Vitamins C and B_1: Amorphization, Crystallization, and Vitamin Degradation

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ABSTRACT

Crystalline ingredient forms of water soluble essential vitamins C and B_1 may dissolve or melt during food production, and then re-solidify in crystalline or amorphous forms. This study compared the phase transformations, amorphization, and chemical degradation of vitamins C and B_1 in solid dispersions with polymers. Solutions containing controlled vitamin:polymer ratios were lyophilized and stored in controlled temperature and relative humidity environments. While different polymers resulted in different amorphization properties, T_gs, moisture sorption profiles, and vitamin degradation rates, the key findings from this study were: 1) vitamins were more labile when amorphous than when crystalline; 2) lower vitamin proportions in amorphous dispersions resulted in more degradation, thus most degradation was found in the dispersions with the highest T_gs; and 3) intermolecular interactions and pH influenced vitamin physical and chemical stability in amorphous solid dispersions.

***Keywords*:** solid dispersions, thiamine, ascorbic acid, vitamin stability

1 INTRODUCTION

Vitamins C and B_1 (thiamine) are among the most unstable vitamins in foods, being sensitive to heat, pH, light, oxygen, and processing and storage conditions [1-5]. These vitamins are most often distributed as crystalline ingredients, which exhibit very little vitamin degradation in environments below their deliquescence points [1, 6]. When formulating foods, these crystalline vitamin ingredients may dissolve (or melt) and then interact with a variety of polymers, enabling their solidification in the amorphous state [7, 8]. Based on these findings, it is likely that vitamins are amorphous in many low or intermediate moisture food products prepared by processes that rapidly cool or dehydrate solutions or hydrated matrices, especially when polymers are present (e.g., doughs or batters).

Amorphous solids are generally less chemically stable than their crystalline counterparts [9-11]. The degradation of these vitamins is deleterious to product quality and can lead to issues with nutritional quality, browning, labeling infractions, and off-flavor development [12-14]. There are several crystalline forms of vitamins C and B_1 commercially available (ascorbic acid, sodium ascorbate, thiamine chloride hydrochloride, thiamine mononitrate, etc.) and commonly added to foods. These forms are known to differ in both chemical and physical stability [1-5, 15, 16]. The objective of this study was to improve the understanding of the phase transformations and stability of these common vitamin forms by studying co-lyophilized vitamin:polymer matrices stored in controlled temperature and relative humidity (RH) environments.

2 EXPERIMENTAL

2.1 Materials

The vitamins used were: L-ascorbic acid (AA) and thiamine chloride hydrochloride (TClHCl) from Sigma - Aldrich Inc (St. Louis, MO), L-ascorbic acid sodium salt (sodium ascorbate, NaAsc) from Acros Organics (NJ), and thiamine mononitrate (TMN) from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ). The polymers used were: polyvinylpyrrolidone (PVP, MW ~40,000) from Fisher Scientific (Waltham, MA), and pectins (CT = citrus peel, K = esterified with potassium salt) and polyacrylic acid (PAA) from Sigma. Salts (Sigma-Aldrich) and saturated salt solutions were used to control desiccator environments at specific RHs [17] ranging from 0-75%RH. For HPLC mobile phases, trifluoroacetic acid (TFA) was purchased from Sigma- Aldrich Inc. Water was purified with a Barnstead™ E-Pure™ ultrapure water purification system (ThermoScientific, Waltham, MA), yielding Type I water with resistivity of 18.2 (MΩ·cm).

2.2 Sample preparation

Vitamin-polymer solid dispersions (VPSDs) were formulated from 1-10% wt/vol solids in solution prior to lyophilization, with vitamin:polymer ratios ranging from 0:100 to 100:0. After fully solubilizing both polymer and vitamin, prelyophilization pH values were collected for each VPSD solution with an Orion Model SA720 pH meter equipped with an Orion 9157BN Triode pH/ATC probe (Fisher Scientific, Waltham, MA). Samples were then placed into a VirTis Genesis 25ES (SPScientfic, Stone Ridge, NY) shelf freeze drier and lyophilized using the conditions described previously [10, 11].

2.3 Storage treatments

After lyophilization, samples were stored in controlled RH desiccators (0 – 75%RH) in water jacketed incubators (Forma Scientific, Inc., Marietta, OH) set to temperatures ranging from 20 to 60 °C. Samples were removed periodically for analysis to determine vitamin degradation and physical properties.

