

Differentiating between the Effects of Water Activity and Glass Transition Dependent Mobility on a Solid State Chemical Reaction: Aspartame Degradation

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Recent debates have emerged on whether it is water activity (a_w) or the state of the system as dictated by the glass transition temperature (T_g) that impacts the rates of chemical reactions in reduced-moisture solid systems. Previously, model systems could not evaluate the effects of water activity and glass transition independently. By using poly(vinylpyrrolidone) (PVP) of different molecular weights, the effect of water activity and glass transition on chemical reactions can be studied independently and at a constant temperature. The kinetics of aspartame degradation, via its rearrangement into diketopiperazine, was evaluated in the PVP model system. Reaction rates at constant water activity, but different T_g values, were not significantly different. However, rates at a similar distance from T_g , but different water activities, were significantly different. Thus, the rate of aspartame degradation depends upon the water activity rather than upon the state of the system.

Keywords: *Glass transition; water activity; aspartame; mobility; reaction kinetics*

INTRODUCTION

Research in the area of glass transitions has increased during recent years. The applicability of glass transition principles has permeated from the area of polymer science into such areas as textiles, food science, pharmaceuticals, and biotechnology. The desire to improve the stability of foods, drugs, and biological materials is the primary reason for the increased interest in better defining the role of the glass transition and mobility in chemical reaction pathways.

The glass transition is a physical change in an amorphous material promoted by the addition of heat and/or the uptake of low molecular weight substances (i.e., plasticizers). Below the glass transition temperature, a material is in a glassy state which is described as rigid yet brittle with a viscosity on the order of 10^{12} Pa s (Ferry, 1980; Sperling, 1986; Noel et al., 1990). Mobility or diffusion in glassy systems is claimed to be virtually nonexistent (Levine and Slade, 1989; Slade et al., 1989). As the temperature increases through and above the glass transition temperature, the glassy material becomes soft or rubbery and demonstrates a decreased viscosity and an increased mobility. For example, Roozen et al. (1991) showed using electron spin resonance (ESR) that the rotational mobility of tempol increased gradually with increasing temperature in the glassy state; however, the mobility increased by over 2 orders of magnitude as the temperature passed through the glass transition temperature.

The principles of glass transition can explain some physical phenomena rather well. The textural or rheological properties of bread have been explained based on glass transition theory (LeMeste et al., 1992). Similarly, textural softening of snack products can be attributed to adsorbed moisture acting as a plasticizer (Simatos and Karel, 1988). Crystallization of sucrose

in an amorphous solid has been explained by glass transition theory; increased mobility of sucrose at the glass transition and in the rubbery state promotes rapid crystallization as compared to the glassy state (Roos and Karel, 1990). The sticking and caking of free flow powders upon humidification have also been associated with glass transition phenomena (Chuy and Labuza, 1994).

Conflicts exist as to the role of the glass transition phenomena on the rates of chemical reactions; questions arise as to whether the molecular mobility of reactants as dictated by the state of the system or the chemical potential of water (i.e., water activity) controls reaction rates. The importance of water activity versus the glass transition temperature has been the subject of much heated discussion (van den Berg, 1986; Franks, 1991; Best, 1992). It is believed that low mobility in the glassy state due to the high viscosity makes chemical reactions improbable. The applicability of this generalization to the many different mechanisms of chemical reactions is yet to be established. Glass transition theory should help explain the kinetics of reactions requiring the diffusion and collision of two molecules. For example, it has been shown that when a matrix of dense glass forms around an oxygen sensitive compound, the degradation of the compound is inhibited (Shimada et al., 1991; Labrousse et al., 1992). However, oxidation is but one mechanism of degradation and is dependent upon proper matrix formation as well as exclusion of oxygen from the matrix. Karmas et al. (1992) evaluated the rate of nonenzymatic browning as a function of the glass transition. However, their systems had different water activities and moisture contents as well, demonstrating the limitations of past experimental designs. Glass transition theory may or may not explain chemical reactions involving the molecular rearrangement of a single compound. However, as mentioned, the mobility of small compounds in the glassy and rubbery states is quite different (Roozen et al., 1991), so rearrangement reactions may be impacted by glass transition. The influence of moisture and water activity on chemical

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reactions has been widely documented (Duckworth, 1975; Labuza, 1980; Rockland and Stewart, 1981). Many reactions are dependent on water either as reactant or solvent. Thus, the current question remains as to whether reactions are governed by water activity or molecular mobility associated with glass transition or both. The objective of the current research was to systematically evaluate the rates of aspartame degradation in model systems in which the effects of water activity and glass transition can be determined independently.

