The Solid-State Stability of Amorphous Quinapril in the Presence of β-Cyclodextrins

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ABSTRACT: The major objective of this study was to investigate the effects of β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP−β-CD) on the solid-state chemical reactivity of the drug, quinapril, when amorphous samples are prepared by colyophilization of quinapril and each of these β-CDs. For comparison, a physical mixture with β-CD and colyophilized mixtures with trehalose and dextran were also prepared and subjected to a similar chemical stability test at 80°C followed by HPLC analysis. Significant inhibition of degradation was observed only for colyophilized miscible mixtures with β-CD and HP−β-CD at molar ratios in excess of 1:1. Colyophilized mixtures with trehalose and dextran, shown to have phase separated, and the physical mixture with β-CD exhibited no inhibiting effects. This suggests that specific molecular complexation is responsible for the significant inhibition by the β-CDs. The tendency of quinapril to form molecular complexes in solution with the β-CDs was measured by ^{1}H solution NMR, by estimating complexation constants from the chemical shift of specific groups on quinapril. Supporting evidence for solid-state complexation was provided by FTIR analysis. DSC and TSC measurements indicated that the β-CDs do not have high enough glass transition temperatures to reduce reactivity by reducing molecular mobility. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:229–243, 2002

Keywords: quinapril; cyclodextrins; glass transition; complexation; chemical degradation

INTRODUCTION

It is generally recognized that for most drug degradation reactions the rate of solid-state degradation is increased when the crystalline form of the drug is rendered partially or fully amorphous.[1–3] This occurs because of the higher state of energy and molecular mobility of drug molecules in the amorphous state relative to the crystalline state.[4–6] Because processes, such as milling, granulation, compaction, and drying, often used in pharmaceutical development, can induce amorphous structure in a crystalline solid, it is not surprising that they also can have a significant effect on solid-state chemical reactivity.[7–9]

In an attempt to better understand the potential role of enhanced molecular mobility in an amorphous region of a solid, it would be useful to prepare a completely amorphous material, characterize its various structural and dynamic properties, and then relate this to its rates of chemical degradation. Recent studies from this laboratory have been carried out along these lines with a model compound, the ACE inhibitor, quinapril hydrochloride (QHCl).[10] QHCl in the solid-state undergoes intramolecular cyclization with the loss of HCl and H_2O to form the corresponding diketopiperazine (DKP) (see Scheme 1).[10] Its value as a model system is that the reaction

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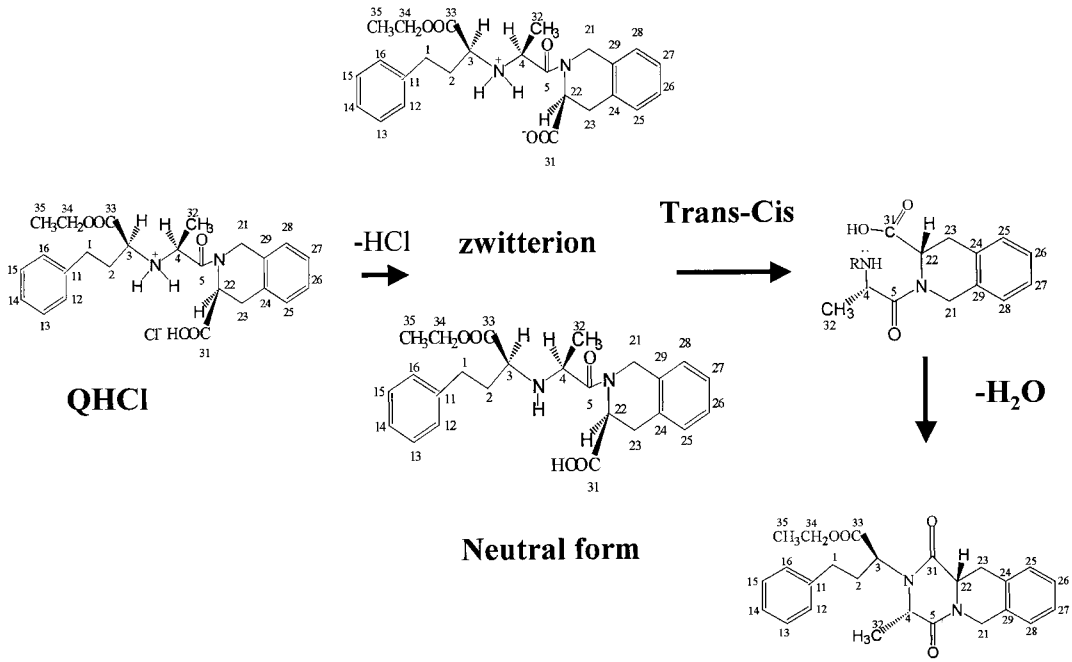
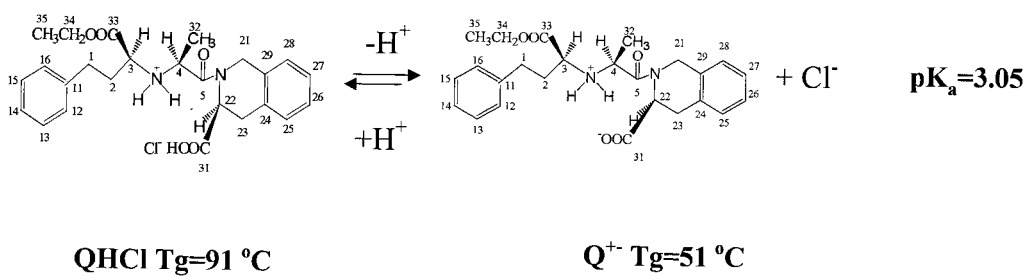


