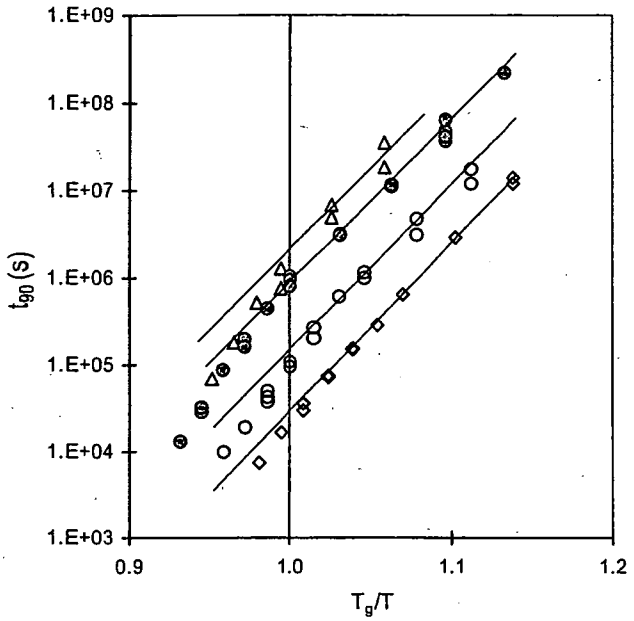


Fig. 3. T/T_g-dependence of t_{90} for aggregation of β-GA lyophilized with sucrose (△), trehalose (○●) or stachyose (◇). The weight fraction of excipient : 0.33 (△○◇) and 0.5 (●).

spectra between the sucrose, trehalose and stachyose formulations. Significant differences in spectra were not observed between the excipients, and the differences in β-GA aggregation rate observed between the excipients could not be attributed to differences in protein secondary structure. It is known that changes in the tertiary structure of protein molecules created during freeze-drying processes can lead to

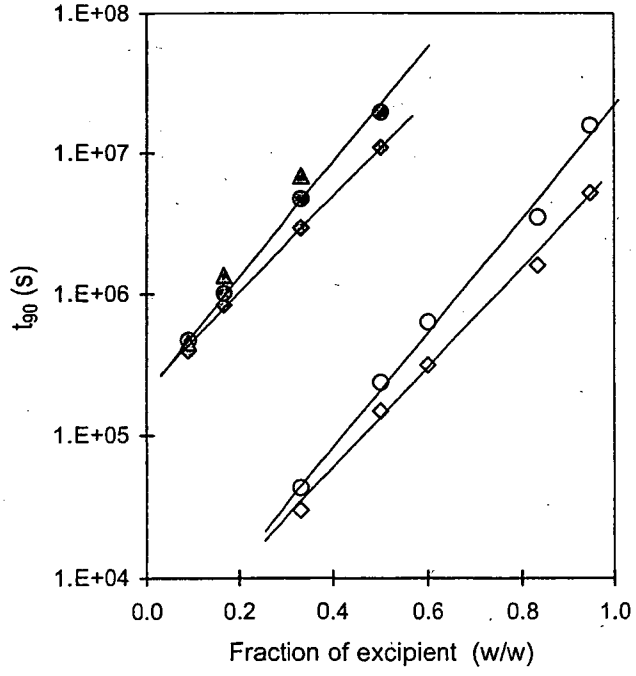
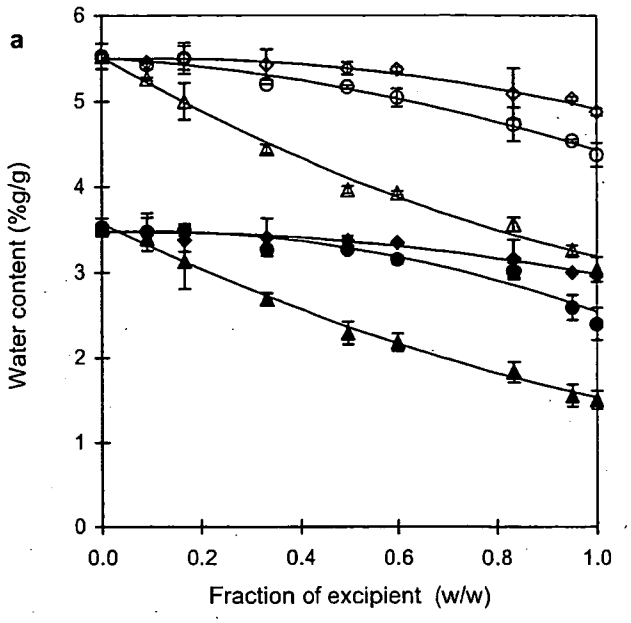


Fig. 4. Dependence of t_{90} on the weight fraction of excipient. The value of t_{90} was determined at 80°C and 12%RH for trehalose (○) and stachyose (◇), and at 50°C and 12%RH for trehalose (●), sucrose (▲) and stachyose (♦).



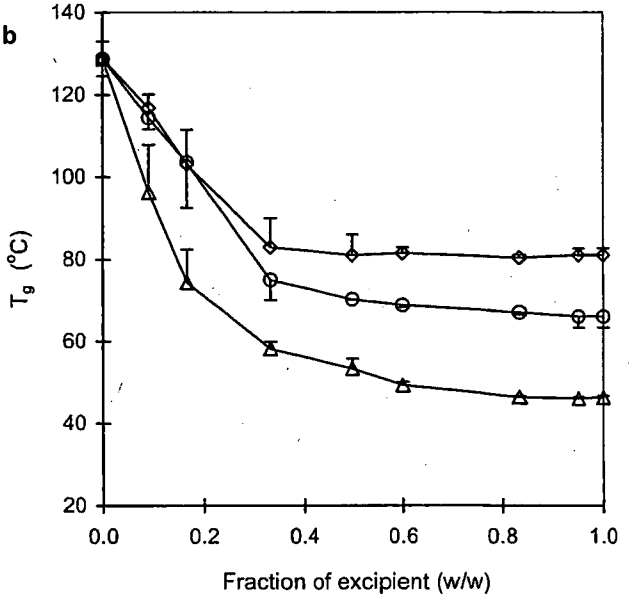


Fig. 5. Water content (**a**) and T_g (**b**) of lyophilized β-GA formulations containing trehalose (○●), sucrose (△▲), or stachyose (◇♦) as a function of the weight fraction of excipient. (**a**) *closed symbols*: 10%RH; *open symbols*: 20%RH. 25°C. (**b**) 12%RH. sd (n=3).

protein aggregation during storage. A possibility that a tertiary structural change is responsible for the differences in β-GA aggregation rate observed between the excipients cannot be excluded.

Significance of Local Mobility, as Determined by $T_{1\rho}$ of β-GA Carbonyl Carbon, and Structural Relaxation in Protein Aggregation

It is generally considered that the rate of protein aggregation, an intermolecular reaction, is mainly determined by structural relaxation that allows for large-scale

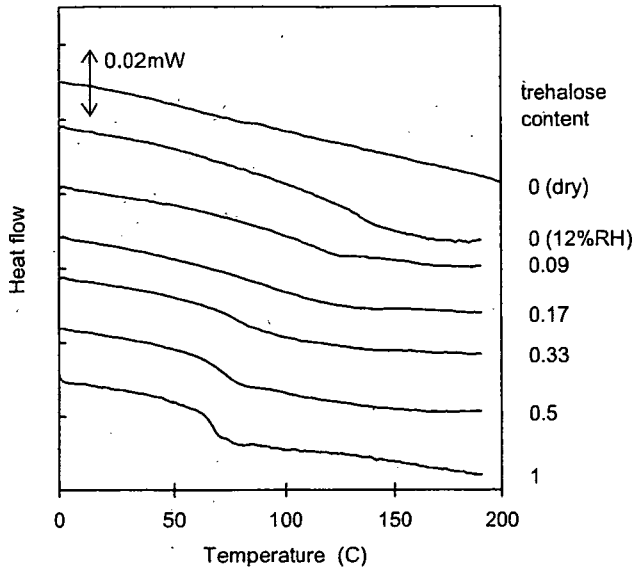


Fig. 6. DSC thermogram for β-GA lyophilized with various weight fractions of trehalose.

diffusion of reactants. From the finding that the t_{90} versus T_g/T plots for the lyophilized β-GA formulations exhibited a change in slope around T_g, β-GA aggregation rate appeared to correlate with structural relaxation. Although β-GA aggregation rate was not related to $(T-T_g)$, this may be explained by assuming that the fragility and fictive temperature of the formulation vary with the excipient. Because the structural relaxation times of the β-GA formulations were not determined in this study, correlations between β-GA aggregation rate and structural relaxation could not be elucidated.

Meanwhile, the local mobility of β-GA was determined by $T_{1\rho}$ of β-GA carbonyl carbon. Figure 9 shows the time course of rotating-frame spin-lattice relaxation at 25°C and 12% RH for the carbonyl carbon of β-GA lyophilized with sucrose, trehalose or stachyose at an excipient fraction of 0.5.

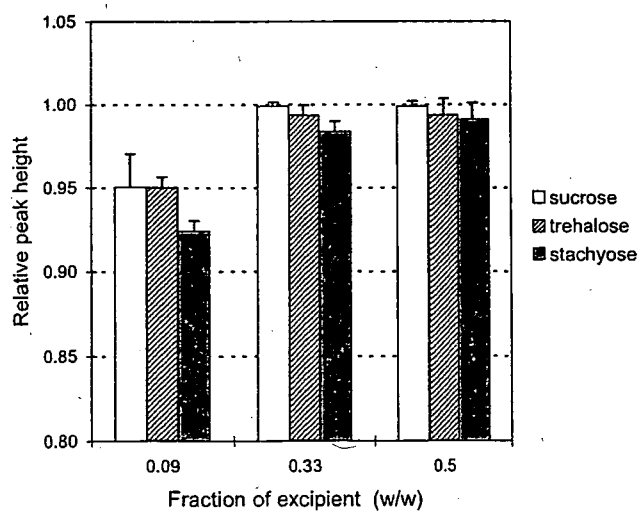
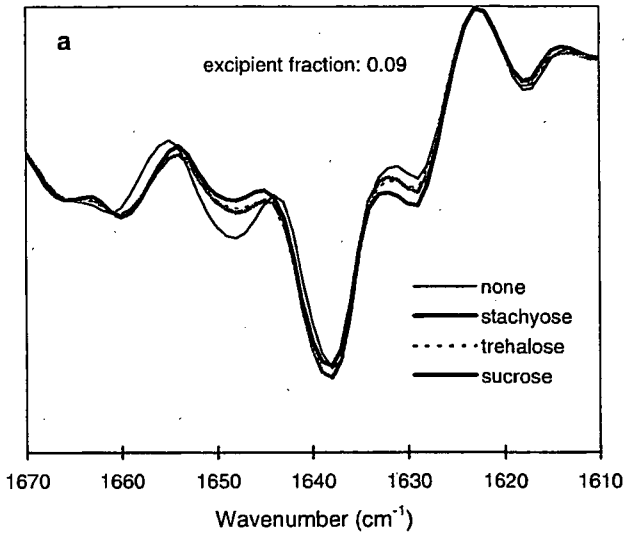


Fig. 7. Ratio of monomeric β-GA remaining after freeze drying with sucrose, trehalose or stachyose. *Bars* represent standard deviation (n=3).

