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Ascorbic Acid Degradation in PVP Solid Dispersions and the Effects of Water and Pre-lyophilization Solution pH On its Chemical Stability

Juan Oscar Sanchez
Purdue University

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By Juan Oscar Sanchez

Entitled
AMORPHOUS ASCORBIC ACID DEGRADATION IN PVP SOLID DISPERSIONS AND THE EFFECTS OF WATER AND
PRE-LYOPHILIZATION SOLUTION PH ON ITS CHEMICAL STABILITY

For the degree of Master of Science

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Head of the Departmental Graduate Program Date
AMORPHOUS ASCORBIC ACID DEGRADATION IN PVP SOLID DISPERSIONS
AND THE EFFECTS OF WATER AND PRE-LYOPHILIZATION SOLUTION PH ON
ITS CHEMICAL STABILITY

A Thesis
Submitted to the Faculty
of
Purdue University
by
Juan O. Sanchez

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

December 2016
Purdue University
West Lafayette, Indiana
For days of good faith.
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Dr. Suzanne Nielsen’s support of the Industry Fellows program led me to apply to Purdue. For that reason I owe her a very special thanks. When I arrived to Purdue, many faculty and staff made me feel at home, and none more than Dr. Mauer. Her support made my graduate school experience an enjoyable one. Then in lab I met Matt Allan; without Matthew and Mikaela both, my two years in Indiana would have felt truly impoverished. And without E. A., well, I just don’t know.
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ABSTRACT

Sanchez, Juan O. M.S., Purdue University, December 2016. Amorphous Ascorbic Acid Degradation in PVP Solid Dispersions and the Effects of Water and Pre-Lyophilization Solution pH on its Chemical Stability. Major Professor: Lisa Mauer.

Ascorbic acid (vitamin C) is an essential micronutrient found both naturally in foods as well as supplemented in fortified foods and dietary supplements. In foods, ascorbic acid typically exists in the aqueous or crystalline solid state, but the lyophilization of ascorbic acid polymer solutions has been proven to render ascorbic acid into the amorphous solid state. This study investigated the importance of two matrix effects – the weight ratio of ascorbic acid to PVP and the pre-lyophilization solution pH – on the chemical degradation of ascorbic acid in the amorphous solid state. Amorphous ascorbic acid was generated via lyophilization with PVP and stored at several controlled temperature conditions and 11% relative humidity. Low pH lyophiles (pH 3.0 and pH 3.5) displayed strongly non-additive moisture sorption behavior while higher pH lyophiles (pH 4.0 and pH 4.5) displayed additive moisture sorption. Changes in the pre-lyophilization solution pH caused up to four-fold increases in the extent of ascorbic acid degradation in lyophiles in spite of similar glass transition temperatures and equal relative humidity storage conditions. Modification of the weight ratio of ascorbic acid to PVP caused over six-fold increases in loss of ascorbic acid during storage. Recrystallized ascorbic acid dispersions showed no significant differences in loss during storage regardless of weight ratio,
supporting the importance of the physical state of small molecules on their chemical stability. Given the large differences in chemical stability caused by ascorbic acid to PVP weight ratio and pre-lyophilization solution pH, these two matrix effects should be considered important factors in the chemical stability of small molecules in amorphous solid dispersions.
CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

The interaction of water with food is a complex subject in food science in part because it can be approached using different theoretical principles. At the most basic level, the amount of water present in a food can be measured via gravimetric and drying techniques to determine its moisture content and is routinely measured in the food industry (Bradley, 2010). A limitation of moisture content as an analytical parameter is that it does not coincide with general food quality characteristics across a wide range of foods (David Reid & Fennema, 2008). One conceptual approach towards water in foods beyond moisture content applies the concept of water activity to food systems. Water activity, unlike moisture content, attempts to measure the availability or the physicochemical state of water present in foods. Section 1.2 discusses the thermodynamic basis of water activity and the theoretical approach it applies to foods. Concepts from the field of polymer science have also been applied to foods in order to understand the interaction of water with food. Unlike the thermodynamic approach of water activity, concepts from polymer science often rely on kinetic parameters. Section 1.4 discusses amorphous solids and the parameter of glass transition temperature often used to characterize amorphous solids. Section 1.5 reviews the degradation mechanism of
ascorbic acid and past research on ascorbic acid degradation in model systems and whole foods.

1.2 The Thermodynamics of Water in Foods and Moisture Sorption Isotherms

Chemical activity as a definition and concept was developed by Gilbert N. Lewis in 1907 in order to model non-ideal aqueous solutions using formal thermodynamic theory (Lewis, 1907). Chemical activity and other ideas of G. N. Lewis gained popularity with the 1923 publication of his textbook *Thermodynamics and the Free Energy of Chemical Substances* together with Merle Randall (Hildebrand, 1958; Lewis & Randall, 1923). In 1953, the microbiologist William James Scott published a study that proved the growth of *Staphylococcus aureus* to occur only in growth media and foods within a defined range of water activities (Scott, 1953). Follow-up studies led to his publication of a review, “Water Relations of Food Spoilage Microorganisms,” relating water activity to spoilage microorganisms in foods (Scott, 1956). In the review, Scott likened foods to aqueous solutions in order to predict their spoilage properties (Scott, 1956) and in doing so introduced the concept of water activity into the field of food science (Chirife & Fontana, 2008; Labuza, 1968). The principles of water activity and their application to food systems are presented in detail below.

When a solute is added to a pure solvent to make a solution, the solute alters four physical properties of the solvent known as its colligative properties. The added solute lowers the vapor pressure of the solvent, depresses its freezing point, elevates its boiling point, and increases its osmotic pressure (Spencer, Bodner, & Rickard, 2008). In theory – that is, for ideal solutions – the molar amount of solute is the *only* variable that changes
the solvent properties. The kind of solute, whether it be an ionic solid like sodium chloride or a polymer like pectin, is ignored in ideal solutions. As expected, the quantitative predictions of colligative properties work best for dilute solutions of small molecules – for example, a 0.1M aqueous solution of sucrose (Labuza & Altunakar, 2008; Winn, 1995).

Mathematically, these concepts are explained for the case of aqueous solutions as follows. If $P^o$ is the vapor pressure of pure water, and $\chi_{water}$ is the mole fraction of water in the solution, then the vapor pressure $P$ of the solution is (Spencer et al., 2008):

$$P = P^o \chi_{water} \quad (1)$$

This expression is known as Raoult’s law for ideal solutions. For dilute solutions, $\chi_{water}$ is very near unity, and so the vapor pressure $P$ of the solution is only slightly less than pure water. Since the mole fraction is always less than one for a solution, the vapor pressure of an aqueous solution will always be lower than that of pure water (Spencer et al., 2008).

Raoult’s law can be divided by $P^o$ to isolate the mole fraction of water:

$$\chi_{water} = \frac{P}{P^o} \quad (2)$$

As $\chi_{water}$ decreases and the solution is more concentrated, its vapor pressure drops further below that of pure water. However, when this happens in real concentrated solutions, Raoult’s law rarely holds true (Winn, 1995). Solutions that break Raoult’s law are known as non-ideal, or real solutions. In addition to high solute concentration, the nature of a solute, or its composition, also violates Raoult’s law and causes non-ideal solution behavior.
Despite a solution’s non-ideal behavior, physical chemists can still model real solutions by modifying Raoult’s law. Since the equation above does not hold true for concentrated solutions, an activity coefficient $\gamma$ can “fix” the equation above when solutions in the laboratory behave non-ideally. The corrected Raoult’s law for real solutions is written as (Winn, 1995):

$$
\gamma \chi_{water} = \frac{P}{P^o} 
$$

(3)

Where the activity coefficient $\gamma$ takes on whatever value is necessary to validate the equation (given experimental knowledge of $\chi_{water}$, $P$, and $P^o$). For ideal solutions, the activity coefficient is unity and the equation reduces to Raoult’s law. As solutions behave non-ideally, $\gamma$ deviates from unity. In other words, the activity coefficient quantifies the non-ideality of the solution. The product $\gamma \chi_{water}$ is defined as the water activity (Labuza & Altunakar, 2008):

$$
a_W = \gamma \chi_{water} 
$$

(4)

And so the corrected Raoult’s law for water can be written using the vapor pressure of water to define water activity (D. Reid, 2008):

$$
a_W = \frac{P}{P^o} 
$$

(5)

Water activity is the parameter measured via vapor pressure in the laboratory.

Early methods to determine the water activities of foods relied on the use of multiple sealed chambers equilibrated with saturated salt solutions that maintained the relative humidity at precise values. Gravimetric measurement of the food over time was used to identify the relative humidity at which the food gained or lost the lowest amount of moisture, which corresponded to a value near its water activity (Labuza, 1968). This
practice took several days to complete, during which mold growth was a common experimental problem for foods with high water activities (X. Yu, Martin, & Schmidt, 2008).

Modern experimental techniques are able to rapidly calculate the vapor pressure in the headspace of a small instrumental chamber using electronic hygrometers such as chilled mirror dew point sensors, electrolytic resistors, and tunable diode lasers (Decagon Devices, 2016; Novasina, 2012). For example, the AquaLab TDL manufactured by Decagon Devices uses a laser at infrared frequencies to quantify the loss in signal strength that results when water vapor is present in the experimental chamber. Loss of signal can then be mathematically related to the vapor pressure of water, which produces a water activity measurement (Decagon Devices, 2016).

If water activity does not equate with mole fraction in real solutions (i.e. all foods) because of the activity coefficient, then what does water activity really measure? The interpretation of water activity requires a discussion of energetics, chemical equilibrium, and the physical structure of water – that is, of what the water molecules are doing in pure water instead of in solutions. In his 1956 paper, J. W. Scott explains the colligative properties of solutions by discussing the energetics resulting from water’s physical structure (Scott, 1956).

When solutes dissolve in water, the water molecules, on average, order themselves better around solute molecules than they do around other water molecules. The new solute ordering increases the average intermolecular attraction between water molecules by reducing water-water repulsion. Reduced repulsion, consequently, leads fewer water molecules to escape into the gas phase. Since fewer molecules equilibrate
into the gas phase, the vapor pressure of solutions drops below that of pure water (Spencer et al., 2008).

From a thermodynamic perspective, solutes lower the vapor pressure and therefore the free energy of solutions relative to that of pure water. Liquid/vapor equilibrium in a solution implies that the free energy of water molecules in the gas phase is equal to that in the liquid phase. Therefore the free energy, or chemical potential, of water in the liquid phase is knowable given the vapor pressure of a solution. The expression for the chemical potential of solutions is hard to derive (Winn, 1995); an analogy works best in this case.

For an ideal gas reaction equilibrium, its free energy is related to its equilibrium constant through the expression (Spencer et al., 2008):

\[
\Delta G^\circ = -RT \ln K
\]

This is the well-known expression for the equilibrium constant. The equilibrium constant \( K \) is a ratio of the reaction products over reactants. In the same way, the molar free energy of a solution could be written as (Winn, 1995):

\[
\mu = RT \ln \chi
\]

where the chemical potential and mole fraction, \( \mu \) and \( \chi \), in a way replace the Gibbs free energy and equilibrium constant, \( \Delta G^\circ \) and \( K \). Instead of a ratio of products and reactants, the mole fraction is a ratio of molar components or, by Raoult’s law, a ratio of vapor pressures. That is to say, if \( \chi \) is replaced by \( P/ P^o \), then

\[
\mu_w = RT \ln \frac{P}{P^o}
\]
In this way, the vapor pressure lowering effect of solutes is related to the thermodynamic concept of chemical potential (Decagon Devices, 2006; Labuza & Altunakar, 2008). Written as an activity,

\[ \mu_w = RT \ln a_w \]  

what this means is that the chemical potential of water in a solution is directly related to the water activity of that solution. As the water activity or chemical potential of water decreases in foods, the increased thermodynamic stability of water results in its inability to promote bacterial growth, stabilize protein structure and enzyme function, and participate in hydrolytic degradation reactions – all of which are phenomena associated with the spoilage of food (Labuza, Tannenba.Sr, & Karel, 1970).

The theory of solutions sounds applicable to foods such as wine, Gatorade, or even milk. But to assume foods like cheese, cookies, and candy behave like solutions is less intuitive. Nevertheless, the water activity of all foods can be measured, and even very dry foods (like beef jerky or potato chips, for instance) have water activities above zero. What such water activities mean has been debated, as will be discussed in Section 1.3.

In spite of academic debate, the application of the water activity concept to foods has been extremely well received and is certainly a great intellectual achievement. Together with pH, the water activity of a food defines its regulatory standing in the Code of Federal Regulations because of its central importance to food safety ("Food Code," 2013).

Two types of diagrams extended the use of water activity beyond a simple single-unit measure of microbiological safety. The first was the food stability map developed by
T. P. Labuza. Just as J. W. Scott investigated minimum water activities for the growth of bacteria in molds in foods (Decagon Devices, 2006; Scott, 1956), Labuza expanded the scope of water activity to chemical reactions. Other workers investigated the relationship between water activity and biochemical reactions, such as enzyme activity (Schwimmer, 1980), and physical conditions, such as crispiness (Heiss, 1958; Katz, 1981). Labuza gathered these disparate phenomena into a single diagram to create a food stability map (Labuza, Hawkes, Gallagher, Hurtado, & McNally, 1972). This map popularized the idea of water activity as the experimental parameter to measure in foods in order to predict a wide range of food spoilage phenomena. Particularly powerful was the idea of threshold water activities, also defined as critical water activities or $a_c$, below which microbial growth, powder caking, or loss of crispness are not observed in foods (Labuza, 1980).

The second addition to the water activity concept was the development of moisture sorption isotherms (MSIs). MSIs are plots of the amount of water present in a food across a range of relative humidities or water activities (Labuza, 1968). Moisture sorption profiles were first empirically calculated for foods by placing food samples inside vacuum desiccators containing saturated salt solutions at several water activity conditions and then recording the amount of moisture present in the food after a steady state in weight change had been reached. Plots of MSIs for different foods vary in shape and have been classified into three types common to food applications, type I (Langmuir), type II (sigmoidal), and type III (J-type or exponential) (Labuza & Altunakar, 2008).

The development of the theoretical description of isotherms was first conducted in the field of physical chemistry (Labuza, 1968). The most relevant theoretical approach to foods was developed by Stephen Brunauer, P. H. Emmet, and Edward Teller in 1938.
(Brunauer, Emmett, & Teller, 1938). Their work studied the adsorption of multi-molecular levels inert gases (mainly nitrogen) onto carbon, metal, and metal catalyst surfaces. When applied to foods, a valuable result from the BET equation was the monolayer value of water, or the grams of water present in the food per gram of solid. Because the BET model was not developed for foods, the BET monolayer value of water was not considered to provide a measurement of the actual surface area of solid food (Labuza, 1968). Studies with nitrogen gas adsorption onto food showed that adsorption values for nitrogen were orders of magnitude less than those for water (Fox, 1963). Water was considered to occupy the polar sites of proteins and polymer chains present in foods, which was not accounted for in the BET model. Nevertheless, the BET model yielded acceptable fits to the MSIs of foods for water activity values between 0.1 and 0.5 (Labuza, 1968).

Another limitation of the BET model to foods was the practice of hysteresis seen in the desorption of water from foods (David Reid & Fennema, 2008). In other words, MSIs generated by water sorption differed from those generated by water desorption. In general, the presence of hysteresis in physical systems implies that systems do not exist in equilibrium or that processes are not thermodynamically reversible (Winn, 1995). In practice, equilibrium weight change is hard to reach. Even simple model systems of maltodextrins stored in saturated salt solution desiccators below their glass transition temperatures do not reach equilibrium after 21 days of storage at 25°C (Dupas-Langlet et al., 2016). In 1968, Labuza reported that the reasons for hysteresis in foods remained unclear because the proposed explanations for hysteresis in foods at the time (which relied on the effects of capillary condensation) did not account for all of the observed
deviations from MSI models. He suggested the possible effects of food composition as a reason for hysteresis, writing that “the effect of composition of a food on the shape of the isotherm has not been studied very much” (Labuza, 1968). In the 1980’s, two scientists, Harry Levine and Louise Slade, published a large amount of work that shifted the research of water-food interactions in the direction of food composition by applying theories and concepts from the field of polymer science, discussed in detail in Section 1.3.

1.3 Levine and Slade and the Food Polymer Science Approach

Some limitations of the water activity and moisture sorption isotherm concepts have already been discussed above. Water activity measurements and moisture sorption isotherms generated very useful information, but they made assumptions about equilibrium criteria and food composition that Levine and Slade considered too unrealistic for food applications. Levine and Slade were highly critical of what they considered the care-free use of water activities and MSIs in food science and technology research and practice (Levine, 1988).

One assumption in particular bothered Levine and Slade more than any other. It was the idea of treating experimentally measured water activities of foods as equilibrium thermodynamic activities. This practice assumed foods to be solutions at equilibrium. Foods were no such thing. Proteins and carbohydrates, two of the food macromolecules, were mostly polymeric molecules. Those that weren’t, such as simple sugars, displayed many of the same experimental behaviors of polymers anyways (Slade & Levine, 1991). Levine and Slade argued that foods deserved a new theoretical treatment based on the principles of the kinetic theories of polymers Much of their theoretical background on
the thermodynamics of water in non-ideal systems was drawn from the work of Felix Franks, a cryobiologist that published extensively on the properties of frozen water in biological systems, lyophilization of proteins, and the effects of polyhydroxyl compounds on the physical properties of water (F. Franks, 1991; F. Franks & Ives, 1966; F. A. Franks, T., 2007). For their understanding of polymer science, they drew from the work of P. J. Flory and J. D. Ferry (Ferry, 1980; Flory, 1953).

Levine and Slade published too many papers and generated too much debate to cover all of their work in this section. A better approach than a summary of that work is to present their overall contribution to the field of water-food interactions. Given the perspective of early water activity research and about twenty years after their own major publications, the summary is this: Levine and Slade pushed the field of water-food interactions into the realm of materials science. They switched the focus from a study of thermodynamic bulk system properties to a study of transient kinetic properties (Aguilera & Lillford, 2008). One way of looking at it is that Levine and Slade asked what food actually looks like or really is. From a more scientific perspective, they pushed the research concepts and theories away from thermodynamics and towards kinetics.