Scheme 1.

mechanism is well understood, it has no tendency to crystallize under various treatments over experimental timescales, an HPLC assay is available, and it can easily be rendered amorphous by a variety of techniques, including grinding, precipitation from solution, and lyophilization.[10,11] To date, it has been shown that QHCl has a glass transition temperature (T_g) of 91°C and that significant thermal degradation can be measured over the range of 70 to 100°C. It has been further shown that degradation rates at temperatures below T_g correlate reasonably well with the effect of temperature based on independently estimated structural relaxation times (molecular mobility), indicating the importance of molecular mobility as a rate-determining step in the reaction. At temperatures above T_g, where the sample tends to soften and aggregate, the rate appears to be controlled by the diffusion of HCl, produced during the reaction, away from the solid into the vapor state.[10]

In a more recent study of lyophilized amorphous samples of QHCl[11] it has been shown that, depending on the initial solution pH, various proportions of a zwitterionic form, Q^{+-}, can be produced (see Scheme 2), with enhanced degradation occurring as the amount of Q^{+-} present increases. Isolation of amorphous Q^{+-} revealed that it has a T_g of 51°C, 40°C lower than that of QHCl, and therefore, a greater degree of molecular mobility than QHCl under the same conditions.

Drug molecules are most often mixed with excipients. If the drug and excipients are rendered amorphous, the possibility exists for the



Scheme 2.

formation of an amorphous molecular dispersion or a "solid solution" of both species. In such cases the dispersion, if miscible, will have one glass transition temperature intermediate to the individual T_g values and dependent on the composition.[12] Depending on the new T_g values, there is the possibility of a change in the molecular mobility and hence a change in the rate of chemical reactivity. It is also possible for the excipient in such cases to produce more specific effects, for example, acid-base equilibria or molecular complexation, giving rise to a "drug-excipient" interaction. Preliminary experiments with a colyophilized sample of quinapril and β-cyclodextrin, known to complex with drugs in solution,[13] indicated a sharp reduction in the rate of chemical degradation at molar ratios of β-cyclodextrin to quinapril greater than 1:1. This study, therefore, was initiated using β-cyclodextrin and hydroxypropyl-β-cyclodextrin to examine this effect more thoroughly and to sort out effects that might be due to "host/guest complexation" as opposed to changes in the amorphous structure and dynamics brought about by the formation of an amorphous molecular dispersion. Colyophilization of quinapril and subsequent stability studies with other carbohydrates, i.e., trehalose and dextran, not capable of forming a host/guest complex, also appeared to provide a basis for interpretation of such effects. To our knowledge, although drug–cyclodextrin complexes in solution have been studied widely, relatively few studies have focused on the stability of drug–cyclodextrin complexes in the solid state.[14–17]