Spin-lattice relaxation was significantly retarded by the addition of excipient. Sucrose resulted in the largest degree of retardation, and there were no significant differences in the degree of retardation between the trehalose and stachyose formulations. The time course of spin-lattice relaxation was describable with a bi-exponential equation including two different $T_{1\rho}$ values. The longer $T_{1\rho}$ value was estimated by curve fitting using a shorter $T_{1\rho}$ of 9 ms and a proportion of 13% for carbonyl carbons with the shorter $T_{1\rho}$. Figure 10 shows the estimates for the longer $T_{1\rho}$ of the dominating proportion, plotted as a function of the excipient fraction. The $T_{1\rho}$ for the sucrose formulation increased significantly with excipient fraction. For the stachyose formulation, in contrast, increases in $T_{1\rho}$ were not significant at an excipient fraction of 0.09, and $T_{1\rho}$ was less than in the sucrose formulation at higher excipient fractions. $T_{1\rho}$ for the trehalose formulation exhibited intermediate behavior when compared to the sucrose and stachyose formulations. The rank order of the ability of excipients to decrease the local



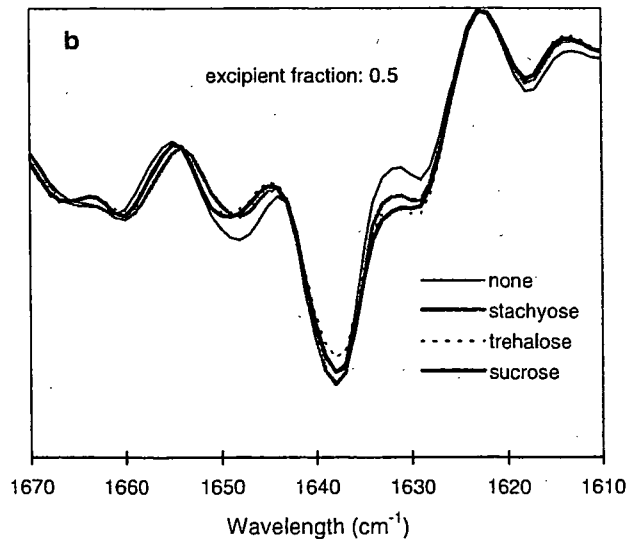


Fig. 8. Second derivative FT-IR spectra for β-GA lyophilized with sucrose, trehalose or stachyose of 0.09 (**a**) and 0.5 fractions (**b**).

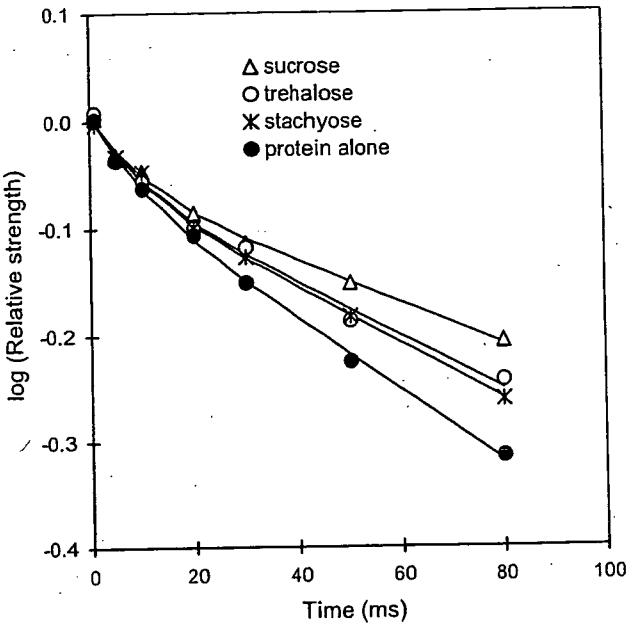


Fig. 9. Time course of spin-lattice relaxation at 25°C and 12%RH for carbonyl carbon of β-GA lyophilized with sucrose, trehalose or stachyose. The weight fraction of excipient : 0.5.

mobility of β-GA appeared to be the same as the rank order of their ability to decrease aggregation rate. This finding suggests that local mobility is a primary factor that affects the stability of lyophilized β-GA formulations; sucrose more potently inhibits local mobility of β-GA, and thus more strongly inhibits β-GA aggregation.

Local mobility is generally considered to follow Arrhenius kinetics. If local mobility is mainly responsible for β-GA aggregation, the temperature dependence of t_{90} should not show a change in slope around T_g. The non-Arrhenius temperature dependence observed for the t_{90} of β-GA aggregation, which is considered to be governed by local mobility, may be explained by assuming that local mobility of protein is coupled with structural relaxation. For bovine serum γ-globulin, the local mobility of protein, as measured by the laboratory-frame spin-lattice relaxation time (T_1) of protein carbonyl carbon, exhibited Arrhenius temperature dependence when lyophilized without excipient (18). When lyophilized with dextran, in contrast, the local mobility of protein exhibited a change in the slope of temperature dependence around the T_{mc} (T_g determined by NMR relaxation measurement), as did local mobility of dextran, as measured by T_1 of dextran methine carbon. These findings suggested that the local mobility of protein was coupled with the structural relaxation of lyophilized solids. The same may be said for the local mobility of protein and structural relaxation of β-GA lyophilized with sucrose, trahalose or stachyose. The local mobility of β-GA may exhibit Arrhenius temperature dependence in the absence of excipient. Upon the addition of excipient, local mobility may become to be coupled with structural relaxation, and the temperature dependence of protein local mobility may become to deviate from Arrhenius behavior.

The great increase in t_{90} with increasing excipient fraction observed for β-GA aggregation rate, as indicated by log-linear dependence on the excipient fraction, may be attributed mainly to the effect of excipient inhibiting protein local mobility in addition to the effect of excipient diluting protein molecules.

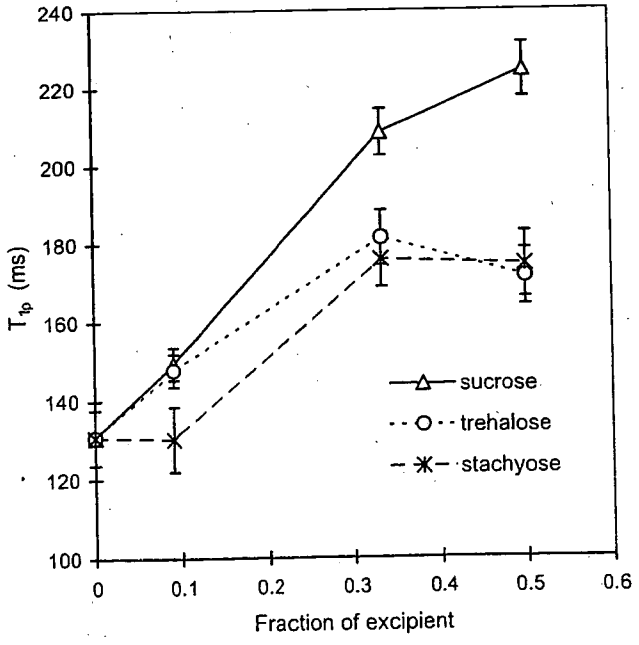


Fig. 10. Effect of weight fraction of excipient on $T_{1\rho}$ of carbonyl carbon at 25°C and 12%RH for β-GA lyophilized with sucrose, trehalose or stachyose

CONCLUSION

The aggregation rate of β-GA lyophilized with sucrose, trehalose or stachyose unexpectedly correlated with the local mobility of β-GA rather than with ($T-T_g$). An increase in the weight fraction of excipient appeared to increase the effects of excipient decreasing local mobility, resulting in increases in the stability of β-GA. Sucrose exhibited the most intense stabilizing effect due to the most intense ability to inhibit local protein mobility during storage.

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平成17年度「日本薬局方の試験法に関する研究」研究報告**

—水分吸着等温線の解析による局方収載添加剤の吸湿性に関する研究—

吉岡　澄江，阿曽　幸男，川西　　徹*

1. はじめに

日本薬局方の各条収載品目には，性状の項に，取り扱う際の有益な情報として「本品は吸湿性である」と記載されている品目がある．各条にこの記載を行うか否かの基準は明確にされていないが，「25℃，75%相対湿度（RH）の条件に7日間保存したときに観察される水分吸着量が3.0%以上かそれ未満か」を基準とすることが綱川ら[1,2]によって提言されている．具体的には，各品目の乾燥減量あるいは水分の規格に適合する検体を用いて，予備乾燥等は行わずに，塩化ナトリウムの飽和水溶液を加えたデシケータあるいは恒温恒湿装置内で25℃，75% RHの条件に7日間保存した後の重量変化を測定し，3.0%以上の水分吸着量が見られた品目については「本品は吸湿性である」と記載する方法である（以後，75% RH重量変化法と称す）．性状の項に記載する「吸湿性」は，本品の試験や保管に際して吸湿に特別に注意を払うべきかどうかの情報を与えることが目的であると考えれば，この75% RH重量変化法によって判断する「吸湿性」は目的に沿った意味を持つと思われる．実際，化学薬品の多くは，30% RH～60% RH程度の領域では，湿度による水分吸着量の変化があまり大きくないことから，75% RH重量変化法によって「吸湿性」を判断することは妥当であると考えられる．しかし，医薬品添加剤として用いられる高分子の中には，化学薬品の多くとは異なり，30% RH～60% RHの範囲で水分吸着量が大きく変化する特異的な水分吸着特性を示すものが数多くあり，これらの品目については，75% RH重量変化法で決定される「吸湿性」の情報は正確さに欠ける恐れがある．すなわち，判断結果が試験に用いた検体の履歴に大きく依存するという問題点がある．例えば，試験前にそれぞれ30% RH及び60% RHに保存されていた2つの試料がいずれも乾燥減量あるいは水分の規格に適合する場合においても，いずれの検体で「吸湿性」の評価をするかによって，異なる評価結果が得られる場合も少なくないと考えられる．したがって，各品目共通の物差しで「吸湿性」を正確に判断するためには，75% RH一点ではなく，複数の湿度条件下の吸着量に基づく方法が必要であると考えられる．