METHODOLOGY

Description of Model System. Poly(vinylpyrrolidone) (PVP), a polar, water soluble polymer, was used as the inert matrix for evaluating the impact of the glass transition temperature (T_g) and water activity on aspartame degradation. Several molecular weights of PVP are available. PVP-K15 has an average molecular weight of approximately 10 000, while PVP-K30 has an average molecular weight of around 40 000. Both types of PVP were obtained from Sigma (St. Louis, MO). Bell and Hageman (1993) showed the moisture sorption isotherms for PVP-K30, PVP-K15, and a low molecular weight PVP (MW < 3500) were virtually identical (i.e., at a given water activity all three molecular weight polymers had similar moisture contents). The glass transition temperatures for these dry polymer systems were 180, 100, and 50 °C, respectively. At a given moisture content, each system will have a different glass transition temperature. Thus, at 25 °C, systems with glass transition temperatures above 25 °C are glassy, whereas systems with glass transition temperatures below 25 °C are rubbery. By evaluation of the chemical reactions in systems such as PVP in which moisture content and water activity can be maintained while the glass transition is altered via molecular weight, the independent effects of water activity and glass transition on reactions can be observed.

Preparation of Model System. The poly(vinylpyrrolidone)s (PVP-K15 and PVP-K30) were dissolved into water and neutralized to pH 7 with NaOH. The solutions were dialyzed through 3500 MWCO dialysis bags to remove low molecular weight components. The bags were placed together into a single 3 L volume of water again neutralized to pH 7 with NaOH. The dialyzed PVP and dialysate (i.e., low molecular weight PVP) were lyophilized for 4 days at 30 °C under a vacuum of less than 20 mTorr; the lyophilized material was stored over anhydrous calcium sulfate.

The lyophilized PVP-K30, PVP-K15, and PVP-LMW (MW < 3500) powders were dissolved in purified water. Aspartame was dissolved into water separately. A given volume of 0.1 M phosphate buffer at pH 7 was added to the PVP solution, such that the final internal buffer concentration after dehydration and moisture sorption would be close to 0.1 M, eliminating the different reaction rates attributed to different buffer concentrations (Bell and Labuza, 1991b; Tsoubeli and Labuza, 1991). Immediately prior to lyophilizing, the solutions of PVP and aspartame were mixed. The resultant solution was flash frozen by dripping into liquid nitrogen using a 22 gauge needle. The frozen pellets were lyophilized for 4 days at 30 °C under a vacuum of less than 20 mTorr. After drying, the pellets were stored in a desiccator containing anhydrous calcium sulfate. Table 1 lists the composition of the model systems.

Analysis and Kinetic Study. Multiple 200 mg samples were equilibrated in closed desiccators at 25 °C for 1 week over saturated salt solutions at the following water activities: 0.33 [MgCl₂], 0.54 [Mg(NO₃)₂], and 0.76 [NaCl]. At various time intervals after equilibration, samples were removed from the desiccators for analysis, with the first sample being designated time zero. The moisture contents were determined gravimetrically as moisture sorbed into the dry system. At least eight samples were analyzed to a minimum of 35% aspartame loss.

To each vial containing the 200 mg sample was added 10 mL of 0.1 M phosphate buffer at pH 4.4. After complete dissolution of the sample, 2 mL of the resulting solution was

Table 1. Composition of Model Systems (PVP with 0.1 M Phosphate Buffer, pH 7)

sample	a _w	moisture (% db)	PVP (g)	APM ^a (mg)	water ^b (g)	0.1 M buffer (μL)	concn ^c (M)
PVP-LMW	0.33	10.6	3.99	15.5	0.423	384	0.091
PVP-K15	0.33	10.1	4.03	18.0	0.407	460	0.113
PVP-K30	0.33	11.1	3.01	13.8	0.335	345	0.103
PVP-LMW	0.54	20.3	4.03	16.4	0.818	760	0.093
PVP-K15	0.54	19.5	4.02	15.5	0.784	788	0.101
PVP-K30	0.54	20.0	3.01	11.5	0.602	651	0.108
PVP-LMW	0.76	38.4	4.03	15.6	1.548	1508	0.097
PVP-K15	0.76	37.1	4.03	17.5	1.495	1456	0.097
PVP-K30	0.76	36.3	3.02	13.8	1.096	1155	0.105

^a APM, aspartame. ^b Sorbed water. ^c Internal buffer concentration after moisture sorption assuming complete dissolution in aqueous phase.

placed in a Centriscart ultrafiltration centrifuge tube (Sartorius, Bohemia, NY) with a molecular weight cutoff of 5000. The tubes were centrifuged for 30 min at 3000 rpm (1900g). The filtrate was then analyzed using high performance liquid chromatography.

