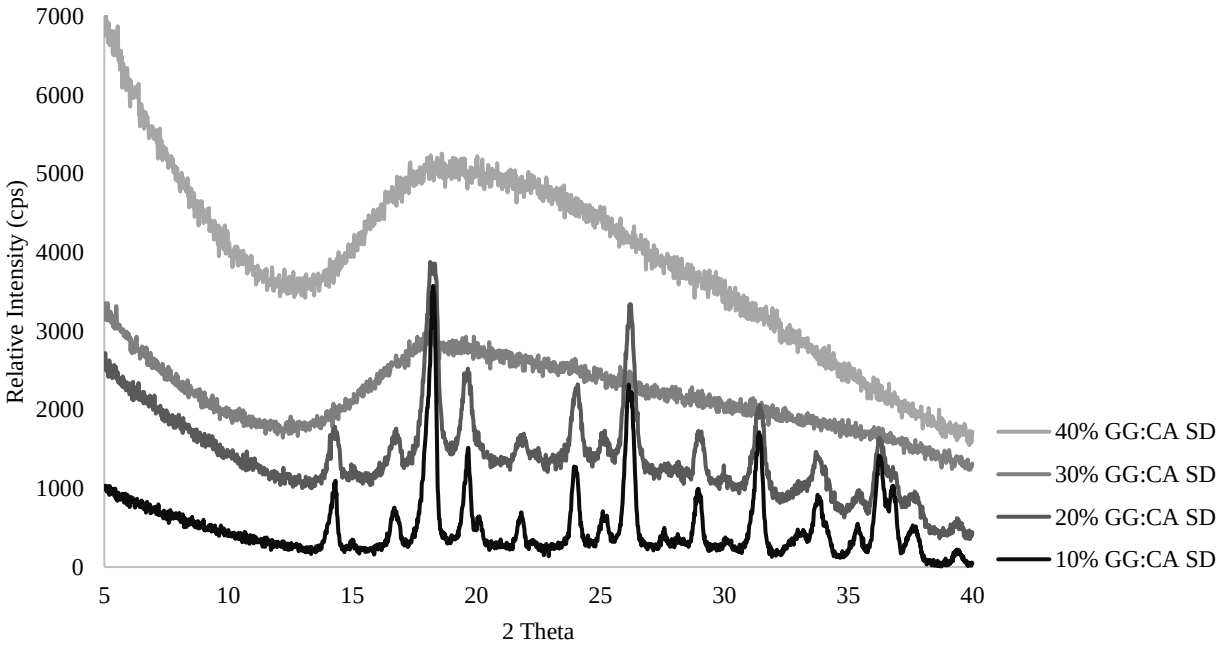
Supplementary Figure 5.1 PXRD patterns of various ratios of CA to polymer: **A)** CA: PEC solid dispersion, **B)** CA: CMC-Na solid dispersion, **C)** CA: GEL solid dispersion, **D)** CA: GG solid dispersion, **E)** CA: HPMC solid dispersion, **F)** CA: KG solid dispersion.

Supplementary Figure 5.1 continued

C)

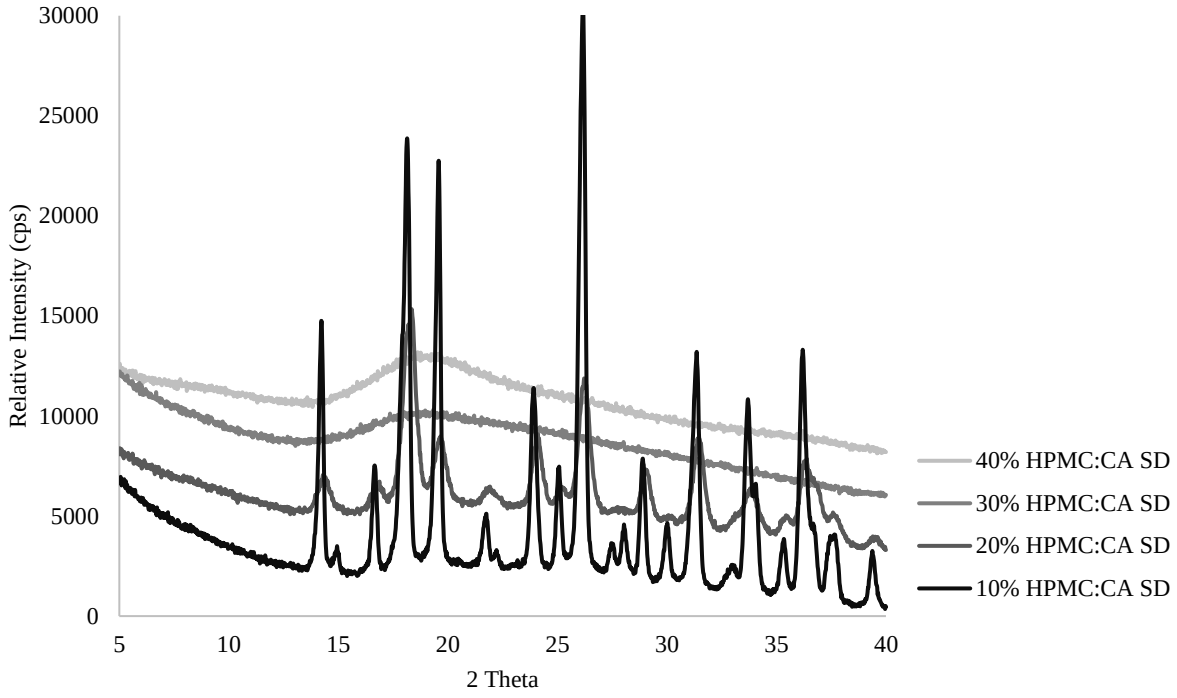


D)

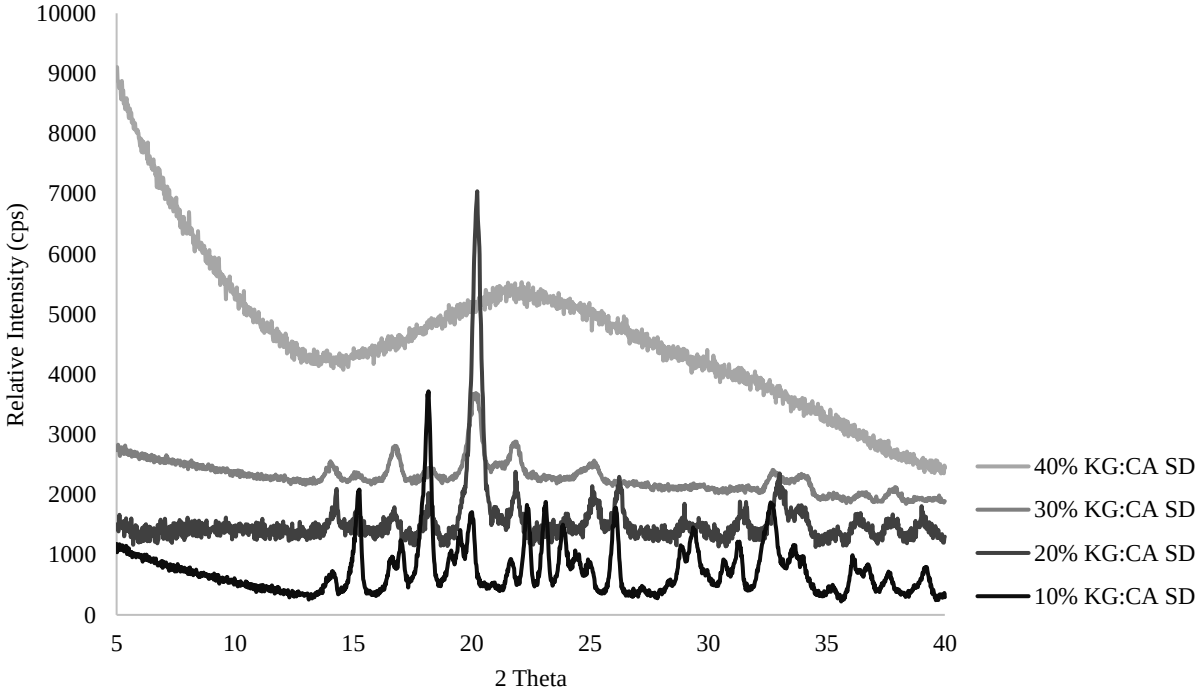


Supplementary Figure 5.1 continued

E)



F)



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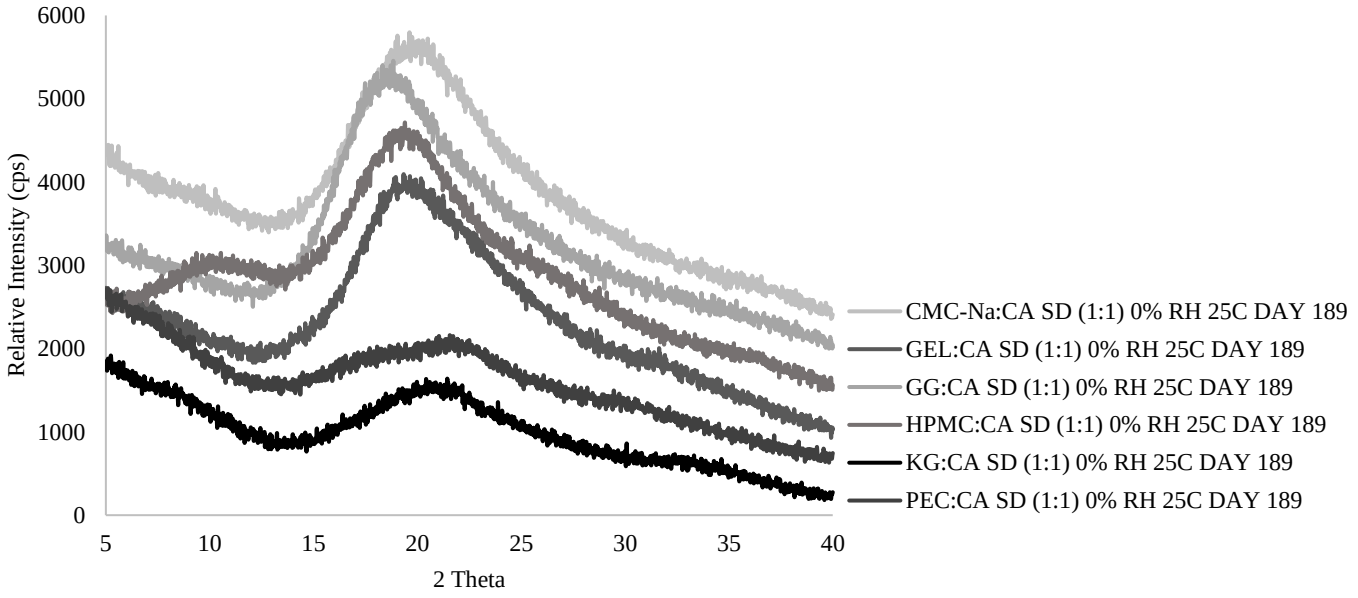