本研究は，75%重量変化法とは異なり，特異的な水分吸着特性を示す高分子添加剤の「吸湿性」の評価にも適用できる試験法として，最近普及が進んでいる真空水分吸脱着測定装置を用いて測定される吸着等温線に基づいて「吸湿性」を評価する方法（吸着等温線法）を検討した．「吸湿性」の局方記載をより科学的根拠に基づいて行うための試験法としての吸着等温線法の有用性を，多様な水分吸着パターンを示す局方収載添加剤について測定して得られた吸着等温線データに基づいて考察した．同時に，この方法で得られるデータに基づいて「本品は吸湿性

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である」の記載の判定をするための基準を考察した．

2．実験方法

局方収載添加剤の中で，水分吸着によって品質に問題が生じる可能性のある品目及び吸湿性の高い品目などの高分子添加剤について，真空水分吸脱着測定装置（MB-300G 型 Gravimetric sorption analyzer，VTI）を用いて 0% RH から 80% RH の湿度領域で平衡水分吸着量を測定した．検討した添加剤は，結晶セルロース（MCC），粉末セルロース（Powdered cellulose），メチルセルロース（MC），ヒドロキシプロピルセルロース（HPC），低置換度ヒドロキシプロピルセルロース（L-HPC），ヒプロメロース（HPMC），カルメロース（CMC），カルメロースカルシウム（CMC-Ca），カルメロースナトリウム（CMC-Na），クロスカルメロースナトリウム（cros-CMC-Na），トウモロコシデンプン（Corn starch），バレイショデンプン（Potato starch），デキストリン（Dextrin），ポビドン K90（PVP），プルラン（Pullulan）の第十五改正日本薬局方収載品目，カルボキシメチルスターチナトリウム（CMS-Na），アルファ化トウモロコシデンプン（α-Corn starch），アルファ化バレイショデンプン（α-Potato starch），クロスポビドン（cros-PVP），コポビドン（PVP/VA）の 5 薬添規収載品目，及び酵素分解デキストリン（Dextrin (enz)）の計 22 品目である．ポビドンは㈱シグマから購入したが，それ以外の添加剤は日本医薬品添加剤協会から提供を受けた．

試料を真空水分吸脱着測定装置に入れたのち，0.0% RH に相当する圧力まで減圧にして水分脱着量を測定した．重量減少が 10 分間で 1 μg 以下になる時点を終点と判断した．その後，相対湿度を 10～80%の範囲で段階的に上昇させ水分吸着量を測定した．終点は重量増加が 10 分間で 1 μg 以下になる時点とした．

3．実験結果

0% RH から 80% RH の各湿度条件下における試料の重量変化の代表例を Fig.1 に示す．ポビドンは各湿度で短期間に平衡吸着量に達するのに対して，トウモロコシデンプンでは平衡吸着量に達するのに比較的長い時間を要したが，それでもすべての検体は 2 日以内で測定が完了した．

セルロース系の添加剤，デンプン系添加剤及びその他の添加剤について測定した吸着等温線を，それぞれ，Fig. 2，3 及び 4 に示す．セルロース系の添加剤（Fig. 2）では，カルメロースで例示されるように，塩を形成すると水分吸着量は増大し，その効果はナトリウム塩で大きかった．また，セルロースへのメチル基の導入やメチルセルロースへのヒドロキシプロピル基の導入は吸着量に大きな変化をもたらさなかった．それに対して，セルロースへのヒドロキシプロピル基の導入では，置換度が低い場合に

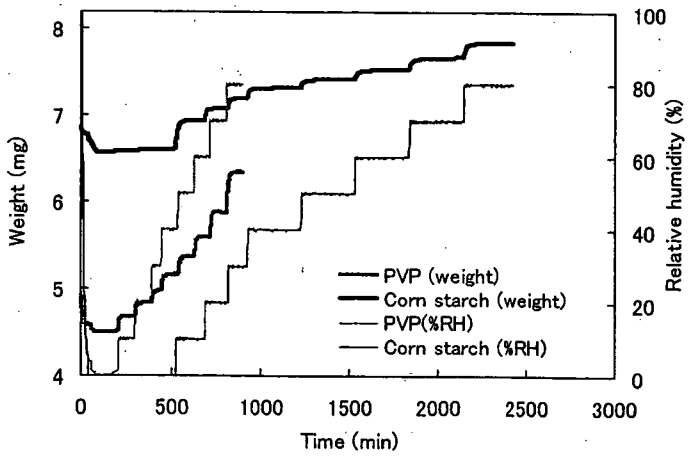

Fig. 1　ポビドン及びトウモロコシデンプンの相対湿度変化に伴う重量変化

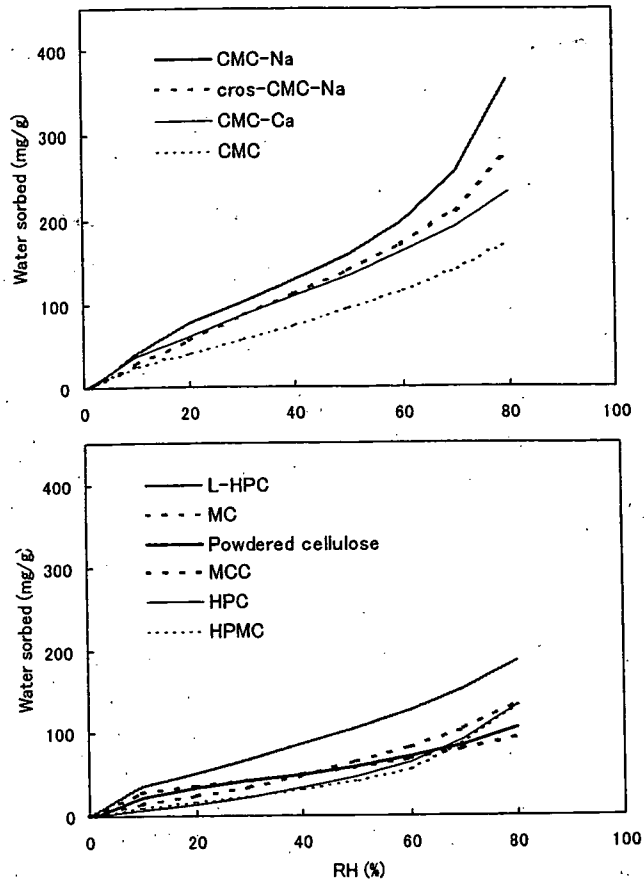

Fig. 2　セルロース系添加剤の水分吸着等温線

著しい吸着量の増大が見られた．デンプン系添加剤(Fig. 3) では，基原間やアルファ化によって吸着量に大きな差は観察されなかったが，加水分解されたデキストリンでは吸着量が減少した．その他の添加剤 (Fig. 4) では，ポビドンはビニルピロリドンと酢酸ビニルの共重合体であるコポビドンより著しく大きい吸着量を示した．

10% RH 及び 60% RH の水分吸着量を各添加剤間で比較すると，Fig. 5 に示すように，60%相対湿度条件における吸湿量は約 5 mg/g～25 mg/g の範囲で大きく変動することが明らかになった．60% RH ではポビドンが最も多くの水分吸着量を示したが，10% RH ではトウモロコシデンプンが最も多くの水分を吸着した．すなわち，Fig. 6 に示すように，トウモロコシデンプンは，低湿度条件で比較的高い吸着量を示すが湿度上昇による吸湿量の増加が小さく，Brunauer-Emmett-Teller (BET) の分類によるII型に近い吸着等温線を示した．それに対して，ポビドンは，低湿度条件で比較的低い吸着量を示すが湿度上昇とともに吸湿量が急激に増加し，III型に近い吸着等温線を示した．同様に，アルファ化トウモロコシデンプン及びクロスカルメロースナトリウムも，それぞれII型及びIII型に近い吸着等温線を示した．

真空水分吸脱着測定装置内で試料を減圧下 0.0% RH になるまで水分を脱着したときの水分脱着量を Fig. 7 に示し，60% RH における水分吸着量及び局方の乾燥減量の規格値と比較した．今回試験に用いた検体は，種々の入手先及び種々の包装形態のものであるが，クロスポビドンでは，検体の水分脱着量が 60% RH における水分吸着量とほぼ等しく，60 % RH 保存と同等の水分を含有しており，水分の

