Effects of a Citrate Buffer System on the Solid-State Chemical Stability of Lyophilized Quinapril Preparations

Jinjiang Li,[1] Yushen Guo,[1,2] and George Zografi[1,3]

Received October 3, 2001; accepted October 8, 2001

Purpose. The objective of this study was to examine the effect of a citric acid-citrate buffer system on the chemical instability of lyophilized amorphous samples of quinapril hydrochloride (QHCl).

Methods. Molecular dispersions of QHCl and citric acid were prepared by colyophilization from their corresponding aqueous solutions with a molar ratio of QHCl to citric acid from 1:1 to 6:1 and solution pH from 2.49 to 3.05. Solid samples were subjected to a temperature of 80°C and were analyzed for degradation using high-performance liquid chromatography. The glass transition temperature, Tg, of all samples was measured by differential scanning calorimetry.

Results. Samples were first examined by varying the Tg and maintaining the initial solution pH constant. At pH 2.49 the rate of reaction was found to be less dependent on the sample Tg, whereas at pH ≥2.75 the rate decreased with an increase in Tg. In a second set of experiments at a constant Tg of ~70°C, the reaction rate increased as the pH increased.

Conclusion. The overall solid-state chemical reactivity of amorphous quinapril depends on the relative amount of QHCl and Q^{+-}, the zwitterionic form of quinapril. At high proportions of Q^{+-} (higher pH values) the reaction rate seems to be strongly influenced by the Tg of the mixture, and hence the molecular mobility, whereas at higher proportions of QHCl (lower pH) the reaction rate is less sensitive to Tg, presumably because of different mechanistic rate determining steps for the two sets of conditions.

KEY WORDS: amorphous state; chemical instability; citric acid; molecular mobility; pH; quinapril.

INTRODUCTION

Many drugs, including small molecules and proteins, in aqueous solution exhibit significant chemical instability over the timescales of storage and use (1,2). In such cases, it is often possible to lyophilize the solution to produce powders that can be reconstituted just before use (3). It is well recognized, however, that lyophilization most often produces solids that are fully or partially amorphous, and that under certain conditions significant instability can still occur (4,5). This is so because molecules in the amorphous state are super-cooled liquids or glasses that can retain sufficient molecular mobility, i.e., translational and rotational motion, to support chemical reactivity (6,7). Control of molecular mobility in such cases requires attention to storage temperature, relative to the glass transition temperature, Tg, water content, and the presence of other ingredients that also affect Tg (8). Such solid-state chemical reactions can be affected more directly by interaction with other ingredients, including water, and by acid-base environments that might favor or inhibit reactivity. The presence of buffers in lyophilized powders, for example, clearly has been shown to have an effect on the overall solid-state reactivity, which seems related to the initial solution pH (9).

In this study, we wish to consider the situation where the rate of a chemical reaction in the dry amorphous state is measured in the presence of a buffer system that can directly affect the chemical reaction via acid-base equilibria, while also itself having an effect on the Tg, and hence molecular mobility under a given set of conditions. In previous studies, we have prepared the amorphous form of the drug, quinapril hydrochloride (QHCl), and have studied its chemical degradation to form the corresponding diketopiperazine (DKP) as outlined in Scheme 1 (10). During the course of such studies, it was noted that lyophilization from unbuffered QHCl solutions produced solid samples with highly variable reaction rates, depending on the initial solution concentration of QHCl and the resulting solution pH (11). Subsequently, it was recognized that in this pH range it was possible for QHCl to be converted in part to its zwitterionic form, Q^{+-}, and that these lyophilized samples were actually mixtures of QHCl and Q^{+-}. Isolation of pure amorphous QHCl and Q^{+-} revealed Tg values of 91°C and 51°C, respectively (11). Because Q^{+-} under identical conditions exhibited much greater reaction rates than QHCl, it was suggested that this occurred primarily because of its much lower Tg and, hence, greater molecular mobility under the same conditions.

In view of these earlier observations, we chose to form amorphous molecular dispersions of QHCl and citric acid by lyophilization from aqueous solution of known initial pH. From the acid-base equilibria shown for QHCl (QHCl $\rightarrow$ Q^{+-} + Cl^-, pK_a = 3.05) and citric acid (citric acid $\rightarrow$ monosodium citrate, pK_{a1} = 3.12) we were able to know the composition of QHCl, Q^{+-}, citric acid, and monosodium citrate in solution at each initial pH chosen. Based on previous studies with lyophilized protein solutions that seem to retain their initial state of ionization when lyophilized to an amorphous solid, i.e., "pH memory" (12,13), we began our studies by varying the initial solution pH of citric acid-QHCl combinations and estimated the composition of various species in the solid state from their solution equilibria based on the pK_a values. In the case of quinapril, however, we could not be sure whether the zwitterionic form was retained or whether the removal of water had produced the neutral form of quinapril, as shown in Scheme 1 (10). Because we knew the Tg values for the various species (QHCl, 91°C; Q^{+-}, 51°C; citric acid, 11°C; and monosodium citrate, 69°C), we also were in a position to attempt to account for the changes in Tg of the lyophilized solid, and therefore, the possible role of any changes in molecular mobility due to various components. In the first series of experiments, we have maintained the pH constant for various systems while systematically altering the Tg of the lyophilized solid. In the second series of experiments, we systematically titrated the pH of our solutions in a certain range of pH, which maintained the system Tg essentially constant.