The HPLC system consisted of a pump and data acquisition system (Dionex Corp., Sunnyvale, CA), an autosampler (Perkin-Elmer Corp., Norwalk, CT), an ABI UV detector (Bodman Industries, Aston, PA) set at 214 nm, and a 3.9 × 150 mm Nova-Pak C₁₈ column (Millipore, Marlborough, MA). The mobile phase consisted of 20% methanol and 80% 0.02 M aqueous sodium phosphate monobasic; the pH was adjusted to a value between 2.8 and 3 with phosphoric acid. The injection volume was 100 μL, and the flow rate was 1 mL/min.

The extraction and HPLC analysis were verified by analyzing aspartame solutions and comparing to the same solution with added PVP. Aspartame was also extracted from lyophilized PVP/aspartame mixtures and compared to the theoretically derived concentrations. The extraction procedure achieved an 81% average extractability with a coefficient of variation of 6.2%.

Aspartame has been shown to degrade via pseudo-first-order kinetics in solution and reduced-moisture solids (Homler, 1984; Prudel and Davidkova, 1981; Bell and Labuza, 1991b) and was modeled as such. The 95% confidence limits of the rate constants were calculated.

Differential Scanning Calorimetry. The glass transition temperatures for the PVP systems were determined using a Perkin-Elmer System 4 differential scanning calorimeter. Three to 8 mg of sample was hermetically sealed into aluminum DSC pans. The scan was run at 5 °C/min over an appropriate temperature range. Rescans verified the endothermic baseline shift associated with the glass transition temperature. The results were reported as the onset temperature and were consistent with those obtained previously (Karmas et al., 1992; Buera et al., 1992; Bell and Hageman, 1993).

RESULTS AND DISCUSSION

As mentioned previously, the major difficulty encountered by researchers in the water activity/glass transition area is the interdependence between water activity, moisture, and the glass transition. To shift into a different state as dictated by the glass transition temperature, one would change either the moisture content or the temperature. However, this also changes the water activity, so that the impact on a chemical reaction could be due to water activity, glass transition, or both. The poly(vinylpyrrolidone) system used in this study allows the glass transition to be altered at constant temperature without significantly changing the moisture content or water activity (Table 2). Thus, the

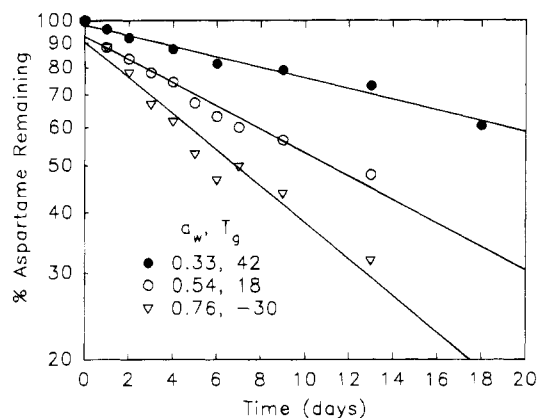


Figure 1. Pseudo-first-order plot of aspartame degradation in poly(vinylpyrrolidone) (MW < 3500) at pH 7, 0.1 M phosphate buffer, and 25 °C.

Table 2. Aspartame Stability in PVP Systems As Influenced by Water Activity (a_w), Moisture, and Glass Transition Temperature (T_g)

system	a_w	moisture (% db)	T_g (°C)	state of the system at 25 °C	rate constant with 95% CL (days ⁻¹)	R^2
PVP-LMW	0.33	10.6	42	glassy	0.026 ± 0.004	0.979
PVP-K15	0.33	10.1	52	glassy	0.017 ± 0.004	0.937
PVP-K30	0.33	11.1	63	glassy	0.021 ± 0.004	0.958
PVP-LMW	0.54	20.3	18	rubbery	0.056 ± 0.009	0.964
PVP-K15	0.54	19.5	23	at T_g	0.046 ± 0.011	0.921
PVP-K30	0.54	20.0	50	glassy	0.047 ± 0.007	0.970
PVP-LMW	0.76	38.4	-30	rubbery	0.086 ± 0.017	0.948
PVP-K15	0.76	37.1	-9	rubbery	0.090 ± 0.021	0.924
PVP-K30	0.76	36.3	0	rubbery	0.10 ± 0.02	0.934

individual effects of water activity and the glass transition temperature on chemical reactions can be deconvoluted.

This study also attempted to control the internal pH of the reduced-moisture solid because it has been shown that the initial hydrated pH is quite different from the pH in the reduced-moisture state and this pH difference affects chemical reaction rates and mechanisms (Bell and Labuza, 1991c). Phosphate buffer was added such that the final pH would be 7 and the final buffer concentration upon moisture sorption would be 0.1 M, relative to the total moisture content. The degradation of aspartame at pH 7 involves intramolecular rearrangement into the diketopiperazine (DKP) with the release of methanol (Bell and Labuza, 1991c). The carbonyl carbon associated with the methyl ester undergoes nucleophilic attack from the free amine of the aspartyl residue, yielding the cyclic DKP. This pathway of degradation requires some extent of molecular mobility and, based on the results of Roozen et al. (1991), may be dependent upon the state of the system. The other major pathway of aspartame degradation, hydrolysis of the methyl ester to produce α -aspartylphenylalanine, is negligible at pH 7 (Bell and Labuza, 1991c).