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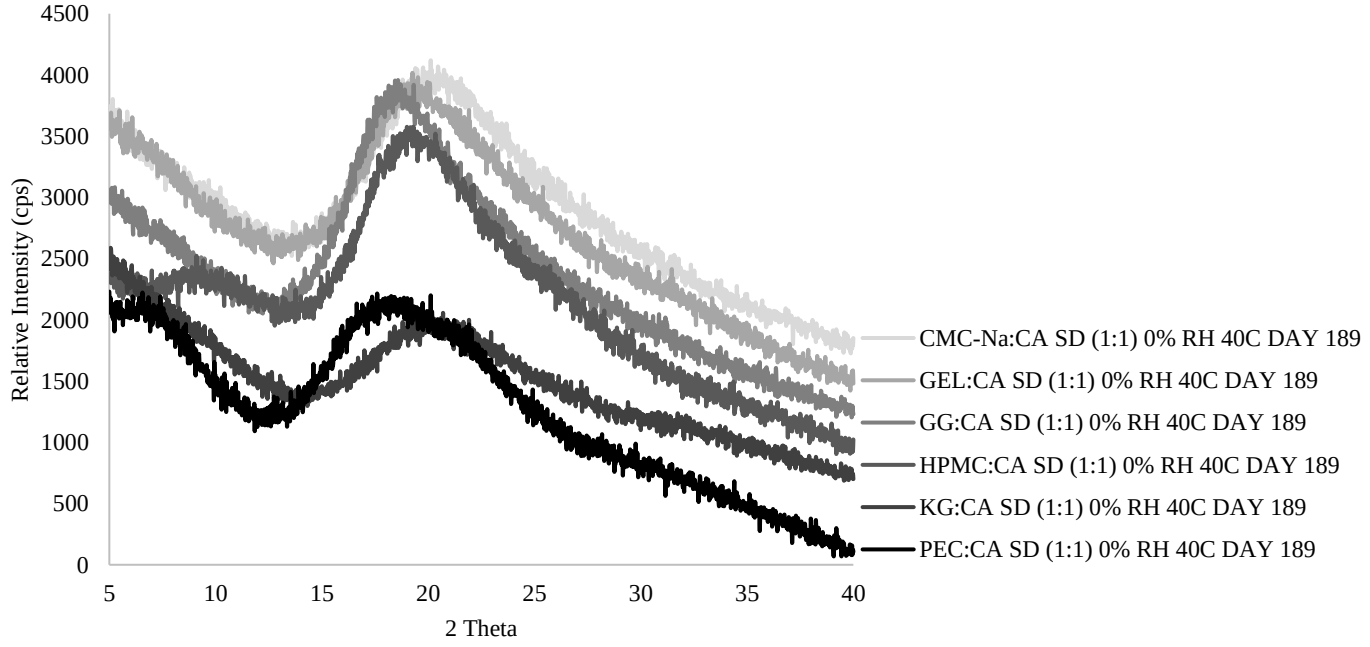
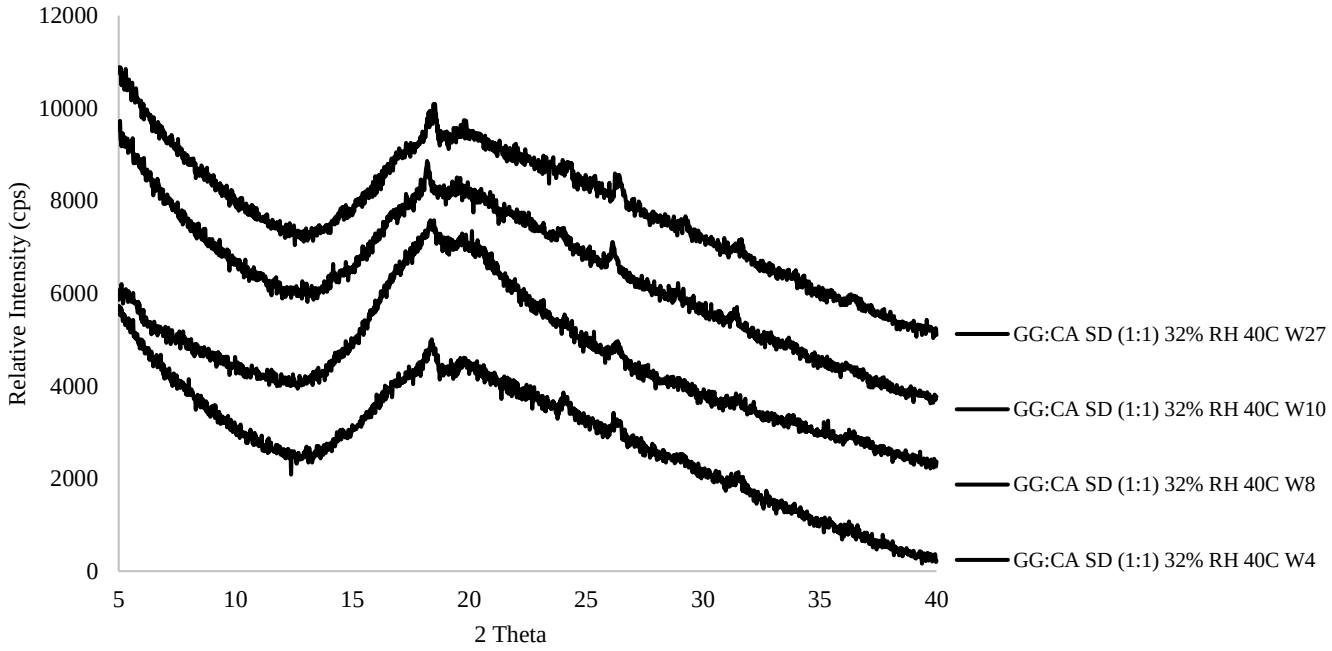


Supplementary Figure 5.2 PXRD patterns of: **A)** 1:1 CA: polymer solid dispersions at 0% RH and 25ºC on day 189, **B)** 1:1 CA: polymer solid dispersions at 0% RH and 40ºC on day 189, **C)** 1:1 CA: GG solid dispersions.

Supplementary Figure 5.2 continued

C)