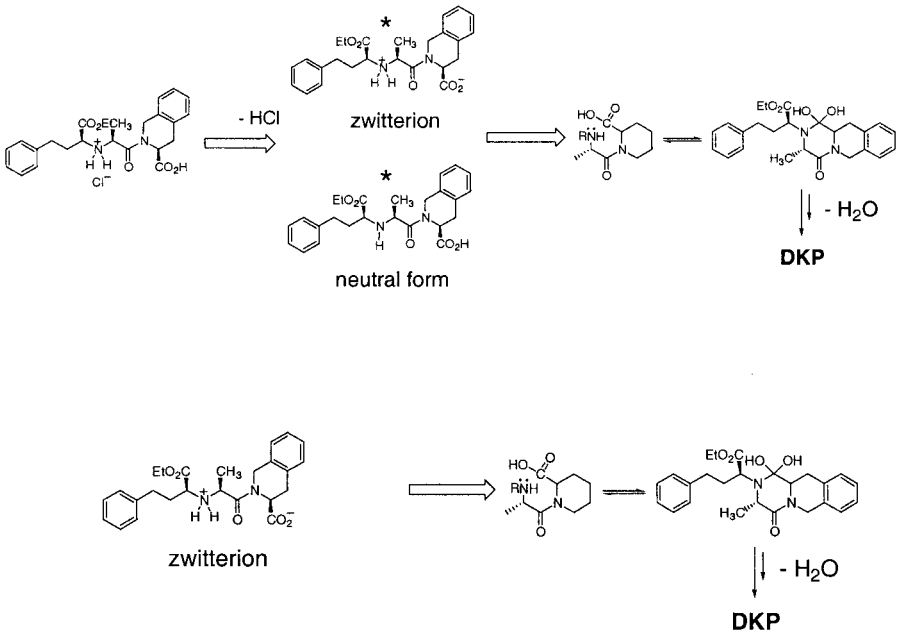
[1] School of Pharmacy, The University of Wisconsin, Madison, Wisconsin 53705.

[2] Present address: Drug Delivery Development, Aventis Pharmaceuticals, Bridgewater, New Jersey 08807.

[3] To whom correspondence should be addressed. (e-mail: gzografi@facstaff.wisc.edu)

ABBREVIATIONS: DKP, diketopiperazine; DSC, differential scanning calorimetry; HPLC, high-performance liquid chromatography; k_{app}, first-order rate constant; PXRD, powder x-ray diffraction; QHCl, quinapril hydrochloride; Tg, glass transition temperature.

0724-8741/02/0100-0020/0 © 2002 Plenum Publishing Corporation



Scheme 1. *Represents the two possible solid-state intermediates generated during the escape of HCl. This differs from the reaction in solution where only the zwitterion is produced.

MATERIALS AND METHODS

Materials

QHCl [3S-[2[R*(R*)], 3R*]]-2-[2-[[(1-ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, hydrochloride was received from the Chemical Processing Division of the Warner-Lambert Co. (Holland, MI) as a gift. The zwitterionic form of quinapril, Q^{+-}, was prepared according to the following procedure. A combination of 0.1 N NaOH and 0.1 N sodium bicarbonate solutions was added to an aqueous solution of QHCl. The precipitate was first filtered and then washed more than three times with deionized water followed by drying in a desiccator containing P_2O_5 under vacuum. There was no Cl^- present in the product based on ion chromatographic analysis. High-performance liquid chromatography (HPLC) measurement showed a single peak corresponding to the retention time of quinapril, and no degradation product was detected. Both citric acid and monosodium citrate were purchased from Mallinckrodt Chemical Co. (Paris, KY). HPLC-grade acetonitrile and methanol were purchased from EM Scientific Co. (Gibbstown, NJ). Other chemicals used including sodium hydroxide and hydrochloric acid were all analytical grade. Deionized water was obtained using a SYBRON/Barnstead pressure cartridge purification system (Pressurized Cartridge System, Boston, MA).

Methods

Lyophilization

All solution samples with quinapril concentration of 1.05×10^{-2} M were lyophilized using a commercial tray dryer (Dura-Stop, FTS Systems, Stone Ridge, NY) in combination with a condenser module (Dura-Dry-MP, FTS Systems). The vials used were liquid scintillation vials from Research Products International Co. (Mount Prospect, IL) with a volume of 20 ml (diameter, 27–28 mm; and height, 57.5 ± 0.1 mm). First, solution samples were transferred into scintillation vials, about 8 ml for each vial, followed by transferring the sample containing vials to a freeze-dryer, which was then frozen to −40°C and kept at this temperature for more than 24 h before applying a vacuum. After 24 h under vacuum, the temperature was raised to −30°C, −20°C, −10°C, and 0°C, respectively every 12 h, and secondary drying was performed at 25°C for 24 h. After lyophilization, samples were pulverized in a glove-box under N_2 atmosphere followed by vacuum-oven drying for 24 h. The water content of all samples was found to be <0.2% by Karl Fischer titration.

pH Measurement

A Denver Instrument pH meter (model 225, Arvada, Co., Chicago, Illinois) equipped with a Fisher Scientific Accumet glass body pH electrode was used for all pH measurements. The pH meter was calibrated using standard buffer solutions (Aldrich Chemical Co., Milwaukee, WI) of pH 1.00, 2.00, and 4.00 (±0.01).