MATERIALS AND METHODS

Materials

Quinapril hydrochloride (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 obtained as a gift from the Chemical Processing Division of the Warner-Lambert Co. (Holland, MI). The major degradation product, quinapril diketopiperazine (DKP, m.p. = 121–123°C), [3S-[2(R*), 3α,11-αβ]]-1,3,4,6,11,11α-hexahydro-3-methyl-1, 4-dioxo-α-(2-phenylethyl)-2H-pyrazino [1,2-*b*] isoquinoline-2-acetic acid, ethyl ester, and quinapril diacid (DA, m.p. = 166–168°C), [3S-[2[R*(R*)], 3R*]]-2-[2-[[(1-carboxy)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, were prepared according to the methods reported in the literature.[18] Both β-cyclodextrin hydrate (β-CD) (MW = 1135, m.p. > 260°C, dec.) and hydroxypropyl-β-cyclodextrin (HP–β-CD) (MW ≅ 1540, MS = 1.0) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and used as received. D (+) trehalose dihydrate (MW = 378) was obtained from Sigma Chemical Co., Inc. (St. Louis, MO), and dextran T10, a linear chain of glucose molecules with a molecular weight of 10,000, was purchased from Pharmacia Biotech AB Co. (Uppsala, Sweden). Trifluoroacetic acid (99+%, spectrophotometric grade) and FTIR grade potassium chloride were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). High-performance liquid chromatography grade methanol (99.8%) and acetonitrile (99.9%) were obtained from Fisher Scientific Inc. (Fair Lawn, NJ). Other chemicals used, including sodium hydroxide and hydrochloric acid, were all analytical grade. For all experiments, water purified by a SYBRON/Barnstead pressure cartridge system (PCS) (Boston, MA) was used.

Methods

Preparation of Amorphous Samples

First, aqueous solutions of QHCl were prepared by dissolving 5 g of QHCl in one liter of deionized water followed by adjusting the solution pH to 3 using 0.1 N NaOH solution. This pH was necessary because at lower pH values degradation of the cyclodextrin was observed during lyophilization and subsequent oven drying. At this pH, we estimated the molar ratio between Q^{+-} and QHCl to be about 0.90:1.0. Consequently, in all subsequent studies the initial solutions used to prepare amorphous β-CD/quinapril mixtures consisted of Q^{+-} and QHCl at this ratio. This mixture of species will be designated quinapril throughout this study. Then, 100 mL of the solution was transferred into a 100 mL volumetric flask followed by addition of β-CD with corresponding β-CD/quinapril molar ratios ranging from 0.2 to 1.8. The same procedure was used to prepare an amorphous dispersion at a 1:1 molar ratio of quinapril to HP–β-CD, trehalose, and dextran. Subsequently, the various aqueous solutions of different molar ratios were quickly transferred to scintillation vials (diameter 27–28 mm and a height of 57.5 ± 0.1 mm) for lyophilization. Each vial contained about 10 mL of the solution. A commercial tray dryer (Dura-Stop, FTS Systems, Stone

Ridge, NY) in combination with a condenser module (Dura-Dry-MP, FTS Systems, Stone Ridge, NY) was used to freeze dry the aqueous solutions. The solution samples were first frozen to -40°C and maintained at this temperature for over 24 h before applying a vacuum. After 24 h under vacuum, the temperature was increased to -30, -20, -10, and 0°C every 12 h. The secondary drying was performed at 25°C for 24 h. The freeze-dried samples were immediately pulverized in a glove box under N_2 atmosphere followed by further drying in a vacuum-oven for another 24 h. For the preparation of a physical mixture of β-CD/quinapril at a 1:1 molar ratio, the lyophilized β-CD and quinapril prepared from its pH 3 aqueous solution by lyophilization were first mixed followed by shaking for about 1/2 h. For all solid samples, water content was determined to be below 0.2% by Karl Fischer titration.

PXRD and Optical Microscopic Analysis

The powder X-ray diffraction patterns of both the colyophilized mixtures at various molar ratios and physical mixtures 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 Cu Kα radiation. Samples were transferred to a quartz sample holder and the scan range of 2θ was from 5° to 40°, with a step size of 0.02° and scanning rate of 5° min^{-1}. All samples were examined using an Olympus BH-2 optical microscope equipped with polarized light (Olympus Optical Co., LTD, Tokyo, Japan).