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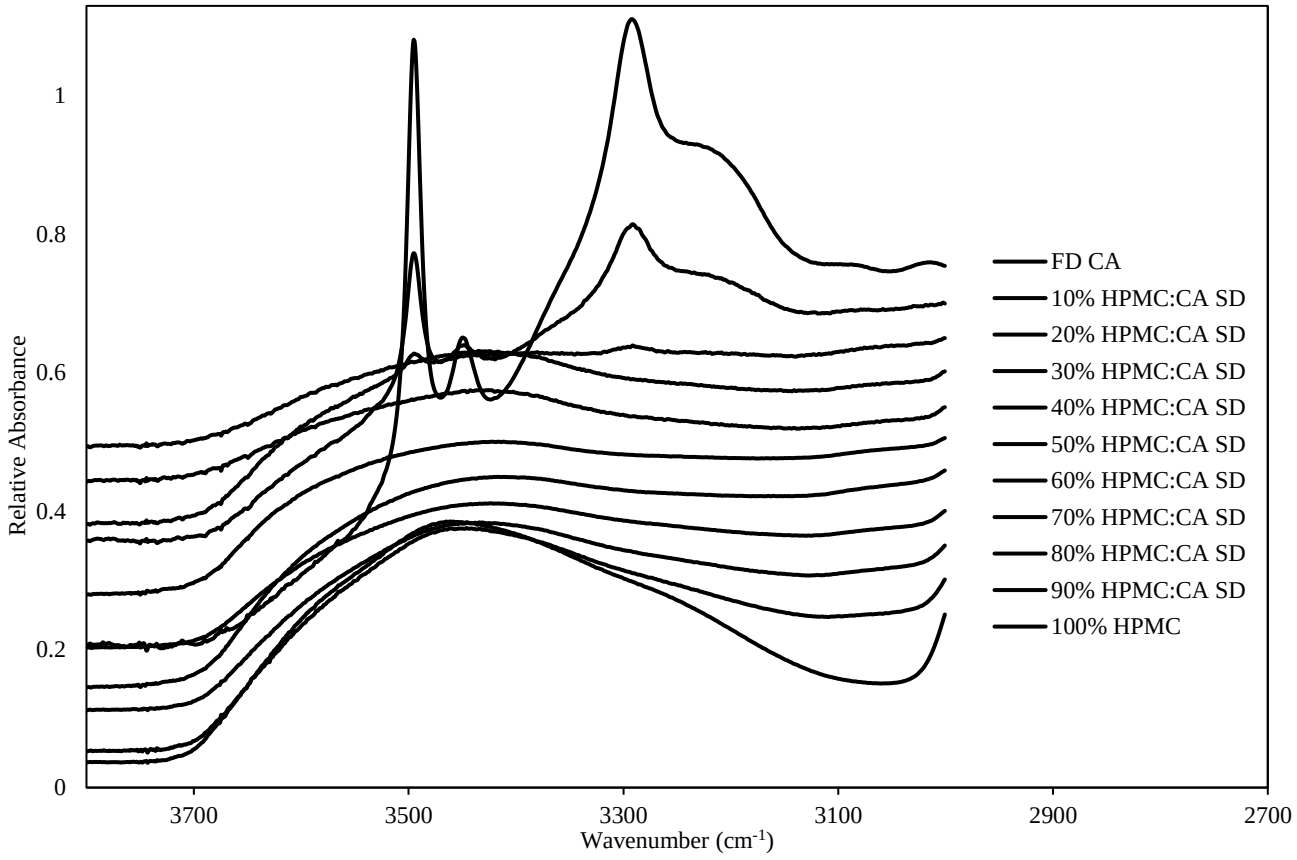
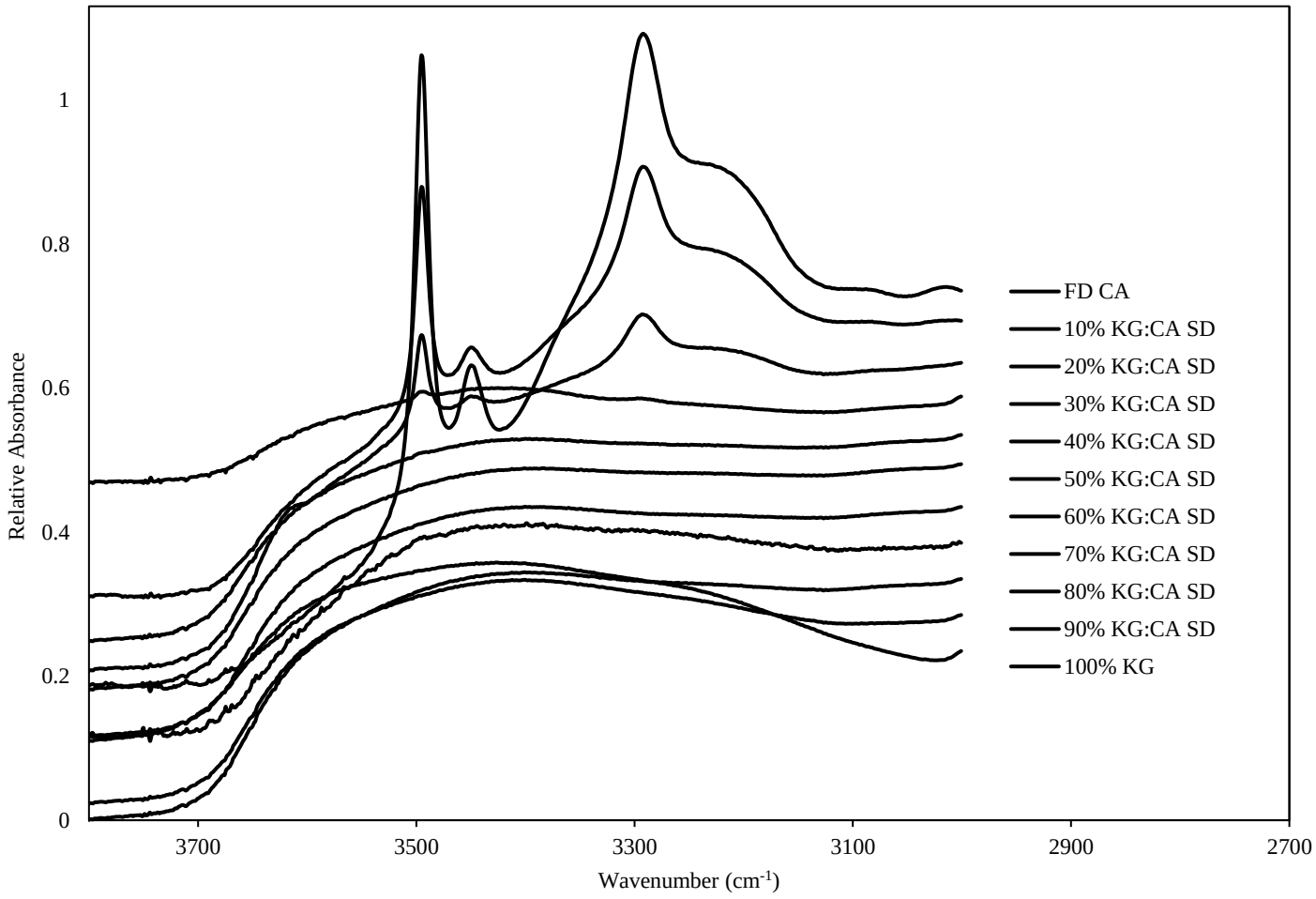


Supplementary Figure 5.3 Mid infrared spectra of: **A)** CA: HMPC solid dispersions (SD), **B)** CA: KG solid dispersions (SD), **C)** CA: CMC-Na solid dispersions (SD), made with various ratios (NH/OH region is shown).

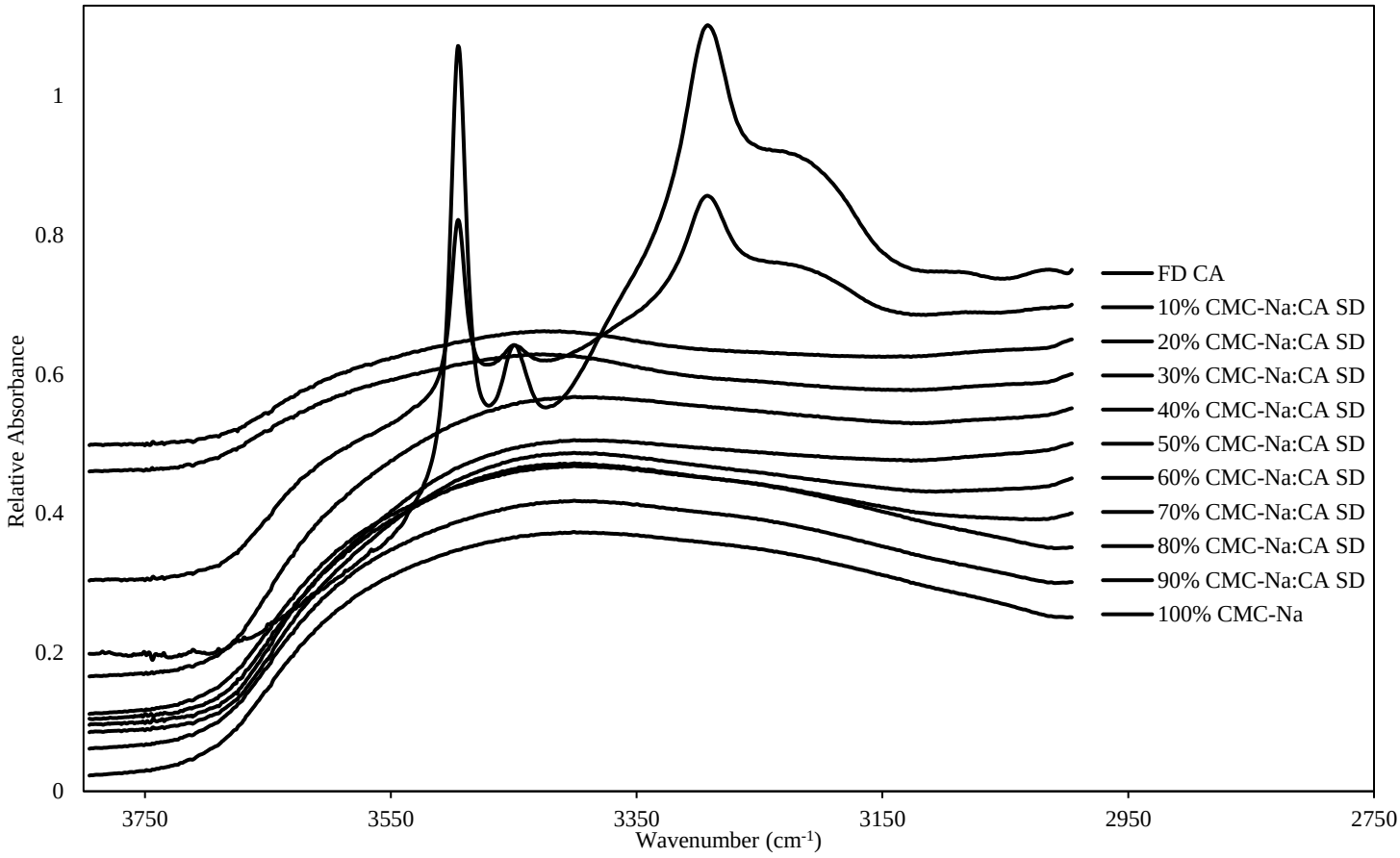
Supplementary Figure 5.3 continued

B)



Supplementary Figure 5.3 continued

C)