Ion Chromatography

A Shimadzu LC-10AT liquid chromatograph instrument (Columbia, Maryland) equipped with a Shimadzu CDD-6A conductivity detector was used to measure chloride ion concentration in both the initial aqueous solutions and the reconstituted solutions. The system consisted of an Alltech (Deerfield, Illinois) Durasep A, 27-μm column (internal diameter, 4.6 mm; length, 100 mm) for separation and an Alltech anion suppressor cartridge for improving sensitivity. The instrument was controlled by a computer via a Shimadzu SCL-10 A controller. A mobile phase consisting of 1 mM sodium bicarbonate and 1 mM sodium carbonate solutions (50:50) was used. A typical flow rate was 1.0 ml/min. Quantitative analysis of chloride ion was based on the response factor of peak area relative to that of standard NaCl solutions.

Powder X-Ray Diffraction Analysis and Optical Microscopic Analysis

The powder x-ray diffraction (PXRD) patterns of all samples used in this study were taken at ambient temperature using a Scintag PadV powder x-ray diffractometer (Scintag Inc., Santa Clara, CA) at 40 mA and 35 kV with CuKa radiation. The scan range of 2θ was from 5° to 40° with a step size of 0.02° and a scanning rate of 5°/min. All samples were also examined using an Olympus BH-2 optical microscope equipped with polarized light (Olympus Optical Co., Tokyo, Japan).

Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) thermograms of amorphous samples consisting of quinapril and citric acid were recorded using a Seiko I SSC/5200 differential scanning calorimeter (Seiko Instruments, Horsham, Pennsylvania) equipped with a Hewlett Packard model 712/60 data station (Palo Alto, California). Dry nitrogen was used as the purge gas (85 ml/min) and liquid nitrogen as the coolant. High-purity indium and biphenyl were used for temperature calibration at a heating rate of 20 K/min. Typically, 5–10 mg of sample was transferred to an aluminum pan in a glove-box under N_2 atmosphere followed by sealing the pan nonhermetically. Measurement of the Tg was performed by placing the sample pan with a pinhole in the lid in a DSC furnace with reference to an empty pan, followed by heating at 20 K/min. The Tg was determined by constructing tangents to the DSC thermogram baseline before and after the glass transition. The intersection of these tangents to the tangent at the inflection point gives the extrapolated onset temperatures. For measuring the Tg of monosodium citrate, an amorphous sample of monosodium citrate was first prepared by lyophilizing 5% of monosodium citrate solution followed by measuring Tg according to the above procedure.

Density Determination

The density of amorphous solids was measured at ambient temperature using a Quantachrome Multipycnometer (Boynton Beach, FL). The sample cell volume was calibrated using standard steel balls and was verified using crystalline sucrose ($\rho = 1.59$ g/cm^3). The density values for QHCl, Q^{+-}, citric acid, and monosodium citrate are 1.18 g/cm^3, 1.21g/cm^3, 1.58 g/cm^3, and 1.80 g/cm^3, respectively.

Solid-State Chemical Stability

Measurements of the solid-state chemical stability of quinapril in the lyophilized samples containing quinapril and citric acid was carried out by transferring 10 mg of the sample into open glass vials (Fisherbrand) followed by their placement in a desiccator that contained P_2O_5 for maintaining dryness. A Fisher Scientific Isotemp Premium Oven (model 750G) was used to maintain the reaction temperature constant at 80 ± 0.5°C with reaction time ranging from 5 to 60 h. The sample temperature was monitored using an Omega microprocessor thermometer (model HH23, Stamford, Connecticut) with a type-K thermocouple directly contacted with the solid sample. At periodic time intervals, the sample was removed from the oven and cooled down immediately before HPLC analysis.

HPLC Analysis

A Thermoseparation Products HPLC system (Spectra-Physics, Fremont, CA) was used to separate and identify quinapril and its degradation products. It consisted of a Spectra SYSTEM P1000 pump, a Spectra SYSTEM UV1000 detector, and a ChemJet integrator. An Altex Ultrasphere-ODS reverse-phase column (4.6 mm internal diameter × 25 cm, Alltech) and an ODS guard column cartridge (2.0 mm internal diameter × 1.0 cm) (Upchurch Scientific Co., Oak Harbor, WA) were used. The mobile phase consisted of a mixture of acetonitrile in water (50%) with an additional 0.1% (v/v) trifluoroacetic acid. The flow rate was 1.0 ml/min, and the detection wavelength was 220 nm. Peak identification was based on the retention time of standard materials and quantitative analysis was performed based on the response factors of peak areas relative to those obtained by measuring authentic samples. Because HPLC cannot differentiate between QHCl and Q^{+-}, the remaining fraction measured was a mixture of both Q^{+-} and QHCl.