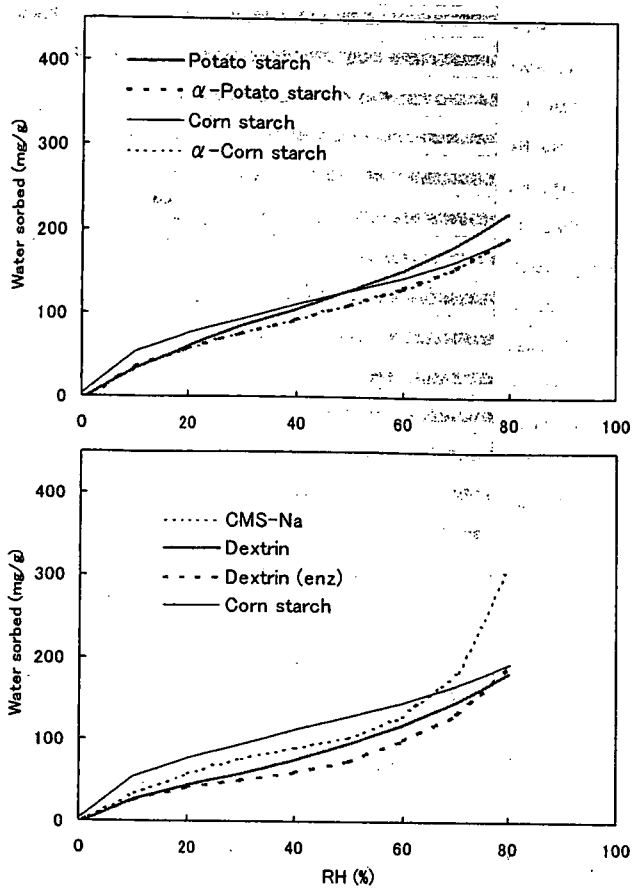

Fig. 3　デンプン系添加剤の水分吸着等温線

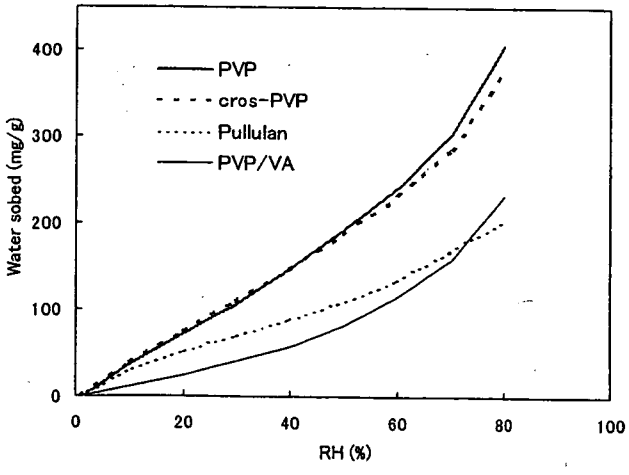

Fig. 4　その他の添加剤の水分吸着等温線

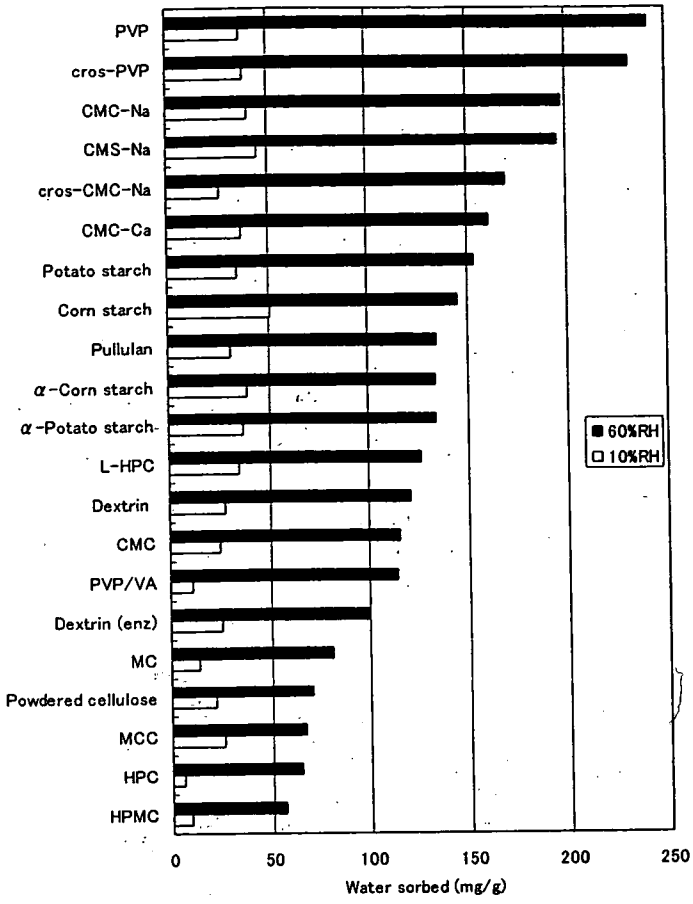

Fig. 5　高分子添加剤の 10% RH 及び 60% RH における水分吸着量

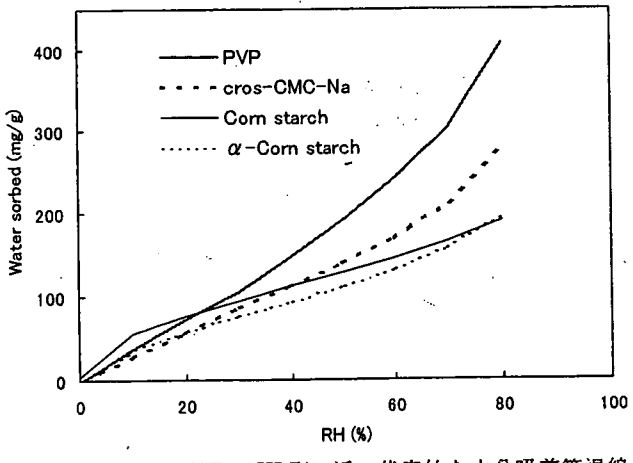

Fig. 6　BET II 型及び III 型に近い代表的な水分吸着等温線

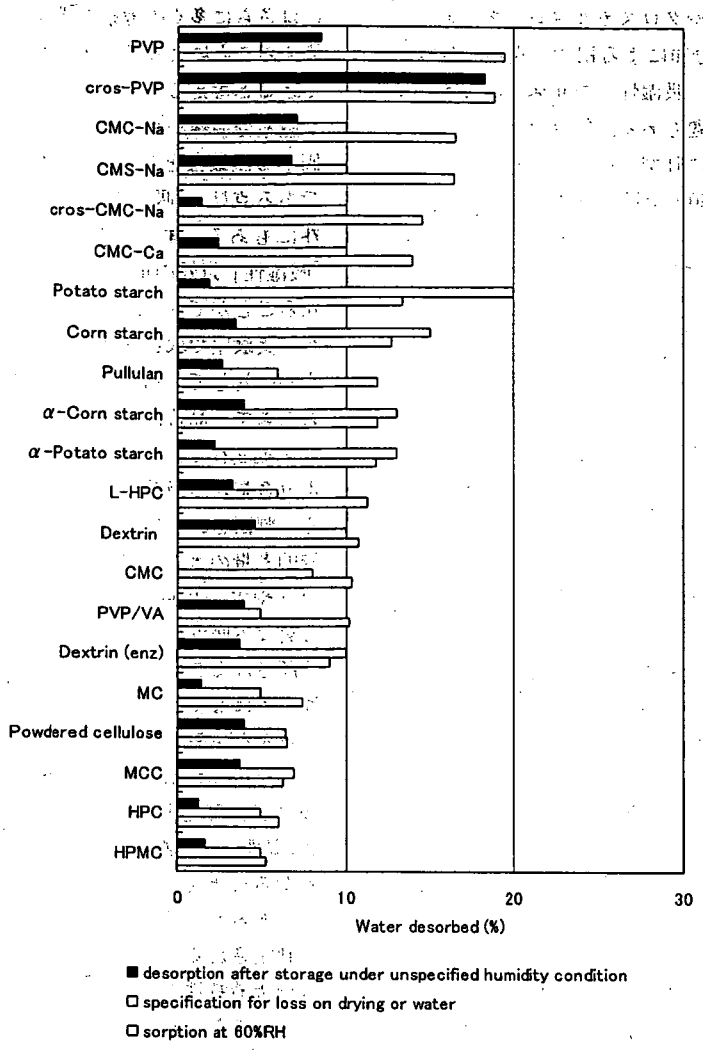

Fig. 7　高分子添加剤の乾燥減量あるいは水分の規格値と比較した水分脱着量

規格値を著しく超過していた．ポビドンの水分脱着量も水分の規格値より大きな値を示した．また，コポビドンでは，規格値より大きくはないが，それに近い脱着量を示した．

4．考　察

綱川らによって提言されている「吸湿性」の基準，すなわち25℃，75% RHの条件に7日間保存したときに観察される水分吸着量が3.0% RH以上か未満かに基づいて「本品は吸湿性である」の記載の有無を判断する方法は，ほとんどの化学薬品については大きな問題なく適用できると考えられる．しかし，局方に収載されている医薬品添加剤には，この75% RH重量変化法による「吸湿性」の評価が適切でないと考えられる品目が数多くあることが分かった．すなわち，高分子添加剤はFig. 2～4に示すように多様な水分吸着等温線を示すため，75% RH保存前後の重量変化を測定するだけでは「吸湿性」の評価が困難である．第一に，75% RH重量変化法の結果は，試験に用いた検体の容器包装形態や試験前におかれていた湿度条件など，検体の履歴によって変動する問題がある．更に，75% RH重量変

化法では，ポビドンやクロスカルメロースナトリウムのようにBETの分類によるIII型に近い水分吸着を示す品目について「吸湿性」を正確に評価することが難しいという問題もある．結晶セルロースや粉末セルロースのようにII型に近い水分吸着を示す品目では，30% RH～60% RHの湿度領域での吸着量の変化が比較的小さいので，75% RH重量変化法によっても，実験室の湿度条件に影響されずに「吸湿性」の評価を行うことができるが，III型に近い水分吸着を示す品目では，0% RH～60% RHの湿度領域で吸着量が大きく変化するため，実験室における秤量操作中の吸着の影響を受けやすく，75% RH重量変化法で吸着量を正確に測定することは難しいからである．

75% RH重量変化法でみられるこれらの問題点を解決できる試験法として，真空水分吸脱着測定装置を用いる吸着等温線法が有用であると考えられる．この試験法では水分吸着量の測定前に試料の乾燥を行うため，試験結果が検体の履歴によって影響されることがない．また，閉鎖系で測定を行うため実験室の湿度条件にも影響されない．更に広範囲の湿度条件における水分吸着パターンを簡便に測定することができる．吸着等温線の測定に要する時間は品目の吸湿特性に大きく依存するが，トウモロコシデンプンのように比較的長い時間を要する品目でも2日以内であり，重量変化測定法の7日間に比較して，試験期間を大幅に短縮することができる．

性状の項に「本品は吸湿性である」を記載するか否かの判定を吸着等温線法で行う場合に判定基準をどうするかについては更なる検討が必要であるが，次のような基準が考えられる．①乾燥減量や水分の規格値は本来，品質保証の観点から設定されるべきものであり，また，実験室の湿度条件は高くても60% RH程度であると考えて，60% RHにおける水分吸着量が乾燥減量や水分の規格値より大きいときに「本品は吸湿性である」と記載する．②吸着等温線の60% RHにおける勾配（湿度が10%上昇するときの水分増加量）に基づいて判断する．すなわち，勾配がある一定値以上のときには，実験室湿度の影響を受けやすいことを意味するので「本品は吸湿性である」の記載を行う．基準の具体的な数値の設定にはさらに多くの考察を要するが，吸着等温線を評価することによって「吸湿性」をより科学的に評価できると考える．