Thermodynamics measures bulk macroscopic properties of systems like pressure, volume, and temperature. It demands very little knowledge of what is “actually happening” in systems at the microscopic level. Thus simple technology (water activity meter, gravimetric analysis) can provide very useful predictions. Kinetics, on the other hand, ultimately deals with the microscopic properties of molecular motion in time and space. It demands knowledge of what is “actually happening,” like diffusion, relaxation, and molecular mobility at the microscopic level. Thus only complex technology (NMR,
neutron scattering) can measure these parameters to provide useful predictions. Considering the complexity of real food, its complete kinetic understanding is incredibly ambitious. Nevertheless, that was the goal that Levine and Slade set (Levine, 2002).

1.4 Crystalline and Amorphous Solid States

The previous section stated that Levine and Slade shifted the research direction of water-food interactions towards a materials-focused study of food. They were certainly not alone. Advances in polymer science, microscopy, separations science, and other fields allowed food scientists to study the physical nature and structure of food in far greater detail than had been previously possible (Angell, 1991; Flory, 1953; Gordon, 1952). The preface to the recent Food Materials Science states: “It has been in the last 20 years that the study of foods as materials has become a field in its own” (Aguilera & Lillford, 2008). One advantage of a material-science approach to food systems is the ability to categorize the physical state of individual components in complex systems. Distinction of physical states also allows for better characterization of the mechanisms of water-solid interactions (Mauer & Taylor, 2010).

Foods can be classified into two distinct kinds of solids: crystalline or amorphous. Crystalline solids are regularly repeating arrangements of atoms or molecules. The microscopic arrangement of crystals is obvious at the macroscopic level – crystals often have obvious geometric shapes and neat edges. Crystalline solids are also thermodynamically well-defined. The crystalline state is an equilibrium state; thus crystalline solids have sharp melting points, precise vapor pressures, and theoretically
infinite lifetimes (Moynihan & Crean, 2009). Organic acids, sugar, salt, and ice are food ingredients often present in the crystalline state in foods.

In contrast, freeze-dried beverage powders, food maltodextrins, and food ingredients composed of complex polymers such as proteins and polysaccharides are often purely amorphous ingredients or contain amorphous phases. Amorphous solids are randomly oriented arrangements of atoms or molecules with regions of molecular order that do not exceed 1 nm (Meille et al., 2011). Amorphous solids are not thermodynamically well-defined (Moynihan & Crean, 2009). The amorphous state is a kinetic state; thus amorphous solids have broad changes in texture and flow instead of sharp melting points between solid and liquid phases. Unlike crystalline solids, amorphous solid structures have theoretically finite lifetimes. Thermodynamically speaking, amorphous solids should eventually reach the crystalline state (Hancock & Zografi, 1997). In practice, amorphous glasses may not recrystallize in observable time periods. Taylor and Van Eerdenbrugh developed a three-tier classification system for the glass-forming ability and glass stability of small molecules based on a large sample set of 51 organic pharmaceutical compounds to distinguish between melt-quenched compounds that recrystallized before $T_g$, recrystallized after reheating past $T_g$, or failed to recrystallize before $T_m$ (Baird, Van Eerdenbrugh, & Taylor, 2010). For small molecules, glass stability (or resistance to crystallization) increased with both molecular weight and molecular complexity.

One common way to think about amorphous solids is to consider them as “frozen” liquids, or liquids with extremely limited molecular mobility. There are several ways to create amorphous solids or to reach the so-called amorphous state. The simplest means is
to cool a liquid very quickly to a temperature below its thermodynamic freezing point, a process known as melt-quenching (L. Yu, 2001). Very fast cooling draws energy from the liquid so quickly that the molecules in the liquid have no time to order themselves to form crystals. The resultant solid is thermodynamically unstable and is often called a supercooled liquid because it exists as a liquid below its freezing point. However, since the molecules in the liquid lack the necessary energy to quickly recrystallize, supercooled liquids are considered meta-stable states (Debenedetti & Stillinger, 2001). Their existence as supercooled liquids depends on kinetic processes, or on the motion of molecules in the liquid. With time, crystal nuclei eventually form, and supercooled liquids revert to the energetically favored crystalline state. It is because of that time-dependent process that amorphous solids are often described with kinetic models such as Williams-Landel-Ferry kinetics and the Kohlraush-Williams-Watts enthalpy relaxation equation (Shamblin, Hancock, & Pikal, 2006; Williams, Landel, & Ferry, 1955).

To further complicate this state, amorphous solids have their own analogue of a melting temperature called the glass transition temperature. Above the glass transition temperature, amorphous solids are described as supercooled liquids because they have some limited movement similar to those materials. Their ability to move is best described by their viscosities, which may be high (between $10^{-3}$ to $10^{12}$ Pa·s) but still allow for some movement and flow (Hancock & Zografi, 1997). Below the glass transition temperature, amorphous solids are described as glassy because their limited movement ceases and they become hard and brittle like a glass. Glassy amorphous solids have higher viscosities of over $10^{12}$ or $10^{13}$ Pa·s (Debenedetti & Stillinger, 2001; Hancock & Zografi, 1997). The glass transition temperature is the temperature at which the
amorphous material experiences a sudden significant increase in its viscosity of at least three orders of magnitude, transforming it from the rubbery state into the glassy state (Hancock & Zografi, 1997).

Surprisingly, debate continues over the exact nature of the glass transition temperature and the amorphous glassy state. The glassy state remains one of the great unsolved problems of condensed matter physics (Angell, 1995). Although the general public may not be as familiar with the amorphous solid state as with the crystalline state, the scientific community is well aware of the problem (Ngai, 2011). A 2008 article in the *New York Times* titled “The Nature of Glass Remains Anything but Clear” tried to popularize the ideas and importance of amorphous solids and the glassy state (Chang, 2008).

For food materials science, the importance of the amorphous solid state lies in its distinction from the crystalline state and how those differences affect the physical properties of food, in particular its water sorption properties, that leads to physical changes such as clumping, caking, melting, stickiness, and so on. Given this background on common physical states of matter in foods, the research of Levine and Slade can be placed in better context. Levine and Slade’s attack on water activity was, in a sense, an attack of the view of foods as equilibrium systems subject to equilibrium conditions such as vapor pressures. Their different view of foods as polymer systems meant that they focused on the non-equilibrium or kinetic behavior of foods, and used their glass transition temperatures as ways of characterizing them.

Both before and after the papers of Levine and Slade, the importance of water in the degradation of ascorbic acid was well accepted. However, instead of basing
conclusions about food stability on water activity as had been done in the past (without much thought into food structure), Levine and Slade preferred a description of water plasticization of food polymer systems to explain food stability. Though a bit of an oversimplification, a debate developed as to which experimental parameter – the water activity, \( a_w \), or the glass transition temperature, \( T_g \) – was the most useful indicator of food stability \((The\ Water\ in\ Foods\ Panel,\ 2001)\).

Most likely, food stability is too complex to describe with a single experimental parameter. This is partly because there are different kinds of stability in foods – chemical stability of small molecules, biochemical stability of enzymes, physical stability of polymer structures, microbiological stability of bacteria – each of which can depend on different system variables. Most workers in the field of food stability understand this, but the promise of a single parameter of food stability remains a tempting possibility \((Schmidt,\ 2004)\).

The related field of pharmaceutical science has studied edible polymer systems for some time and has mostly avoided oversimplification of drug system stability and moisture sorption. Part of the reason is because pharmaceutical systems are nowhere near as complex as whole food systems, thus require less reductionism and simplification to model and to understand. Many drug delivery systems may not exceed three or four ingredients, and thus the effects of each ingredient can be studied in far greater detail. For example, a study on the effectiveness of the BET model to describe the moisture sorption of PVP led to the rejection of the BET model in favor of the Flory-Huggins model for moisture sorption \((Hancock\ &\ Zografi,\ 1993)\) that contains terms unique to each ingredient in the amorphous solid. Such an approach is possible for a single-compound
system such as amorphous PVP, but impossible for a food system. A well-cited paper from the pharmaceutical literature concluded that while, for single compounds, $T_g$ is a reliable indicator of stability, it is not reliably so for multi-compound systems or for solid dispersions (systems of small molecules and polymers) (Shamblin et al., 2006). In solid dispersions, chemical stability is still believed to be dependent on the molecular mobility of the molecules of interest. If molecular motion is highly limited, degradation reactions will not occur. However, while this is probably true, solid dispersions are more nuanced because molecular mobility varies for each compound in a system (Labuza, 2010). Compounds with low molecular weights on average move more than those with higher ones. The result is that water remains highly mobile even in systems well below their glass transition temperatures (Yoshioka & Aso, 2007). Since chemical degradations that occur via hydrolysis (like ascorbic acid) require little molecular mobility, the $T_g$ of solid dispersion correlates poorly with the chemical stability of water-sensitive compounds (Bell & Hageman, 1994; Ohtake & Shalaev, 2013; Shalaev & Zografi, 1996; Shamblin et al., 2006).

More nuanced view of amorphous solid dispersions take into account the polarity of the media, the intermolecular interactions between small molecules and polymers, and the mixing behavior of small molecules with polymers. Taylor and workers have approached drug-polymer solubility with a pseudo-equilibrium approach using Flory-Huggins theory to predict the miscibility of small molecules with PVP in solid dispersions (Marsac, Shamblin, & Taylor, 2006). The interaction parameters in the Flory-Huggins mixing equation have also been applied to PVP-sucrose and PVP-trehalose.
systems with some success in predicting their deviations from ideal and non-ideal moisture sorption from environmental relative humidity (Zhang & Zografi, 2001).

The chemical stability of small molecules in the amorphous solid state has been well studied in pharmaceutical research because poorly water soluble drugs are often formulated as amorphous solids to increase their bioavailability in human dosage forms (Hancock & Zografi, 1997). Similar principles of amorphous stability can also apply to solid state dietary supplements or functional foods such as green tea powder in which antioxidants such as (−)-epigallocatechin gallate (EGCG) exist in the amorphous solid state and degrade more easily than their crystalline forms (Na Li, Taylor, Ferruzzi, & Mauer, 2013). Water activity and glass transition temperature – as well as the moisture sorption properties of the solid forms – have been shown to have important effects on the physical and chemical stability of amorphous food systems (N. Li, Taylor, & Mauer, 2011; Zhou & Roos, 2012). Since ascorbic acid has been shown to readily amorphize in various food polymers via lyophilization (Christina, Taylor, & Mauer, 2015), its presence in the amorphous solid state in spray dried fruit powders, freeze dried whole fruits, and other dried foods with native vitamin C is probable and its stability would be expected to depend on the factors discussed in this review (water activity, moisture sorption properties, glass transition temperature, physical state). In determining which of these factors plays the most important role in the chemical stability of ascorbic acid, the chemical properties of vitamin C are fundamentally relevant. The degradation mechanism, degradation rate, and other chemical properties of vitamin C are discussed in Section 1.5 below.
1.5 The Chemical Degradation of Vitamin C

Vitamin C refers to two physiologically active forms of an organic acid – its reduced form, ascorbic acid, and its oxidized form, dehydroascorbic acid (Figure 1).

Ascorbic acid exists in the crystalline and aqueous state as a lactone (a closed-ring ester) (Bauernfeind, 1982). The source of its chemical instability lies in the presence of the 2,3-dienol in the lactone, which is an example of a class of redox-active organic compounds known as reductones (Yasuo Abe, 1986). The 2,3-dienol moiety allows the hydroxyl group at C3 to be easily deprotonated because of the resultant resonance stabilization in the lactone. This ease of deprotonation is why ascorbic acid behaves as a weak organic acid (Figure 2).
Deprotonated ascorbic acid is redox active (Buettner, 1993). It can readily lose and electron to form a radical – ascorbyl radical – because the resultant radical is also resonance stabilized.

![Figure 3 - One-electron oxidation of ascorbate results in ascorbyl radical, which is resonance stabilized](image)

Loss of a second electron, whether at C3 with simultaneous tautomerization at C2 or via deprotonation at C2 followed by electron loss, forms dehydroascorbic acid (Yasuo Abe, 1986).

![Figure 4 - Loss of a second electron via either tautomerization at C2 (above) or deprotonation at C2 (below) forms dehydroascorbic acid](image)

Dehydroascorbic acid contains three adjacent carbonyl groups in the lactone. This arrangement of neighboring carbonyl groups aligned on the same place is energetically
disfavored. For this reason, the most common conformational form of dehydroascorbic acid in solution is the hemiketal form (Ward, 1982).

Figure 5 – The hydroxyl group at C6 hydrates the carbonyl group at C3 to form a hemiketal, the most stable conformational form of dehydroascorbic acid.

Because of the carbonyl ring strain described in Figure 5, dehydroascorbic acid is susceptible to nucleophilic attack by water at C1 – i.e. to lactone hydrolysis (Sakurai, 1967). The resultant open form compound, 2,3-diketogulonic acid, can freely rotate to alleviate the repulsive interactions between the adjacent carbonyl groups (Carey, 2002).

Figure 6 – Hydrolysis at C1 produces 2,3-diketogulonic acid.

At this point, 2,3-diketogulonic acid degrades via several pathways that vary according to the reaction conditions (downstream degradation products will be discussed further on). Nevertheless, during storage ascorbic acid in foods degrades mostly via the oxidative pathway explained above (Serpen & Gokmen, 2007; Smuda & Glomb, 2013). Examples of reduced-form degradation of ascorbic acid are found in shelf life studies of canned fruits and vegetables. After losses incurred during heat processing, both classical
and modern shelf life studies show anywhere from no significant losses to losses less than 15% after one year of storage at room temperature (Rickman, Barrett, & Bruhn, 2007). Losses during canning, however, when reactive oxygen is dissolved in the medium, have been recorded as high as 80%.

Ascorbic acid degradation in simple aqueous systems with added reducing and oxidizing agents further shows the dramatic effects of oxidation on ascorbic acid stability. Degradation of aqueous ascorbic acid at 90°C for 6 hours resulted in 72.5% loss. When the degradation was performed in the presence of 20 mg/L of cysteine, a reducing agent, ascorbic acid loss dropped to only 20%. Addition of 20 mg/L of iron (III) nitrate, a metal catalyst, increased the loss to up to 95% (Serpen & Gokmen, 2007).

The downstream products of ascorbic acid degradation have been characterized in a variety of model and real food systems. Since no two studies degrade ascorbic acid in identical reaction conditions, many late-stage degradation products have been recorded. In spite of the differences between reaction conditions in separate studies, a few trends emerge. Early downstream degradation products after the formation of 2,3-diketogulonic acid include oxalic acid, threonic acid, and other polyhydroxylic acid fragments of 2,3-diketogulonic acid (Sakurai, 1967; Smuda & Glomb, 2013; Szultka, Buszewska-Forajta, Kaliszan, & Buszewski, 2014). Continued degradation dehydrates these fragments to produce unsaturated aromatic compounds such as furfural, 5-hydroxymethylfurfural, 2-furoic acid, 2,5-dihydro-2-furoic acid, and 2-methylfuran (Coggiola, 1963; Limacher, Kerler, Conde-Petit, & Blank, 2007; Sakurai, 1967; Tatum, Shaw, & Berry, 1969; Yuan, 1999).
Ascorbic acid degradation also increases in the presence of sugars and amino acids because the hydrolyzed lactone is easily susceptible to nucleophilic attack and thus participates in non-enzymatic browning (Rojas & Gerschenson, 2001; Smuda & Glomb, 2013). Ample research exists in this aspect of ascorbic acid degradation because of the non-enzymatic browning that occurs in orange juice and processed orange juice products (Tikekar, Anantheswaran, Elias, & LaBorde, 2011)

Since the molecular mechanism for the initial degradation of ascorbic acid is well known, the reaction kinetics of degradation can be theoretically modeled using rate law kinetics. The simplest kinetic models in the literature have modeled the reaction as pseudo-first order – pseudo because water participates in stoichiometric amounts in the reaction but its concentration is incorporated into the rate constant (Kirk, Dennison, Kokoczka, & Heldman, 1977; Y. C. Lee, Kirk, Bedford, & Heldman, 1977):

\[
\frac{d[AA]}{dt} = -k[AA]
\]

(9)

More sophisticated degradation kinetics that include the oxidation into dehydroascorbic acid before hydrolysis have also been successfully applied \( (r^2 > 0.99) \) to aqueous degradations (Serpen & Gokmen, 2007):

\[
\frac{d[AA]}{dt} = -k_1[AA] + k_2[DHAA]
\]

(10)

\[
\frac{d[DHAA]}{dt} = k_1[AA] - k_2[DHAA] - k_3[DHAA]
\]

(11)

In spite of the complexity of accurately modeling ascorbic acid degradation (the model above requires Laplace transformations), the literature commonly reports good fits to ascorbic acid degradation using first order kinetics in both model aqueous systems and
real food systems (Assiry, Sastry, & Samaranayake, 2003; De'Nobili, 2013; Giannakourou & Taoukis, 2003; Kirk et al., 1977; Manso, Oliveira, Oliveira, & Frias, 2001; Riemer & Karel, 1978; Serpen & Gokmen, 2007), although a few zero-order degradation rates have also been reported in crystalline and saturated aqueous ascorbic acid (Shephard, Nichols, & Braithwaite, 1999a) and infant formula (Sablani, Al-Belushi, Al-Marhubi, & Al-Belushi, 2007). In cases of ascorbic acid degradation in dehydrated systems, some literature has reported degradation kinetics that do not follow simple rate laws. These systems included air-dried ascorbic acid/maltodextrin slurries (Frias, Oliveira, Cunha, & Oliveira, 1998), encapsulated ascorbic acid in disaccharide and polysaccharides (Hung, Horagai, Kimura, & Adachi, 2007), lyophilized grapefruit juice (Moraga, Igual, Garcia-Martinez, Mosquera, & Martinez-Navarrete, 2012), and hot air dried papaya (Kurozawa, Terng, Hubinger, & Park, 2014). The variation in ascorbic acid degradation reports is likely a result of the differences present in the reaction media (oxidizing or reducing agents; pH; enzyme activity; physical state), storage conditions (relative humidity; temperature), and analysis method (time scale of analysis; analytical detection technique).