RESULTS

As mentioned in the Introduction, the Tg values of QHCl, Q^{+-}, citric acid, and monosodium citrate are 91°C, 51°C, 11°C, and 69°C, respectively. The mixture of QHCl and Q^{+-} is referred to as quinapril, and the mixture of citric acid and monosodium citrate is expressed as citric acid. The first three values are in agreement with those obtained in earlier studies (10,11,14), whereas the value for monosodium citrate is reported here for the first time. To better establish the most likely reference pH to be used in this study, in Table I pH values for various solutions before and after lyophilization are presented, along with the concentration of Cl^-. As shown in Table I, the pH values of the reconstituted solutions are greater than those of the corresponding initial solutions, and there is also an accompanying loss of Cl^-. Thus, the change in pH is attributed to the loss of volatile HCl during the lyophilization process. However, the extent of Cl^- loss is much greater than can be accounted for by the pH change. This difference is most likely attributed to the buffering effect of the citric acid system. In view of these results, any mention of pH in this paper will be given in reference to the reconstituted solution pH because this most likely reflects the situation in the amorphous solid sample.

All samples were shown to be amorphous by PXRD

Table I. pH and Chloride Ion Concentration before and after Lyophilization[a]

Initial solution pH (± 0.01)	The pH of reconstituted solution (± 0.01)	[Cl^-] in the initial solution (M) (± 0.001)	[Cl^-] in the reconstituted solution (M) (± 0.001)
2.31	2.36	0.022	0.020
2.45	2.49	0.022	0.020
2.52	2.60	0.022	0.019
2.60	2.65	0.022	0.019
2.65	2.70	0.022	0.019
2.73	2.77	0.022	0.020
2.80	2.82	0.022	0.018
2.90	2.95	0.022	0.018

[a] The molar ratio of quinapril to citric is 1:1.

(data not shown) and were confirmed by the absence of birefringence using a polarizing optical microscope. In Table II we show measured Tg values for samples prepared with four different molar ratios of quinapril to citric acid and then adjusted to four specific pH values in the range of 2.49–3.05. Also included in Table II for each pH is the molar ratio of Q^{+-} to QHCl that is believed to exist at each pH as well as the estimated Tg. As might be expected with increasing amounts of QHCl (Tg, 91°C) to citric acid (Tg, 11°C), the Tg of the mixture increases. The extent of increase in the overall Tg is less at the higher pH values presumably because of the enhanced amount of monosodium citrate (Tg, 69°C), which increases with increasing pH is offset by the plasticizing effect of increasing Q^{+-} (Tg, 51°C). Based on the Tg values of individual components and their composition change with pH, we expect an increase in pH to produce a higher Tg from the increased amount of the monocitrate ion and a lower Tg because of the increase in the amount of Q^{+-}. For such a four-component system, it is possible to estimate an approximate overall Tg value by assuming reasonably close densities, which allows for the application of the Fox equation (15) as shown below:

$$\frac{1}{Tg} = \frac{w_1}{Tg_1} + \frac{w_2}{Tg_2} + \frac{w_3}{Tg_3} + \frac{w_4}{Tg_4} \quad (1)$$

where w_1, w_2, w_3, and w_4 represent the weight fractions of components with corresponding Tg values of Tg_1, Tg_2, Tg_3, and Tg_4. The estimated Tg values from Eq. 1 are considered as only approximate because Eq. 1 assumes ideal mixing and equal densities. In Table II, the Tg is predicted to increase with the molar ratio of quinapril to citric acid at constant pH, although the magnitude of change in Tg varies with pH. It is interesting to note by comparison with measured Tg values that, although the values are close in some cases, generally the measured values are lower, in particular at higher pH values, indicating some nonideality in the mixing of the various ingredients. Still, the trends for increasing pH or an increasing ratio of quinapril to citric acid at constant pH seem to be as predicted.

Effect of Tg

In Fig. 1, we present the extent of reaction for quinapril as a function of time at 80°C for two representative pH values of 2.49 and 2.95 at various molar ratios of quinapril to citric acid, as shown in Table 2. Here we note in Fig. 1a that at pH 2.49, where the amount of Q^{+-} is relatively low (Q^{+-}/QHCl = 0.28), the reaction rate does not seem to be significantly affected by the overall Tg of the system. Thus, at this pH, and hence at the given constant molar ratio of Q^{+-} to QHCl, any reduced molecular mobility due to an increase in Tg does not seem to significantly affect the reaction rate. This is interesting because the values of temperature difference between the reaction temperature (80°C) and the Tg at these molar ratios varies from almost zero to about 26°C (1:1). In Fig. 1b, we note that the reaction rate at pH 2.95 (Q^{+-}/QHCl = 0.79), on the other hand, systematically increases as the molar ratio of quinapril to citric acid changes from 6:1 to 1:1, or as Tg decreases and T-Tg increases. This is shown in another way in Fig. 2 where the first-order rate constants for reactions at pH values from 2.75 to 3.05 and for all molar ratios of quinapril to citric acid seem to decrease with increasing Tg, whereas a much lower reaction rate, essentially independent of Tg, has