吸着等温線法が高分子添加剤の水分吸着特性を評価する試験法として75% RH重量変化法より有用である点は，「吸湿性」の評価法としての有用性以外にもある．すなわち，吸着等温線法によって，「吸湿性」以外の重要な情報として「水分吸着能」を知ることができる．「水分吸着能」とは，「吸湿性」が「実験室で取扱うときに吸湿しやすい性質」を表すのに対して，物質自体が有する水分を吸着する能力を表す．品質保証の面から乾燥減量又は水分が低く規定されている品目については，規格通りの低い水分含量から吸着した量に基づいて「吸湿性」があると判断されるのに対して，通常の湿度条件下で比較的多量の水分を吸着して安定した水分吸着状態になりやすい品目については，75% RHにおかれても更なる吸湿はしにくいために「吸湿性」はないと判断されることもある．後者の品目の場合は「吸湿性」ではなく，「水分吸着能」が水分吸着特性として重要な情報となる．例えば，同じII型の水分吸着を示し，75% RH保存前後の重量変化が等しい場合には，75% RH重量変化法によって同程度の「吸湿性」と判断されても，「水分吸着能」は大きく異なることもありうる．乾燥減量規格が20.0%以下であるバレイショデンプンは，「吸湿性」はないと判定されるが，「水分吸着能」は大きく，それが水分吸着特性を表す重要な特性となる．「水分吸着能」を測るためには，低い湿度条件における吸着量を測定する必要があるが，その操作は従来の重量変化測定法では非常に難しいのに対し，吸着等温線法によれば簡便に行うことができる．

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REVIEW

Correlations between Molecular Mobility and Chemical Stability During Storage of Amorphous Pharmaceuticals

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ABSTRACT: Recent studies have demonstrated that molecular mobility is an important factor affecting the chemical stability of amorphous pharmaceuticals, including small-molecular-weight drugs, peptides and proteins. However, quantitative correlations between molecular mobility and chemical stability have not yet been elucidated. The purpose of this article is to review literature describing the effect of molecular mobility on chemical stability during storage of amorphous pharmaceuticals, and to seek a better understanding of the relative significance of molecular mobility and other factors for chemical reactivity. We first consider the feature of chemical stability often observed for amorphous pharmaceuticals; changes in temperature dependence of chemical stability around matrix glass transition temperature (T_g), and greater stability associated with higher T_g. Secondly, we review papers which quantitatively studied the effects of the global mobility (often referred to as structural relaxation or α-relaxation) of amorphous pharmaceuticals on chemical stability, and discuss correlations between chemical stability and global mobility using various equations that have thus far been proposed. Thirdly, the significance of local mobility of drug and excipient molecules in chemical reactivity is discussed in comparison with that of global mobility. Furthermore, we review literature reports which show no relationship between chemical stability and molecular mobility. The lack of apparent relationship is discussed in terms of the effects of the contribution of excipient molecules as reactants, the specific effects of water molecules, the heterogeneity of the matrix, and so on. The following summary has been obtained; the chemical stability of amorphous pharmaceuticals is affected by global mobility and/or local mobility, depending on the length scale of molecular mobility responsible for the chemical reactivity. In some cases, when activation energy for degradation processes is high and when other factors such as the specific effects of water and/or excipients contribute the degradation rate, stability seems to be largely independent of molecular mobility. J Pharm Sci 96:960–981, 2007

Keywords: chemical stability; solid state stability; glass transition; amorphous; molecular mobility; local mobility; matrix mobility

INTRODUCTION

Molecular mobility is thought to be one of the most important factors determining the chemical

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stability of amorphous pharmaceuticals. Many studies have demonstrated that the chemical reactivity of amorphous pharmaceuticals, ranging from small molecules to high-molecular-weight molecules such as peptides and proteins, is related to molecular mobility, in that increased molecular mobility leads to enhanced chemical degradation.[1–5] Therefore, reducing the molecular mobility of amorphous pharmaceuticals is considered to be a useful strategy for improving their storage stability. However, a reduction in molecular mobility does not necessarily result in increased storage stability, because storage stability also depends on various other factors. Chemical reactions with minor contributions from molecular mobility cannot be inhibited by reducing molecular mobility. An understanding of the quantitative relationship between chemical reactivity and molecular mobility will not only provide useful information contributing to stabilization strategies but will also allow development of an advanced method for predicting storage stability. If the chemical reactivity of a certain amorphous pharmaceutical is found to be affected mainly by the molecular mobility related to glass transition, its storage stability may be predicted based on that factor, but it cannot be predicted by accelerated testing without explicit knowledge of temperature dependence of molecular mobility because of possible non-linear temperature dependence around the glass transition temperature (T_g). If chemical reactivity is found not to be affected by molecular mobility related to glass transition, storage stability cannot be predicted based on this, but it may be predicted by the extrapolation of data obtained under accelerated conditions because of the lack of change in the temperature-dependent slope around T_g.

This work aims to review literature describing correlations between molecular mobility and chemical stability during storage of amorphous pharmaceuticals, and to seek a better understanding of the relative significance of molecular mobility and other factors in chemical reactivity. This review covers the chemical stability of amorphous pharmaceuticals and food components ranging from low-molecular-weight molecules to proteins. The primary focus is placed on chemical degradation involving covalent modification, but protein degradation such as aggregation, which does not necessarily involve covalent modification, is also included. The scope of this review is limited to correlations between molecular mobility and chemical stability during storage. The correlation between molecular mobility and stability during freeze-drying processes, as reported for various proteins,[6] is outside the scope of this review.

In this review, we first consider the feature of chemical stability often observed for amorphous pharmaceuticals; changes in temperature dependence of chemical stability around matrix T_g, and greater stability associated with higher matrix T_g. Secondly, we review papers which quantitatively studied the effects of the global mobility of amorphous pharmaceuticals on chemical stability, and discuss correlations between chemical stability and global mobility using various equations that have thus far been proposed. Here, we use the term "global mobility" to represent large-scale mobility, often referred to as structural relaxation or α-relaxation. Thirdly, the significance of local mobility (or β-relaxation, small-scale mobility) of drug and excipient molecules in chemical reactivity, which has recently attracted attention as an important factor in chemical reactivity, is discussed in comparison with that of global mobility. Furthermore, we review literature reports which show no relationship between chemical stability and molecular mobility. The lack of apparent relationship is discussed in terms of the effects of the contribution of excipient molecules as reactants, the specific effects of water molecules, the heterogeneity of the matrix, and so on.

OFTEN-OBSERVED FEATURE OF CHEMICAL STABILITY OF AMORPHOUS PHARMACEUTICALS

Temperature Dependence of Stability Often Changes Around Matrix T_g

Amorphous pharmaceuticals generally exhibit greater molecular mobility and chemical reactivity than the corresponding crystalline forms, and the degradation rate around T_g often shows nonlinear Arrhenius plots, as reported for a range of β-lactam antibiotics.[7,8] Similar temperature dependence of degradation rates has been demonstrated for various amorphous pharmaceuticals. The hydrolysis rate of aspirin lyophilized with (hydroxypropyl)-β-cyclodextrin exhibited S-shaped temperature dependence around T_g, with activation energies (E_a) of 9.1 and 14.4 kcal/mol, respectively, in the glassy and rubbery states (Fig. 1).[9] The rate of acetyl transfer between aspirin and sulfadiazine, a bimolecular reaction, in mixtures lyophilized with dextran was also

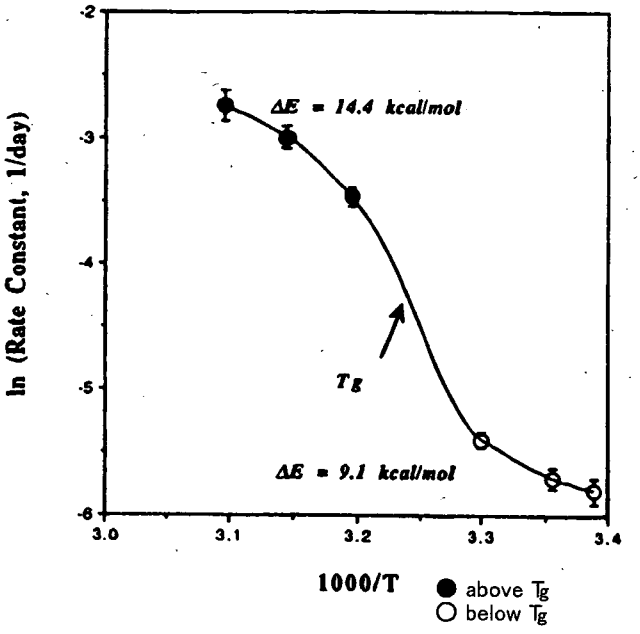

Figure 1. The Arrhenius plots for degradation of aspirin lyophilized with (hydroxypropyl)-β-cyclodextrin. (Produced using data reported in Reference [9]).

found to be affected by glass transition: the temperature dependence of the reaction rate changed at the critical mobility temperature (T_{mc}), at which Lorentzian relaxation takes place due to protons with high mobility (Fig. 2).[10] T_{mc} is the

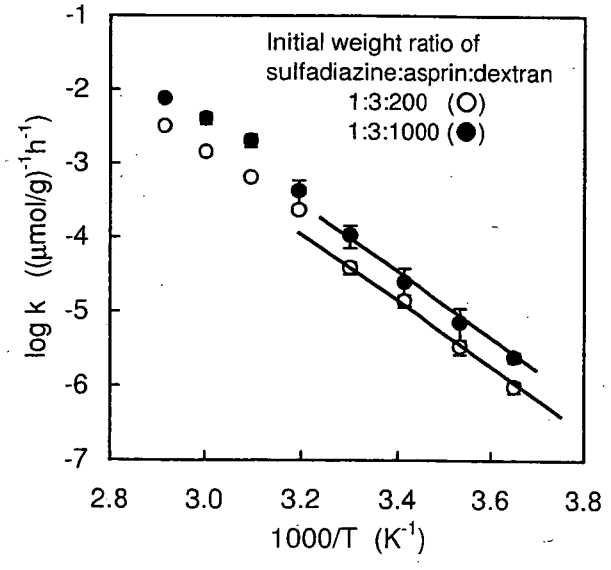

Figure 2. The Arrhenius plots for acetyl transfer between aspirin and sulfadiazine lyophilized with dextran at 60% RH. (Produced using data reported in Reference [10]).

temperature at which glass transition is first detected by NMR relaxation measurement. For amorphous solids containing polymers, T_{mc} may be the critical temperature at which local sites of polymer molecules begin to show higher mobility, and this value was found to be lower than the corresponding T_g (determined by DSC) by approximately 25°C.[11] Similarly to T_{mc}, a critical water content, at which the slower NMR relaxation component (corresponding to Lorentzian relaxation) increased abruptly, was observed for lyophilized human growth hormone.[12]