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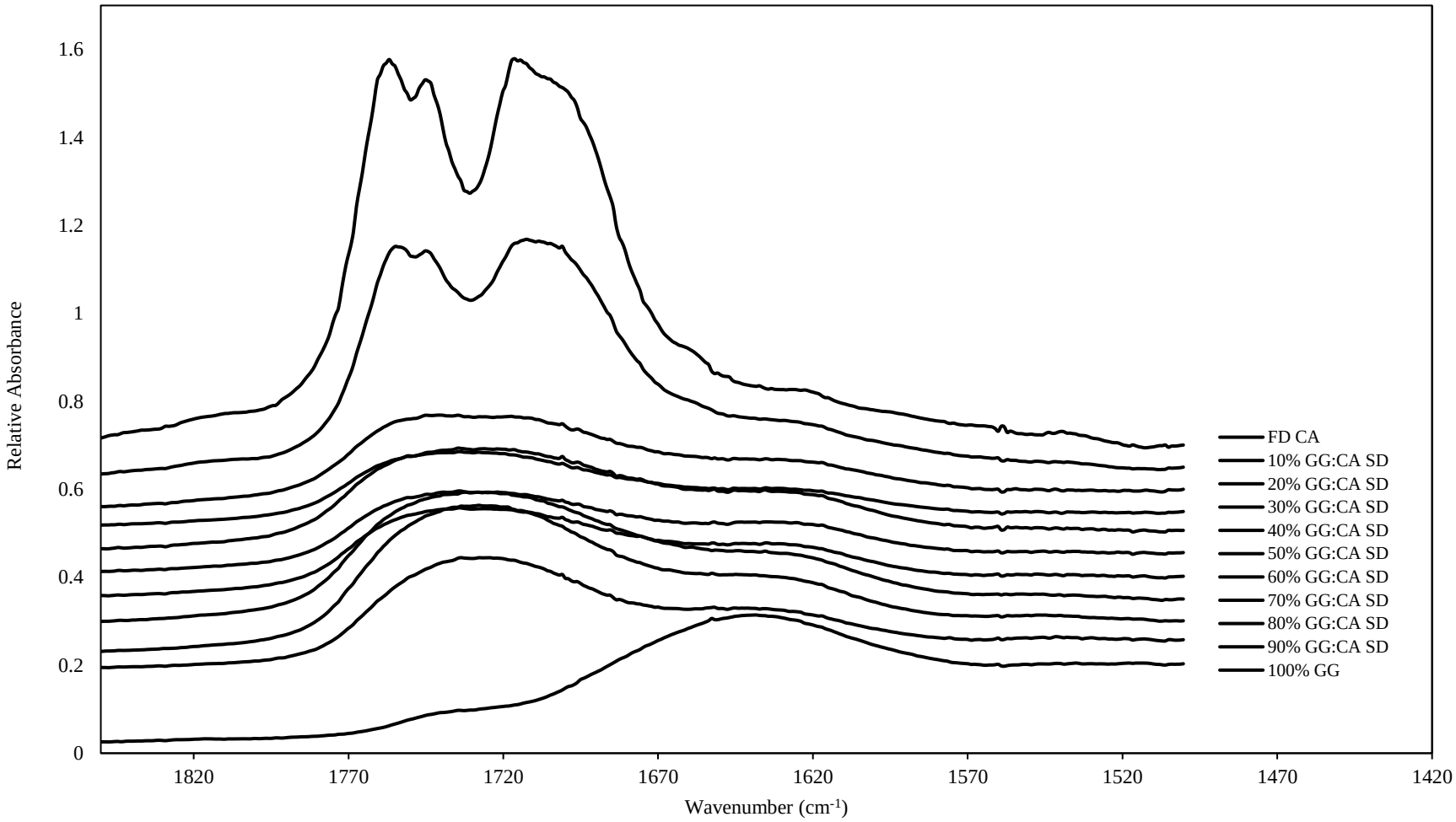
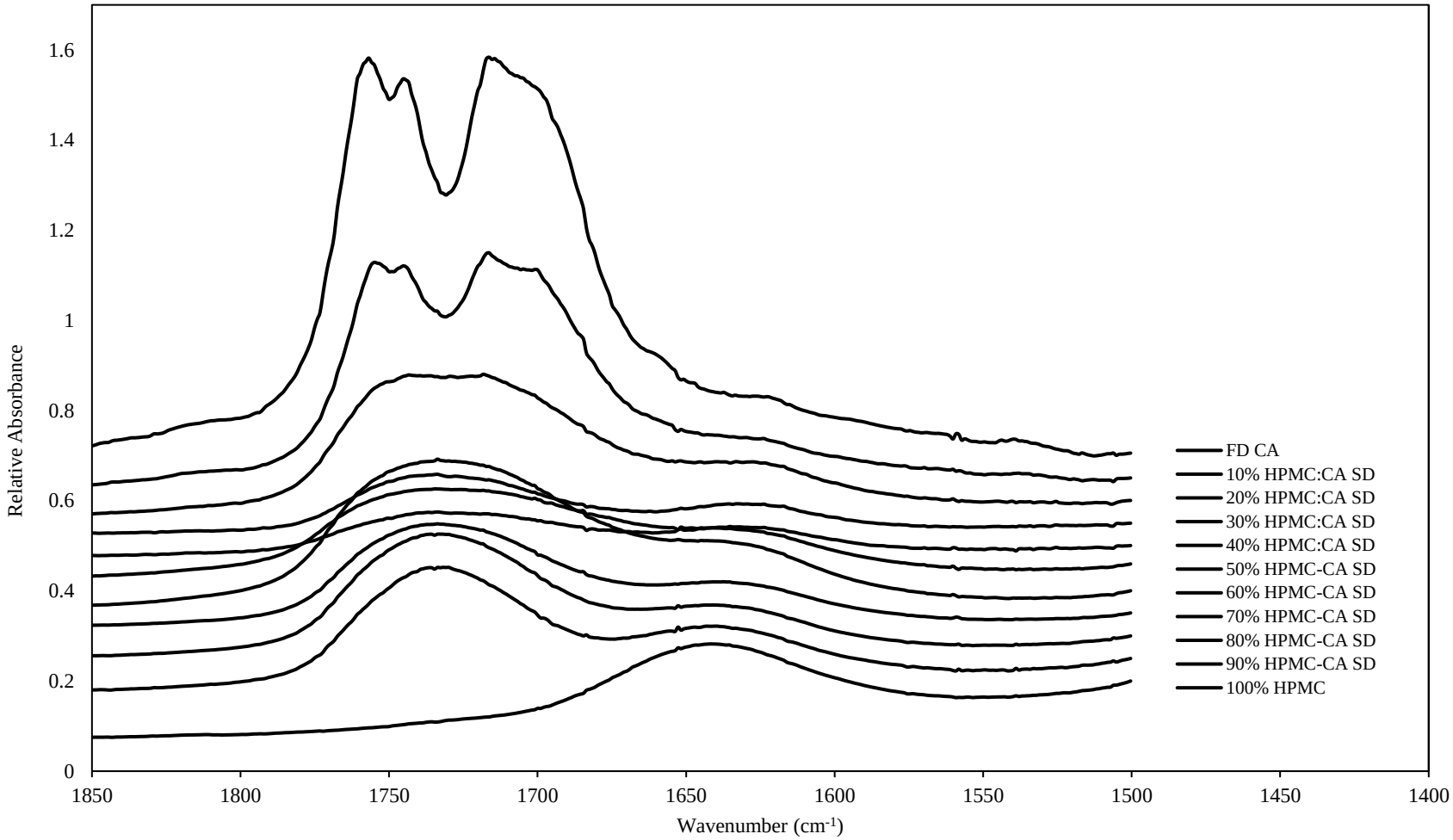


Supplementary Figure 5.4 Mid infrared spectra of: **A)** CA: GG solid dispersion (SD), **B)** CA:HPMC solid dispersion (SD), **C)** CA: KG solid dispersion (SD), made with various ratios, (carbonyl region is shown), **D)** Speciation plot of CA.