The experiments discussed in Chapters 3 and 4 sought to study the chemical degradation of ascorbic acid in the amorphous solid state in the context of the concepts discussed in this literature review: the storage relative humidity and moisture sorption properties (Section 1.2), the effects of the glass transition temperature versus water activity conditions (Section 1.3) the amorphous versus crystalline solid state (Section 1.4), and the reaction mechanism of ascorbic acid (Section 1.5). All systems used in this study were solutions of ascorbic acid and PVP lyophilized to form solid dispersions and stored
at 11% relative humidity. Two sample preparation variables were explored: (1) the weight ratio of ascorbic acid to PVP in the dispersions (Chapter 3), and (2) the pre-lyophilization solution pH of the ascorbic acid/PVP solutions (Chapter 4).

The experimental results in chapter 3 provide evidence for the stability of crystalline ascorbic acid relative to amorphous ascorbic acid in PVP solid dispersions. In the case of solid dispersions where ascorbic acid exists in the amorphous solid state, ascorbic acid stability is proven to decrease with increased dispersion T_g in the systems studied. The moisture sorption properties of the dispersions are suggested to best explain the observed behavior of ascorbic acid degradation in the amorphous solid state. The data presented in chapter 4 proves how the degree of ascorbic acid ionization, modified by buffering ascorbic acid with sodium ascorbate prior to lyophilization, affects the chemical degradation of ascorbic acid in the amorphous glassy state. Pre-lyophilization solution pH is proven to strongly affect the extent of ascorbic acid degradation in the solid dispersions but weakly affect their glass transition temperatures. Through modeling of the moisture sorption profiles, intermolecular interactions and the differences in the moisture contents of the dispersions are argued to account for the large observed differences in chemical stability that resulted from changes in the degree of ionization of ascorbic acid.
CHAPTER 2. METHOD DEVELOPMENT

2.1 Overview

The study of amorphous ascorbic acid degradation in PVP solid dispersions required the implementation or development of new experimental methods. Preliminary results on the chemical degradation of ascorbic acid solid dispersions had indicated no ascorbic acid loss over time in spite of color development in ascorbic acid lyophiles (Christina, 2014). Those experiments relied on a bench-top freeze drier to formulate lyophiles as well as a UV-Vis plate reader assay to quantify ascorbic acid. Limitations in both of those experimental methods led to the development of an HPLC method for the quantification of ascorbic acid (Section 2.2) and a lyophilization method using a pilot-scale shelf freeze drier (Section 2.3.1).

Before beginning the two studies discussed in Chapters 3 and 4, preliminary experiments of amorphous ascorbic acid degradation were done (Section 2.3.1 and Section 2.3.2) using the newly developed methods in order to confirm the degradation of amorphous ascorbic acid in the solid dispersions and to validate the measurement of ascorbic acid loss. Since the new lyophilization method generated lyophiles quite different from those studied in previous solid dispersions, PXRD experiments were performed in order to establish a detection threshold for crystalline ascorbic acid in the formulated lyophiles and in physical blends (Section 2.4).
The experiments discussed in Chapter 4 required modification of the pH of ascorbic acid/PVP solutions prior to lyophilization. In order to achieve pH modification given the lyophile preparation method described in Section 2.3.1 and using only sodium ascorbate as an additional reagent, a special preparation method was developed for buffered lyophiles (Section 2.5).

Results from the preliminary experiments of amorphous ascorbic acid degradation informed the selection of the storage conditions used for the experiments in Chapters 3 and 4. The methods discussed in this chapter for the preparation of lyophiles and the quantification of ascorbic acid were also exclusively used for the experiments presented in Chapters 3 and 4.

2.2 HPLC Techniques for the Quantitative Detection of Ascorbic Acid

The official analytical technique for ascorbic acid quantitation as approved by the AOAC remains a titration assay with indophenol (Fontannaz, Kilinc, & Heudi, 2006). Most modern techniques, however – both in academic research and in industrial practice – use HPLC techniques for ascorbic acid quantitation (Heudi, Kilinc, & Fontannaz, 2005; Novakova, Solich, & Solichova, 2008). Because of the popularity and ease of use of reverse-phase C18 columns in chromatography, RP-C18 is the most widely applied separation phase in HPLC analysis on vitamin C. A review on modern HPLC methods published after 1999 for vitamin C analysis counts RP-C18 used in 33 out of 40 publications, with the remainder using mostly ion-pair phases (Novakova et al., 2008). Although RP-C18 is the most widely used stationary phase, it is not the best suited because of the low retention of ascorbic acid in non-polar phase (Novakova et al., 2008).
However, for quantitation of ascorbic acid from simple sample matrices, RP-C18 separation in an isocratic mode has been widely used together with two chromatographic techniques to minimize the disadvantage of C18 columns (Assiry et al., 2003; Chebrolu, Jayaprakasha, Yoo, Jifon, & Patil, 2012; Shephard et al., 1999a; Yuan, 1999). First, the mobile phase is maintained as aqueous as possible, often at 95% and even 100% water. Second, the mobile phase is kept at low pH (from 2.0 to 2.6), often with meta-phosphoric acid, chloroacetic acid, or trifluoroacetic acid, to maintain ascorbic acid in the protonated state to help improve retention onto non-polar media and sharpen peak shape (McMaster, 2007). The method used for this study followed the approach from the above cited publications that used RP-C18 columns in isocratic mode with a high aqueous phase at low pH. The final conditions used were 95% water, 5% methanol, and .025% trifluoroacetic acid (TFA) to maintain the mobile phase at pH~2.0. Standard curves generated with these chromatographic conditions always resulted in linear fits with an $r^2$ of at least 0.990 in all cases and usually with $r^2 > 0.995$. Fresh ascorbic acid standard solutions and calibration curves were prepared before each experimental run. The use of sodium ascorbate as opposed to ascorbic acid in standard curves, as expected, did not affect the outcome of the calibration curves because of the TFA used to maintain a low mobile phase pH.

Isolation and quantitation of ascorbic acid from more complex sample matrices often require more involved chromatographic conditions such as gradient elution and ion-pairing reagents (Fontannaz et al., 2006; Tikekar et al., 2011). These conditions were not considered necessary for the experiments in this work considering the simplicity of the
sample matrix (limited to ascorbic acid and PVP) and the high native concentrations of ascorbic acid in the experimental samples.

Detection of dehydroascorbic acid as well as ascorbic acid to quantify total vitamin C content requires chemical derivitization prior to analysis since dehydroascorbic acid is not UV-active (Novakova et al., 2008). A reducing agent is used to reduce dehydroascorbic acid to ascorbic acid, and the additive method is used to determine the total vitamin C content. The reagent tris-(2-carboxymethyl) phosphine (TCEP) is the most widely used reducing agent for dehydroascorbic acid reduction (Lykkesfeldt, 2000; Wechtersbach & Cigic, 2007).

Apart from HPLC methods, plate reader, AOAC titration, and even bioassay methods have been used for quantitative ascorbic acid detection (Hung et al., 2007; Rekha & Murthy, 2010; Stevens, 2006; Zhou & Roos, 2012), but HPLC has become the most popular method for vitamin C detection as evidenced by the large number of publications that employ the technique.

2.3 The Amorphization of Vitamin C and Preliminary Degradation Studies

2.3.1 Formulation of Amorphous Ascorbic Acid Solid Dispersions

Amorphous solid dispersions of ascorbic acid had previously been produced by lyophilizing ascorbic acid and polymer solutions using a VirTis benchtop 2K XL system with a two liter condenser capacity and condenser temperature of -75°C (SP Industries; Gardiner, NY) (Christina et al., 2015). While these samples were useful for physical characterization techniques such as PXRD, FT-IR, and moisture sorption analysis, they presented two potential problems for quantitative chemical analysis. First, the moisture
sorption behavior of the lyophiles over time confounded the theoretical amount of ascorbic acid in any weighed sample. Second, the very low solids content (1.1% weight) resulted in extremely light lyophiles that proved difficult to weigh and transfer with acceptable precision.

A new lyophilization method was developed based on that of Shalaev et al and advice from Dr. Taylor (Shalaev, Lu, Shalaeva, & Zografi, 2000). The new method increased the weight solids content to 10% weight and used a VirTis 25 ES shelf freeze-drier. Samples were frozen directly in the freeze drier at -40°C. The chamber temperature climbed five degrees Celsius every 230 minutes and the pressure was maintained at 330 mtorr for a total run time of 52 hours. After the cycle, the chamber remained under vacuum until the samples were removed.

PVP was selected as the carrier polymer for both its experimental advantages and its well-studied physical properties. The pectins and PAA used in ascorbic acid physical stability experiments (Christina, 2014) were unable to form solutions with solids content greater than 2% weight. PVP solutions could readily reach 10% and even 20% solids contents, which allowed for better formation of lyophiles. A solids content of 10% weight allowed for better experimental technique in several analyses, most importantly in DSC (to pack sample pans with at least 5 mg sample) and SPS (to record changes in moisture sorption above the instrumental limit of detection). The high T_g of PVP also allowed for the formulation of amorphous ascorbic acid at high ratios of ascorbic acid. Carriers such as disaccharides, which possess lower glass transition temperatures than polymers, would have narrowed the range of weight ratios able to form amorphous ascorbic acid.
The new lyophilization method used one 20 mL glass scintillation vial for each HPLC analysis to avoid any weighing and transfer errors. Three vial methods were tested. First, in the batch test method, a 200 mL solution of the aqueous dispersion was formulated on a stir plate under gentle heat and stirring. Once the PVP and ascorbic acid (Sigma Aldrich, St. Louis, MO) were dissolved, 5 mL aliquots of solution were pipetted into glass vials and placed on the freeze drier tray. In this way, each vial was assumed to have the exact same concentration of reagents. The other two methods weighed out reagents into each individual glass vial. Individual reagent weights were recorded and 5 mL of water were added to the vials. Because of the low wettability of PVP, the vials required agitation on a vortex to fully dissolve the polymer. In the no cap method, the caps used for each vial were discarded after vortex agitation even though a small amount of the solution remained on the inside surface of each cap. In the cap method, these caps were labeled and freeze dried upside down alongside the vials.

After lyophilization, the lyophiles were reconstituted with 15 mL of mobile phase (95% water, 5% methanol, .025% TFA; pH ~ 2.0). For the cap method vials, the labeled caps were screwed onto the corresponding vials before vortex agitation to fully dissolve the lyophiles. For the other two methods, new caps were used. The reconstituted solutions were pipetted into volumetric flasks and diluted with mobile phase down to appropriate concentrations for HPLC analysis in both HPLC-PDA (Waters Corp., Milford, MA and HPLC-ECD (ESA, Chelmsford, MA). Peak maxima for the HPLC-PDA were about 1.5 AU, and peak maxima for the HPLC-ECD were about 1 μA (at 280 mV), the commonly suggested range for both detectors.
Two trends emerged from this methodology experiment. The first was that PDA detection provided more precise results since the standard deviation for all three sample types were much lower than for the ECD detection method (2.3, 4.8, and 5.2 versus 6.7, 10.8, and 7.7 percent change in loss, respectively). The second was that the cap method provided the most precise measurement of ascorbic acid in the samples. Within each of the three sample types (ascorbic acid, ascorbic and citric acid, and ascorbic acid with sodium citrate), the cap method resulted in the least observed degradation. For this reason, together with the slightly greater precision of the capped method, the capped method was adopted as the method for all further chemical degradation experiments.

2.3.2 Qualitative Visual Study on the pH Dependence of Ascorbic Acid Degradation in the Solid State

The objective of this study was to investigate the pH-dependent degradation behavior of amorphous ascorbic acid in polymer solid dispersions. Solid dispersion or lyophiles were prepared using the batch method described in Section 2.2.1. Quantification of ascorbic acid loss was carried out after 27 days of storage using the HPLC-ECD.

Ascorbic acid, polyvinylpyrrolidone (PVP) (average molecular weight ~40,000), and citric acid (Sigma Aldrich; St. Louis, MO and J.T. Baker; Central Valley, PA) had been previously opened and used. Three sample types were prepared and the pH of the batched solutions were recorded before being aliquoted into 20 mL glass scintillation vials for lyophilization. Citric acid was used in order to follow the experimental procedure studied by Shalaev et al (Shalaev et al., 2000). Citric acid is also a practical acidulant for lyophilization since the acid does not sublimate during the lyophilization
process. For all three batches of different pH, 2g of ascorbic acid and 18g of PVP were used to maintain the ascorbic acid:PVP weight ratio at 10% w/w. The percent weight solids increased slightly with the addition of citric acid, to about 11% and 12% w/w, respectively. Samples acidulated with citric acid resulted in pre-lyophilization solution pHs of 2.3 and 2.0, while the solution of ascorbic acid had a pH of 3.0.

After lyophilization, samples were stored uncapped in three water-jacketed incubators kept at 40, 50, and 60°C. The relative humidities in the three incubators were monitored using an electronic hygrometer and remained below 8% RH. Photographs of the color development at different temperatures make the effect of citric acid on ascorbic acid degradation quite clear (Figure 7).

![Figure 7](image)

Figure 7 – Color development in ascorbic acid lyophiles formulated with added citric acid and stored in uncontrolled but low (< 8% RH) relative humidity conditions over time at 40, 50, and 60°C.
Lower pre-lyophilization pH caused by citric acid seems to have stabilized the ascorbic acid in the amorphous solid state. However, the stabilization may have also been due to the secondary antioxidant properties of citric acid, which chelate heavy metals present in trace amounts in either the water or the reagents. Additional methodological experiments with sodium citrate, discussed in Section 2.2.3, imply that a solid-state pH does appear to affect ascorbic acid chemical stability.

2.3.3 Accelerated Degradation at 60°C and Two Low Relative Humidity Conditions

The purpose of this study was to track the accelerated degradation of amorphous ascorbic acid using HPLC-PDA detection in order to determine 1) the feasibility of HPLC-PDA detection for long-term degradation studies, 2) the effects of controlled relative humidity on chemical degradation, and 3) the approximate rate of degradation in order to predict degradation rates at lower temperatures for long-term studies.

Lyophiles were prepared in triplicate following the capped method described in Section 2.2.1. Four types of lyophiles were formulated: ascorbic acid with citric acid, ascorbic acid, ascorbic acid with water treated with Chelex, and ascorbic acid with sodium citrate.

Chelex is a chelating ion-exchange resin with a high affinity for divalent ions and metal ions such as mercury, copper, and iron. A set of samples was treated with Chelex using the suggested treatment concentrations in order to remove potential trace metals from the water since these can catalyze the oxidation of ascorbic acid. Such catalysis was undesired in this experiment in order to distinguish the mechanism of action behind citric acid stabilization of ascorbic acid. Since citric acid is an acidulant and a chelating agent, the stability of ascorbic acid/citric acid lyophiles could be caused by the chelating
property of citric acid instead of its acidulating property. Total removal of metal ions from the pre-lyophilization solution would test whether or not citric acid inhibits metal-catalyzed degradation or not.

Sample analysis followed that outlined in Section 2.2.1. Samples were analyzed after three and 13 days of storage at 60°C at either ~0% RH (Dri-Rite™) and 29% (MgCl₂) conditions. In all experiments, lyophiles were pure white after lyophilization and only changed color when stored at high temperatures or relative humidities. The photographs below contrast the color of the four different prepared lyophiles.

Figure 8 – Color development in ascorbic acid lyophiles formulated with citric acid, sodium citrate, or water treated with Chelex.

It was assumed, for calculations of ascorbic acid loss, that samples lost an average of 9.4% ascorbic acid during the lyophilization process. This was the average loss calculated in the experiment described in Section 2.2.1. Accounting for these initial losses, the losses of ascorbic acid during storage are summarized in the table below. The standard deviations for all samples were less than 4% loss.
Table 1 – Ascorbic acid loss in lyophiles formulated with citric acid, sodium citrate, and water treated with Chelex after storage at two relative humidity conditions at 60°C

<table>
<thead>
<tr>
<th></th>
<th>0% Relative Humidity (Dri-Rite)</th>
<th>29% Relative Humidity (MgCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascorbic acid and citric acid</td>
<td>Ascorbic acid treated with Chelex</td>
</tr>
<tr>
<td>Day 3</td>
<td>9.1%</td>
<td>23.7%</td>
</tr>
<tr>
<td>Day 13</td>
<td>33.4%</td>
<td>52.2%</td>
</tr>
</tbody>
</table>

Citric acid stabilized ascorbic acid and sodium citrate destabilized ascorbic acid.

The relative humidity condition also greatly increased the rate of ascorbic acid degradation. A comparison between the two conditions reveals that ascorbic acid remains relatively stable at very low moisture content, although substantial degradation still occurs at very low levels of water.

2.4 The Detection Threshold for Ascorbic Acid Using Powder X-ray Diffraction (PXRD)

Early attempts to establish the detection threshold of crystalline ascorbic acid with PXRD relied on physical blends of crystalline ascorbic acid and PVP polymer. Ascorbic acid was blended at different weight ratios with PVP in individual 20 mL glass scintillation vials, vortexed, and analyzed using a Shimadzu LabX XRD-6000 (Shimadzu Corporation, Kyoto, Japan) equipped with a Cu-Kα source set in Bragg-Brentano geometry. Samples were analyzed at a scan speed of 4°/min, a step size of 0.04°, and a scan range between 5° and 35° 2θ. To mimic the crystal size of potentially recrystallized
ascorbic acid in lyophiles, one set of samples did not use ascorbic acid as purchased.
Instead, solutions of ascorbic acid were lyophilized, and the resulting crystals were gently
ground with a mortar and pestle. This ascorbic acid was then blended with PVP.

Neither of these physical blends – reagent-grade ascorbic acid or lyophilized
ascorbic acid – yielded acceptable standard curves. Poor standard curves may have been
expected given the imprecision of powder blending using the Vortex method described
earlier. Reagent-grade crystals exhibited a crystalline diffraction peak at concentrations
as low as 1.25% w/w ascorbic acid to PVP. Recrystallized (lyophilized) ascorbic acid
proved harder to detect, with a detection threshold of 10% w/w ascorbic acid to PVP.
Based on these results, it seemed that the smaller the crystal size, the harder it became for
the PXRD to detect crystalline solids.