As in the degradation of small molecules, changes in the slope of temperature dependence around T_g have also been shown for protein aggregation. The aggregation rate of lyophilized monoclonal antibody exhibited an abrupt change in temperature dependence around T_g, as determined by DSC and dielectric relaxation spectroscopy (DRS).[13] The aggregation rates of lyophilized bovine serum albumin (BSA) and bovine serum γ-globulin (BGG) also increased abruptly when the temperature rose above T_{mc}.[14,15] An abrupt change in temperature dependence around T_{mc} was also observed for aggregation of BGG lyophilized with dextran or methylcellulose. The values of mean aggregation time and stretched exponential constant were calculated according to the Kohlrausch–Williams–Watts stretched exponential function (KWW equation) by assuming that the time required for protein molecules to aggregate varies because the rate of protein aggregation depends on the degree of protein deformation resulting from stresses created during freeze-drying; these values have been shown to exhibit temperature dependence with an abrupt change around T_{mc}.[16] Similarly, the slope of the temperature dependence of aggregation for β-galactosidase lyophilized with polyvinyl alcohol (PVA) or methylcellulose significantly changed around T_{mc} (Fig. 3).[17]

Higher Matrix T_g Often Brings About Greater Stability

The matrix T_g of amorphous pharmaceuticals is a critical parameter for chemical stability. The storage stability of amorphous pharmaceuticals largely depends on the formulation,[18] and the use of an excipient with a higher T_g is generally considered to be important in the preparation of stable amorphous pharmaceuticals. The rate of the Maillard reaction between glycine and glucose in mixtures with poly(vinylpyrrolidone) (PVP)

DOI 10.1002/jps

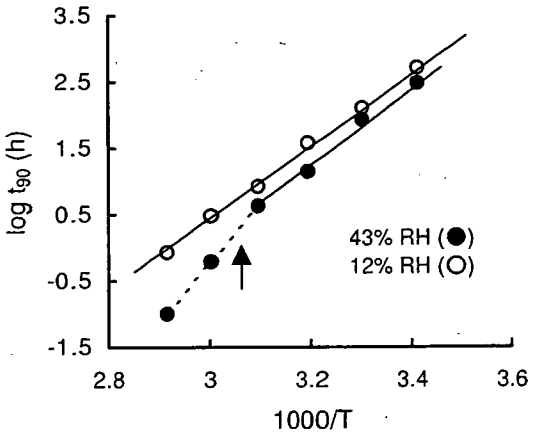

Figure 3. The Arrhenius plots for aggregation of β-galactosidase lyophilized with methylcellulose. t_{90} (time required for 10% aggregation) was calculated according to the KWW equation. Arrow indicates glass transition temperature determined by NMR relaxation (T_{mc}) at 43%RH. (Reproduced from Reference [17] with permission of copyright owner.)

was found to decrease, as the T_g of PVP rose in association with increases in its molecular weight.[19] The degradation rate of lyophilized quinapril hydrochloride was found to increase, as T_g decreased in association with increases in the fraction of the neutralized form (Fig. 4).[20,21] This correlation between chemical reactivity and T_g was eliminated at lower pH, probably due to a change in the rate-determining step.

Greater stability resulting from the use of excipients with higher T_g values has also been reported for lyophilized proteins. The stability of

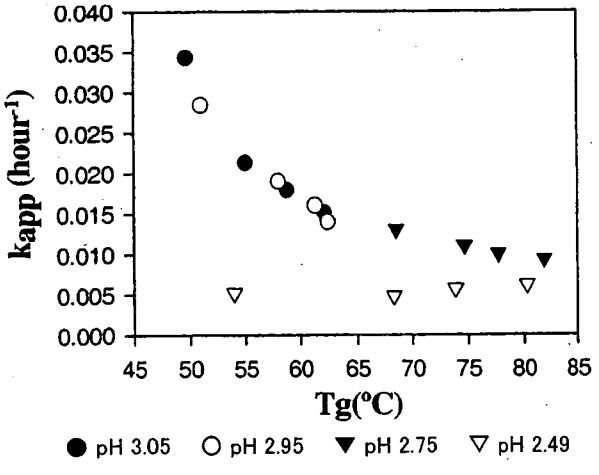

Figure 4. The T_g-rate profile for degradation of quinapril lyophilized with citrate buffer. (Reproduced from Reference [21] with permission of copyright owner.)

lyophilized invertase during storage at 90°C increased with increasing PVP molecular weight (Fig. 5).[22] The storage stability of lyophilized recombinant human interleukin-2 increased when combined with excipients of increasing T_g (sucrose < trehalose < raffinose < stachyose).[23] The aggregation rate of BGG lyophilized with dextran was found to decrease as the molecular weight of dextran increased (i.e., with increasing T_{mc}; Fig. 6).[24]

Although excipients with high T_g stabilize amorphous pharmaceuticals in many cases, they are often inefficient in preventing unfolding during freeze-drying. In order to prevent this and also improve storage stability, the use of a disaccharide in addition to a high-T_g excipient has been attempted, as exemplified by the stabilization of actin with sucrose and dextran[25] and the stabilization of recombinant human interleukin-11 with sucrose or trehalose and hydroxyethyl starch.[26]

The matrix T_g, which is closely related to the chemical stability of amorphous pharmaceuticals, is mainly determined by the T_g values of the drug and excipients, but is also affected by water sorption. It is well known that water lowers the matrix T_g through plasticization. The plasticizing effect of water has been reported for various systems comprised of sugars and polymers.[27–30] Plasticization of amorphous pharmaceuticals due to water sorption has been reported to lead to decreased stability.[31–36] The deamidation and dimerization rates of recombinant bovine somatotropin solids were found to increase with increasing water content (Fig. 7).[37] Salmon calcitonin spray-dried powders containing mannitol exhibited water sorption-enhanced aggregation, which accompanied crystallization of mannitol at higher humidities.[38] The extent of covalent aggregation of lyophilized insulin was found to be directly correlated with water uptake, and the critical role of protein conformational mobility in the aggregation process was speculated.[39]

The extent of aggregation in BSA, ovalbumin, glucose oxidase and β-lactoglobulin exhibited a bell-shaped relationship with water content,[40] showing increases due to plasticization in the lower range and decreases due to dilution of the reactants in the higher range of water content (Fig. 8).[41] Similar bell-shaped relationships between water content and the extent of protein aggregation have been reported for various systems such as lyophilized BSA,[42] recombinant human albumin,[43] tetanus toxoid,[44,45] bovine and human insulin,[46] albumins,[47] γ-globulin,[47] and

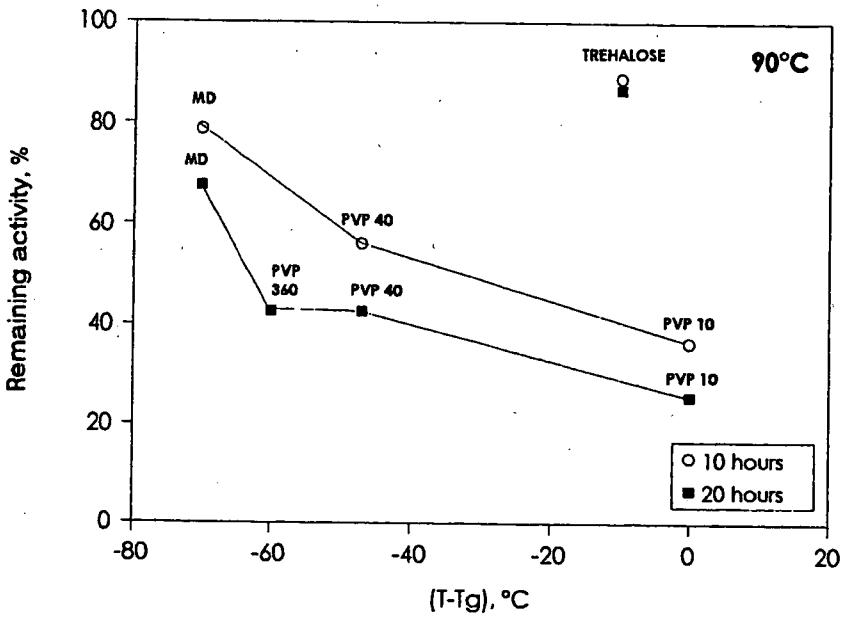

Figure 5. The activity loss of invertase lyophilized with trehalose, maltodextrin or PVP after storage at 90°C as a function of $(T-T_g)$. (Reproduced from Reference [22] with permission of copyright owner).

recombinant human interleukin-1 receptor antagonist.[48]

The enhancement of degradation associated with greater global mobility due to increased water content has also been reported for degradation reactions of small molecules, such as oxidation of vitamin A, peptides and steroids, as well as cyclization reactions of spirapril hydrochloride, quinapril hydrochloride and moexipril.[49] The rate of hydrolysis of methylprednisolone sodium succinate was found to be greater in the freeze-dried solid containing mannitol than in a solid with the same water content containing lactose, because of increased water content in the microenvironment of the drug due to mannitol crystallization.[50]

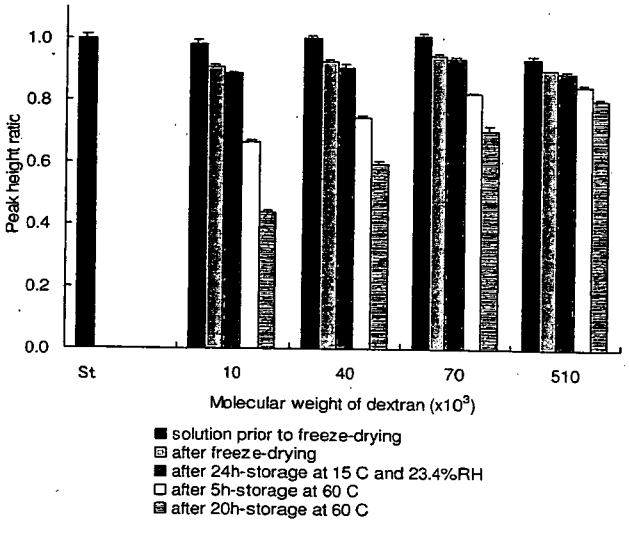

Figure 6. The effect of dextran molecular weight on the ratio of BGG remaining after storage. St represents BGG standard solution without dextran. (Reproduced from Reference [24] with permission of copyright owner.)

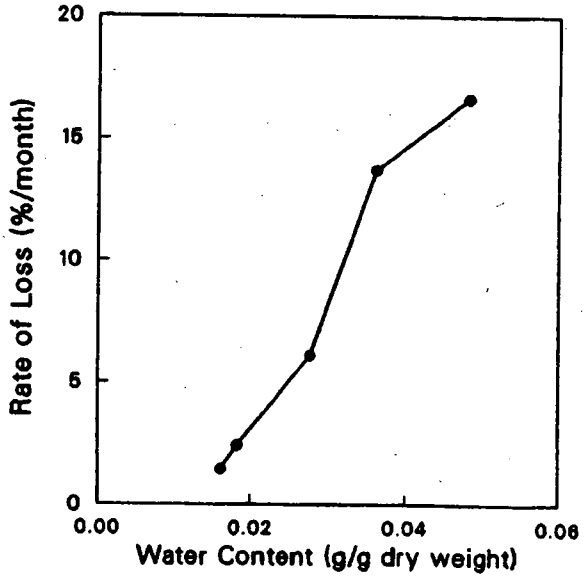

Figure 7. The effect of water content on the degradation rate of lyophilized reconbinant bovine somatotropin at 47°C. (Reproduced from Reference [37] with permission of copyright owner.)