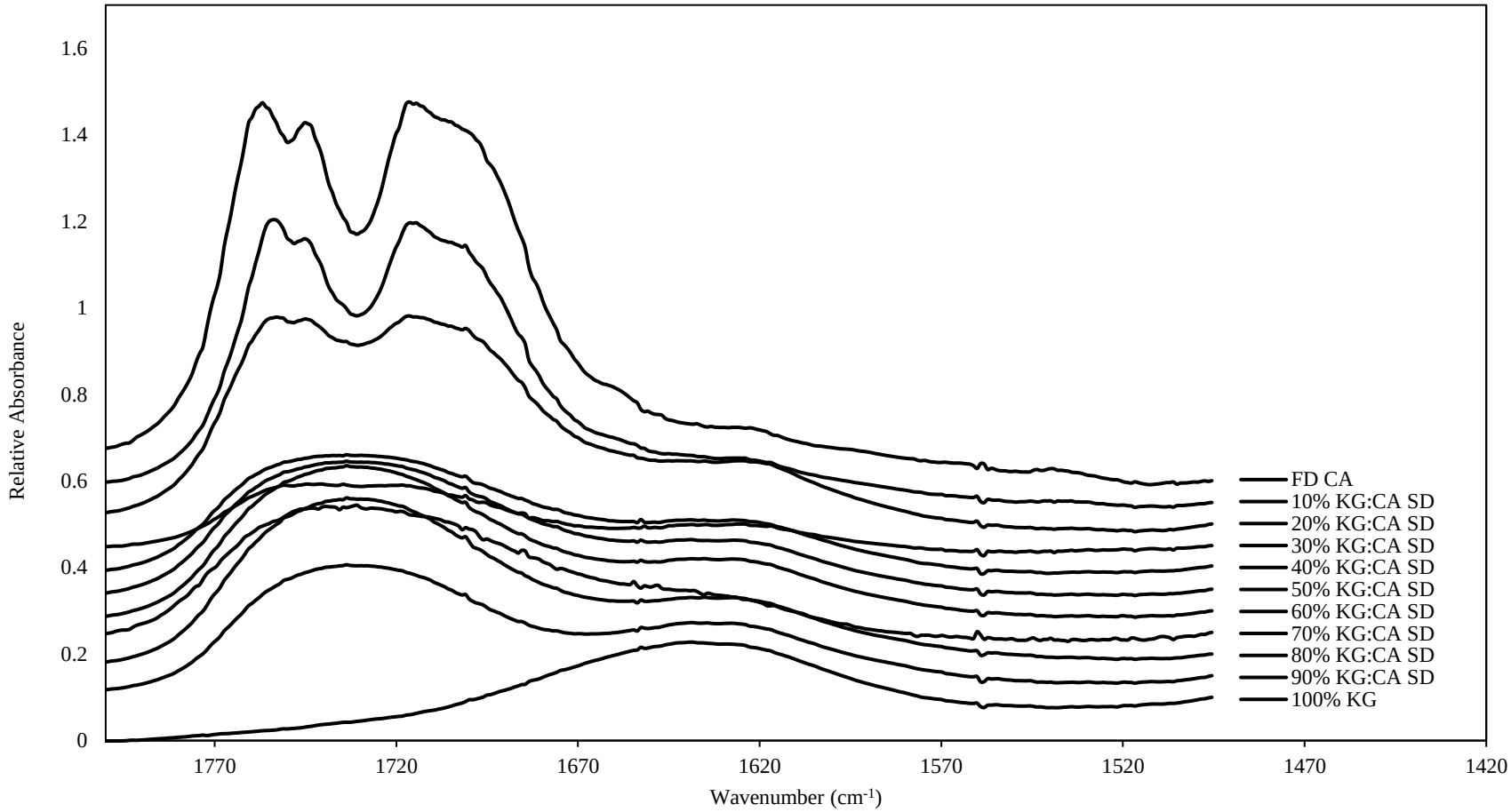
Supplementary Figure 5.4 continued

B)



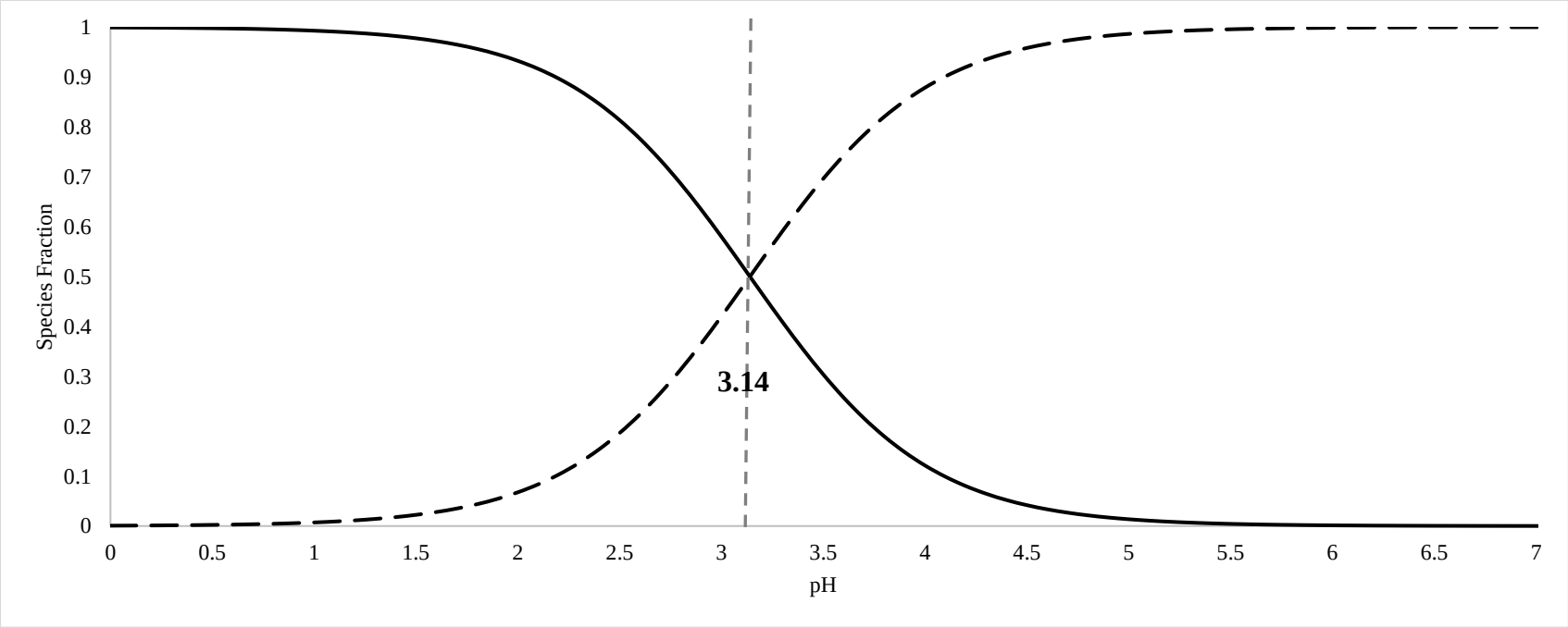
Supplementary Figure 5.4 continued

C)



Supplementary Figure 5.4 continued

D)



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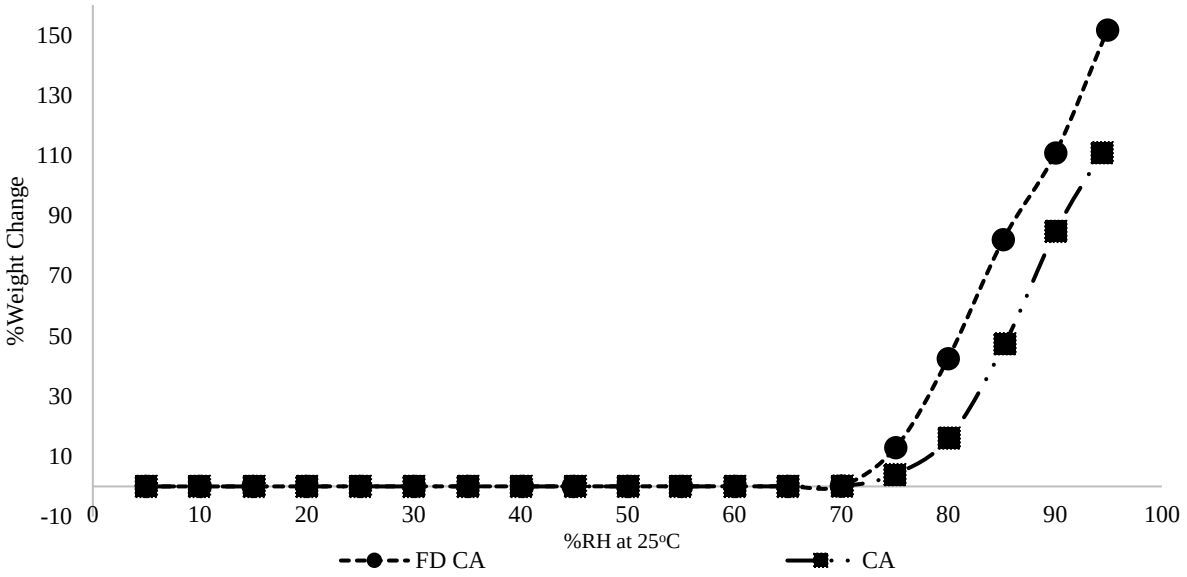

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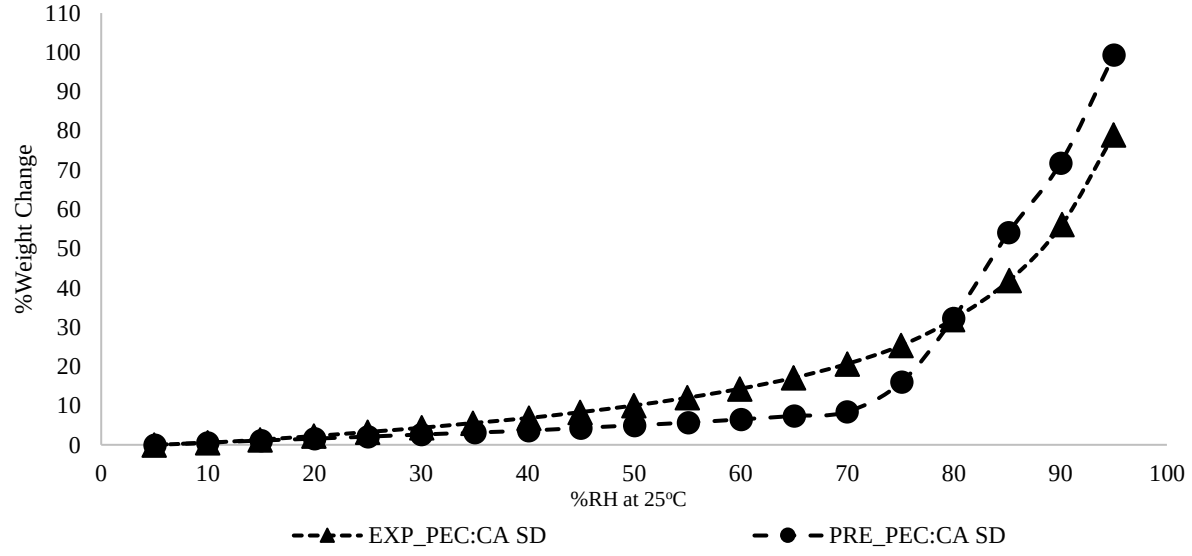
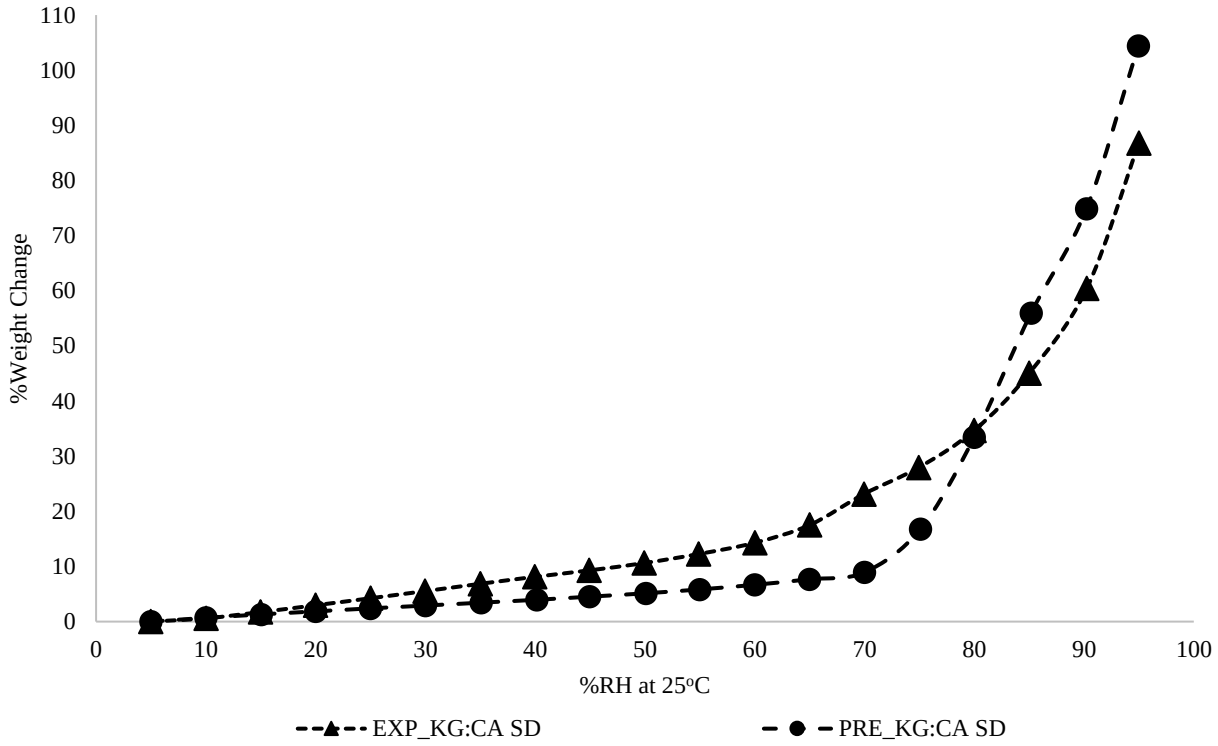