A later experiment analyzed lyophilized ascorbic acid buffer solutions (no added
polymer; held at 60°C for 24 hours after lyophilization) at pHs 3.0, 3.5, 4.0, and 4.5
formulated at 10% weight solids. The intensity of a strong ascorbic acid diffraction peak
at 2θ = 30.1° was recorded for each pH sample and plotted versus ascorbic acid weight to
test for a correlation. Unlike the physical blends, these samples yielded a clear pattern
(though not a linear fit). Solid dispersions with weight percentages of ascorbic acid
greater than 80% AA/PVP, by which point ascorbic acid had recrystallized, also
displayed a pattern of increasing peak intensity with increasing ascorbic acid. A linear fit
was inappropriate for this set of samples as well.

Both sets of samples in these experiments provided consistent and reliable results,
probably due to the complete and homogenous mixing of ascorbic acid that took place
during sample preparation and lyophilization. These results appeared to have some
predictive power over the concentration of crystallized ascorbic acid in lyophiles, but only when the concentrations of ascorbic acid were high (above 10% weight crystalline material).

2.5 The Ascorbic Acid Buffer System

Early attempts to adjust the sample pH before lyophilization relied on citric acid or sodium citrate to adjust the sample pH. This approach allowed for a pH range from about 2.0 to 4.6. Acidulation with citric acid had a few disadvantages: it increased the complexity of the solid dispersions to ternary systems and introduced the effects of a secondary antioxidant in the solid matrix. Previous works on pre-lyophilization pH have used sodium hydroxide, hydrochloric acid, and citric acid as pH adjusters. Hydrochloric acid was reported by Strickley et al. to be a poor acidulant for lyophilization since acid loss could take place during the lyophilization process (Strickley & Anderson, 1996), although Li et al. did not report changes in post-lyophilization pH using hydrochloric acid as an acidulant (J. J. Li, Guo, & Zografi, 2002). Citric acid or other non-volatile organic acids must be added in appreciable amounts in order to affect the pre-lyophilization pH (Shalaev et al., 2000), but this addition increases the complexity of the resultant solid dispersion. Acidulation of a basic species with a positive counterion could also result in small amounts of salt formation, as reported by Strickley et al. In the reported case, the generated sodium chloride salt drastically changed the moisture sorption profiles of the formulated lyophiles (Strickley & Anderson, 1996).

In the case of ascorbic acid and sodium ascorbate, pH adjustment of sodium ascorbate solutions using hydrochloric acid would be the least recommended choice since
this approach would also generate sodium chloride in the solid dispersions. pH adjustment of ascorbic acid solutions with concentrated sodium hydroxide would not generate salts, but this approach would have required sample preparation using the batch method described in Section 2.3.1.

To improve the experimental precision, the pH was adjusted by creating ascorbic acid buffer systems using only reagent grade ascorbic acid and sodium ascorbate (Fisher Scientific; Waltham, MA). This simplified the system to a binary system, although by necessity it also narrowed the attainable pH range from about 3.0 to 4.6. The validity of ascorbate buffer systems at the concentrations used for sample preparation was tested repeatedly in the lab to ensure that the theoretical pHs were in agreement with the experimentally recorded pHs. The ascorbate buffer system is reviewed in the remainder of this section.

The acid-base equilibrium of ascorbic acid in solution can be written as follows:

\[ \text{AOH} + \text{H}_2\text{O} \overset{\text{equilibrium}}{\longrightarrow} \text{AO}^- + \text{H}_3\text{O}^+ \]  

(12)

With an equilibrium constant:

\[ K_a = \frac{[\text{AO}^-][\text{H}_3\text{O}^+]}{[\text{AOH}]} \]  

(13)

Ascorbic acid, with a pK\(_a\) of 4.1, must be treated as a weak acid. Since not all of the ascorbic acid dissociates, then only an amount \( x \) dissociates, and this amount is equal to the concentration of ascorbate ion and also of hydronium ion. Given an initial concentration \( a \) of ascorbic acid, the equilibrium expression can be written as:
Equation 14 can be solved for $x$ to yield the hydronium concentration in solution, from which the pH can be readily calculated. If $x$ is taken to be small, then the value of the term $x - a$ is approximately equal to $x$, and so the expression for the pH of an ascorbic acid solution is simply

$$pH = -\log \sqrt{aK_a}$$  \hspace{1cm} (15)

The predicted pH from Equation 15 agrees with experimental results in lab using an Orion SA720 pH meter (Thermo Orion, Beverly, MA) to the nearest two decimal places. In other words, the assumption that $x - a \approx x$ is a good one for the ascorbic acid concentrations used in the lyophiles studied for this work.

A buffer system of ascorbic acid exhibits the same chemical equilibrium as Equation 12. The only difference is that the concentration of ascorbate ion in solution is not equal to $x$ (the concentration of hydronium ion). Instead, if the solution is made with an initial concentration $b$ of ascorbate ion, then the equilibrium expression becomes

$$K_a = \frac{(b + x)x}{a - x}$$  \hspace{1cm} (16)

Which, is solved explicitly for $x$, results in a cumbersome but unnecessary expression. If the approximation is made that $b + x \approx b$ then the equilibrium expression of a buffered solution of ascorbic acid becomes

$$K_a = \frac{bx}{a}$$  \hspace{1cm} (17)

From which the resultant solution pH is equal to
Despite the approximations, Equation 18 is surprisingly accurate for solutions of reasonable molarity. Given this equation, the molar ratios of ascorbic acid and sodium ascorbate were calculated in order to formulate lyophiles at given pHs. For example, the pHs at 3.0, 3.5, 4.0, and 4.5 were used in this work. To calculate the amounts of ascorbic acid and sodium ascorbate to use, the equation can be rearranged to solve for the ratio $a/b$ at each desired pH. From the ratio

$$a/b = \frac{10^{-pH}}{K_a}$$  \hspace{1cm} (19)

Table 2 was easily generated:

<table>
<thead>
<tr>
<th>Desired pH</th>
<th>Ratio of reagents $a/b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>12.59</td>
</tr>
<tr>
<td>3.5</td>
<td>3.98</td>
</tr>
<tr>
<td>4.0</td>
<td>1.26</td>
</tr>
<tr>
<td>4.5</td>
<td>0.398</td>
</tr>
</tbody>
</table>

This ratio, together with a desired final concentration of ascorbate ion, allowed for the preparation of individual sample vials at the desired pHs. In other words, each sample formulated in 20 mL scintillation vials was a pre-weighed buffer system, no different in preparation than any standard buffer solution. An experiment was carried out where numerous samples at given pHs were formulated and, immediately after lyophilization, were reconstituted to test their post-lyophilization pHs. The recorded pHs agreed with the theoretically calculated pHs to the nearest 0.05 pH units.
2.6 Summary

The developed methods presented in this chapter made three improvements on the previous published methods for formulating amorphous ascorbic acid solid dispersions and quantifying their ascorbic acid content over time. First, the use of HPLC-PDA for quantification of ascorbic acid resolved earlier complications of a UV-Vis plate reader assay that arose from color development in samples with degraded ascorbic acid (Christina, 2014). Second, sample preparation in 20 mL glass scintillation vials and lyophilization using a pilot-scale shelf freeze drier allowed for the formation of dry, stiff lyophiles with well-known ascorbic acid contents that could be used for chemical degradation experiments. Third, the use of the Henderson-Hasselbalch equation to determine precise levels of ascorbic acid and sodium ascorbate necessary to reach desired solution pHs allowed for the formulation of buffered lyophiles without the use of additional acids or bases. The HPLC, lyophilization, and buffered lyophile preparation methods were all used for the experiments presented in Chapters 3 and 4.
CHAPTER 3. EFFECT OF ASCORBIC ACID TO PVP WEIGHT RATIO ON FORMATION AND DEGRADATION OF ASCORBIC ACID LYOPHILES

3.1 Abstract

This study reports the changes in the physical and chemical properties of ascorbic acid that result upon modifying the weight ratio of ascorbic acid and PVP used to formulate ascorbic acid/PVP lyophiles. Lyophiles of ascorbic acid and PVP were prepared at 14 different weight ratios of ascorbic acid to PVP, from 1% to 100% ascorbic acid by weight. After lyophilization, lyophiles were stored under controlled conditions at 60°C and 11% relative humidity for one month. Physical and chemical stability of the lyophiles was analyzed over one month using photography, gravimetric analysis, powder X-ray diffraction (PXRD), polarized light microscopy (PLM), and HPLC to track the physical appearance, change in moisture content, physical state, and loss of ascorbic acid over time. For select amorphous lyophiles, the glass transition temperatures and moisture sorption profiles were recorded using differential scanning calorimetry (DSC) and dynamic vapor sorption. The results were then modeled using the Gordon-Taylor equation and the Guggenheim-Anderson-DeBoer equation in order to calculate the glass transition temperature of amorphous ascorbic acid and the GAB monolayer value of the amorphous solid dispersions. Lyophiles at 70% weight ascorbic acid or more recrystallized during lyophilization. During storage, amorphous lyophiles did not
recrystallize, and lyophiles with 20% weight ascorbic acid or less gained and retained significantly \( (p < .05) \) more moisture than lyophiles at greater ratios of ascorbic acid by weight. Recrystallized lyophiles did not lose a significantly different \( (p < .05) \) amount of ascorbic acid during storage. In amorphous lyophiles, the relative loss of ascorbic acid significantly increased \( (p < .05) \) as the weight ratio of ascorbic acid decreased.

3.2 Introduction

Ascorbic acid is an essential micronutrient found both naturally in foods as well as supplemented in fortified foods and dietary supplements. Relative to the other vitamins, ascorbic acid poses the greatest challenges to chemical stability and product shelf life (Riaz, Asif, & Ali, 2009; Rickman et al., 2007). Its sensitivity to moisture, temperature, light, oxygen, and pH have long made its degradation during food processing and storage a continual subject of study, particularly in the aqueous state, as in juices and beverages, and the crystalline state in single ingredients and powdered vitamin pre-mixes (Bauernfeind, 1953; Bauernfeind & Pinkert, 1970).

Recent work from our lab has proven that ascorbic acid can be formed in the amorphous solid state when lyophilized with several polymers, including pectins and synthetic polymers (Christina et al., 2015). Given the ease of amorphization of ascorbic acid with polymers, even at ratios of vitamin to polymer greater than 50% w/w, ascorbic acid may readily exist in the amorphous solid state in foods such as freeze dried fruits and vegetables, spray-dried fruit and vegetable juices, and fortified foods. Amorphous solids usually exhibit greater chemical instability than crystalline solids, as well as higher moisture sorption at relative humidities below the deliquescence point of crystalline
solids (Hancock & Zografi, 1997; L. Yu, 2001). This higher hygroscopicity can lead to further chemical instability as well as to undesired physical phenomena such as clumping and caking (Hiatt, Taylor, & Mauer, 2010; Mauer & Taylor, 2010). Amorphous solids may also crystallize during storage, a phase transformation leading to water redistribution and related changes in a product. The glass transition temperature is often considered an important parameter to predict both the physical and chemical stability of small molecules in the amorphous solid state (Shamblin et al., 2006).

The physical stability of amorphous ascorbic acid in polymer solid dispersions has been documented as a function of temperature and relative humidity by Christina et al. (Christina et al., 2015). Of the polymers used in that study (citrus pectin, apple pectin, pectins with varying degrees of esterification, polyvinylpyrrolidone (PVP), and polyacrylic acid (PAA)), the more stable amorphous dispersions were formed by polymers with more intermolecular hydrogen bonding between the ascorbic acid and polymer, and not with the least hygroscopic polymer nor the polymer with the highest glass transition temperature. PVP participated most strongly in hydrogen bonding with ascorbic acid. Together with its good ability to inhibit ascorbic acid recrystallization relative to the other assayed polymers, PVP promised to form the most stable amorphous solid dispersions at ratios of ascorbic acid to polymer of 50% w/w or less.

The objective of this study was to examine the chemical degradation of ascorbic acid as confirmed to occur in the amorphous state. Previous studies of ascorbic acid degradation in lyophilized systems most likely observed the degradation of ascorbic acid in the amorphous solid state (De' Nobili, 2013; Kirk et al., 1977; Kurozawa et al., 2014; Moraga et al., 2012; Zhou & Roos, 2012). However, the physical state of the vitamin
during degradation has not been experimentally verified. This study is the first to report
the experimentally confirmed chemical degradation of ascorbic acid in the amorphous
solid state. Experiments were designed to determine: 1) the effects of the ascorbic acid to
polymer (PVP) weight ratio on the chemical stability of amorphous ascorbic acid over
time, 2) how the ascorbic acid:PVP weight ratio affected the moisture sorption of the
dispersions and the effects of moisture sorption on vitamin stability, and 3) the
relationship between the $T_g$ and the vitamin chemical stability.

3.3 Materials and Methods

3.3.1 Materials

Ascorbic acid and PVP (MW ~ 40,000) (Fisher Scientific; Waltham, MA) were
used as is for all experiments. Lithium chloride used to create saturated salt solutions (11%
RH) was purchased from EMD (EMD Millipore; Billerica, MA) and Drie-Rite™ used to
create dry (< 3% RH) conditions in desiccators was purchased from W. A. Hammond
Drierite Co. (Xenia, OH). Methanol and trifluoroacetic acid used in liquid
chromatography were purchased from Fisher (Fisher Scientific; Waltham, MA). Any
water used in the experiment was purified using a Milli-Q system (EMD Millipore;
Billerica, MA) to reach a resistivity at 25°C of greater than 18 MΩ·cm.

3.3.2 Sample preparation

Lyophiles of ascorbic acid (Fisher Sci, Waltham, MA) and PVP K40 (Fisher Sci,
Waltham, MA) were prepared in 20 mL glass scintillation vials across 14 different weight
ratios of ascorbic acid to PVP, from 100% w/w (pure ascorbic acid) to 1% w/w. The low
viscosity of PVP allowed for the formulation of lyophiles at 10% weight solids, which
increased the precision of the experimental gravimetric techniques. After ascorbic acid and PVP were added to the vials, 5 mL of water were pipetted into the vials to reach the desired 10% weight solids. Vials were capped and vortexed to fully dissolve the solids. Together with their upturned lids, the sample vials were quickly transferred on a tray into a VirTis Genesis 25ES (SPScientific, Stone Ridge, NY) shelf freeze drier.

Lyophilization proceeded as follows: samples were frozen inside the freeze drier at -40°C and 400 torr for four hours. The pressure was then reduced to 300 mtorr to begin the drying cycle. The temperature was raised to -25°C and increased 20°C in five degree increments with 300 minutes at each step and then raised to 30°C for two hours to finish the drying cycle. Once the drying cycle was complete, vacuum was maintained at 300 mtorr and the temperature was held at 20°C until samples were collected from the freeze drier.

Physical blend controls of ascorbic acid and PVP were formulated at every lyophile weight ratio in 20 mL glass scintillation vials. After PVP and crystalline ascorbic acid were added, vials were capped and vortexed to blend the reagents. Immediately after preparation, samples were transferred to the same storage conditions used for lyophiles described below.

3.3.3 Sample Storage

Samples for chemical degradation, gravimetric analysis, microscopy, and X-ray diffraction experiments were stored in desiccators at 11% RH as controlled by a saturated solution of lithium chloride. The desiccators were stored in water jacketed incubators maintained at 60°C. At the time of analysis, samples were removed from the desiccators, capped, weighed, and photographed prior to chemical analysis.
Samples for DSC analysis were stored for four days at 20°C in desiccators filled with Dri-Rite™ to maintain the relative humidity near 0% RH. Samples for moisture sorption profile analysis were stored in the same conditions for one day prior to the moisture sorption experiment. Their preparation method varied slightly from other samples as explained in Section 3.3.10 below.

3.3.4 Photography

Lyophiles were photographed immediately after lyophilization and at the times of chemical analysis in an Elviros light box with a black background (1,300 lumen/m²). An iPhone 6 was placed one inch above the top of the scintillation vial and the smartphone camera was used to take the photograph.

3.3.5 Gravimetric Analysis

Immediately before chemical analysis, lyophiles were weighed to record the changes in weight during storage. Any changes in weight after lyophilization or during storage were considered to be caused solely due to moisture gain or loss.

3.3.6 Polarized Light Microscopy

Polarized light microscopy (PLM) was used alongside powder X-ray diffraction (PXRD) to monitor the physical state of the lyophiles over time. Small amounts of sample were placed on glass slides and observed under polarized light at 100x magnification with an Omano OM349P polarized light microscope (The Microscope Store LLC, Roanoke, VA). The microscope was equipped with an iPhone 6 adapter to take sample photographs. Any observed birefringence was taken to indicate ascorbic acid recrystallization. Samples that lacked birefringence were considered PLM amorphous.
3.3.7 Powder X-ray Diffraction

Powder X-ray diffraction (PXRD) was used to detect the presence of ascorbic acid crystals in lyophiles. Samples were prepared for analysis by crushing lyophiles in their vials with a metal spatula to obtain a uniform powder. Enough powder was transferred onto an aluminum PXRD sample holder to completely fill the slide, and the powder was smoothed out with a glass slide. A Shimadzu LabX XRD-6000 (Shimadzu Corporation, Kyoto, Japan) equipped with a Cu-Kα source set in Bragg-Brentano geometry was used to analyze the samples. The scan range was set from 5° and 35° 2θ and the scan speed was set at 4°/min with a 0.04° step size. Samples with diffraction patterns that displayed no peaks above the baseline were considered PXRD amorphous.

3.3.8 High-Pressure Liquid Chromatography

The amount of ascorbic acid in lyophiles over time was quantified using a Waters 2695 SM (Waters Corp., Milford, MA) equipped with a Waters 2996 photodiode array detector (Waters Corp., Milford, MA). A mobile phase of 95% water, 5% methanol, and .025% trifluoroacetic acid was used with an XTerra RP-C18 column (Waters Corp., Milford, MA) to quantify ascorbic acid at 244 nm as adapted from published methods (Assiry et al., 2003; Van de Velde, Pirovani, Camara, Gueemes, & Bernardi, 2012). Before each analysis, lyophiles were reconstituted in water, diluted in mobile phase, and filtered through a 0.2 um syringe filter. Standard curves of ascorbic acid ($r^2 > 0.99$) were prepared for each analysis.