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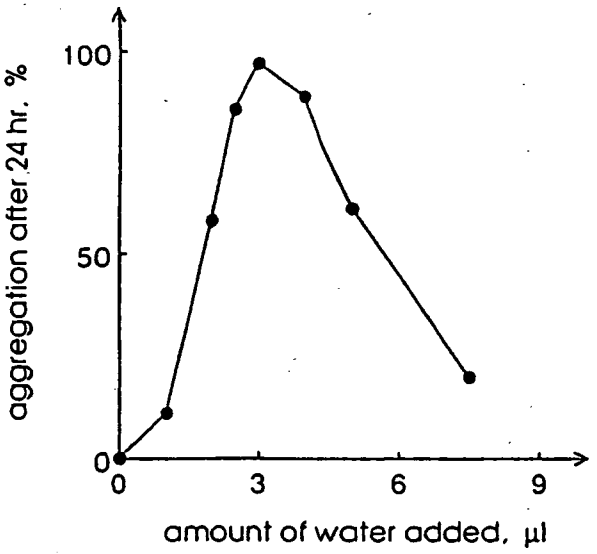

Figure 8. The effect of the amount of water on the extent of aggregation of lyophilized bovine serum albumin at 37°C. (Reproduced from Reference [41] with permission of copyright owner.)

The effect of water in enhancing diffusion-controlled degradation as a plasticizer may be much greater than its effect as a reactant. A 10-fold increase in water content, from 0.1 to 1%, may increase the rate constant by a factor of 10 as a reactant, but by a factor of $10-10^6$ as a plasticizer, depending on fragility and the Gordon–Taylor constants.[51] Increases in the molecular mobility of lyophilized solids due to the plasticizing effect of water have been confirmed by changes in the spin-lattice relaxation time (T_1) as measured by NMR. The T_1 of protein carbons in lyophilized lysozyme was found to decrease with increasing water content, indicating increased molecular mobility in the protein, which led to enhanced aggregation.[52,53]

QUANTITATIVE RELATIONSHIP BETWEEN CHEMICAL REACTIVITY AND GLOBAL MOBILITY

Amorphous pharmaceuticals often exhibit chemical stability that is related to matrix T_g, and the temperature dependence of stability often changes around T_g, as described above. These observations suggest that the global mobility of amorphous pharmaceuticals is one of the critical factors that determine chemical stability. In order to gain a further understanding of the significance of global mobility for chemical stability, this section describes the quantitative relationship between global mobility and the chemical stability of amorphous pharmaceuticals.

Dependence of Chemical Reactivity on $(T-T_g)$

The temperature dependence of chemical degradation rates at temperatures above T_g can generally be analyzed using the Williams–Landel–Ferry (WLF) model:

$$\log\left(\frac{k_{T_g}}{k_T}\right) = \log\left(\frac{\tau_T}{\tau_{T_g}}\right) = \frac{-C_1(T-T_g)}{C_2+T-T_g} \quad (1)$$

where k_{T_g} and k_T represent the rate constants at T_g and T, respectively, and τ_{T_g} and τ_T represent structural relaxation times at T_g and T, respectively. C_1 and C_2 are constants depending on the system. The reaction rate of monoclonal antibody-vinca alkaloid conjugate[54] and the inactivation rate of glucose-6-phosphate dehydrogenase[55] were correlated with the value of $(T-T_g)$ according to the WLF equation (Figs. 9 and 10). The temperature dependence of the chemical degradation rate of human growth hormone can also be described using the WLF equation.[4] The aggregation rate of BGG lyophilized with dextran was correlated with T_{mc}, the glass transition temperature determined by NMR relaxation measurement, according to the WLF equation in which T_g is replaced by T_{mc} (Fig. 11).[56]

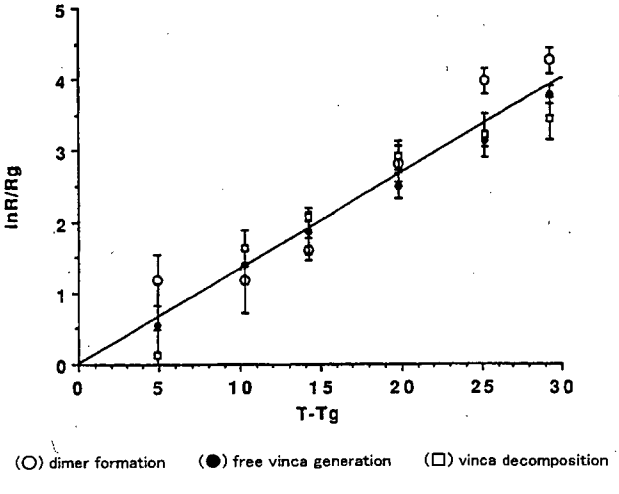

Figure 9. The WLF plots for degradation of lyophilized monoclonal antibody-vinca alkaloid conjugate. R and R_g are degradation rates (%/month) at a given temperature and at T_g, respectively. (Reproduced from Reference [54] with permission of copyright owner).

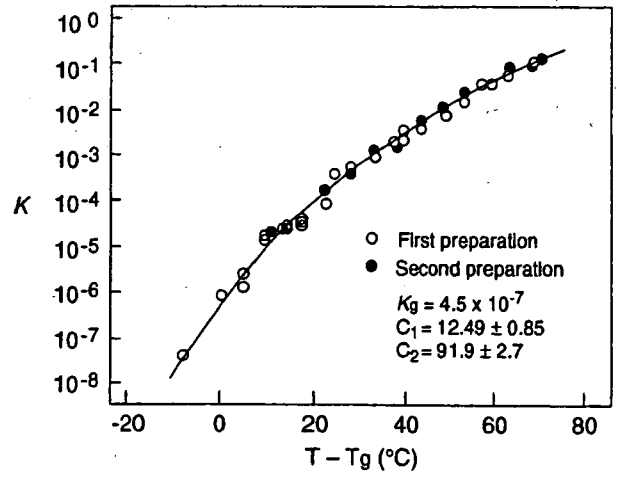

Figure 10. The WLF plots for inactivation of lyophilized glucose-6-phosphate dehydrogenase. K_g corresponds to k_{T_g} in Eq. (1). (Reproduced from Reference [55] with permission of copyright owner.)

For deamidation of an asparagine-containing hexapeptide in lyophilized PVA or PVP, which occurs via a cyclic imide intermediate, the rates at various water content levels were correlated with the value of $(T-T_g)$, indicating a close relationship between chemical reactivity and the degree of plasticization by water (Fig. 12).[57] However, the dependence on temperature and water content of the deamidation rate for the PVP formulation was different from that for the PVA formulation, which suggests that there are factors other than

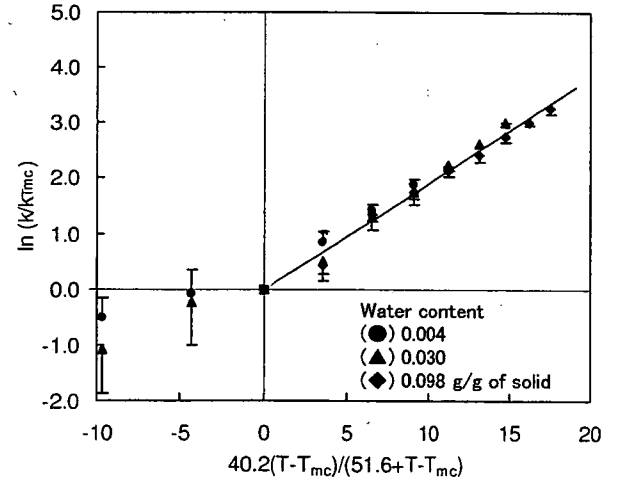

Figure 11. The WLF plots for aggregation of BGG lyophilized with dextran 40 k. Line represents curve-fitting according to Eq. (1) using C_1 of 17.44 and C_2 of 51.6. (Reproduced from Reference [56] with permission of copyright owner.)

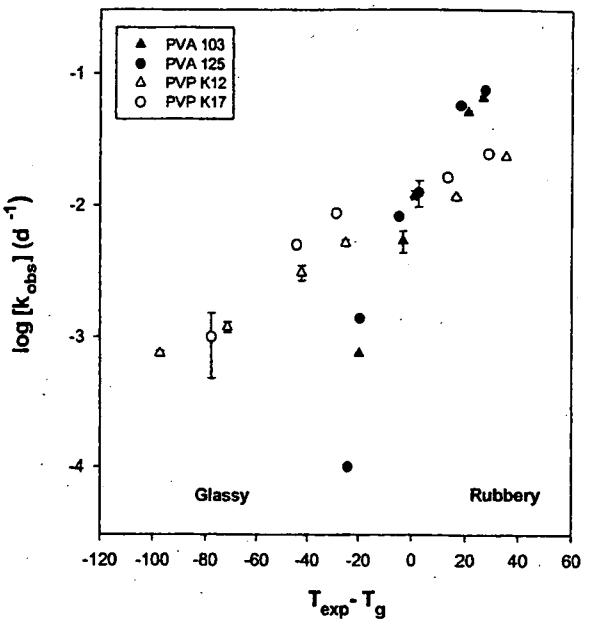

Figure 12. The WLF plots for deamidation of Asn-hexapeptide lyophilized with PVA and PVP at 50°C. (Reproduced from Reference [57] with permission of copyright owner.)

plasticization. Deamidation rates in PVP-glycerol formulations were correlated with changes in $(T-T_g)$, either associated with varying amounts of glycerol or with varying water contents, at temperatures above T_g (Fig. 13).[58] Thus, global mobility is thought to determine the deamidation rate. At temperatures below T_g, in contrast, the rate increased with increasing water content even at the same value of $(T-T_g)$, suggesting that water has an effect as a reactant or a solvent/medium in addition to its effect as a plasticizer.