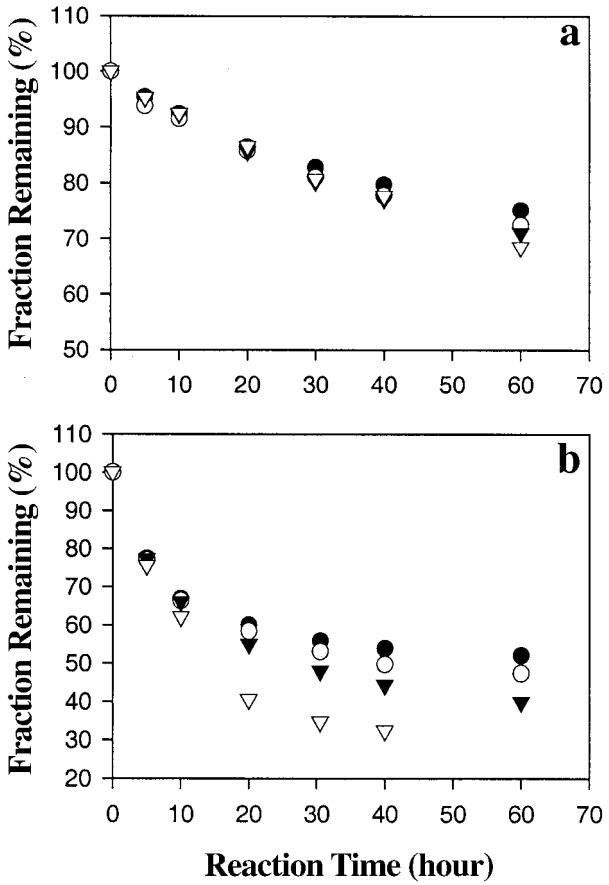


Fig. 1. The reaction profile of quinapril for intramolecular cyclization in lyophilized mixtures of quinapril and citric acid with molar ratios of 6:1 (●), 3:1 (o), 2:1(▼), and 1:1(∇) at pH 2.49 (a) and with molar ratios of 6:1 (●), 3:1 (○), 2;1 (▼), and 1:1 (∇) at pH 2.95 (b).

Table II. Measured Tg Values (Tg^m) and Those Estimated from Eq. 1 (Tg^e) for Samples Consisting of Quinapril and Citric Acid in Various Molar Ratios over the pH Range of 2.49–3.05

Composition (Quinapril/Citric)	Tg (°C) pH = 2.49 Q^+/QHCl = 0.28		Tg (°C) pH = 2.75 Q^{+-}/QHCl = 0.50		Tg (°C) pH = 2.95 Q^{+-}/QHCl = 0.79		Tg (°C) pH = 3.05 Q^{+-}/QHCl = 1.0	
	Tg^m	Tg^e	Tg^m	Tg^e	Tg^m	Tg^e	Tg^m	Tg^e
1:1	54.0	61.7	68.5	60.8	51.0	60.0	49.7	59.6
2:1	68.3	69.9	74.7	67.4	58.0	65.2	55.0	64.0
3:1	73.9	73.4	77.7	70.2	61.3	67.4	58.8	65.9
6:1	80.3	77.4	81.9	73.5	62.5	70.0	62.2	68.1

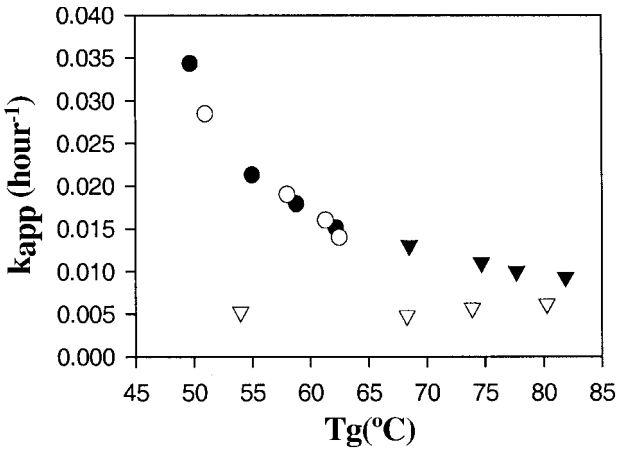


Fig. 2. The Tg-rate profile for lyophilized mixtures of quinapril and citric acid: pH 3.05 (●), pH 2.95 (o), pH 2.75 (▼), and pH 2.49 (▽). k_{app} is the apparent first-order rate constant.

been observed for the samples with a solution pH of 2.49. Thus, it seems that at pH 2.49 enhanced molecular mobility is not as important a factor in favoring chemical reactivity, whereas at pH 2.95 reactivity is clearly enhanced as T-Tg increases and the overall molecular mobility of the system is enhanced.