3.3.9 Differential Scanning Calorimetry

Thermal analysis of selected lyophiles was carried out using a TA Discovery Series DSC (TE Instruments, New Castle, DE). The instrument was calibrated with
indium and sapphire and purged with nitrogen gas. To prepare the samples, lyophiles were gently crushed in their 20 mL glass vials using a metal spatula. Roughly 5 to 10 mg of lyophile was then transferred into a TZero pan and hermetically sealed. The onset glass transition temperatures were determined by heating the samples from 25°C to 150°C at a rate of 10°C/minute. TRIOS software (Texas Instruments, New Castle, DE) was used to calculate the onset glass transition temperature, which was defined as the intersection between the baseline tangent line and the tangent line at the point of inflection of the T_g event.

Lyophiles of ascorbic acid and PVP at three weight ratios, 10%, 20%, and 30% w/w stored at 20°C for three days after lyophilization in Dri-Rite™ were analyzed using the above method to experimentally determine their onset glass transition temperatures. The Gordon-Taylor equation:

\[
T_{g,\text{mix}} = \frac{w_1 T_{g,1} + k w_2 T_{g,2}}{w_1 + k w_2}
\]

was then used to calculate the predicted glass transition temperature of ascorbic acid (Hancock & Zografi, 1994). The moisture contents of the lyophiles used were assumed to be roughly equivalent.

3.3.10 Dynamic Vapor Sorption

Lyophiles for dynamic moisture sorption analysis were first prepared in 20 mL glass scintillation vials as described above. After the solids were fully dissolved, 2 mL of the ascorbic acid/PVP solution was transferred onto 30 mm aluminum pans and lyophilized using the same program as all other lyophiles. After lyophilization, the resultant lyophiles all had a uniform surface area, shape, and weight of about 185 mg.
Lyophiles were stored in Dri-Rite™ at 20°C for one day before beginning the moisture analysis experiment.

Lyophiles in their 30 mm aluminum pans were placed inside an SPS Dynamic Vapor Sorption Analyzer (Projekt Messtechnik, Ulm, Germany) to generate their moisture sorption profiles at 25°C. The moisture sorption program began at 5% RH and increased up to 90% RH in 5% RH increments with a maximum RH step time of five hours or an equilibrium condition of .01%dm per 15 minutes. The final %dm at each RH condition was taken to be the equilibrium moisture content of the lyophile, which was then used to plot the moisture sorption profile of the lyophile.

The Guggenheim-Anderson-DeBoer (GAB) equation:

\[
m_{w} = \frac{m_{0}CKa_{w}}{(1 - Ka_{w})(1 - Ka_{w} + CKa_{w})}
\]  

(21)

was used to calculate the monolayer values for the lyophiles given their experimental moisture sorption profiles (Andrade, 2011). Non-linear regression (MATLAB, Natick, MA) was used to optimize the two constants \(c\) and \(k\).

The differences in moisture sorption between lyophiles were analyzed with a linear model. The moisture sorption of a lyophile was assumed to equal the weighted average of the moisture sorption of lyophilized PVP plus some deviation, represented by phi:

\[
m_{\text{blend}} = cm_{\text{PVP}} + \varphi
\]

(22)

where \(m = \% \Delta \text{weight}\)

The variable phi represents the moisture sorption effect of amorphous ascorbic acid in the lyophile with no assumption as to its predicted value. In another sense, phi equals the
difference in %dm between the ascorbic-PVP solid dispersion and pure lyophilized PVP corrected for the weight of PVP replaced with ascorbic acid.

3.3.11 Statistical Analysis

Lyophiles formulated at 14 different weight ratios of ascorbic acid and stored at 60°C and 11% RH were prepared in triplicate in order to perform statistical analysis on gravimetric and chemical degradation measurements. Single-variable ANOVA using Minitab 16 (Minitab Inc; State College, PA) was performed for each lyophile composition at each time point of analysis: at 4 and 29 days for gravimetric analysis and at weeks 1, 2, and 4 for chemical analysis. A significance level of $\alpha = .05$ was used in all cases.

3.4 Results and Discussion

3.4.1 Lyophile Appearance

Lyophilization of ascorbic acid and PVP (hereafter AA/PVP) solutions across the full range of AA/PVP weight ratio produced lyophiles of varying physical structure (Figure 9).
Figure 9 – Photographs of ascorbic acid and PVP lyophiles across a wide range of vitamin to polymer ratios. Photographs were taken immediately after lyophilization (week 0) and after one, two, and four weeks of storage at 60°C and 11% RH.

Ratios of AA/PVP from 1% w/w up to 70% w/w produced crisp, uniform white cakes.

Above 70% w/w AA/PVP, lyophiles appeared collapsed and took on distinct structures.

Color development appeared in some lyophiles after storage for one week at 60°C and 11%RH. At AA/PVP ratios past 80% w/w, lyophiles developed light yellow hues. The lyophile at 70% w/w collapsed after one week and also developed a light yellow color. Lyophiles at 60% and 50% peeled away from the sides of the vials but did not collapse after one week.

After two weeks of storage, yellow hues developed in all lyophiles above 20% w/w AA/PVP. The lyophile at 60% w/w collapsed after two weeks of storage similar to the 70% lyophile at week one. The lyophile at 50% continued to peel away from the sides of the glass vial. Below 20%, lyophiles developed light pink hues as seen in Figure 10 that are hard to see in the previous photographs because of the strong overhead light.
Figure 10 – Photographs of lyophiles formulated at varying ratios of ascorbic acid to PVP. From left to right, ratios at: 50, 40, 30, 20, 10, 5, and 1% AA/PVP. The three samples on the right developed a slight pink color different from the yellow colors developed in samples with greater amounts of ascorbic acid.

Four weeks of storage intensified the yellow hues in lyophiles above 20% w/w AA/PVP. Lyophiles at 100% w/w AA/PVP (i.e., pure ascorbic acid) and 99% developed brown hues. The lyophile at 50% shrank but did not collapse like the lyophiles at 60% and 70% w/w AA/PVP.

3.4.2 Lyophile Physical State

Two methods, PXRD and PLM, were compared in their ability to determine any crystalline ascorbic acid content in lyophiles immediately after lyophilization. Under polarized light at 100x magnification, lyophiles at low weight ratios of AA/PVP were clear and lacked any birefringence (Figure 11).
Figure 11 – Ascorbic acid and PVP lyophiles under polarized light and 100x magnification. The lyophile at 70% AA appeared PLM crystalline, whereas the 60% lyophile appeared PLM amorphous.

Their appearance was virtually identical to that of lyophilized PVP with no added ascorbic acid. Lyophiles with this appearance were considered PLM amorphous.

Lyophiles at 60% w/w AA/PVP and below were all PLM amorphous. The lyophile at 70% w/w, despite having formed a cake, displayed birefringence, as did lyophiles at all higher weight ratios. Their appearance was virtually identical to that of lyophilized ascorbic acid with no added PVP, so they were considered PLM crystalline.

When analyzed with PXRD, samples at ratios of 70% w/w AA/PVP resulted in an amorphous halo with an absence of any peaks (Figure 12). These samples were considered PXRD amorphous. At ratios of 80% and above, lyophiles displayed peaks that coincided with those of lyophilized ascorbic acid in the absence of PVP as well as crystalline ascorbic acid standards. These lyophiles were considered PXRD crystalline.
Formulation across the entire range of AA/PVP ratio yielded both amorphous and recrystallized ascorbic acid. Physical characterization of lyophiles with PXRD and PLM led to two main observations: 1) lyophiles of up to 60% w/w AA/PVP were amorphous after lyophilization while lyophiles at 70% and above were at least partially crystalline, and 2) four weeks of storage at 60°C and 11%RH did not lead to any detected ascorbic acid recrystallization in amorphous lyophiles. Considered alongside the physical shapes of the lyophiles, it appeared that ascorbic acid remained amorphous so long as the lyophilized cake maintained its structure. Cake collapse was a rough visual indicator of ascorbic acid recrystallization, though obviously not a reliable method to determine the physical state.
3.4.3 Chemical Stability During Storage at 60°C and 11% RH

The ascorbic acid and PVP lyophiles that were physically characterized during storage at 60°C and 11%RH were also analyzed with HPLC to track chemical loss over time. The high temperature/low relative humidity storage condition in this study was chosen based on preliminary data collected on ascorbic acid/PVP lyophiles stored under milder conditions and analyzed with a UV-Vis plate reader assay. Based on those results, amorphous AA/PVP lyophiles at 50% w/w AA/PVP showed no loss of ascorbic acid over time at 40°C and 23% RH after 10 weeks of storage (Christina, 2014). Higher relative humidity conditions led to ascorbic acid recrystallization for those lyophiles, so a hotter and drier condition was chosen in the hope of observing degradation at 50% w/w AA/PVP without ascorbic acid recrystallization.

Lyophiles were analyzed for ascorbic acid loss after one week, two weeks, and four weeks of storage at 60°C and 11%RH. Physical blends of ascorbic acid and PVP were also stored under the same conditions and analyzed after one month of storage. The combined results of the three time points of analysis for all lyophiles are reported in Figure 13. The x-axis denotes the weight ratio of ascorbic acid to PVP in the lyophile and aligns with the axis of the lyophile photographs in Section 4.3.1. The shading separates the lyophiles with crystalline ascorbic acid (left) from those with amorphous ascorbic acid (right).
Figure 13 – The loss of ascorbic acid during storage at 60°C and 11% RH in all formulated lyophiles after one, two, and four weeks of storage. Lyophiles with crystalline ascorbic acid did not lose a significant amount of ascorbic acid relative to each other at any time of analysis. Degradation occurred fastest in lyophiles with low ratios of ascorbic acid.

After one week of storage, nearly all amorphous AA/PVP lyophiles lost a significant ($p < .05$) amount of ascorbic acid relative to any recrystallized lyophiles. Only the amorphous lyophiles at 50% and 60% w/w AA/PVP resulted in no significant loss of ascorbic acid relative to the crystalline lyophiles. After two weeks, the 50% w/w lyophile did lose a significant amount of ascorbic acid, but the 60% lyophile did not. After four weeks of storage, the 60% w/w AA/PVP lyophile still did not lose a significant amount of ascorbic acid relative to the recrystallized lyophiles. Physical blend controls of ascorbic acid and PVP at the same ratios showed no statistically significant difference in ascorbic acid loss after one month of storage regardless of AA/PVP weight ratio. Recrystallized
lyophiles showed no significant differences in loss between themselves at any time point of analysis.

In the amorphous lyophiles, ascorbic acid loss increased with decreased ratios of ascorbic acid to PVP. The average rate of loss also increased with decreased ascorbic acid. The lyophile with 1% w/w AA/PVP lost 85% of its ascorbic acid after the first week of storage. After two weeks, essentially all of the ascorbic acid had been lost.

In summary, the chemical stability of amorphous ascorbic acid increased with greater weight ratios of ascorbic acid to PVP. Lyophiles with recrystallized ascorbic acid showed no significant differences in loss over time, and physical blend controls lost no significant amount of ascorbic acid after one month of storage. While dilute ascorbic acid dispersions proved very unstable, dispersions with high weight ratios of ascorbic acid to PVP proved surprisingly stable given the high temperature storage conditions. These results confirm the preliminary findings from our lab that recorded no loss of amorphous ascorbic acid after 10 weeks of storage at 40°C and 23% RH (Christina, 2014).

A possible explanation for the trend of high losses at low ascorbic acid contents could be due to the presence of trace oxidative species that occur in PVP at concentrations of roughly 200 ppm as an impurity from its manufacturing process (Waterman, Adami, & Hong, 2004). The relative ratio of oxidative impurities from PVP would be greatest in lyophiles at the lowest ascorbic acid weight ratios. However, the molar amount of ascorbic acid degradation in even the 1% w/w ascorbic acid lyophile far exceeds the reported amount of oxidative degradation caused by PVP impurities and would require their concentration to be at least two orders of magnitude greater than the reported values (Hartauer et al., 2000; Laicher, Junger, & Klemm, 1997). Although
oxidative impurities may promote ascorbic acid oxidation, their presence cannot explain the magnitude of ascorbic acid loss observed in the formulated lyophiles at even 1% weight ascorbic acid and even more so at higher weight ratios.

A kinetic model for the degradation of crystalline drugs as a function of excipient dilution was recently published by Waterman et al. whereby degradation increases as a function of the surface area contact of crystalline small molecule to excipient molecule (Waterman, Gerst, & Dai, 2012). If a similar concept of surface area contact between small molecule and excipient is applied to the case of amorphous ascorbic acid and PVP, the degradation rate would be expected to increase at higher ratios of surrounding molecules (or monomeric units) of PVP, which occur in lyophiles with low concentrations of ascorbic acid. At high weight ratios of ascorbic acid, the surface area or neighboring interaction of ascorbic acid to PVP would decrease and potentially result in greater ascorbic acid stability.

3.4.4 Glass Transition Temperature of Lyophiles

The onset glass transition temperatures of select amorphous lyophiles of varying AA/PVP weight ratios are reported in Table 3.

<table>
<thead>
<tr>
<th>Lyophile Composition</th>
<th>Onset T$_g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>105</td>
</tr>
<tr>
<td>10% AA</td>
<td>87</td>
</tr>
<tr>
<td>20% AA</td>
<td>78</td>
</tr>
<tr>
<td>30% AA</td>
<td>67</td>
</tr>
<tr>
<td>40% AA</td>
<td>53</td>
</tr>
</tbody>
</table>
After lyophilization, the lyophiles were stored over Dri-Rite™ at 20°C for three days before DSC analysis. First scan values are reported because the glass transition events of the second scan were very weak. The experimentally obtained onset glass transition temperature for pure lyophilized PVP obtained during the second scan, 134°C, agrees with the reported values for PVP K40 (Lu, Ewing, Gatlin, Suryanarayanan, & Shalaev, 2009). Ascorbic acid acted as a low molecular weight plasticizer when replaced with PVP in the lyophiles; the glass transition temperatures of the solid dispersions decreased as the weight ratio of ascorbic acid increased.

Experimentally determined glass transition temperatures were fitted to the Gordon-Taylor equation using non-linear regression in order to calculate the theoretical glass transition temperature of ascorbic acid. The Gordon Taylor model (adjusted \( r^2 = 0.98 \)) predicted the glass transition temperature of ascorbic acid to be 3.1°C, ten degrees away from our previous estimate of -7.1°C (Christina et al., 2015).

The glass transition temperature of an amorphous solid has often been considered as a factor in the chemical stability of small molecules, both in pure solids and in amorphous solid dispersions (Hancock & Zografi, 1997). In the glassy state, molecular mobility is thought to slow down enough to inhibit chemical degradation, which in most cases requires an appreciable amount of molecular mobility depending on the degradation mechanism. In practice, chemical stability has sometimes, but not always, been linked to glass transition temperature. An important distinction concerning the molecular mobility of glasses is the heterogeneity of the amorphous matrix and the molecular mobility of each component in the solid dispersion (Ohtake & Shalaev, 2013; Yoshioka & Aso, 2007). The molecular mobility of water in PVP as affected by the \( T_g, W_g, \) and \( M_w \) (the
BET monolayer value) has been studied using proton and carbon-13 NMR and translational diffusion techniques (Oksanen & Zografi, 1993). The translational diffusional coefficient and effective $T_1$ relaxation times for water made no sudden or discontinuous changes before or after any of the three physical parameters of PVP ($T_g$, $W_g$, and $M_w$). Even below the BET monolayer value water remained readily mobile, with a calculated ability to move a distance of one millimeter in less than one second.

In this study, the glass transition temperature of AA/PVP lyophiles did not predict the stability of ascorbic acid in the amorphous solid state during storage at 11% RH and 60°C. Ascorbic acid stability increased at lower glass transition temperatures. Even fully amorphous lyophiles stored above their glass transition temperatures (40, 50, and 60% w/w AA/PVP) exhibited far less degradation than those stored below their glass transition temperatures (30% w/w AA/PVP and below). These results may be due to the free mobility of water in the lyophiles together with the very limited molecular mobility of ascorbic acid necessary to hydrolyze or oxidize the ascorbic acid lactone structure. The differences in moisture sorption and water content observed between lyophiles supports this hypothesis.

### 3.4.5 Moisture Sorption of Lyophiles During Storage

An important difference between lyophiles was the amount of moisture that they gained and lost during storage at 11% RH and 60°C. Any changes in weight during storage were attributed to either gains or losses in moisture. Ascorbic acid degradation could possibly result in release of carbon dioxide as well as water from downstream degradation products (Limacher et al., 2007; Shephard, Nichols, & Braithwaite, 1999b).
However, had these losses been significant enough to detect with gravimetric means, lyophiles with substantial amounts of amorphous ascorbic acid degradation after one month of storage (30, 40, and 50% w/w AA/PVP) would have lost up to 10% of their total weight in water or carbon dioxide (assuming only stoichiometric loss of one water or CO$_2$ molecule per ascorbic acid molecule). Observed losses in weight were also statistically insignificant for lyophiles that contained ten times the amount of ascorbic acid (1% versus 10% w/w AA/PVP, for example). Given these calculations, the observed losses in moisture during storage of roughly 1 to 2% weight for the stored lyophiles were attributed to moisture desorption. Similar moisture gains followed by losses have been observed during the equilibration of maltodextrins in saturated salt solution desiccators over 21 days of storage at 25°C at 23% and 33% RH conditions (Dupas-Langlet et al., 2016). After four days of storage, all lyophiles either gained weight from moisture uptake or maintained their post-lyophilization weights (Figure 14). Lyophiles composed mostly or entirely of recrystallized ascorbic acid predictably maintained their initial weights. Amorphous lyophiles, on the other hand, or recrystallized lyophiles with at least 70% weight ascorbic acid, gained moisture after four days of storage. Weight gain increased with higher amounts of PVP, with up to about 3% gains in weight after four days of storage.
Figure 14 – Change in weight of lyophiles after four and 29 days of storage at 60°C and 11% RH. Lyophiles initially absorbed moisture but desorbed moisture after extended storage. While lyophiles at 30% w/w ascorbic acid or greater returned to their initial post-lyophilization weights, lyophiles at 20% w/w ascorbic acid or less retained a net gain in moisture of about 2% total weight.