Figure 1 shows the pseudo-first-order plots for aspartame degradation in PVP-LMW (MW < 3500) as influenced by water activity. The linearity of the plots is typical for aspartame loss in the other PVP systems. The effect of water activity on the reaction is similar to that reported previously (Bell and Labuza, 1991a). Table 2 lists the pseudo-first-order rate constants with the 95% confidence limits (95% CL) as determined from the kinetic plots. As shown in this table, the rates of aspartame degradation are generally not significantly different ($p > 0.05$) at a given water activity despite the

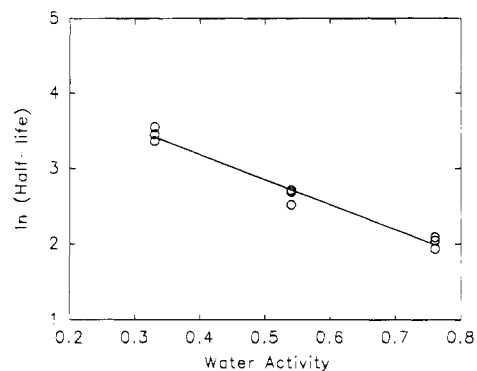


Figure 2. Q_A plot for aspartame degradation in poly(vinylpyrrolidone) at pH 7, 0.1 M phosphate buffer, and 25 °C.

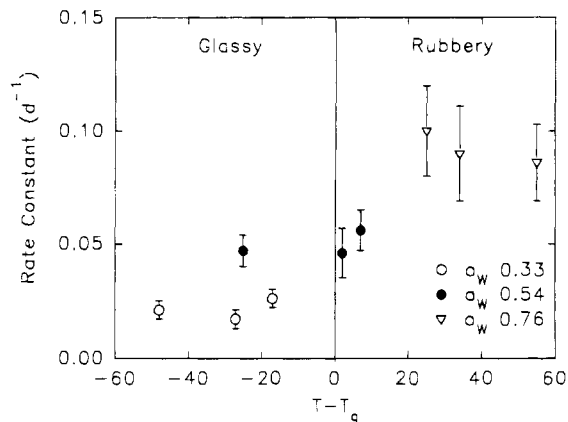


Figure 3. Rate constants for aspartame degradation as a function of the distance from the glass transition temperature.

differences in glass transition temperature. At a water activity of 0.33, it is important to note that the reaction occurs in the glassy state. Furthermore, at a water activity of 0.54, the rate of degradation is similar in both the glassy and rubbery states. However, the two systems having a glass transition temperature near 50 °C have significantly different ($p < 0.05$) reaction rates, with the faster rate occurring in the higher water activity system.

Figure 2 shows a Q_A plot for aspartame degradation, in which the natural log of the half-life is plotted as a function of water activity. The slope of this plot gives an indication of the effect water activity has on a reaction. Using the composite data, the Q_A was determined to be 1.4, which is similar to that reported previously for the degradation of aspartame (Bell and Labuza, 1991a), ascorbic acid (Lee and Labuza, 1975), and thiamin (Kamman et al., 1981). A Q_A of 1.4 means that for a 0.1 water activity increase, the rate of aspartame degradation increases by 40%. However, this type of model does not account for changes in the glass transition at the different water activities.

Roozen et al. (1991) showed the mobility of tempol, a small ESR probe, increased by over 2 orders of magnitude at the glass transition temperature. Because the intramolecular rearrangement of aspartame requires mobility, it was expected that the rate of degradation would show a dramatic increase at the glass transition temperature. Thus, a plot of the rate constant for aspartame degradation as a function of the distance from the glass transition temperature (i.e., $T - T_g$) would show a break at $T - T_g = 0$ (i.e., $T = T_g = 25$ °C). However, Figure 3 shows that the rate of aspartame degradation changes by only 4-fold on either side of the glass transition temperature, with no distinct break in

the curve, as compared to over a 100-fold deviation for the mobility of tempol.

These results suggest that the intramolecular rearrangement of aspartame appears to be more dependent on water activity than on the glass transition temperature. It is possible, however, that the free volume, even within the glassy system, exceeds that necessary for aspartame to rearrange. Hence, the free volume, and therefore mobility, as dictated by the glass transition temperature of the system is not rate limiting; rather, some factor influenced primarily by the water activity predominates. The kinetics of other reactions need to be studied to more completely differentiate between the effects of water activity and glass transition on chemical reaction rates.

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