Effect of Solution pH

In Fig. 3a, we present the reaction profiles for quinapril at 80°C, where the remaining fraction is plotted as a function of time, for a situation where the molar ratio of quinapril to citric acid is maintained as 1:1 over the pH range of 2.60–2.82. In this case, the overall Tg remains fairly constant in the vicinity of 67–70°C (see Table III), but the reaction rate increases systematically with increasing pH. Presumably at a constant ratio of quinapril to citric acid, the amount of Q^{+-} increases sufficiently as the pH is raised to enhance the overall reaction rate despite a fairly constant overall Tg for the system (see Fig. 3b). In Fig. 3b, the first-order kinetic rate constants obtained based on the experimental data in Fig. 3a are plotted as a function of the mole fraction of Q^{+-}in the sample, indicating a consistent increase of rate constant with the amount of Q^{+-} present.

DISCUSSION

From the experimental degradation rates observed for different systems a number of interesting observations have been made. Clearly, for samples at pH 2.75 and higher the reaction rate seems to decrease when the overall Tg value is increased from a T-Tg of about 26°C to a T-Tg of about 0°C, as shown in Fig. 2. Thus, in these cases the presence of more Q^{+-}, generally showing a greater extent of reactivity than QHCl due to a higher pH, is responsible for the rate change with a change in the molecular mobility of the system. On the other hand, at pH 2.49, where the estimated ratio of Q^{+-} to QHCl is about 0.28 to 1.0, we see lower reactivity relative to higher pH systems and the reaction rate shows little dependence on an increase in the overall Tg of the system. It would seem, therefore, that the reaction rate at this pH is controlled by the presence of the great amount of QHCl in a manner not dictated by molecular mobility. From such observations it is

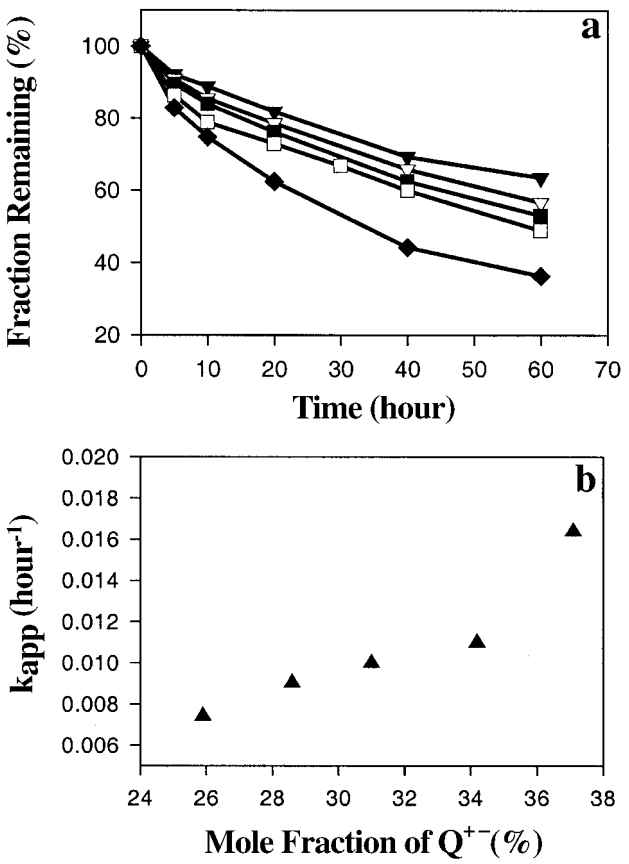


Fig. 3. (a) The reaction profile of quinapril for intramolecular cyclization in lyophilized mixtures of quinapril and citric acid at a 1:1 molar ratio at different pH values: 2.60 (▼), 2.65 (▽), 2.70 (▽), 2.75 (□), and 2.82 (■). (b) A plot showing k_{app} as a function of mole fraction of Q^{+-} for the lyophilized mixtures of quinapril and citric acid at a 1:1 molar ratio at 80°C.

concluded that the rate-limiting step for this reaction that determines the overall rate constant may vary with the molar ratio of Q^{+-} to QHCl. As shown in Scheme 1, the critical part of the reaction at low levels of Q^{+-} is the loss of HCl by QHCl, followed by a trans-to-cis conformational change. This conformational change allows an intramolecular nucleophilic attack (-NH-) at the carboxylic acid group attached to the isoquinoline ring to form a zwitterionic tetrahedral intermediate followed by the expulsion of H_2O from the intermediate (9,10,16). Also shown in Scheme 1, the intermediate produced due to the loss of HCl from QHCl is most likely different from the zwitterion produced from solution. In the solid state for systems containing QHCl only, it has been shown that the