Continued storage at 11% RH and 60°C led not to further gains in moisture but to losses – all lyophiles lost weight after 29 days of storage. Lyophiles composed mostly of ascorbic acid (greater than 80% w/w AA) lost about 1% weight. At AA/PVP ratios between 70% and 30%, lyophiles returned to their initial post-lyophilization weights. In other words, these lyophiles gained about 2% weight moisture in the early stages of storage and then lost that moisture over time. Lyophiles at 20% AA/PVP and below,
composed mostly of PVP, also lost moisture but still retained a net increase in moisture after 29 days of storage.

When the moisture sorption data is considered together with the chemical stability of the respective lyophiles, the result is that the least chemically stable lyophiles gained and retained the most moisture during storage. Given that ascorbic acid degradation proceeds through a hydrolysis step, increased moisture content may have contributed to the increased degradation of ascorbic acid in low AA/PVP weight ratio lyophiles.

3.4.6 Moisture Sorption Profiles

The moisture sorption profiles of AA/PVP lyophiles at weight ratios of 10, 20, 30, 40, and 50% were generated at 25°C along with a pure PVP control in order to compare the differences in moisture sorption across a much wider range of relative humidities than one storage condition. Samples were lyophilized and exposed to moisture conditions in the same sample pans in order to generate precise sorption profiles that consistently differentiated the lyophiles at each relative humidity and moisture content as seen in the figure below.

The moisture sorption profiles all resulted in Type III moisture sorption isotherms (Figure 15). Lyophilized PVP absorbed the greatest amount of moisture at all relative humidity conditions. As the ratio of PVP in lyophiles decreased, so did their moisture sorption at any given RH.
Figure 15 – Moisture sorption profiles generated at 25°C for ascorbic acid and PVP lyophiles at varying weight ratios of AA to PVP. Moisture sorption decreased with added ascorbic acid.

Moisture sorption profiles for the 30%, 40%, and 50% AA/PVP lyophiles displayed a small point of inflection at RHs between 50% to 70% caused by recrystallization of ascorbic acid during the experiment (as confirmed with PLM after the experiment). The trend towards lower moisture sorption with greater ascorbic acid content seen in the sorption profiles in Figure 15 followed that of the one month gravimetric study during storage at 11% RH and 60°C. At the relative humidity condition of 60%, lyophiles at 10, 20, and 30% w/w AA/PVP differed in percent weight change by about 6% weight.

Thanks to the high instrumental precision and experimental setup, the GAB model produced very good fits for all of the moisture sorption profiles, with adjusted r-squared
values of 0.995 or higher. As a result, the calculated GAB monolayer values were significantly different \( (p < .05) \) between all of the solid dispersions that did not recrystallize during the experiment (Table 4). As expected from plots of the moisture sorption profiles, PVP had the highest monolayer value of 0.177. Monolayer values decreased with added ascorbic acid down to .045 for 50% w/w AA/PVP lyophiles.

Table 4 – GAB Monolayer values for select lyophiles at varying weight ratio of ascorbic acid to PVP. Values with different superscript letters are significantly different from each other as calculated from the non-linear regression model.

<table>
<thead>
<tr>
<th>Lyophile Composition</th>
<th>GAB Monolayer Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>.177(^a)</td>
</tr>
<tr>
<td>10% AA</td>
<td>.113(^b)</td>
</tr>
<tr>
<td>20% AA</td>
<td>.080(^c)</td>
</tr>
<tr>
<td>30% AA</td>
<td>.060(^d)</td>
</tr>
<tr>
<td>40% AA</td>
<td>.046(^d)</td>
</tr>
<tr>
<td>50% AA</td>
<td>.045(^d)</td>
</tr>
</tbody>
</table>

Together, the GAB monolayer values and the moisture sorption profiles of the solid dispersions indicate that amorphous ascorbic acid exhibits less hygroscopic behavior than PVP. The amorphous ascorbic acid present in lyophiles dominated by PVP (less than 20% weight ascorbic acid) was likely exposed to more water than the lyophiles dominated by ascorbic acid. This increased hygroscopicity in high-PVP lyophiles may have created more favorable conditions for the lactone hydrolysis of ascorbic acid that resulted in greater ascorbic acid loss.

The results from the gravimetric experiment and GAB modeling quantify the behavior of the solid dispersions as a whole. Another approach is to consider the moisture sorption of the dispersions as a combination of moisture sorption from each individual ingredient, in this case ascorbic acid and PVP. An additive linear model of the moisture
The sorption of each amorphous component is the simplest approach, although more complex models exist (Crowley & Zografi, 2002; Rumondor, Konno, Marsac, & Taylor, 2010). An additive linear model assumes a linear addition of the moisture sorption of each amorphous ingredient weighted by the weight ratio of each ingredient in the dispersion or blend. Deviations from the linear model in amorphous solid dispersions can be interpreted as the result of intermolecular interactions between each amorphous ingredient that then change the way the dispersion absorbs and interacts with water (Zhang & Zografi, 2001).

Since ascorbic acid cannot be easily vitrified in the absence of a polymer, a moisture sorption profile of its pure amorphous form cannot be experimentally generated. An alternative approach was developed to work around this experimental challenge. The linear model from Section 4.2.9 represents the moisture sorption of amorphous ascorbic acid with the variable phi. The sign and magnitude of phi indicates the deviation from additive moisture sorption in the solid dispersions. Positive values indicate potentially additive moisture sorption, whereas zero or negative values indicate highly non-additive moisture sorption.

An example of how the value of phi is calculated at each RH to generate a plot is diagrammed in Figure 16.
Figure 16 – Plots of moisture sorption profiles of 10% ascorbic acid:PVP lyophile, PVP lyophile, and the theoretical weighted sorption of PVP in the AA/PVP lyophile. In this case, since the sorption curve of the solid dispersion is less than that of the weighted PVP, the value of phi is negative at all relative humidities.

The moisture sorption profile of a solid dispersion of ascorbic acid and PVP, in this case 10% w/w AA/PVP, can be plotted alongside the moisture sorption profile of PVP alone. Since the dispersion contains 10% weight ascorbic acid, the predicted linear moisture sorption contribution of PVP would be \(0.9 m_{PVP}\) (also plotted in Figure 16). In this case, the moisture sorption profile of the 10% w/w AA/PVP dispersion falls below the predicted sorption profile of \(0.9 m_{PVP}\). The difference between the two curves equals phi, which in this case is negative at all RHs but changes depending on the RH.

Plots of phi for the AA/PVP lyophiles at 10, 20, 30, 40, and 50% w/w AA/PVP yielded negative values of phi for all RHs below 85% RH (Figure 17).
Figure 17 – Plots of phi for AA/PVP lyophiles of 10, 20, 30, 40, and 50% AA/PVP. The axis into the page plots each different lyophile. Phi increases in magnitude up to 30% w/w AA/PVP, after which it increases.

In other words, amorphous ascorbic acid displayed highly non-additive moisture sorption in dispersions with PVP. Negative values of phi indicate that amorphous ascorbic acid actually decreased the hygroscopicity of PVP, possibly as a result of strong hydrogen bonding occurring between PVP and ascorbic acid that could reduce the available hydrogen bonding sites for water (Christina et al., 2015; Rumondor et al., 2010).

Also apparent from the plots of phi is that the deviation from ideal moisture sorption varied between lyophiles and displayed a consistent trend. At any given RH, a cross section of the phi plot resulted in a U-shaped pattern across AA/PVP weight ratio. The greatest negative value occurred in the lyophile at 30% w/w AA/PVP. At this ratio,
the solid dispersion may have reached a maximum amount of ascorbic acid/PVP
intermolecular interaction. Ratios above 30% w/w AA/PVP resulted in decreased
magnitudes of phi that trended back towards zero. At 40% w/w AA/PVP and above, the
solid dispersions likely contained greater amounts of ascorbic acid/ascorbic acid
intermolecular interactions. Perhaps because of the stronger hydrogen bonding of these
interactions, or weaker hydrogen bonding interactions between ascorbic acid and water,
dispersions with high amorphous ascorbic acid content absorbed less moisture than those
with greater amounts of PVP.

Conclusions drawn from the moisture sorption profiles of the lyophiles can be
related to the physical and chemical stability of the lyophiles stored at 11% RH and 60°C.
The moisture sorption profiles of the lyophiles confirmed the results from the desiccator
storage study. With increasing ratio of amorphous ascorbic acid, dispersions absorbed
less moisture decreased in hygroscopicity.

3.5 Conclusion

The purpose of this study was to understand the importance of the ascorbic acid to
PVP weight ratio in formulating amorphous ascorbic acid solid dispersions. Changes to
the AA/PVP weight ratio significantly affected the chemical stability of amorphous
ascorbic acid, the glass transition temperatures of the lyophiles, and the moisture sorption
properties of the dispersions. Solid dispersions of 50% weight or more ascorbic acid
allowed the vitamin to remain quite chemically and physically stable in the amorphous
solid state at 11% relative humidity. In contrast, solid dispersions with less ascorbic acid
showed greater chemical instability at the same storage conditions. Given that the
dispersions with the smallest amount of ascorbic acid had the highest glass transition temperatures, the $T_g$ of ascorbic acid/PVP dispersions was proven not to be an important factor in the chemical stability of amorphous ascorbic acid in PVP lyophiles. The moisture sorption properties of the dispersions did coincide with trends of ascorbic acid chemical stability. Dispersions low in ascorbic acid and high in PVP sorbed and retained the greatest amount of water during one month of storage at 60°C and 11% RH. Low ascorbic acid dispersions also displayed higher GAB monolayer values closer to that of PVP than dispersions with more ascorbic acid. Dispersions at high ascorbic acid ratios, on the other hand, absorbed the least amount of water and sorbed less water than predicted from an additive moisture sorption model. These results indicate that the hygroscopicity of PVP had a deleterious effect on the chemical stability of ascorbic acid, possibly by absorbing more water into the solid dispersions that could subsequently hydrolyze ascorbic acid. As the ratio of ascorbic acid in the dispersions increased, the hygroscopicity decreased and chemical stability increased in spite of significantly lower glass transition temperatures.

The chemical stability of crystalline versus amorphous small molecules has been amply documented and was confirmed in this study for the case of ascorbic acid in PVP solid dispersions. However, less information is easily available concerning the effects of weight ratio or concentration of labile molecules in solid dispersions on their chemical stability. Given the strong effect of weight ratio on amorphous ascorbic acid stability documented in this study, the simple parameter of weight ratio should be more often considered when formulating or studying amorphous solid dispersions with chemically labile molecules. Common stability parameters such as the glass transition temperature
may not necessarily predict the stability of small molecules in the amorphous solid state when the stability of those molecules, like in the case of ascorbic acid, depends on factors that $T_g$ cannot easily represent.
CHAPTER 4. THE EFFECTS OF PRE-LYOPHILIZATION SOLUTION pH ON THE DEGRADATION OF ASCORBIC ACID IN THE AMORPHOUS GLASSY STATE IN PVP SOLID DISPERSIONS

4.1 Abstract

The pH of solutions of small molecules, proteins, and small molecule/polymer solutions prior to lyophilization has been reported to alter the chemical stability of chemically labile compounds present in the lyophile, as well as the moisture sorption properties of the lyophile during storage. In this study, the pre-lyophilization solution pH of ascorbic acid and PVP solutions was adjusted to four pH conditions (3.0, 3.5, 4.0, and 4.5) prior to lyophilization. After lyophilization, the lyophiles were characterized with powder X-ray diffraction (PXRD) and polarized light microscopy (PLM) to confirm the presence of amorphous ascorbic acid in the PVP solid dispersions. Lyophiles were further physically characterized using differential scanning calorimetry (DSC), gravimetric analysis, and dynamic vapor sorption to determine their glass transition temperatures and moisture sorption properties. Chemical degradation of the amorphous ascorbic acid present in lyophiles was carried out for one month at four temperature conditions (20, 30, 40, and 50°C) and 11% relative humidity and the loss of ascorbic acid was quantified over time using HPLC-PDA. The pre-lyophilization solution pH did not alter the $T_g$ of
lyophiles by more than 5°C. The moisture content of lyophiles at pH 4.0 and pH 4.5 were significantly higher \((p < .05)\) than those at pH 3.0 and pH 3.5. Analysis of the moisture sorption profiles of the dispersions suggested that the hygroscopicity of amorphous ascorbic acid increased with pre-lyophilization solution pH. The rate and extent of ascorbic acid degradation during storage was strongly affected by the pre-lyophilization pH; lyophiles at pH 4.5 lost roughly four times the ascorbic acid as lyophiles at pH 3.0 after five days of storage at 20°C and 11% RH.

4.2 Introduction

The pH of foods has long been used as a crucial indicator of food safety, stability, and overall quality. In the case of dry and low moisture foods, the food industry has traditionally determined the pH of foods by measuring the aqueous pH of a food slurry or paste (Bell & Labuza, 1992). The development and spread of freeze-drying as a method for stabilizing proteins and microorganisms led to observations and questions on the nature of solid-state acidity or so-called “pH-memory” (Costantino, Griebenow, Langer, & Klibanov, 1997). Despite ongoing research in the field of solid-state pH, no clear formal or mathematical definition of its meaning has been widely adopted (Govindarajan, Chatterjee, Gatlin, Suryanarayanan, & Shalaev, 2006). Nevertheless, the importance of “pH-memory,” pre-lyophilization pH, or solid-state acidity in the chemical stability of small molecules has been repeatedly proven (Hailu & Bogner, 2010; J. J. Li et al., 2002; Song, Schowen, Borchardt, & Topp, 2001).

Past research in the field of solid-state pH has focused primarily on very low moisture (i.e. “dry”) small molecule glasses (Chatterjee, Shalaev, Suryanarayanan, & Govindarajan, 2008; J. J. Li et al., 2002; Shalaev et al., 2000). Amorphous organic salts
have been shown to have higher glass transition temperatures relative to their free bases (Towler et al., 2008). However, when the pH of small molecule glasses has been modified over a narrow range, little to no change in the $T_g$ of the resultant glasses has been found (J. J. Li et al., 2002; Shalaev et al., 2000). These results imply that solid-state acidity plays a complex role in small molecule stability that is not fully reflected by the glass transition temperature. Amorphous organic salts also display higher hygroscopicity than their neutral forms. The effect appears to extend into changes in pH. Lyophilized human insulin, for example, absorbs less water with increasing pre-lyophilization solution pH (Strickley & Anderson, 1996). Thus, even partial ionization (i.e. a change in pH) seems to affect the hygroscopicity of amorphous glasses.

Amorphous solid dispersions further complicate the effects of solid-state pH because of the altered intermolecular interactions between small molecule and polymer that may result from small molecule ionization. In one of several studies on the effects of solid-state pH on amorphous sucrose inversion in glasses, Shalaev et al. compared sucrose inversion kinetics in PVP versus dextran lyophiles (Chatterjee et al., 2008). At the same pre-lyophilization solution pH, sucrose degraded more rapidly in dextran than in PVP in spite of the higher $T_g$ of dextran lyophiles. Although the pre-lyophilization solution pH was equivalent for both dispersions, the solid-state pH measured through spectrophotometric means was lower in dextran lyophiles than in PVP. Thus, in solid dispersions, medium effects and intermolecular interactions may both change as a function of solid-state pH in ways that have yet to be studied in detail.

The purpose of this study was to investigate the role of pre-lyophilization solution pH on the chemical stability of amorphous ascorbic acid in solid dispersions. Previous
work from this lab identified the solid-state degradation of ascorbic acid in lyophilized anthocyanin extracts from purple corn and black rice (West & Mauer, 2013). Further work in model food polymers, including several pectins, confirmed the ability of polymers to easily form amorphous ascorbic acid via lyophilization (Christina et al., 2015). These findings have implications for the shelf life of dehydrated foods that contain native vitamin C including freeze dried whole fruits and spray-dried juice powders, as well as for foods and dietary supplements in which ascorbic acid is an additive. As with any amorphous small molecule, the stability of the amorphous form would be expected to vary significantly from that of the crystalline form (Na Li et al., 2013).

Since pH is an easily measured and modified property in foods, and the chemical stability of ascorbic acid exhibits a well-known pH dependence (Y. C. Lee et al., 1977), this study sought to learn how the pre-lyophilization solution pH affected the stability of amorphous ascorbic acid in the glassy state. The moisture sorption properties of ascorbic acid dispersions were studied in detail because ascorbic acid degradation proceeds through a hydrolysis reaction (Limacher et al., 2007). Given the importance of both pH and water on ascorbic acid stability in solution, it was hypothesized that ascorbic acid degradation in the amorphous glassy state would mirror degradation in solution, so stability would decrease with increased pre-lyophilization solution pH. Also in accordance with recent research in solid-state pH (J. J. Li et al., 2002; Shalaev et al., 2000), the glass transition temperature of the dispersions were predicted to serve as poor indicators of the physical and chemical stability of amorphous ascorbic acid relative to their moisture sorption properties.
4.3 Materials and Methods

4.3.1 Materials

Ascorbic acid, sodium ascorbate (Acros Organics), and poly(vinylpyrrolidone) (PVP; MW ~ 40,000) were purchased from Fisher Scientific (Fisher Scientific; Waltham, MA). Lithium chloride used to create saturated salt solutions (11% RH) for storage in desiccators was purchased from EMD (EMD Millipore; Billerica, MA). Drie-Rite™ used to generate dry (< 3% RH) conditions in desiccators was purchased from W. A. Hammond Drierite Co. (Xenia, OH). Methanol and trifluoroacetic acid used for HPLC analyses were purchased from Fisher (Fisher Scientific; Waltham, MA). Any water used in sample preparation or chromatography was purified to reach a resistivity of at least 18 MΩ·cm at 25°C using a Milli-Q purification system (EMD Millipore; Billerica, MA).