2.4 Moisture sorption profiling

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

2.5 Powder X-ray diffraction

A Shimadzu LabX XRD-6000 (Shimadzu Corporation, Kyoto, Japan) with a Cu-Kα source was set in Bragg-Brentano geometry and calibrated each day of analysis with a silicon standard. Approximately 1 gram of sample was transferred onto a PXRD aluminum slide and scanned from 5 to 40 degrees 2θ with a step size of 0.02-0.04° and a rate of 4° per minute. Crystallinity was identified by peak location and height.

2.6 Differential scanning calorimetry

Thermal analyses of the samples were conducted using a Perkin Elmer DSC 4000 (Waltham, MA) that was calibrated with indium, tin, and ice and purged with nitrogen gas. Approximately 7-12 mg of sample was weighted and sealed into aluminum DSC pans. Samples were heated from -20ºC to a temperature 20-30ºC higher than the expected T_g values at a rate of 20ºC/min, followed by cooling to -40ºC at a rate of 50ºC/min. Then, the second heating scan was applied to 150-200ºC at 20ºC/min. T_g was determined in this second heating step (where a baseline shift occurred in the endothermic direction) using Pyris software (Perkin Elmer).

2.7 High performance liquid chromatography

A Waters 2690SM (Waters Corp., Milford, MA) HPLC with a Waters Xterra RP-C_{18} column and a Waters 2996 photodiode array detector were used for vitamin quantification, following methods optimized for each vitamin previously [10, 14]. The amount of vitamin remaining was quantified by standard curves prepared prior to analysis (r^2=0.9997-1.0000).

2.8 Fourier transform infrared spectroscopy

Intermolecular interactions in VPSDs were examined by collecting spectra with a ThermoNicolet Nexus 670 FTIR (Madison, WI) that was equipped with a mercury cadmium telluride A detector, a KBr beam splitter, and a diffuse reflectance accessory, as described previously [7, 8, 11].

2.9 Statistical analysis

Data were collected in at least duplicate at all timepoints. All statistical analyses (ANOVA models and Tukey multiple comparisons) were conducted using PC SAS 9.4 (SAS Institute Inc., Cary, NC) with $\alpha = 0.05$.

3 RESULTS AND DISCUSSION

3.1 Vitamin C amorphization and physical stability

It was possible to create amorphous solid dispersions of both vitamin C forms in all polymers studied, based on PXRD diffractograms, although the minimum amount of polymer needed to amorphize vitamin C varied by polymer type and vitamin form. All vitamin C VPSDs were initially amorphous when at least 50% w/w polymer was present. The physical stability of AA VPSDs followed the order PAA > CT/K > PVP, similar to a previous report [7]. Crystallization tendencies of NaAsc VPSDs were surprisingly found to be opposite of the AA VPSDs (PVP > K > CT/PAA), a difference attributed to the deprotonation of the C_3 hydroxyl in the ascorbate molecule. The relative polymer ability to inhibit vitamin crystallization was consistent with the magnitude of peak shifts in FTIR spectra attributed to intermolecular hydrogen bonding [7]. All VPSDs that were initially amorphous remained so for at least 30 days when stored at low RHs (0 – 23%RH) but exhibited increasing crystallization tendencies as the RH increased to ≥54%RH. VPSDs with higher vitamin weight proportions exhibited faster and lower RH crystallization tendencies than the VPSDs containing lesser amounts of vitamin (as illustrated in Figure 1 by the onset of mass loss in the moisture sorption profiles and verified by PXRD analyses).

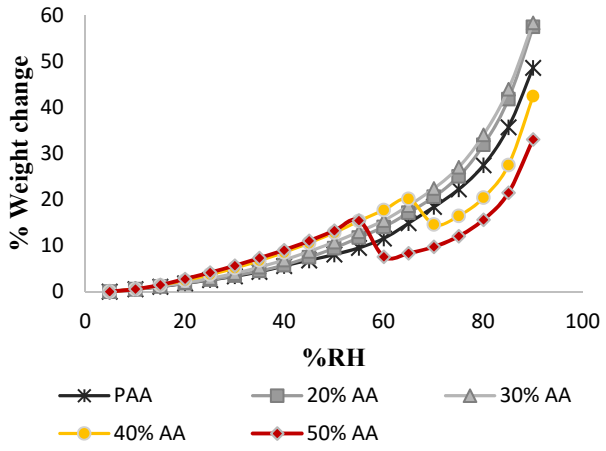

Figure 1: Moisture sorption profiles of AA:PAA vitamin:polymer lyophilized solid dispersions containing varying ratios of AA (20-50% w/w), collected at 25° C.

