Solid State ^{13}C NMR Investigation of Impact of Annealing in Lyophilized Glasses

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ABSTRACT: The purpose of this study was to investigate the impact of annealing on molecular mobility in lyophilized glasses, composed of a saccharide excipient and a small concentration of aspartame as a model "drug." Changes in molecular dynamics during annealing were monitored through carbon (^{13}C) T_1 and $T_{1\rho}$ nuclear magnetic resonance relaxation times of the aspartame and the saccharides. Two different saccharides were studied, sucrose and trehalose. The local mobility of the aspartame guest was found to correlate closely with the overall structural relaxation monitored through calorimetric methods in the aspartame: sucrose formulation. In general terms, annealing leads to longer NMR relaxation times, indicating a slowing of the local dynamics. By contrast, annealing had only a minimal effect on the NMR relaxation times in aspartame: trehalose. Specificity of solid state NMR in detecting molecular mobility in guest and host molecules showed that sucrose provided a homogenous matrix for the guest drug as compared to the trehalose. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:4336–4346, 2008

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INTRODUCTION

Stabilization of proteins in carbohydrate glasses is a common practice in pharmaceutics, but the critical stabilization factors are only partially understood, thereby limiting the efficient design of quality into the product. The protection of proteins against degradation is proposed to be due to the substitution of closely bound water by the saccharide,[1] and/or the slowing of the dynamics of the guest molecule in the glassy matrix.[2] This research is an investigation of the link between molecular mobility, in particular that reflected by NMR relaxation times, and pharmaceutical stability. The focus is on the impact of thermal history, or annealing, on mobility, and on stability.

According to the glass dynamics hypothesis, the molecular mobility is strongly coupled to the viscosity which is very high in glassy state. The principle of stabilization in the glassy state has been applied to small molecule systems, in addition to proteins, and the decrease in overall mobility in the glass, as quantified by the glass transition temperature T_g[3,4] and structural relaxation time τ or τ^{β},[5,6] has been correlated with the chemical stability. There have been reports of systems in which stability does not correlate with global mobility.[5,7] The reason of

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this discrepancy could be that local faster motions are most critical for instability or that some feature of the interaction of the drug with the host matrix affects stability, but yet is unrelated to dynamics, such as "structure" or phase separation. Examples that illustrate the importance of faster mobility are lyophilized horseradish peroxidase (HRP) and alcohol dehydrogenase (ADH) proteins in trehalose matrices with added small molecule plasticizers like DMSO and glycerol, where MHz–GHz dynamics measured using neutron scattering correlates with stability very well.[8–10]

Glasses are not in thermodynamic equilibrium, and contain excess volume, enthalpy and entropy. In general, glasses therefore are unstable compared to the stable crystalline and metastable supercooled liquid state, and gradually transition to lower energy states. This is known as thermal aging or annealing.[11] Annealing releases excess enthalpy and tends to slow down relaxation and mass transport processes. It is therefore plausible that annealing should stabilize the system against any change that requires molecular mobility, including chemical degradation. The broad objective of our research was to determine the impact of thermal annealing on chemical stability. In the present study, the glasses were prepared by lyophilization, which involves temperature and composition changes during freezing, and then composition changes during drying below T_g.[12] This tends to produce glasses with large amounts of excess enthalpy.[13,14] A brief account of the impact of annealing achieved through variation of the freeze drying process was given a number of years ago[15] and briefly discussed again in a review.[16] Recently, Abdul Fattah et al.[6] observed a small increase in the chemical stability as well as a corresponding increase in the structural relaxation time when Moxalactam freeze-dried with 12% mannitol was aged at various temperatures below T_g. The results are interesting since they open the perspective of enhancing the shelf-life of drugs through heat treatment.

Encouraged by these findings, we have systematically investigated the impact of thermal annealing on the chemical stability of lyophilized systems composed of a saccharide excipient and a small concentration of aspartame as a model "drug." The aim of the present work was to quantify the impact of the annealing conditions on structural relaxation, molecular mobility,[17] and chemical stability. The present article reports results on molecular mobility obtained using nuclear magnetic resonance spectroscopy, while findings pertaining to chemical stability and calorimetry will be published separately.[18]

Various techniques have been used to study local dynamics in glasses including neutron scattering,[8] NMR,[19] and thermally stimulated current (TSC).[20] While nonspecific techniques like neutron scattering and TSC usually cannot distinguish between mobility of the host and the guest species, ^{13}C NMR is a specific technique, hence making it possible to study motion in selected parts of either molecule. The model compound used in this research, aspartame, undergoes a single step intramolecular cyclization in the solid state to form 3-carboxymethyl-6-benzyl-2,5-dioxopiperazine (DKP).[21–23] In the present research, the carbons active in this degradation process were selectively labeled with ^{13}C, and the impact of thermal annealing on ^{13}C nuclear relaxation was observed on the aspartame molecule as well as the disaccharide matrix.

Solid state NMR is well established as a tool to study molecular dynamics.[24,25] Thermal vibrations lead to stochastic modulations of the nuclear spin Hamiltonian and thus drive nuclear spin relaxation processes. In particular, the spin-lattice relaxation time in the laboratory frame (T_1) and in the rotating frame ($T_{1\rho}$) are sensitive to motion processes of the order 100–500 MHz, and 20–100 kHz, respectively.[26] Several authors have applied this concept to study mobility in food[27,28] and pharmaceutical systems.[29–31] For a review of relaxation measurements in supercooled liquids and glasses, see Ref. 32. Aso et al.[33] have used ^{2}H spin-lattice relaxation to investigate the effect of mobile water on the decomposition rate of solid drugs. The same group also studied the localized molecular mobility of drug and Poly (vinyl pyrrolidone; PVP) in amorphous nifedipine—PVP and phenobarbital—PVP solid dispersions using T_1 relaxation times.[34] The authors found that T_1 of the PVP carbonyl carbon in the solid dispersions increased with the drug content, suggesting that the local mobility of the PVP carbonyl carbon was hindered by hydrogen bond interactions with the drug. Yoshioka et al.[35] recently reported a correlation between storage stability and ^{13}C $T_{1\rho}$ of freeze-dried insulin formulations. The effect of trehalose and dextran on $T_{1\rho}$ was compared at various humidity levels. The authors attributed the improvement in stability with trehalose to the inhibition of β-relaxations, as evidenced by higher $T_{1\rho}$ values.