Table III. Measured Tg Values for the Lyophilized Mixtures of Quinapril and Citric in 1:1 Molar Ratio at Different pH and the Corresponding Molar Ratio of Q^{+-} to QHCl

pH of the reconstituted solution	Measured Tg (°C)	Q^{+-}/QHCl
2.60	67.0	0.35
2.65	68.0	0.40
2.70	67.0	0.45
2.77	69.0	0.52
2.82	70.0	0.59

escape of HCl vapor is the rate-limiting step (10), and thus kinetically this reaction as seen in Scheme 1 can be simplified as: QHCl → DKP. For Q^{+-} to form DKP in the solid state, Q^{+-} probably goes through one or more proton transfer steps followed by the same mechanism to form DKP (9). Because water is a good leaving group at 80°C, the rate-limiting step for the formation of DKP from Q^{+-} is most likely the conformational change from trans to cis (16), which would be expected to be molecular mobility dependent. From a kinetic point of view, this reaction can be written as: $Q^{+-} \rightarrow$ DKP. In the case of lyophilized solid consisting of both QHCl and Q^{+-}, the reaction rate for production of DKP or consumption of quinapril can be considered as a combination of these two reactions with the rate given by the following equation:

$$\frac{-d[DKP]}{dt} = \frac{-d[quinapril]}{dt} = (k_1[QHCl] + k_2[Q^{+-}]) \qquad (2)$$

where k_1 and k_2 are the apparent rate constants for reactions forming DKP from QHCl and Q^{+-}, respectively. To facilitate our analysis, we can express [QHCl] and $[Q^{+-}]$ in terms of the total quinapril concentration in the sample and the molar ratio of Q^{+-} to QHCl (r), and thereby Eq. 2 can be rearranged to the following form.

$$\frac{-d[quinapril]}{dt} = \left(k_1 \frac{1}{1+r} + k_2 \frac{r}{1+r}\right)[quinapril] = k_{app}[quinapril] \qquad (3)$$

$$\text{where } \frac{1}{1+r}[quinapril] = [QHCl] \text{ and } \frac{r}{1+r}[quinapril] = [Q^{+-}]$$

Equation 3 indicates that the apparent first-order rate constant (k_{app}) for quinapril degradation depends on both the intrinsic reactivity of Q^{+-} and QHCl , and their molar ratio, r, which changes with reaction time. However, the molar ratio (r_0) at the initial stage of the reaction, to which r is proportional, can be estimated from the pH (see Tables II and III). Thus, in this article r_0 is used to qualitatively interpret our experimental results and to estimate the initial rate constant. Given that Q^{+-} is more reactive than QHCl ($k_2 >> k_1$) under the same experimental conditions (11), it would be expected that the overall reactivity for quinapril in the solid state increases with r_0 and in turn with pH. This is consistent with our experimental observation (Fig. 3). To account for the effect of molecular mobility on the reaction rate, two situations are considered: (1) pH = 2.49; and (2) 3.05 ≥ pH ≥ 2.75. Because at pH 2.49 the amount of Q^{+-}, relative to QHCl, is small (r_0 = 0.28), the rate constant for quinapril degradation is predominantly influenced by the reactivity of QHCl, as shown in Eq. 3. Mechanistically, the reaction of QHCl to form DKP is controlled by the escape of HCl (10) and thus the rate constant, k_{app}, seems to be less Tg dependent (see Fig. 2). In the pH range of 2.75–3.05, the amount of Q^{+-} becomes substantial, as reflected by a higher molar ratio of Q^{+-} to QHCl (r_0 > 0.50), therefore k_{app} is mainly contributed to by the reaction of Q^{+-} as seen in Eq. 3 due to $k_2 >> k_1$. Because k_2 is not only greater than k_1 but also molecular mobility dependent, k_{app} is higher and Tg dependent (Fig. 2). To further test this hypothesis, the rate constant at the initial stage of the reaction, calculated based on Eq. 3 using the k_{app} for QHCl at 80°C as k_1 and the k_{app} for Q^{+-} scaled to a Tg of 70°C and a reaction temperature of 80°C (Tg /T = 0.97) as k_2, is plotted as a function of pH and compared with that estimated from experimental data by considering a reaction time up to 10 h (see Fig. 4). Figure 4 indicates that the predicted change of the rate constant with pH generally agrees with that obtained experimentally, although there is some discrepancy between these two rate constants possibly in part due to an under- or overestimation of the experimental initial rate constant or an effect of the chemical structure of the citrate ion, e.g., steric effect or citrate salt formation (17). Nonetheless, these results seem to support our hypothesis that the distribution of the ionization state in solution can be essentially carried over to the solid state after lyophilization and that the solid-state stability can be correlated to the composition of the prelyophilized solution.

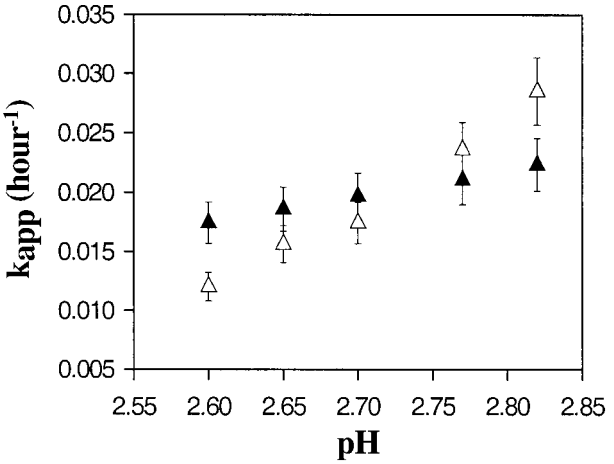


Fig. 4. A plot showing a comparison between the calculated pH-rate profile (see text) (▼) and the experimental pH-rate profile (∇).