Solid-State Stability

The solid-state stability of quinapril in both the colyophilized mixtures and physical mixtures was measured by transferring 10 mg of samples into open glass vials (15×45 mm, Fisherbrand) followed by placing these vials in a desiccator with P_2O_5 for maintaining dryness. A Fisher Scientific Isotemp® Premium Oven (Model 750G) was used to keep the reaction temperature constant. The intramolecular cyclization reaction was followed at 80°C, and the reaction time typically ranged from 5 to 60 h. The sample temperature was monitored using a Type-K thermocouple and was found to be $80 \pm 0.5^{\circ}$C. Upon completion of each reaction, the reaction product was taken out, cooled down immediately, followed by dissolving in a solution of methanol and water (50:50) before HPLC analysis.

HPLC Analysis

HPLC analysis of quinapril and its degradation products was performed using a Thermoseparation Products HPLC system (Spectra-Physics Analytical, Fremont, CA), equipped with a Spectra SYSTEM P1000 pump, a Spectra SYSTEM UV 1000 detector, and a ChemJet integrator. An Altex Ultrasphere-ODS reverse phase column (4.6 mm I.D. $\times$ 5 cm, Alltech, Deerfield, IL) connected with ODS guard column cartridge (2.0 mm I.D. $\times$ 1 cm, Upchurch Scientific, Oak Harbor, WA) was used for separation. The mobile phase consisted of a mixture of acetonitrile in water (50% v/v) with an additional 0.1% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL/min, and the detection wavelength was 220 nm. Quantitative analysis is based on the response factors of peak area relative to those obtained by measuring authentic pure samples. The assay was not able to differentiate between QHCl and Q^{+-}, and hence, the amount of quinapril remaining represents a mixture of QHCl and Q^{+-}.

Solution NMR Analysis

To determine any tendencies for quinapril to complex with β-CDs in the amorphous solid state, it was deemed useful to first establish if such a tendency would exist in the aqueous solutions from which the solids were lyophilized. Hence, solution NMR measurements were carried out at pH 1.0 and 3.0 to measure possible complexation constants and to identify groups on the quinapril molecule that might be involved in such a complex. For this purpose, a Bruker DMX 500 NMR spectrometer equipped with a QNP probe was used to obtain spectra at 25°C, pH 1.0 and pH 3.0. All 1D ^{1}H spectra were recorded at 500 ± 0.1 MHz. The stock solution of QHCl was prepared by dissolving 500 mg in 100 mL of D_2O followed by adjustment to either pH 1.0 or 3.0 using 0.1 N HCl and NaOH solutions. Then, 5 mL of quinapril–D_2O solution was pipetted into a 20 mL vial followed by the addition of β-CD or HP–β-CD. The amount of β-CD added to the quinapril–D_2O solution gave β-CD:quinapril molar ratios ranging from 0 to 1.8. All solutions were freshly prepared before NMR spectra were recorded. Similar solutions of quinapril and HP–β-CD were prepared at HP–β-CD:quinapril molar ratios of 0 to 5.0. In addition, trimethylamine hydrochloride (2 mg) was added into all stock solutions as an internal standard. All samples were well agitated to ensure complete dissolution. To obtain a NMR

spectrum, about 2 mL of the above solutions were transferred to a 5 mm NMR tube (Wilmad/Lab Glass, Buena, NJ) followed by transporting the tube into the NMR instrument. A standard tuning, matching and acquiring procedure was followed.

To assign any NMR proton peaks for quinapril dissolved in D_2O that might undergo a chemical shift change, an indication of possible interaction with the β-CDs, the 2D HMBC ^{1}H-^{13}C NMR spectrum for quinapril was recorded at 25°C on a Bruker DMX 500 spectrometer equipped with a triple-resonance $^{1}H/^{13}C/^{15}N$ probe using a HMBC pulse sequence. All spectra collected at the National Magnetic Resonance Facility at Madison (NMRFAM, University of Wisconsin–Madison) made use of digital filtering capabilities.