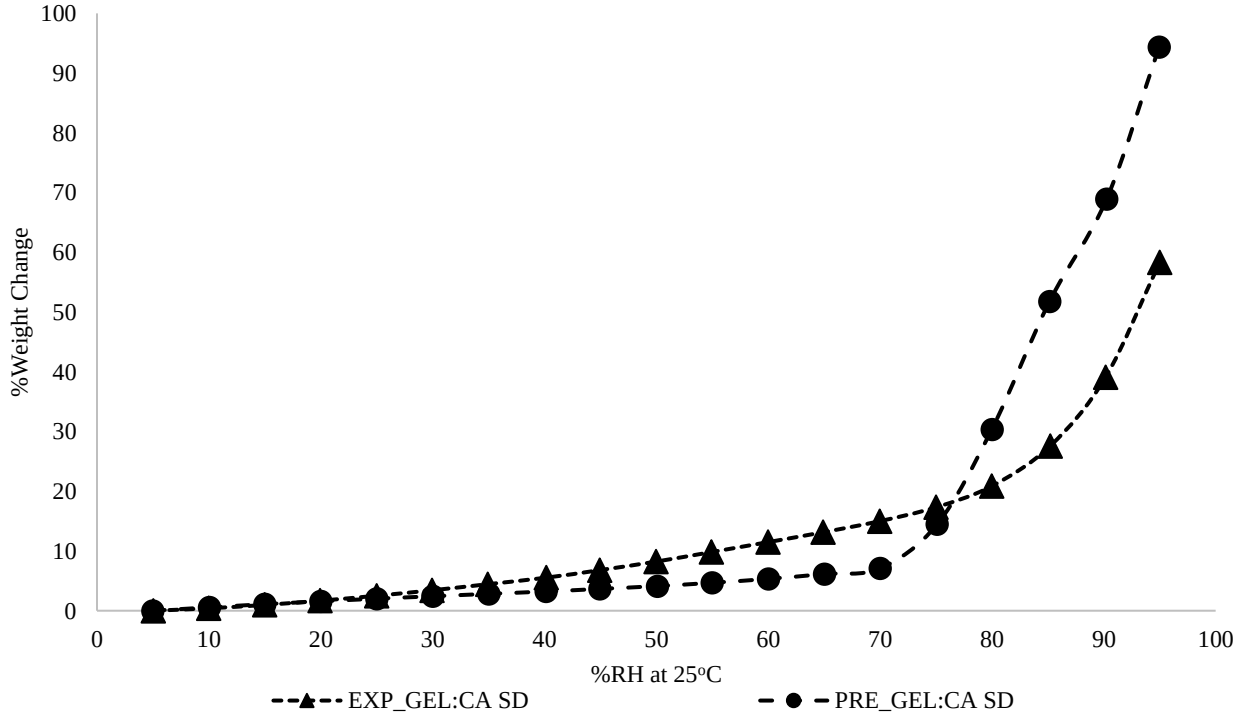
Supplementary Figure 5.5 **A)** Moisture sorption profiles of samples CA and FD CA at 25ºC. Experimental (EXP) moisture sorption profile of 1:1 CA: polymer solid dispersions (SD) vs. predicted (PRE) moisture sorption profile, **B)** CA: PEC solid dispersions (SD) **C)** CA: KG solid dispersions (SD), **D)** CA: GEL solid dispersions (SD), **E)** CA: GG solid dispersions (SD), **F)** CA: HPMC solid dispersions (SD), **G)** CA: CMC-Na solid dispersions (SD).

Supplementary Figure 5.5 continued

C)

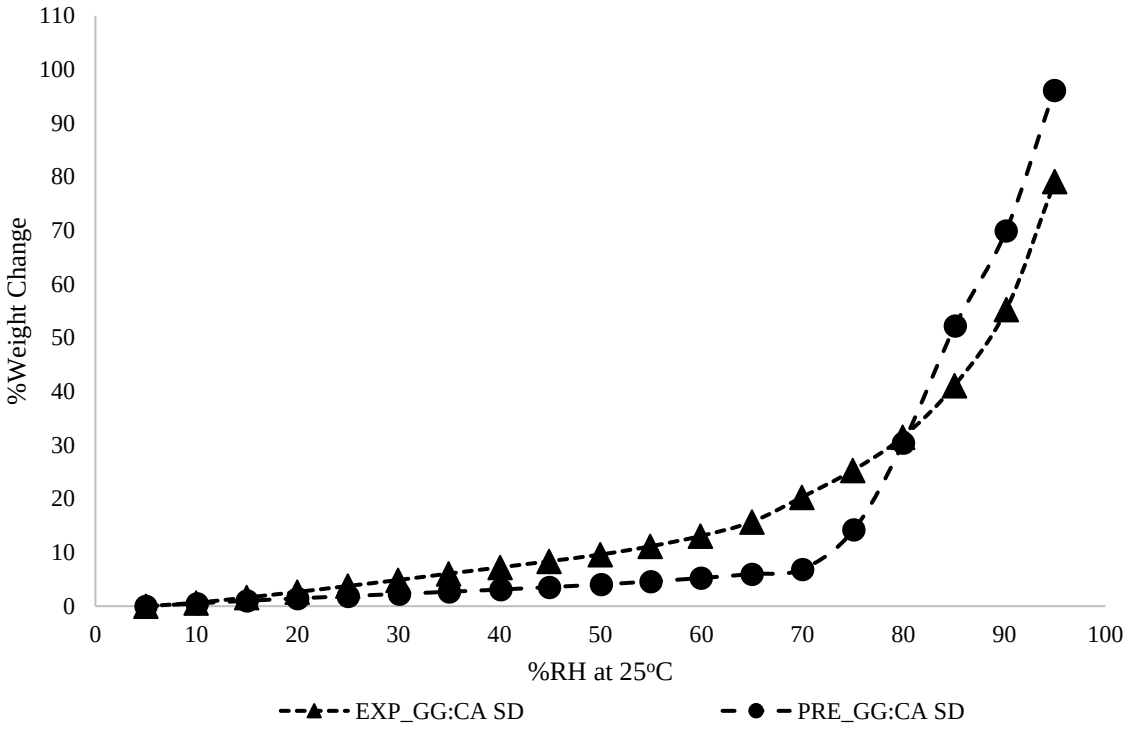


D)