The glass transition temperatures (T_gs) of amorphous vitamins were expected to be less than the T_gs of amorphous polymers, due to the molecular weight differences. Based on the averaging effect of blending, the T_gs of dispersions of

miscible amorphous solids were expected to be a weighted average of the vitamin and polymer (and water) present [7, 9], as illustrated in Figure 2. The dry T_gs of the vitamins were calculated using the Boyer-Beaman rule [18] as $T_{g,AA}$ = 35.6 °C, and $T_{g,NaAsc}$ = 54.3 °C , while the dry T_gs of the polymers were >100 °C (for example, $T_{g,PVP}$ = 160 °C). The T_gs of the VPSDs were between these values. Increasing water content, such as by increasing ambient RH, is known to decrease T_gs, and crystallization often proceeds faster once the T_g falls below the storage temperature [9]. This theory can be used to explain why vitamins were less likely to crystallize from VPSDs that had lower vitamin proportions, and thus higher T_gs, and why increasing the ambient RH, which lowered T_g, resulted in vitamin crystallization from the initially amorphous VPSDs.

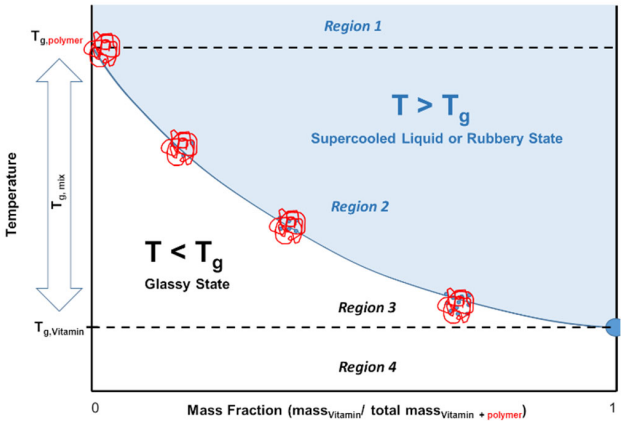

Figure 2: Illustration of formulation effects on the glass transition temperature of amorphous vitamin:polymer solid dispersions.

3.2 Vitamin C chemical stability

Polymer type, vitamin form, and vitamin weight proportion all significantly influenced vitamin C stability over time. No significant degradation occurred when vitamin C was crystalline, but vitamin C was less stable when amorphous in VPSDs. The order of chemical stability relative to polymer type for AA VPSDs was PAA > CT > K > PVP. Unlike the physical stability results, NaAsc VPSDs followed the same trend of vitamin chemical stability relative to polymer type (PAA>CT>K>PVP) (Figure 3); however, NaAsc degraded more than AA in all VPSDs excluding PAA. The more rapid degradation of NaAsc was attributed to the C_3 being in a deprotonated state, which is the first step in the oxidative degradation pathway [19]. The more acidic polymers, or those with more hydrogen bond donor groups, better stabilized vitamin C from oxidation, perhaps by maintaining a higher protonated ratio of vitamin C which created an energy barrier to degradation. Amorphous VPSDs containing a higher weight proportion of vitamin were more stable than those with a lower proportion of vitamin.

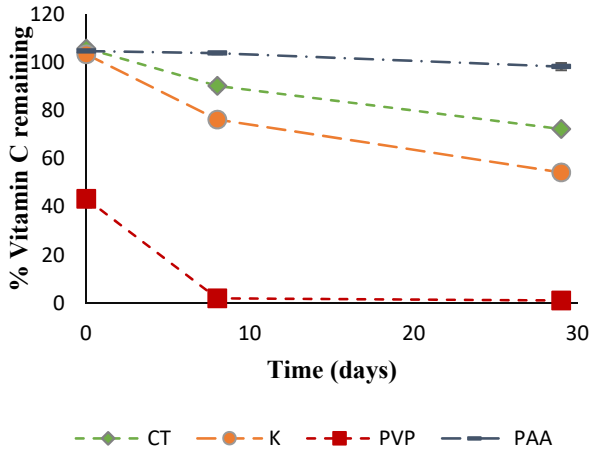

Figure 3: Effects of polymer type on vitamin C degradation in sodium ascorbate:polymer VPSDs containing 10% w/w vitamin and stored at 30°C and 11%RH.