4.3.2 Lyophile Preparation

All lyophiles were formulated at 10-11% weight solids in 20 mL glass scintillation vials. Buffered ascorbate lyophiles at pH 3.0, pH 3.5, pH 4.0, and pH 4.5 were prepared as follows. The Henderson-Hasselbalch equation was used to calculate the amount of ascorbic acid and sodium ascorbate necessary to reach the desired pre-lyophilization solution pH (molar ratios of ascorbic acid:sodium ascorbate equal to 12.59, 3.98, 1.26, and 0.39 for pH 3.0, pH 3.5, pH 4.0, and pH 4.5, respectively). The crystalline forms of both compounds were individually measured into 20 mL glass scintillation vials along with powdered poly(vinylpyrrolidone) (PVP). Five milliliters of water where then pipetted into each vial and the samples were vortexed to fully dissolve all of the components. The pH of the aqueous samples (i.e. the pre-lyophilization solution pH) was confirmed to fall within 0.1 pH units of the theoretically calculated pH using an Orion
SA720 pH meter (Thermo Orion, Beverly, MA). Lyophiles of ascorbic acid or sodium ascorbate followed the same procedure without calculation or measurement of the pH.

After complete dissolution, sample vials along with their upturned lids were immediately transferred onto a tray into a VirTis Genesis 25ES shelf freeze drier (SPScientific, Stone Ridge, NY). The solutions were frozen inside the freeze drier at -40°C and 400 torr for four hours. After freezing, the pressure was reduced to 300 mtorr to begin the drying cycle. The temperature increased from -25°C to 20°C in five degree increments with 300 minutes at each step. The temperature was then increased to 30°C and held for two hours to finish the drying cycle. After the drying cycle, the temperature dropped to 20°C and the vacuum was maintained until samples were removed from the freeze dryer. To determine any changes in pH as a result of lyophilization, buffered ascorbate lyophiles were reconstituted immediately after removal from the freeze drier. The solutions of reconstituted lyophiles retained the pH of the solutions prior to lyophilization to within 0.1 pH units. Lyophiles for gravimetric and chemical analysis were prepared in triplicate. Lyophiles for moisture sorption analysis differed in their preparation as described later on.

4.3.3 Sample Storage

After lyophilization, samples for chemical, gravimetric, and DSC analysis were transferred into desiccators containing a saturated lithium chloride solution to maintain the relative humidity at 11% RH. The desiccators were then stored in a temperature controlled room kept at 20°C or water jacketed incubators kept at 30, 40, and 50°C. Samples for moisture sorption profile analysis or for DSC analysis were stored in desiccators filled with Dri-Rite™ (~0%RH) at 20°C until needed for analysis.
4.3.4 Sample Photography

To document sample physical appearance over time, lyophiles were removed from storage and immediately placed inside an Elviros light box with a black background. An iPhone 6 was placed one inch above the top of the scintillation vial and the smartphone camera was used to take the photograph.

4.3.5 Polarized Light Microscopy

Polarized light microscopy (PLM) was used to determine the presence of crystalline compounds in lyophiles. A small amount of lyophile was transferred onto a glass slide and observed under polarized light at 100x magnification using an Omano OM349P polarized light microscope (The Microscope Store LLC, Roanoke, VA). Samples that lacked birefringence were considered PLM amorphous.

4.3.6 Powder X-ray Diffraction

Powder X-ray Diffraction (PXRD) was used to determine the presence of crystalline ascorbic acid or sodium ascorbate in lyophiles. To analyze samples, lyophiles were ground in their scintillation vials with a metal spatula. The resultant powder was transferred into an aluminum sample holder and smoothed out with a glass slide. Analysis was carried out with a Shimadzu LabX XRD-6000 (Shimadzu Corporation, Kyoto, Japan) equipped with a Cu-Kα source set in Bragg-Brentano geometry. The scan range was set between 5° and 35° 2θ and the scan speed was 4°/min at a 0.04° step size. The absence of any peaks above the baseline was taken to indicate the lack of crystalline content; such samples were considered PXRD amorphous.
4.3.7 Differential Scanning Calorimetry

A TA Discovery Series DSC (TA Instruments, New Castle, DE) calibrated with indium and sapphire and purged with nitrogen gas was used for thermal analysis of lyophiles. About 5 to 10 milligrams of sample were measured and hermetically sealed in aluminum Tzero pans (TA instruments). To determine the onset glass transition temperature, samples were heated at a rate of 10°C/min from 25°C to 150°C. Some samples were scanned twice to erase the thermal history, cooling from 150°C down to 25°C at a rate of 40°C/min and heating back to 150°C at 10°C/min. Onset glass transition temperatures were calculated using TRIOS software (TA Instruments) by identifying the intersection of the baseline tangent and point of inflection tangent lines.

Solid dispersions of ascorbic acid and PVP or sodium ascorbate and PVP were analyzed at three weight ratios of vitamin to polymer (10, 20, and 30% w/w) after three days of storage in Dri-Rite™ at 20°C to calculate the onset Tg of the amorphous dispersions. The Fox equation,

\[ \frac{1}{T_{g \text{ blend}}} = \frac{w_1}{T_{g 1}} + \frac{w_2}{T_{g 2}} \]  

(23)

Was then used to determine the theoretical Tg of both ascorbic acid and sodium ascorbate (Hancock & Zografi, 1994). This calculation assumed that the moisture content of the lyophiles was roughly equivalent.

4.3.8 Chemical Analysis and HPLC

Buffered ascorbate lyophiles were prepared at four different pH conditions (pH 3.0, pH 3.5, pH 4.0, and pH 4.5) for all chemical degradation studies as explained above. Degradation was tracked weekly over one month of storage at 11% RH and 20, 30, 40,
and 50°C by determining ascorbic acid content using a Waters 2690SM (Waters Corp., Milford, MA) with an XTerra reverse-phase C18 column and a Waters 2996 photodiode array detector. Standard curves ($r^2 > 0.99$) of ascorbic acid were freshly prepared before each analysis. Isocratic elution with 95% water, 5% methanol, and .025% trifluoroacetic acid separated ascorbic acid from degradation products that built up with extensive ascorbic acid loss (Assiry et al., 2003; Van de Velde et al., 2012). The flow rate was 1 mL/min and the detection wavelength was 244 nm. Lyophiles were dissolved in mobile phase and filtered through a 0.2 micron filter prior to analysis.

4.3.9 Dynamic Vapor Sorption

To prepare samples for moisture sorption analysis, sample solutions were first prepared in 20 mL scintillation vials as described above. Two milliliters of solution were then transferred into 30 mm pans and lyophilized following the protocol above. The resultant lyophiles all weighed approximately 185 mg. This preparation method minimized the variability in weight, surface area, and particle size between samples. Samples not immediately analyzed following lyophilization were stored in desiccators containing Dri-Rite™ for up to 24 hours at 20°C.

Moisture sorption profiles of lyophiles were measured using an SPS Dynamic Vapor Sorption Analyzer (Projekt Messtechnik, Ulm, Germany) at 25°C. The moisture sorption program began at 5% RH and increased to 90% RH in 5% RH steps with a maximum RH step time of five hours or an equilibrium condition of .01%dm per 15 minutes. The %dm at the end of each RH step was taken as the equilibrium moisture gain at that RH and used to plot the moisture sorption profile for the lyophile.
A linear model was used to model the differences in moisture sorption profiles between lyophiles (Equation 24). The moisture sorption of a solid dispersion was assumed to equal the weighted average of the moisture sorption of lyophilized PVP plus some deviation, represented by the variable phi:

\[
m_{\text{blend}} = cm_{\text{PVP}} + \varphi
\]

\[
where \ m = \%\Delta \text{ weight}
\]  

(24)

Phi represents the moisture sorption effect of the amorphous ascorbate in the system without any assumption as to its predicted value. In another sense, phi is simply the difference in %dm between the ascorbate-PVP solid dispersion and pure PVP corrected for the weight of PVP replaced with ascorbate buffer.

4.4 Results and Discussion

4.4.1 Physical State and Appearance of Lyophiles

To assess the ability of PVP to form amorphous solid dispersions with ascorbate buffers, solid dispersions of ascorbic acid, sodium ascorbate, and PVP were prepared across a range of ratios (100% to 1% w/w) of the vitamin to polymer. Photographs of the resultant polymers are seen below in Figure 18.
Figure 18 – Photographs of ascorbic acid/PVP and sodium ascorbate/PVP lyophiles immediately after lyophilization

At 10% w/w ascorbic acid or sodium ascorbate to polymer – the ratio used for the chemical degradation experiments – all lyophiles formed stiff, dry amorphous cakes as confirmed by both PXRD and PLM. In the case of ascorbic acid, PVP was able to form PXRD and PLM amorphous solid dispersions at ratios of up to 60% w/w ascorbic acid, and these dispersions remained amorphous after storage at 11% RH and up to 60°C for at least one month.

In contrast, amorphous dispersions of sodium ascorbate were harder to form at higher vitamin to polymer ratios. Lyophiles showed signs of collapse at ratios as low as 30% sodium ascorbate:PVP, and crystals were detected by PLM in samples containing 50% sodium ascorbate immediately after lyophilization. Although very small amounts of polymer led to sodium ascorbate recrystallization, sodium ascorbate lyophilized in the absence of polymer formed an amorphous glass that collapsed and recrystallized within minutes unless stored under dry (< 3% RH) conditions. Relative to ascorbic acid
lyophiles, sodium ascorbate lyophiles developed shades of yellow during the lyophilization process and appeared more hygroscopic. Sodium ascorbate lyophiles kept in sealed vials exposed to ambient laboratory conditions either collapsed or appeared to develop beads of moisture on their surfaces and to lose their matte appearance.

4.4.2 Moisture Sorption Profiles as a Function of pH

Although the moisture content differences between lyophiles cannot be controlled in desiccators, the differences in their moisture sorption can be compared relative to PVP in the absence of ascorbic acid. The high capacity of the SPS analyzer allowed for the generation of moisture sorption profiles at five weight ratios of ascorbate buffer to polymer (10, 20, 30, 40, and 50% w/w) and six different pH conditions (ascorbic acid, pH 3.0, pH 3.5, pH 4.0, pH 4.5, and sodium ascorbate). Plotted at any given weight ratio, higher pH lyophiles consistently sorbed more moisture (Figure 19).
Figure 19 – Moisture sorption profiles of lyophiles of ascorbic acid, ascorbate buffers at pH 3.0, pH 3.5, pH 4.0, pH 4.5, and sodium ascorbate. At 10% w/w, lyophiles with greater extent of ionization consistently absorbed more moisture at all relative humidities. The only exception to this trend occurred when lyophiles with high weight ratios of sodium ascorbate (30% w/w sodium ascorbate or greater) recrystallized during the experiment.

At the vitamin:polymer ratio used for chemical degradation experiments – 10% (w/w) ascorbate buffer – the differences in moisture gain between lyophiles were subtle at low and intermediate RHs (a few %dm). As RH increased, the differences in moisture uptake gradually increased as the ratio of ascorbate buffer increased. At 50% (w/w) ascorbate buffer seen in Figure 20, samples varied by 5 to 10%dm or more, depending on the relative humidity.
Figure 20 – Moisture sorption profiles of lyophiles of ascorbic acid, ascorbate buffers at pH 3.0, pH 3.5, pH 4.0, pH 4.5, and sodium ascorbate. At 50% w/w vitamin to polymer, all lyophiles except pH 4.0 recrystallized during the experiment. The sodium ascorbate lyophile recrystallized during lyophilization. Prior to crystallization, lyophiles at higher pH absorbed more moisture than those at lower pHs.

Any further trends in the results were hard to discern from plots of the moisture sorption profiles alone. This led to the development of a moisture sorption model to further explore the results. Past analyses of the moisture sorption behavior of drug-polymer blends have assayed several predictive moisture sorption models of varying complexity. The simplest is an additive model where the moisture sorption of an amorphous blend is set to equal the weighted average of the moisture sorption of each amorphous ingredient (Rumondor et al., 2010). Such an analysis is not possible for this
case because ascorbic acid cannot be rendered amorphous alone. Sodium ascorbate, on the other hand, forms a hygroscopic glass that recrystallizes too quickly to develop a moisture sorption profile.

These limitations led to the development of the linear moisture sorption model described in Section 3.2.8. The model continues to assume a linear additive model – i.e. that the moisture sorption of the dispersion equals the weighted average of the moisture sorption of its components. However, the moisture sorption component of amorphous ascorbic acid is replaced with the variable phi since the moisture sorption profile of amorphous ascorbic acid cannot be experimentally determined due to the acid’s strong recrystallization tendency. The sign and magnitude of phi represent the deviation from additive moisture sorption in the solid dispersions. Positive values indicate potentially additive moisture sorption, whereas negative values indicate highly non-additive moisture sorption. Plots of phi across all relative humidities for each pH at a given ascorbate buffer weight ratio yielded both positive and negative values depending on the pH.

Lyophiles of ascorbate buffer at pH 4.0, pH 4.5 or sodium ascorbate at 20% vitamin:PVP yielded positive values of phi at all relative humidities (Figure 21). The value of phi increased as the weight ratio of ascorbate in the lyophiles increased. The lyophile at 50% w/w pH 4.5 buffer to PVP recrystallized during the experiment, which resulted in the local maxima at 65% RH.
Figure 21 – A plot of phi, or the weighted difference in moisture sorption between pH 4.5 lyophiles and PVP, at five weight ratios of ascorbate buffer to polymer across all relative humidities. Positive values indicate that the pH 4.5 ascorbate buffer participates in moisture sorption.

In the plot above of phi for lyophiles at pH 4.5, phi became increasingly positive as the RH increased. Figure 22 displays a cross section of Figure 21 at 50% RH to illustrate this trend.
Figure 22 – A cross section from the plot above at 50% RH reveals a continuous increase in the sorption differences as the ratio of ascorbate buffer increased.

Positive values of phi indicate that the ascorbate buffer dispersions displayed some degree of additive moisture sorption behavior. In general, this would be the expected result, especially given the high observed hygroscopicity of amorphous sodium ascorbate. A cross section of the phi plot at any RH exhibited a gradual increase in phi as the ratio of ascorbate buffer increased (as shown in the figure above at 50% RH). Since the moisture sorption of pure sodium ascorbate is not known, there is no way to determine whether these lyophiles exhibited additive moisture sorption behavior. Nevertheless, the utility of these plots becomes clear when compared to the more acidic lyophiles.

Lyophiles of ascorbic acid or ascorbate buffer at pH 3.0 or pH 3.5 had negative values of phi at all but the highest relative humidities (Figure 23).
Figure 23 – Plots of phi, or the weighted difference in moisture sorption between pH 3.0 lyophiles and PVP, at five weight ratios of ascorbate buffer to polymer across all relative humidities. Negative values indicate that the pH 3.0 ascorbate buffer decreased the hygroscopicity of PVP.

Negative values of phi indicate not only non-additive moisture sorption but that the ascorbic acid in the dispersion decreased the hygroscopicity of PVP. The value of phi decreased as the ratio of ascorbic acid increased in the dispersions, though not indefinitely. A cross section of phi at any RH in pH 3.0 lyophiles had a U-shape with the lowest value at lyophiles of 30% w/w ascorbate buffer (Figure 24). Ratios above 30% w/w increased in hygroscopicity but remained negative.
Figure 24 – A cross section of the figure above at 50% RH reveals a distinct U-shape in the sorption differences with a minimum value at 30% w/w ascorbate buffer to polymer.

The U-shape trend persisted in lyophiles at pH 3.5 and disappeared at pH 4.0, after which the curves continually increased as the ratio of vitamin increased.

Additive moisture sorption in PVP solid dispersions has been studied for various compounds including several hydrophobic pharmaceuticals, sucrose, and trehalose (Rumondor et al., 2010; Rumondor, Wikstroem, Van Eerdenbrugh, & Taylor, 2011; Zhang & Zografi, 2001). Many of these amorphous solid dispersions sorb moisture in accordance to an additive model, whereas others sorb less moisture than predicted. In the cases of decreased moisture sorption, hydrogen bonding between the small molecule and PVP was considered as the most likely reason for the deviation from additive moisture sorption. Strong hydrogen bonding interactions between the small molecule and PVP may decrease the favorability of hydrogen bonding with absorbed water, resulting in reduced moisture sorption in the dispersion.
Based on the strongly non-additive moisture sorption of ascorbic acid and low pH ascorbate buffer dispersions, the protonated form of ascorbic acid appears to exhibit strong hydrogen bonding type interactions with PVP. The minima in phi present at all relative humidities, as seen by the U-shape in the cross section, may correspond to the saturation concentration of ascorbic acid with hydrogen bonding sites in PVP (Rumondor et al., 2010). At concentrations greater than 30% w/w ascorbate buffer, the dispersion may contain enough ascorbic acid for it to contribute a small amount of moisture sorption itself. Too much ascorbic acid, though, quickly resulted in recrystallization, as observed in lyophiles of 50% w/w pH 3.0 ascorbate buffer.

As the pH (or degree of ionization) increased, the hydrogen bonding interactions between ascorbic acid and PVP decreased in favor of hydrogen bond interactions between sodium ascorbate and other ionized ascorbate molecules or with water. Decreased interactions between sodium ascorbate and PVP result in greater moisture sorption and positive values of phi, which indicate moisture sorption behavior closer to an additive model.