The rate of bimolecular reaction in lyophilized aspirin-sulfadiazine formulations containing dextran and isomalto-oligomers of different molecular weights (acetyl transfer between aspirin and sulfadiazine and Maillard reaction between sulfadiazine and the terminal carbonyl group of excipients) was found to increase as the T_g of the formulations decreased, associated either with decreases in the molecular weight of the excipient or with increases in water content. Similar plots of rate versus $(T-T_g)$ were observed for the formulations of different T_g values, suggesting that the degradation rate is quantitatively correlated with global mobility.[59]

Rate constants for chemical reactions (k_{obs}) in which the diffusion rate of the reactants is hindered can be described using the Collins–Kimball equation (Eq. 2), with the activation

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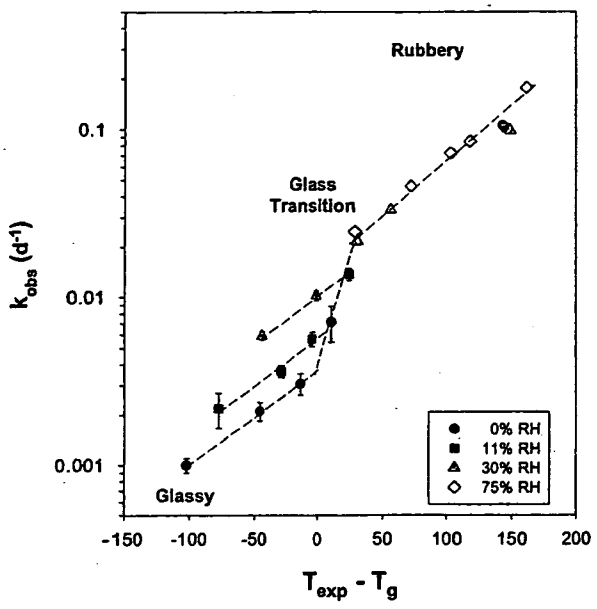

Figure 13. The WLF plots for degradation of Asn-hexapeptide at 50°C (T_{exp}) and different relative humidities (0% RH, 11% RH, 30% RH, and 75% RH). At each relative humidity, formulations with lower T_g were generated by adding increasing amount of glycerol. (Reproduced from Reference [58] with permission of copyright owner.).

energy of the reaction (E_a) and the diffusion coefficient of reactant (D_r).[60,61]

$$k_{obs} = k_{act}\left(\frac{1}{1+\frac{k_{act}}{\alpha' D_r}}\right) = k_{act}\left(\frac{\alpha' D_r}{k_{act}+\alpha' D_r}\right) \quad (2)$$

Here, α' represents the degree of correlation between the diffusion-controlled rate and D_r, such that $\alpha' D_r$ corresponds to diffusion-controlled rate constant. The term k_{act} represents the rate constant without restriction of diffusion, and is described by Eq. (3):

$$k_{act} = A\exp\left(\frac{-E_a}{RT}\right) \quad (3)$$

where A and R are the frequency factor and gas constant, respectively.

The term $\alpha' D_r$ in the Collins–Kimball equation can be represented by $8RT/3\eta$, where η is the viscosity of the medium, according to the Stokes–Einstein relation between η and D_r. The rate of the Maillard reaction between lysine and glucose was found not to be consistent with the diffusion-controlled rate constant calculated based on viscosity, indicating that the reaction rate is determined not only by the diffusivity of reactants but also by the E_a of the reaction (Fig. 14).[62]

Dependence of Chemical Reactivity on Structural Relaxation Time

Correlations between global mobility, represented by $(T-T_g)$, and chemical reactivity have been demonstrated for various amorphous pharmaceuticals, as described above. However, when amorphous pharmaceuticals have different values of fragility and fictive temperature, global mobility cannot be represented by T_g values alone; in this case, it is better represented in terms of structural relaxation time (τ) by incorporating values of fragility and fictive temperature.[63,64] Lyophilized monoclonal antibody containing trehalose with a higher T_g value was found to be more susceptible to aggregation than that containing sucrose at temperatures below T_g, indicating that fragility and fictive temperature should be taken into account in evaluating the global mobility.[65] Compared to cefamandole Na, lyophilized cephalothin Na was found to have a shorter τ by slightly more than a factor of 20 and higher chemical reactivity by approximately a factor of 20, indicating a good correlation between τ and reactivity (Fig. 15).[3b] A good correlation between τ and

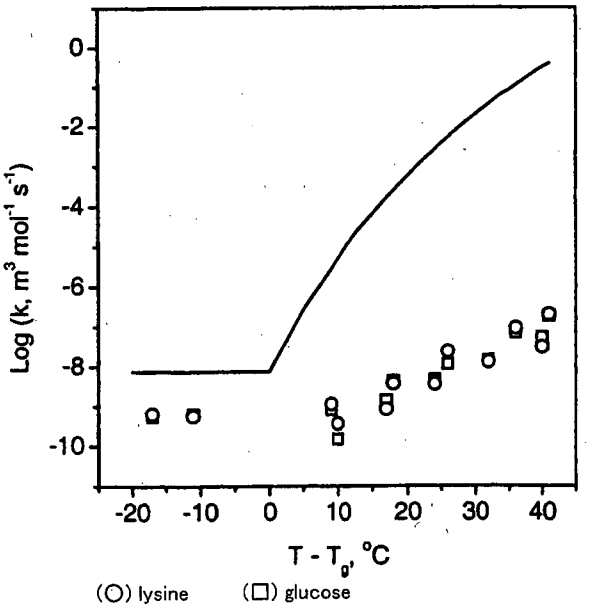

Figure 14. The WLF plots for consumption of lysine and glucose. Solid line represents predicted diffusion-controlled rates (Rates in the glass calculated assuming $\eta = 10^{12}$ Pa). (Reproduced from Reference [62] with permission of copyright owner.)

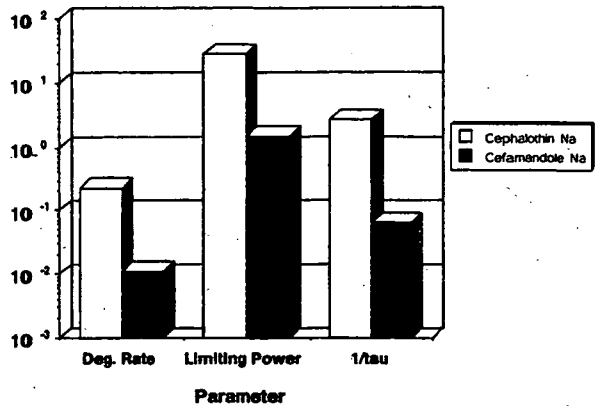

Figure 15. Comparison of relaxation dynamics and degradation rate of freeze-dried cephalosporins at 40°C. (Reproduced from Reference [3b] with permission of copyright owner.)

reactivity was also observed for aggregation of human growth hormone.[3b] Similarly, the aggregation rate of lyophilized IgG1 antibody decreased in conjunction with rises in structural relaxation time associated with increases in the sucrose content up to 0.4 weight fraction. The observation that the degree of retention of native structure, as determined by FTIR, also increased with increasing sucrose content suggests that native protein structure also contributes to stability.[3b]

Guo et al.[66] analyzed the quantitative relationship between the rate constant (k) and the structural relaxation time (τ) of amorphous quinapril hydrochloride using Eq. (4), which is derived from the Stokes–Einstein equation and the Maxwell equation.

$$\frac{k_2}{k_1} \approx \frac{D_{r2}}{D_{r1}} \approx \left(\frac{T_2}{T_1}\right)\left(\frac{\tau_1}{\tau_2}\right)^{\xi} \tag{4}$$

where subscripts 1 and 2 represent parameters at T_1 and T_2, respectively. ξ is a parameter that represents coupling between D_r and τ, with a magnitude of 1 at temperatures well above T_g and a magnitude of 0.75 near or below T_g.[67] The value of τ in Eq. (4) can be calculated according to the Adam–Gibbs–Vogel equation (Eq. 5).

$$\tau = \tau_0 \exp\left(\frac{DT_0}{T - (T/T_f)T_0}\right) \tag{5}$$

where T_f is the fictive temperature. D and T_0 can be calculated from fragility (m) and T_g according to Eqs. (6 and 7), respectively.

$$D = 2.303(m_{min})^2/(m - m_{min}) \tag{6}$$

$$T_0 = T_g(1 - m_{min}/m) \tag{7}$$

$$m_{min} = \log(\tau_{T_g}/\tau_0) \tag{8}$$

It was found that the value of structural relaxation time calculated from the observed rate constant of amorphous quinapril hydrochloride using Eq. (4) was coincident with that calculated using Eq. (5) at temperatures below T_g (Fig. 16), indicating good correlation between k and τ. Substitution of the values 1 or 0.75 for ξ did not cause a significant difference in the fit.

Pikal proposed Eq. (9) to describe the temperature dependence of the rate constant for diffusion-controlled reactions:[4]

$$k = A_k \exp\left(-\frac{gDT_0}{T - (T/T_f)T_0}\right) \tag{9}$$

where A_k is a pre-exponential constant whose value is thought to decrease as the number of diffusional jumps needed to complete a reaction increases. Eq. (9) can be rewritten using τ to give $k = A_k(\tau_0/\tau)^g$; thus, g is a constant representing coupling between k and τ. Compared with Eq. (4), Eq. (9) may be applicable to rate constants obtained in the temperature range in which the value of T_2/T_1 may be approximated to unity.

Eq. (10) was proposed to describe the temperature dependence of the rate constant for reactions that are not completely diffusion-controlled, in

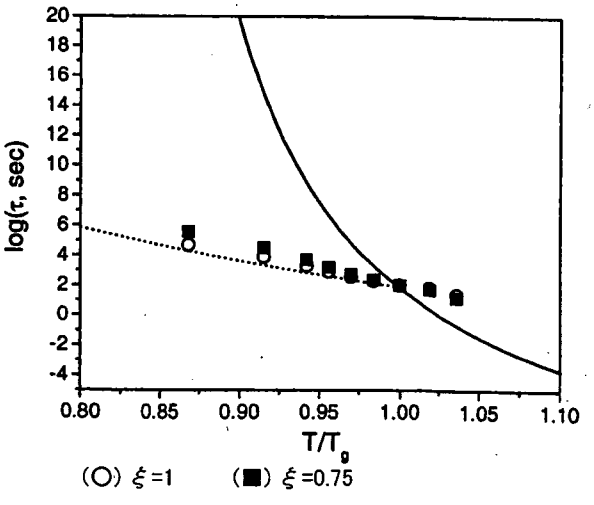

Figure 16. Comparison of the relaxation times calculated from rate constants according to Eq. (4) with $\xi = 1$ and 0.75, and the relaxation times calculated using the VTF equation (solid line) and the AGV equation (dotted line), for the cyclization reaction of amorphous quinapril hydrochloride. (Reproduced from Reference [66] with permission of copyright owner.)

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