3.3 Thiamine amorphization and physical stability

The minimum amount of polymer needed to amorphize thiamine varied more by vitamin form than what was observed in the vitamin C studies. TClHCl was amorphous in VPSDs containing ≥60% PVP or ≥40% pectins; however, ≥90% PVP (Figure 4) or ≥80% pectin was needed to amorphize TMN, indicating both polymer type and the counterion on thiamine influenced amorphization. These vitamin:polymer ratios are still well above the amount of thiamine fortified in foods, and thus in food there is potential for amorphization of either form of thiamine. Trends in physical stability compared to intermolecular interactions and T_g were consistent with those from the vitamin C studies: VPSDs with more intermolecular interaction (FTIR hydroxyl peak shifts) and higher T_gs were less likely to crystallize (data not shown). Unlike vitamin C, different thiamine polymorphs were found in VPSDs containing different polymers upon crystallization (data not shown but consistent with [8]).

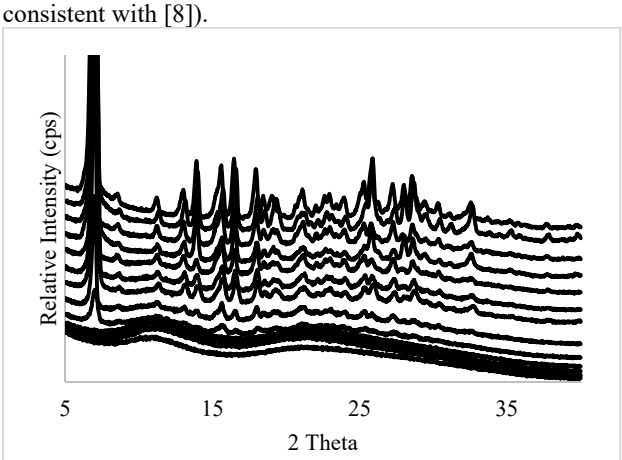

Figure 4: X-ray powder diffraction patterns of TMN:PVP VPSDs containing 1 to 100% w/w TMN (shown from 1% on bottom to 100% on top).

3.4 Thiamine chemical stability

Thiamine degradation increased as vitamin proportion in the amorphous VPSDs decreased, and thiamine was more labile when amorphous than when crystalline (Figure 5). Amorphous TMN VPSDs were often less stable than TClHCl VPSDs, attributed to higher solution pHs in the TMN systems. In solution, thiamine is less stable when the pH exceeds the pKa [14], which seems to extend to stability trends in amorphous thiamine solid dispersions. For all forms of vitamins C and B_1 investigated, it appeared that intermolecular interactions with polymers influenced physical stability, while physical form, matrix pH, and vitamin proportion had greater effects on vitamin degradation. VPSDs that best inhibited vitamin crystallization resulted in the most vitamin degradation.

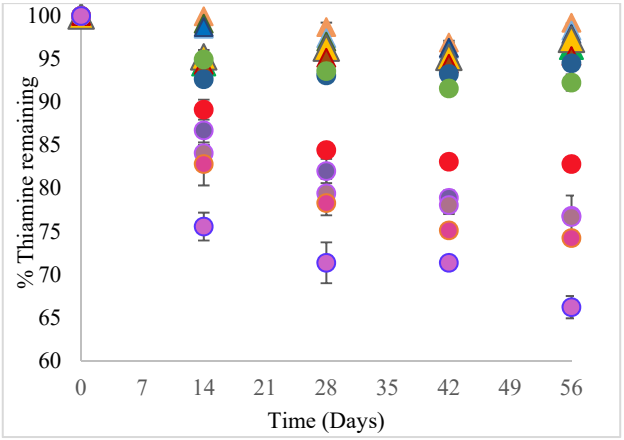

Figure 5: Chemical stability of TMN:PVP VPSDs containing 1 to 100% w/w TMN after storage at 11%RH and 60C for 56 days. From bottom to top the samples contain 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% w/w TMN in PVP. The circles demark amorphous VPSDs, while TMN was crystalline in samples marked with triangles.

4 CONCLUSION

While different polymers resulted in different T_gs, moisture sorption profiles, amorphization and crystallization trends, and vitamin degradation rates in the VPSDs, the key findings from this study were: 1) vitamins C and B_1 were more labile when amorphous than when crystalline; 2) vitamin amorphization was found in the presence of a variety of polymers, with both vitamin form and polymer type influencing the minimum amount of polymer needed; 3) all vitamins degraded significantly more when present at lower amounts in the amorphous solid dispersions, thus most degradation was found in the dispersions with the highest T_gs; and 5) intermolecular interactions and pH influenced vitamin physical and chemical stability. Vitamin degradation was found in storage environments that maintained $T<T_g$ (the glassy state), which is representative of many low-moisture food products.

ACKNOWLEDGMENT

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