Solid-State ^{13}C NMR

In an attempt to observe any ^{13}C chemical shifts for lyophilized samples of quinapril and the β-CDs, solid-state ^{13}C NMR spectra were collected using cross polarization magic angle spinning at 75 MHz on a Chemagnetics CMX-300 spectrometer. Powder samples of QHCl and the colyophilized mixture of β-CD/quinapril were first inserted into a 7.5 mm zirconia rotor. A contact time of 2 ms and recycle delay of 2 s were employed. A magic angle spinning rate of 5 kHz was used. A comparison of spectra for quinapril alone and that colyophilized with β-CD (data not shown) revealed that the spectral peaks of quinapril alone were too broad to indicate any chemical shifts using the solid-state NMR technique.

FTIR Spectra

FTIR spectra for selected lyophilized samples of quinapril and β-CD were obtained using a Galaxy Series FTIR 5000 spectrometer made by ATI Mattson (Madison, WI). The IR spectrometer was controlled by a PC with WinFIRST-Fourier Infrared software for data acquisition and analysis. KCl pellets of solid samples instead of KBr were prepared using a minipress without extra grinding for reasons previously described.[10] The weight ratio of KCl to drug was about 100.

Differential Scanning Calorimetry (DSC) Analysis

The DSC thermograms of all colyophilized and physical mixtures were measured using a Seiko I SSC/5200 differential scanning calorimeter (Seiko Instruments, Horsham, PA) equipped with a Hewlett Packard Model 712/60 data station. 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. The glass transition temperature was determined at a scanning rate of 20 K/min 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 glass transition temperature.

Thermally Stimulated Current (TSC) Analysis

TSC analysis of β-CD, HP–β-CD, and selected colyophilized and physical mixtures of β-CD/quinapril was performed on a TSC 9000 spectrometer (Thermold Partners Inc., Stamford, CT). In contrast to DSC measurements, in which an enthalpic change is measured, TSC measures the depolarization current from dipole relaxation. Typically, about 30 mg of sample was transferred to a sample press followed by pressing the sample into a pellet. This pellet was then quickly transferred to a sample holder insulated using a piece of Teflon film. An electrometer was placed on the top of the pellet to measure the depolarization current.[19] For colyophilized samples, the samples were opened in a dry-box followed by placing a small amount of sample in Teflon-covered DSC Al pans. This pan was crimped followed by placing the pan between electrodes. The sample was first purged using He gas, and then cooled to −100°C using liquid nitrogen. For TSC, a voltage (100 VDC) was applied across the sample while it was heated. Measurement of relaxation temperature was achieved by heating the sample at 7°C/min while monitoring the depolarization current at the same time. The current minimum was determined to be the relaxation temperature. Typically for each sample three runs were carried out.

RESULTS

Powder X-ray Diffraction (PXRD) and Optical Microscopic Analysis

The powder X-ray diffractograms of various β-CD/quinapril samples including the original

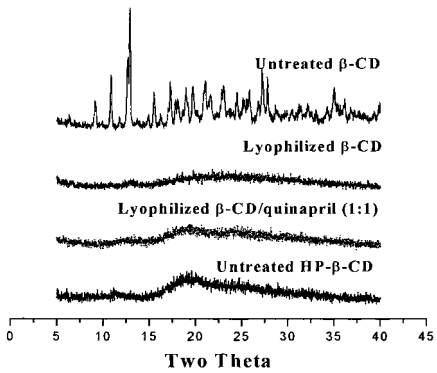


Figure 1. Powder X-ray diffractograms of untreated β-CD,lyophilized β-CD, 1:1 colyophilized β-CD/quinapril mixture, and untreated HP−β-CD.

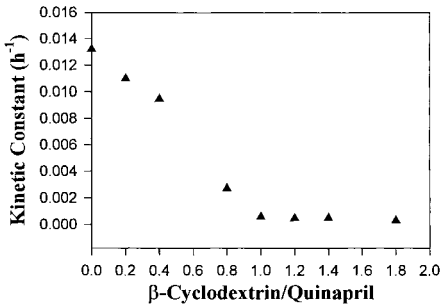


Figure 3. Kinetic rate constants for quinapril intramolecular cyclization as a function of the β-CD/quinapril molar ratio at 80°C.

crystalline β-CD, freeze-dried β-CD, 1:1 colyophilized β-CD/quinapril and the original HP−β-CD are shown in Figure 1. The absence of birefringence under polarized light was also observed for all samples. These results indicate that lyophilization of β-CD and quinapril aqueous solutions generated amorphous materials.