CONCLUSIONS

In this work, it has been demonstrated that the lyophilization of QHCl with citric acid affects both the sample Tg and its corresponding pH, consequently influencing the chemical reactivity of quinapril in the solid state. Controlling pH can regulate the molar ratio of Q^{+-} to QHCl and thus the amount of Q^{+-} in the sample, which ultimately determines the overall reactivity and the rate-limiting step. At a constant Tg, the reaction rate of quinapril increases with pH because the amount of Q^{+-} increases with pH and Q^{+-} is more reactive than QHCl under the same experimental conditions. At low pH, the reaction rate for quinapril seems to be less sensitive to sample Tg and thus the molecular mobility of the system. Mechanistically, at low pH the dominant form of quinapril is QHCl and its reactivity in the solid state is controlled by the escape of HCl vapor (10). In the pH range of 2.75–3.05 (Q^{+-}/QHCl = 0.50–1.0), the chemical reactivity of quinapril seems to decrease with increasing sample Tg. It is hypothesized that, in this pH range, the amount of Q^{+-} in the sample becomes substantial and the rate-limiting step is controlled by the conformational change from the trans to cis forms, which is molecular mobility dependent. The above model seems to be supported by our calculations using Eq. 3.

ACKNOWLEDGMENT

The financial support from The Purdue-Wisconsin Program on the Chemical and Physical Stability of Pharmaceu-

tical Solids is gratefully acknowledged. The authors would like to thank Mr. C. Lieven for technical assistance in carrying out the HPLC analysis.

REFERENCES

1. T. Österberg and T. Wadsten. Physical state of L-histidine after freeze-drying and long term storage. *Eur. J. Pharm. Sci.* **8**:301–308 (1999).
2. E. J. McNally. *Protein Formulation and Delivery*. Marcel Dekker, New York, 2000.
3. W. Wang. Lyophilization and development of solid protein pharmaceuticals. *Int. J. Pharm.* **203**:1–60 (2000).
4. D. Q. M. Craig, P. G. Royall, V. L. Kett, and M. L. Hopton. The relevance of the amorphous state to pharmaceutical dosage forms: glass drugs and freeze-dried systems. *Int. J. Pharm.* **179**: 179–207 (1999).
5. B. C. Hancock and G. Zografi. Characteristics and significance of the amorphous state in pharmaceutical systems. *J. Pharm. Sci.* **86**:1–12 (1997).
6. S. L. Shamblin, X. Tang, L. Chang, B. C. Hancock, and M. J. Pikal. Characterization of the time scales of molecular motion in pharmaceutically important glasses. *J. Phys. Chem. B* **103**:4113–4121 (1999).
7. B. C. Hancock, S. L. Shamblin, and G. Zografi. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. *Pharm. Res.* **12**:799–806 (1995).
8. S. D. Allison, M. C. Manning, T. W. Randolph, K. Middleton, K. A. Davis, and J. F. Carpenter. Optimization of storage stability of lyophilized actin using combination of disaccharide and dextran. *J. Pharm. Sci.* **89**:199–214 (2000).
9. R. G. Strickley, G. C. Visor, L. H. Lin, and L. Gu. An unexpected pH effect on the stability of moexipril lyophilized powder. *Pharm. Res.* **6**:971–975 (1989).
10. Y. Guo, S. R. Byrn, and G. Zografi. Physical characteristics and chemical degradation of amorphous quinapril hydrochloride. *J. Pharm. Sci.* **89**:128–143 (2000).
11. Y. Guo, S. R. Byrn, and G. Zografi. Effects of lyophilization on the physical characteristics and chemical stability of amorphous quinapril hydrochloride. *Pharm. Res.* **17**:930–935 (2000).
12. H. R. Costantino, K. Griebenow, R. Langer, and A. M. Klibanov. On the pH memory of lyophilized compounds containing protein functional groups. *Biotech. Bioeng.* **53**:345–348 (1997).
13. H. T. Vakos, H. Kaplan, B. Black, B. Dawson, and M. A. Hefford. Use of the pH memory effect in lyophilized proteins to achieve preferential methylation of α-amino groups. *J. Protein Chem.* **19**:231–237 (2000).
14. Q. Lu and G. Zografi. Properties of citric acid at the glass transition. *J. Pharm. Sci.* **86**:1374–1378 (1997).
15. T. G. Fox. Influence of diluent and of copolymer composition on the glass temperature of a polymer system. *Bull. Am. Phys. Soc.* **1**:123 (1956).
16. F. A. Carey and R. J. Sundberg. *Advance Organic Chemistry: Part A: Structure and Mechanism*. Plenum Press, New York, 1993.
17. R. G. Strickley and B. D. Anderson. Solid-state stability of human insulin: II. Effect of water on reactive intermediate partitioning in lyophiles from pH 2–5 solutions: Stabilization against covalent dimer formation. *J. Pharm. Sci.* **86**:645–653 (1997).