4.4.3 Glass Transition Temperature of Lyophiles

The onset glass transition temperatures of ascorbic acid, sodium ascorbate, and buffered ascorbate lyophiles that had been equilibrated at 20°C in desiccators over Drie-Rite™ (< 3% RH) and 11% RH, are reported in Table 5 below.
Table 5 – Onset glass transition temperature of lyophiles over varying pH at 10% weight vitamin to PVP after lyophilization and after one month of storage

<table>
<thead>
<tr>
<th>Lyophile Composition</th>
<th>Dry $T_g$ ($^\circ$C)</th>
<th>$T_g$ ($^\circ$C) after one month of storage at 20°C and 11% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP</td>
<td>105</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>pH 3.0</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>92</td>
<td>86</td>
</tr>
<tr>
<td>pH 4.0</td>
<td>87</td>
<td>95</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>87</td>
<td>91</td>
</tr>
<tr>
<td>Sodium ascorbate</td>
<td>90</td>
<td>94</td>
</tr>
</tbody>
</table>

First scan values are reported because the glass transition event during the second scan for some lyophiles was extremely weak. Dry lyophiles all underwent the glass transition at similar temperatures; no difference in glass transition temperature between lyophiles exceeded 5°C. A sample DSC scan from the sodium ascorbate:PVP lyophile is shown below in Figure 25.
Three lyophiles at different pHs showed the same T_g of 87°C: ascorbic acid, pH 4.0, and pH 4.5. The weak, if any, relationship between pre-lyophilization solution pH and solid dispersion T_g in these results align with reports from other workers that attempted to distinguish differences in the T_g of dispersions with slightly modified pHs formulated with citric acid or sucrose (J. J. Li et al., 2002; Shalaev et al., 2000).

The lack of a change in glass transition temperature as a function of lyophile pH can be further explored by considering the glass transition temperatures of pure ascorbic acid and sodium ascorbate. Unfortunately, both of these values prove hard to experimentally obtain. Ascorbic acid cannot be vitrified without the help of a polymer, and sodium ascorbate forms an unstable hygroscopic glass that recrystallizes very quickly at room temperature. Melt-quenching the crystalline compounds in a DSC would result in chemical degradation because both ascorbic acid and sodium ascorbate degrade at their...
melting temperatures. The resultant glasses would contain substantial chemical impurities that would either increase or decrease the observed glass transition temperatures dependent on the sampling conditions (Roos et al., 2013). For example, the \( T_g \) of sucrose as determined by melt-quenching in DSC has been shown to vary by over 30°C as a function of the final heating temperature (Vanhal & Blond, 1999).

An indirect estimate of their glass transition temperatures can be calculated with the Fox equation by obtaining the glass transition temperatures of their solid dispersions at various ratios of vitamin to polymer. This approach was taken for solid dispersions of 10, 20, and 30% w/w ascorbic acid:PVP and sodium ascorbate:PVP. Each lyophile yielded one estimated value for the \( T_g \) of the pure compound, which were then averaged to obtain the best estimate for each compound. The calculated \( T_g \) values were nearly identical: -1.6°C for ascorbic acid and -2.2°C for sodium ascorbate. These results were consistent with the observed glass transition temperatures of the ascorbate buffer dispersions. Since the dispersions showed no consistent trends as the pH increased, the \( T_g \) of pure ascorbic acid or sodium ascorbate lyophiles would not be expected to vary.

Another estimate of the glass transition temperatures of ascorbic acid and its sodium salt can be calculated using the 2/3 \( T_m \) rule, an empirically calculated relationship between the \( T_g \) and \( T_m \) of small pharmaceutical molecules (Fukuoka, Makita, & Nakamura, 1991). Given the similar melting points of 190°C and 218°C for ascorbic acid and sodium ascorbate, respectively, the predicted glass transition temperatures for both compounds (35.4°C for ascorbic acid and 54.0°C for sodium ascorbate) differ by about 20°C. Although the glass transition temperatures from the 2/3 \( T_m \) rule do not agree closely to our Fox equation estimates, the ratios of \( T_g/T_m \) for ascorbic acid and sodium
ascorbate using the Fox estimates are 0.59 and 0.55 – low, but in range to other reported literature values for small molecules. The $T_g/T_m$ of aspirin, for example, has been reported to be 0.59 (Fukuoka, Makita, & Yamamura, 1989).

After one month of storage at 11% RH and 20°C, the glass transition temperatures of the same lyophiles decreased in low pH lyophiles but increased in high pH lyophiles. Storage at 11% RH and 20°C for one month caused lyophiles to absorb about 4% weight water – lyophiles at pH 3.0 and pH 3.5 absorbed significantly less ($p < .05$) water than lyophiles at pH 4.0 and pH 4.5.

Given the gain in moisture content, the glass transition temperatures of the dispersions were expected to decrease. High amounts of chemical degradation in the higher pH lyophiles may explain this trend since degradation byproducts could have potentially formed polymeric compounds of high molecular weight that would increase the glass transition temperature of the dispersion (Vanhal & Blond, 1999). In lyophiles at pH 4.5, for example, less than 10% of the ascorbic acid remained after one month of storage. Pigmented polymeric compounds present in these dispersions may have affected the rise in their glass transition temperatures.

4.4.4 Chemical Degradation of Amorphous Ascorbate Dispersions

The normalized degradation kinetics of buffered ascorbate lyophiles stored at 20°C and 11% RH are plotted in Figure 26.
Figure 26 -- Normalized loss of ascorbic acid in buffered lyophiles at pre-lyophilization solution pHs of pH 3.0, pH 3.5, pH 4.0, and pH 4.5 over 22 days of storage at 20°C and 11% RH. The rate and extent of degradation increased with increasing pre-lyophilization solution pH. The pre-lyophilization solution pH strongly affected the degradation rate – higher pH lyophiles degraded more quickly than lower pH lyophiles. Degradation appeared to proceed in two phases: a fast initial loss followed by a much slower degradation. The initial period of degradation resulted in losses of about 20%, 40%, 60%, and 80% ascorbic acid at pH 3.0, pH 3.5, pH 4.0, and pH 4.5, respectively. After one month, only about 10% more loss was observed at any pH. Although loss appears to plateau, analysis after three and five months of storage (data not shown) confirmed the slow but steady loss of ascorbic acid over time. Samples stored at 30, 40, and 50°C (also at 11% RH)
followed a similar pH stability behavior. At any given temperature, lower pH lyophiles retained more ascorbic acid than higher pH lyophiles.

Normalized degradation curves at varying temperatures for each pH are plotted below.

![Normalized degradation curves of ascorbic acid in buffered lyophiles at pre-lyophilization solution pHs of pH 3.0, pH 3.5, pH 4.0, and pH 4.5 stored at 11% RH and 20, 30, 40, and 50°C for one month. Degradations exhibited a moderate temperature dependence, clearest in the pH 3.0 lyophiles.](image)

Higher temperatures, as expected, generally resulted in greater loss of ascorbic acid. At every time point of analysis, significantly more ($p < .05$) ascorbic acid degraded at 40°C than at 20°C. In half of all points of analysis, every temperature resulted in a significantly different ($p < .05$) loss of ascorbic acid. The points that resulted in no significant difference in loss may have been due in part to the variability in ascorbic acid loss that occurred during sample preparation and lyophilization, which was done separately for
each temperature condition. Immediately after removal from the freeze drier, lyophiles at pH 3.0 lost an average of 6% ascorbic acid, compared to losses of 9%, 15%, and 20% for pH 3.5, pH 4.0, and pH 4.5 lyophiles, respectively, with a standard deviation of about 3% for each pH.

The degradation mechanism of ascorbic acid, even in aqueous systems and much more so in foods, remains a complex topic that is not fully understood (Limacher et al., 2007; Smuda & Glomb, 2013). Ascorbic acid’s sensitivity to oxygen, light, pH, water, and other compounds present in foods adds complexity to its degradation pathways (Rojas & Gerschenson, 2001). In general, ascorbic acid in foods degrades primarily via the aerobic or oxidative pathway (Serpen & Gokmen, 2007; Smuda & Glomb, 2013). For example, in lyophilized grapefruit juice, oxidative degradation was confirmed by detection of dehydroascorbic acid concomitant with ascorbic acid loss during storage (Moraga et al., 2012). Of special interest to our work is the difference between the oxidation of ascorbic acid and sodium ascorbate since the ratio of both species varies with respect to pre-lyophilization solution pH. A detailed study of reductone oxidation in reference to ascorbic acid was published by Yasuo Abe et al (Yasuo Abe, 1986). Ab initio calculations and kinetic experiments indicate that oxidation proceeds most favorably from the deprotonated form of the vitamin (i.e. sodium ascorbate) since electron removal can occur from a conjugated pi system instead of a hydroxyl group. Electron removal from sodium ascorbate was calculated to require 30 kcal/mol as opposed to 188 kcal/mol for ascorbic acid (Yasuo Abe, 1986).

If the ratio of ascorbic acid to sodium ascorbate in the lyophiles studied remained equal to that in solution prior to lyophilization, pH 4.5 lyophiles contained roughly three
times the number of sodium ascorbate molecules as pH 3.0 lyophiles. Under the same assumption, since the pKa of ascorbic acid is 4.17, pH 4.5 lyophiles contained more sodium ascorbate than ascorbic acid, whereas pH 3.0 lyophiles contained more ascorbic acid than sodium ascorbate (roughly 1 in 13). The greater abundance of sodium ascorbate species together with the greater ease of oxidation of sodium ascorbate helps explain the large observed differences in chemical stability between the buffered pH lyophiles.

Pre-lyophilization pH adjustment using an organic acid or other weak acids and bases would have added complexity to the solid dispersion and potentially altered the intermolecular interactions present in the lyophiles studied (and ultimately the chemical stability). However, substantial amounts of degradation products formed during lyophile storage, and the observed trends in pH stability persisted in spite of such physical changes in the solid dispersion. Thus, pH adjustment with other compounds would be predicted to result in similar degradation results, at least in the case of PVP solid dispersions.

The lack of correlation between glass transition temperature and chemical stability in the lyophiles likely resulted from the degradation mechanism of ascorbic acid. Whether ascorbic acid or sodium ascorbate degradation is aerobic or anaerobic, no initial pathway requires substantial molecular mobility on the part of the vitamin. This may explain the lack of a relationship between chemical stability and glass transition temperature in these lyophiles. Molecular mobility of oxygen and/or water is required, yet both of these compounds remain highly mobile in the glassy state (Aso, Yoshioka, Zhang, & Zografi, 2002; Slade & Levine, 1991). Since significant molecular mobility is not necessary for oxidation or hydrolysis, stability would be expected to depend less on
the glass transition temperature. Studies of glassy state degradation of pharmaceuticals via hydrolysis as opposed to dimerization have found weak dependences of chemical stability to molecular motion for hydrolysis as opposed to dimerization (Ohtake & Shalaev, 2013). Since mainly the mobility of water is necessary for ascorbic acid degradation, stability would be expected to depend weakly if at all on the glass transition temperature. A similar lack of dependence between ascorbic acid chemical stability and $T_g$ was reported in 2007 by Adachi and co-workers by comparison of ascorbic acid degradation in six different encapsulated carbohydrate systems ranging from polysaccharides to disaccharides (Hung et al., 2007). Vitamin C degradation more than tripled in the two polymeric systems studied, soluble soybean polysaccharide and gum Arabic, in spite of those systems having glass transition temperatures at least 50°C higher than the other carbohydrate systems studied (maltodextrin, α-cyclodextrin, and two disaccharides).

Moisture content, on the other hand, may be expected to have an effect on chemical stability given that ascorbic acid degradation requires stoichiometric amounts of water for hydrolysis. A recent study of ascorbic acid degradation in lactose and trehalose lyophiles found small losses at 11% RH but much larger losses at 44% RH and 65% RH (over 50% ascorbic acid loss after five days of storage at 65% RH and 24°C) (Zhou & Roos, 2012). These results are consistent with older studies that found definite relationships between ascorbic acid degradation and water activity in model food systems during storage (Kirk et al., 1977; S. H. Lee & Labuza, 1975; Y. C. Lee et al., 1977). In this study, lyophiles at pH 4.0 and pH 4.5 that gained significantly more moisture than lower pH lyophiles also lost significantly more ascorbic acid during storage in the same
conditions. Greater moisture content may have also contributed to the chemical instability of higher pH lyophiles in this study.

4.5 Conclusion

A variable as simple as the pre-lyophilization solution pH resulted in pronounced differences in the physical and chemical stability of ascorbic acid and PVP solid dispersions. As in the aqueous state, higher pre-lyophilization solution pH decreased the chemical stability of ascorbic acid in the glassy state. These differences could not be explained by the glass transition temperatures of the lyophiles, which showed little if any differences across the studied range of pH. The most significant and persistent difference between the physical properties of the lyophiles in addition to their chemical stability was their moisture sorption behavior.

The lyophiles in this study were all equilibrated at the same relative humidity condition of 11% RH, but changes to the pre-lyophilization solution pH altered the moisture content between lyophiles. Gains in moisture content consistently increased with pre-lyophilization solution pH, and the lyophiles at pH 4.0 and pH 4.5 that gained significantly more moisture than lyophiles at lower pHs lost the most ascorbic acid during storage. The large difference in moisture sorption behavior in low versus high pH lyophiles – from anti-hygroscopic and non-additive at pH 3.0 to somewhat additive at pH 4.5 – implied the presence of strong intermolecular interactions between ascorbic acid and PVP that decreased with ascorbic acid ionization. Results from this study support past observations on the importance of the pre-lyophilization solution pH on the chemical stability of small molecule
CHAPTER 5. CONCLUSION

5.1 Summary

Small molecules in the amorphous solid state, as opposed to the crystalline state, are subject to greater physical and chemical instability during storage. In this work, ascorbic acid was lyophilized together with PVP in order to form amorphous ascorbic acid. When stored at 11% relative humidity, amorphous ascorbic acid displayed greater chemical instability than its crystalline form. The weight ratio of ascorbic acid to PVP used to create amorphous ascorbic acid was shown to be an important parameter in its chemical stability. When 50% weight ascorbic acid was used to generate a solid dispersion, the chemical stability of the amorphous form was shown to be quite high, exhibiting only about 10% loss after two weeks of storage at 60°C. In contrast, formulation at 1% weight ascorbic acid resulted in over 95% loss after two weeks. This finding explains the results of preliminary work on the chemical stability of ascorbic acid in amorphous solid dispersions of PVP, pectin, and other polymers, where no ascorbic acid loss was detected after 10 weeks of storage under several different temperature and relative humidity conditions.

The reason for greater chemical stability at high weight ratio of ascorbic acid was argued to be related to the moisture sorption properties of the solid dispersions. Since
PVP is a highly hygroscopic polymer, lyophiles with high weight ratios of PVP absorbed and retained greater amounts of moisture during storage. Amorphous ascorbic acid, on the other hand, was shown to display very low hygroscopicity because of its non-additive moisture sorption behavior in solid dispersions. Thus lyophiles with over 50% weight amorphous ascorbic acid absorbed less moisture than lyophiles richer in PVP, which likely resulted in greater retention of ascorbic acid over time.

Amorphous ascorbic acid was also shown to display high sensitivity to the solution pH prior to lyophilization. Compared to ascorbic acid lyophiles buffered to pH 3.0, lyophiles at pH 4.5 lost roughly four times the amount of ascorbic acid after 22 days of storage. As with lyophiles at different weight ratios of ascorbic acid to PVP, the highest chemical degradation was observed in lyophiles that both 1) absorbed the greatest amount of moisture, and 2) displayed the most hygroscopic moisture sorption behavior. For this reason, the chemical instability seen at higher pH was argued to be caused in part by the greater amount of water present in the dispersions to hydrolyze ascorbic acid.

The glass transition temperature of amorphous solids is often considered an important measure of the physical and chemical stability of small molecules. In the lyophiles studied in this work, the glass transition temperature did not reflect the chemical stability of ascorbic acid in the amorphous solid state. This trend was most pronounced in the comparison of lyophiles formulated at different weight ratios of ascorbic acid to PVP. In those lyophiles, the greatest degradation was observed in lyophiles with the highest $T_g$, opposite to the commonly expected result of higher stability at greater magnitudes of $T - T_g$. This result was considered as a consequence of
the degradation mechanism of ascorbic acid, which requires the molecular mobility of water but very little molecular mobility on the part of ascorbic acid.

In lyophiles buffered at different pre-lyophilization solution pHs, the glass transition temperature between lyophiles did not differ by more than 5°C. Mathematical modeling of $T_g$ values obtained from ascorbic acid and sodium ascorbate dispersions predicted similar glass transition temperatures for both the acid and the sodium salt: -1.6°C and -2.2°C, respectively. As in the experiments of modified weight ratio, the glass transition temperatures did not reflect the large differences in chemical stability caused by altered pre-lyophilization solution pH.

The results from both the weight ratio and the pre-lyophilization solution pH studies provide important practical knowledge for the formulation of amorphous solid dispersions of other small molecules including vitamins, antioxidants, and other micronutrients. The degradation mechanism of the small molecule in the dispersion must be considered alongside the expected properties of the solid dispersion, since factors that increase the degradation of one specific compound may not necessarily affect the degradation of other compounds.

5.2 Future Directions

Future work could be done to better understand the degradation mechanism of ascorbic acid in the amorphous solid state in PVP solid dispersions. A measurement of dehydroascorbic acid content during storage could provide better values for modeling the kinetics of ascorbic acid degradation in lyophiles. Formulation of solid dispersions with antioxidants could also be used to investigate the importance of oxidation on the extent of
ascorbic acid degradation in PVP. To support the conclusions from moisture sorption modeling regarding changes in intermolecular interactions between ascorbic acid and PVP, solid-state FT-IR could be used to detect changes in the hydroxyl and carbonyl group stretching of ascorbic acid that would indicate altered intermolecular interactions as a function of pre-lyophilization solution pH.

The knowledge of weight ratio on chemical stability could also be applied to amorphous solid dispersions beyond that of ascorbic acid and PVP. Similar studies with other small molecule/polymer solid dispersions could be performed as a screening process to determine the weight ratios of maximal chemical stability. A study with other water-sensitive small molecules – for example, glycosylated aromas or bioactive compounds – could be performed in order to further validate the findings from ascorbic acid/PVP solid dispersions presented in this work.

The degradation of amorphous ascorbic acid could also be monitored in whole food systems as opposed to model polymer systems in order to broaden the applications of this work and showcase the possible relevance to industrial food products. For example, lyophilized or spray-dried fruit juices could be formulated with spiked levels of ascorbic acid and monitored for ascorbic acid loss during storage. The pH of fruit juices prior to lyophilization or spray drying could also be modified to further test the importance of pre-lyophilization solution pH on ascorbic acid stability. Many of the same analyses used in this work – gravimetric experiments, physical state characterization, moisture sorption profiles, thermal profiles – could also be readily applied to such systems.
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