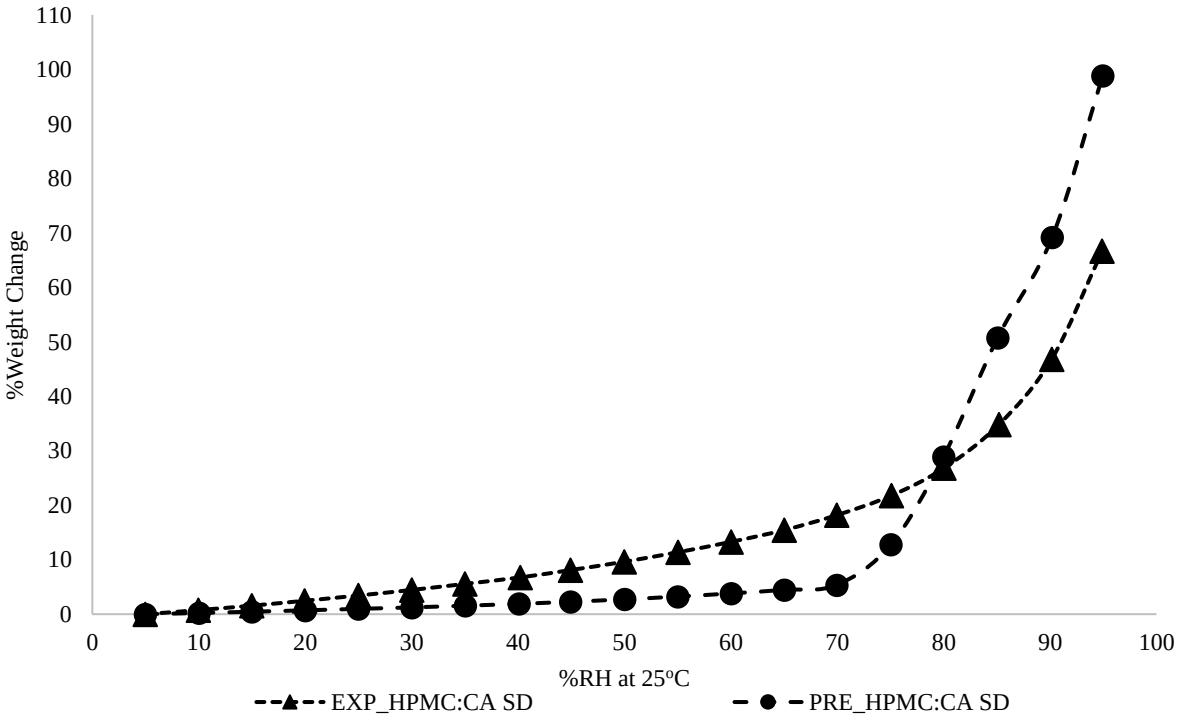


Supplementary Figure 5.5 continued

E)

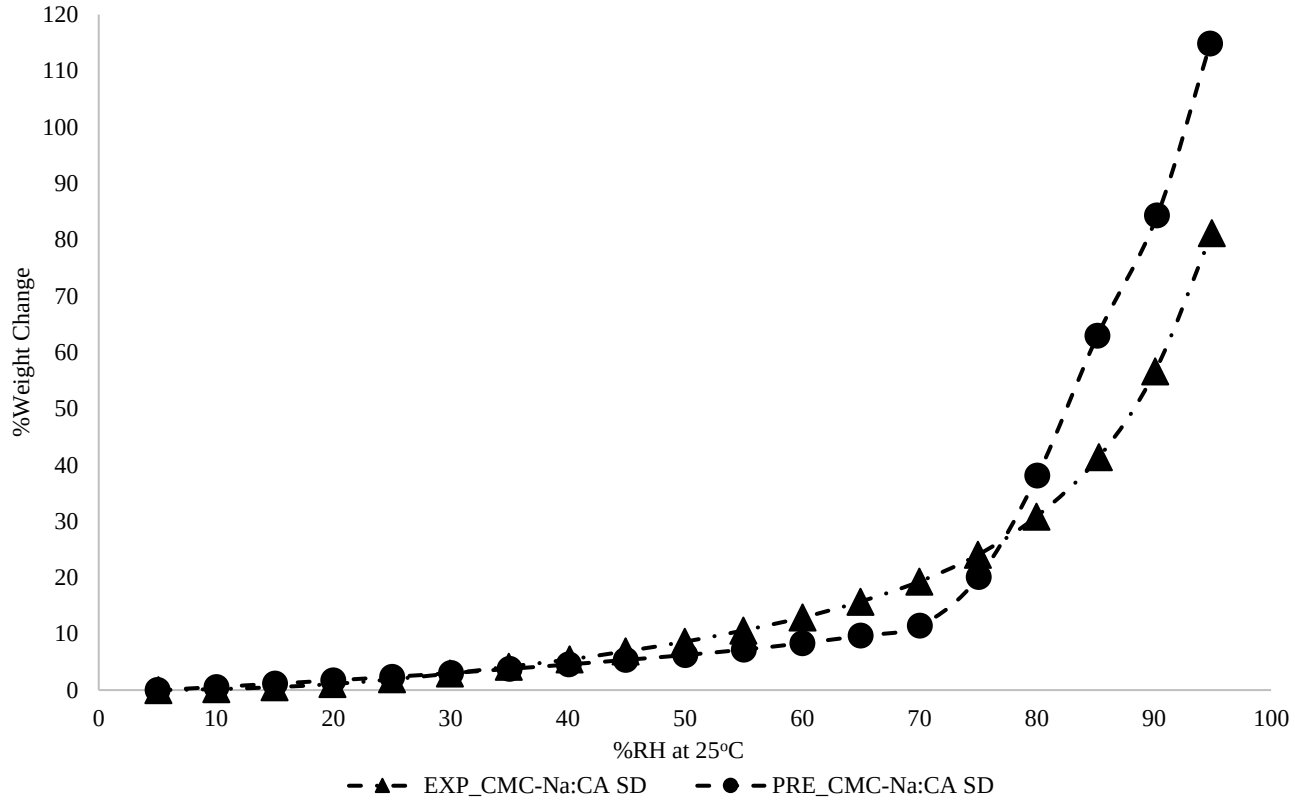


F)



Supplementary Figure 5.5 continued

G)



5.6 References

Allan, M., & Mauer, L. J. (2017). RH-temperature phase diagrams of hydrate forming deliquescent crystalline ingredients. *Food Chemistry, 236*, 21-31. doi:10.1016/j.foodchem.2017.02.110

Ambike, A. A., Mahadik, K. R., & Paradkar, A. (2005). Spray-dried amorphous solid dispersions of simvastatin, a low T-g drug: In vitro and in vivo evaluations. *Pharmaceutical Research, 22*(6), 990-998. doi:10.1007/s11095-005-4594-z

Apelblat, A. (2014). *Citric acid*. Switzerland: Springer International Publishing.

Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International, 99*, 363-374. doi:https://doi.org/10.1016/j.foodres.2017.05.021

Beaman, G. R. (1952). Relation between (apparent) second-order transition temperature and melting point. *Journal of Polymer Science, 9*(5), 470-472.

Bichara, L. C., Lanús, H. E., Ferrer, E. G., Gramajo, M. B., & Brandán, S. A. (2011). Vibrational Study and Force Field of the Citric Acid Dimer Based on the SQM Methodology. *Advances in Physical Chemistry, 2011*, 1-10.

Bochek, A. M., Shevchuk, I. L., Gavrilova, II, Nesterova, N. A., Panarin, E. F., Yudin, V. E., . . . Vlasova, E. N. (2012). Compatibility of Carboxymethyl Cellulose Ionized to Various Degrees with Poly-N-vinylformamide in Composite Films. *Russian Journal of Applied Chemistry, 85*(9), 1413-1421. doi:10.1134/s1070427212090182

Chiou, W. L., & Riegelman, S. (1969). Preparation and dissolution characteristics of several fast-release solid dispersions of griseofulvin. *Journal of Pharmaceutical Sciences, 58*(12), 1505-+. doi:10.1002/jps.2600581218

Chiou, W. L., & Riegelman, S. (1971). Pharmaceutical applications of solid dispersion systems. *Journal of Pharmaceutical Sciences, 60*(9), 1281-+. doi:10.1002/jps.2600600902

Christina, B., Taylor, L. S., & Mauer, L. J. (2015). Physical stability of L-ascorbic acid amorphous solid dispersions in different polymers: A study of polymer crystallization inhibitor properties. *Food Research International, 76*, 867-877. doi:10.1016/j.foodres.2015.08.009

Criquet, J., Nebout, P., & Leitner, N. K. V. (2010). Enhancement of carboxylic acid degradation with sulfate radical generated by persulfate activation. *Water Science and Technology, 61*(5), 1221-1226. doi:10.2166/wst.2010.008

Etter, M. C. (1990). Encoding and decoding hydrogen bond patterns of organic compounds. *Accounts of Chemical Research, 23*(4), 120-126. doi:10.1021/ar00172a005

Franks, F. (2007). Chapter 8 Primary Drying: The Sublimation of Ice. In *Freeze-drying of Pharmaceuticals and Biopharmaceuticals: Principles and Practice* (pp. 105-120): The Royal Society of Chemistry.

Glusker, J. P., Minkin, J. A., & Patterson, A. L. (1969). X-ray Crystal Analysis of the substrates of aconitase. IX.A refinement of the structure of anhydrous citric acid. *Acta Crystallographica Section B, 25*(6), 1066-1072.

Hancock, B. C., & Zografi, G. (1997). Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences, 86*(1), 1-12. doi:10.1021/js9601896

Hoppu, P., Hietala, S., Schantz, S., & Juppo, A. M. (2009). Rheology and molecular mobility of amorphous blends of citric acid and paracetamol. *European Journal of Pharmaceutics and Biopharmaceutics, 71*(1), 55-63. doi:10.1016/j.ejpb.2008.06.029

Hoppu, P., Jouppila, K., Rantanen, J., Schantz, S., & Juppo, A. M. (2007). Characterisation of blends of paracetamol and citric acid. *Journal of Pharmacy and Pharmacology, 59*(3), 373-381. doi:10.1211/jpp.59.3.0006

Hoppu, P., Jouppila, K., Rantanen, J., Schantz, S., & Juppo Anne, M. (2010). Characterisation of blends of paracetamol and citric acid. *Journal of Pharmacy and Pharmacology, 59*(3), 373-381. doi:10.1211/jpp.59.3.0006

Igoe, R. S. (2011). *Dictionary of food ingredients* (5 ed.). New York, NY: Springer.

Kadoya, S., Izutsu, K. I., Yonemochi, E., Terada, K., Yomota, C., & Kawanishi, T. (2008). Glass-state amorphous salt solids formed by freeze-drying of amines and hydroxy carboxylic acids: Effect of hydrogen-bonding and electrostatic interactions. *Chemical & Pharmaceutical Bulletin, 56*(6), 821-826. doi:10.1248/cpb.56.821

Kim, M. S., Jin, S. J., Kim, J. S., Park, H. J., Song, H. S., Neubert, R. H. H., & Hwang, S. J. (2008). Preparation, characterization and in vivo evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. *European Journal of Pharmaceutics and Biopharmaceutics, 69*(2), 454-465. doi:10.1016/j.ejpb.2008.01.007

Kumar, A., De, A., & Mozumdar, S. (2015). Synthesis of acrylate guar-gum for delivery of bio-active molecules. *Bulletin of Materials Science, 38*(4), 1025-1032. doi:10.1007/s12034-015-0930-z

Laurence, C., Brameld, K., A.,, Graton, J., Le Questel, J., Y.,, & Renault, E. (2009). The pK(BHX) Database: Toward a better understanding of hydrogen-bond basicity for medical chemists. *Journal of Medicinal Chemistry, 52*(14), 4073-4086.

Law, D., Schmitt, E. A., Marsh, K. C., Everitt, E. A., Wang, W. L., Fort, J. J., . . . Qiu, Y. H. (2004). Ritonavir-PEG 8000 amorphous solid dispersions: In vitro and in vivo evaluations. *Journal of Pharmaceutical Sciences, 93*(3), 563-570. doi:10.1002/jps.10566

Lu, Q., & Zografi, G. (1997). Properties of citric acid at the glass transition. *Journal of Pharmaceutical Sciences, 86*(12), 1374-1378. doi:10.1021/js970157y

Lu, Q., & Zografi, G. (1998). Phase behavior of binary and ternary amorphous mixtures containing indomethacin, citric acid, and PVP. *Pharmaceutical Research, 15*(8), 1202-1206. doi:10.1023/a:1011983606606

Mahmood, W. A. K., Khan, M. M. R., & Yee, T. C. (2014). Effects of reaction temperature on the synthesis and thermal properties of carrageenan ester. *Journal of Physical Science, 25*(1), 123-138.

Matsumoto, T., & Zografi, G. (1999). Physical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinylacetate) in relation to indomethacin crystallization. *Pharmaceutical Research, 16*(11), 1722-1728. doi:10.1023/a:1018906132279

Mauer, L. J., & Taylor, L. S. (2010). Water-solids interactions: deliquescence. *Food Science and Technology, 1*.

Nordman, C. E., Weldon, A. S., & Patterson, A. L. (1960). X-ray Crystal analysis of the substrates of aconitase . II.Anhydrous citric acid. *Acta Crystallographica, 13*(5), 418-426. doi:10.1107/s0365110x60000996

Ribeiro, A. P. B., Masuchi, M. H., Miyasaki, E. K., Domingues, M. A. F., Stroppa, V. L. Z., de Oliveira, G. M., & Kieckbusch, T. G. (2015). Crystallization modifiers in lipid systems. *Journal of Food Science and Technology-Mysore, 52*(7), 3925-3946. doi:10.1007/s13197-014-1587-0

Roy, L., Lipert, M. P., & Rodriguez-Hornedo, N. (2012). Chapter 11 Co-crystal Solubility and Thermodynamic Stability. In *Pharmaceutical Salts and Co-crystals* (pp. 247-279): The Royal Society of Chemistry.

Salameh, A. K., Mauer, L. J., & Taylor, L. S. (2006). Deliquescence lowering in food ingredient mixtures. *Journal of Food Science, 71*(1), E10-E16.

Sanchez, J., Ismail, Y., Christina, B., & Mauer, J. L. (2018). Degradation of L-Ascorbic Acid in the Amorphous Solid State. *Journal of Food Science, 83*(3), 670-681. doi:10.1111/1750-3841.13998

Socrates, G. (2001). *Infrared and Raman characteristic group frequencies* (3rd ed.). West Sussex, England: John Willey & Sons Ltd.

Summers, M. P. (1978). Glass formation in barbiturate and solid dispersion system of barbiturates with citric acid. *Journal of Pharmaceutical Sciences, 67*(11), 1606-1610. doi:10.1002/jps.2600671126

Summers, M. P., & Enever, R. P. (1976). Preparation and properties of solid dispersion system containing citric acid and primidone. *Journal of Pharmaceutical Sciences, 65*(11), 1613-1617. doi:10.1002/jps.2600651113

Taylor, L. S., & Zografi, G. (1997). Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharmaceutical Research, 14*(12), 1691-1698. doi:10.1023/a:1012167410376

Timko, R. J., & Lordi, N. G. (1979). Thermal characterization of citric acid solid dispersions with benzoic acid and phenobarbital. *Journal of Pharmaceutical Sciences, 68*(5), 601-605. doi:10.1002/jps.2600680523

Van den Mooter, G., Wuyts, M., Blaton, N., Busson, R., Grobet, P., Augustijns, P., & Kinget, R. (2001). Physical stabilization of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25. *European Journal of Pharmaceutical Sciences, 12*(3), 261-269.

Wang, J., Chang, R., Zhao, Y., Zhang, J., Zhang, T., Fu, Q., . . . Zeng, A. (2017). Coamorphous Loratadine-Citric Acid System with Enhanced Physical Stability and Bioavailability. *AAPS PharmSciTech, 18*(7), 2541-2550. doi:10.1208/s12249-017-0734-0

Wegiel, L. A., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2013). Crystallization of amorphous solid dispersions of resveratrol during preparation and storage-Impact of different polymers. *Journal of Pharmaceutical Sciences, 102*(1), 171-184. doi:10.1002/jps.23358

Wegiel, L. A., Zhao, Y. H., Mauer, L. J., Edgar, K. J., & Taylor, L. S. (2014). Curcumin amorphous solid dispersions: the influence of intra and intermolecular bonding on physical stability. *Pharmaceutical Development and Technology, 19*(8), 976-986. doi:10.3109/10837450.2013.846374

Yoshioka, M., Hancock, B. C., & Zografi, G. (1994). Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. *Journal of Pharmaceutical Sciences, 83*(12), 1700-1705.

OVERALL CONCLUSION

Understanding the behavior of individual food ingredients during processing and storage is essential when they are introduced to complex food matrices to ensure the quality of final food products and fulfill intended nutrient delivery. In this comprehensive dissertation, a mechanistic understanding of the factors dictating the physical and chemical stability of crystalline and amorphous solids alone, in binary mixtures as well as in solid dispersions (created by commonly used hydrocolloids) was gained. This was achieved by studying the different physical forms of thiamine and citric acid (crystalline vs. amorphous), as well as different salt forms of thiamine (TClHCl and TMN), which are commonly used for food fortification and enrichment purposes.

More specifically, the first part of this dissertation was an extension of a previous work in which physical stability of amorphous TClHCl was conducted in different solid dispersions prepared by various polymers (Arioglu-Tuncil et al., 2017). Therefore, this part was focused more on the chemical degradation of thiamine in crystalline TClHCl, physical blends of crystalline TClHCl with polymers, and in amorphous TClHCl solid dispersions. Thiamine degradation in TClHCl was not significant when it was in the crystalline form. Similarly, binary blends of crystalline TClHCl with polymers did not induce thiamine degradation in the solid state. However, significantly more thiamine degraded when it was in the amorphous form. Amorphous thiamine stability was also found to be influenced by polymer type with being more stable in pectin than PVP, which was mainly attributed to stronger intermolecular interaction formation between TClHCl and pectin, confirmed with FTIR. Moreover, proportion of TClHCl in the solid dispersions was discovered to have a great impact on thiamine stability: it degraded more when it was found in lower quantities. It was also found that importance of polymer hygroscopicity cannot be rules out when higher RH conditions were used for storage treatments, since solution formation

might be induced. On the other hand, T_g was not a determinant factor for stability, however it was important when samples were stored above the T_g.

In the second study, a detailed assessment of physical and chemical stability of thiamine in TMN was completed. In addition, the findings of this part were compared to those of obtained from the previous study (Chapter 2) to gain a further understanding for the impact of thiamine salt form on chemical loss of thiamine. Similar to previous findings obtained from Chapter 1, significantly more thiamine degraded in the amorphous form compared to crystalline form, depending on the polymer and storage conditions used. In addition, degradation of thiamine was highly affected by the proportion of thiamine relative to polymer mainly due to greater surface area of contact between TMN and polymer. On the other hand, there was no significant differences found between TMN and TClHCl for thiamine degradation in the solid state. However, when the solution formation was induced at high RH storage conditions due to high hygroscopicity of polymer, significantly more thiamine degraded in TMN dispersions than TClHCl dispersions and it was attributed to differences in pH with TMN creating a higher pH than TClHCl where thiamine is reported to be less stable.

To understand the impact of pH of pre-lyophilization solutions on thiamine, a preliminary study was conducted and chemical degradation of thiamine in TClHCl solid dispersions was investigated in three different pH conditions (4, 5, and 6) adjusted with a citrate buffer. Thiamine was more stable when it was stored at lower RH and temperature conditions (11% RH and 30^{o}C), regardless of the pH. However, degradation of thiamine was induced as pH increased, suggesting that thiamine stability was pH dependent.

Lastly, physical stability of citric acid in the amorphous form was evaluated in different storage conditions by accounting for the impact of chemistry and features of the select polymers.

Citric acid was successfully amorphized in the presence of polymers. Crystallization inhibitor properties of polymers were found to be highly dependent on their abilities to interact with citric acid via hydrogen bonding and/or ionic interactions and intermolecular interactions between citric acid and polymers were the most important factor inhibiting the recrystallization of citric acid. Polymer hygroscopicity was important especially at higher RH storage conditions. In contrast, there was no correlation found between the amorphous stability and T_g.

The studies comprised of this dissertation were conducted using an interdisciplinary approach and the findings obtained will help food scientists, nutritionists, and pharmacists to better understand the factors that need to be considered and avoided during each step of productions (decision making, formulations, recommendations, developing shelf-life models etc.). To do so, quality defects in final products could be prevented and required delivery of thiamine to target populations could be achieved.