Chemical Degradation of Quinapril

Chemical stability of quinapril in the presence of β-CDs and other carbohydrate excipients lyophilized from a pH 3.0 solution was studied by heating samples at 80°C. Figure 2 compares the degradation profile of a 1:1 colyophilized β-CD/quinapril mixture with those of a 1:1 physical mixture and quinapril alone, where the remaining fraction of quinapril after degradation is plotted as a function of reaction time. Figure 2 demonstrates that degradation of quinapril was essentially eliminated in the 1:1 colyophilized mixture, whereas quinapril degradation in the 1:1 physical mixture was similar to that of quinapril alone. This indicates that β-CD in the colyophilized mixture is closely associated with quinapril, but that β-CD is not involved in any reaction with quinapril in the physical mixture. To further illustrate this, a series of samples with a variety of β-CD/quinapril molar ratios ranging from 0.2 to 1.8 were prepared from corresponding aqueous solutions by lyophilization and thermally treated at 80°C. The kinetic rate constants for these samples (k_{app}) obtained by treating quinapril degradation as a first-order reaction are plotted as a function of β-CD/quinapril molar ratio (Fig. 3). Figure 3 shows that the extent of quinapril degradation decreases linearly with an increase in the molar ratio until 1.0 followed by a plateau. This linear reduction of rate constant with molar ratio suggests that the protection of quinapril by β-CD in the solid state is stoichiometrically correlated with the β-CD/quinapril molar ratio in solution. Thus, it would appear that specific interactions between β-CD and quinapril in solution might be carried over from solution to the solid state. To clarify this further, quinapril was colyophilized with other carbohydrates including HP−β-CD, trehalose (small molecule) and dextran (polymer) followed by a similar investigation. Table 1 lists the kinetic rate constants for quinapril degraded at 80°C in the presence of all these excipients. The k_{app} value for quinapril alone is also shown for comparison. Table 1 shows that both β-CD and HP−β-CD reduced the degradation of quinapril significantly (low k_{app}), whereas dextran and trehalose did not affect the reaction at all. Therefore, the reduction of quinapril degradation in the presence of β-CD and HP−β-CD appears to be related to the commonality of their chemical structure and their ability to form a guest−host complex.

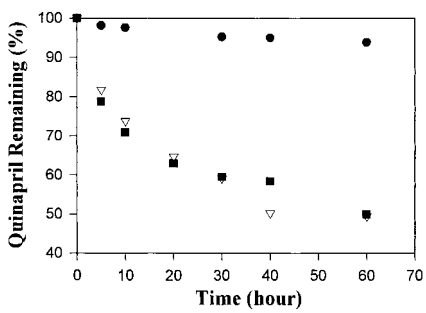


Figure 2. Degradation profile of the 1:1 colyophilized β-CD/quinapril mixture (●), 1:1 physical mixture (▽), and quinapril alone (■) at 80°C.

Table 1. Degradation Kinetic Constants for the Intramolecular Cyclization of Quinapril in the Presence of Different Excipients at 80°C for Samples Lyophilized From a pH 3.0 Solution

Sample	k ($hour^{-1}$) (±5%)
Quinapril	0.013
Physical mixture (1:1)	0.015
β-CD/quinapril (1:1)	0.00058
HP–β-CD/quinapril (1:1)	0.0010
Trehalose/quinapril (1:1)	0.015
Dextran/quinapril (1:1)	0.012

The number in parenthesis represents one average standard deviation.

Solution NMR Analysis

Very often in studying the molecular complexation of substances with cyclodextrins in solution, chemical shifts of internal protons from the cyclodextrin (e.g., H-3 or H-5), are monitored.[20–22] In this work, because of overlapping of the β-CD and quinapril spectra in this region we chose to follow well-defined chemical shifts for quinapril in the various systems containing β-CDs. From these results no new chemical shift was observed in the spectra of β-CD/quinapril, indicating that no chemical reactions, such as hydrolysis, occurred in solution. Figure 4 displays a portion of 1D solution ^{1}H NMR spectrum (2.50 ppm to 3.75 ppm) for both quinapril alone and β-CD/quinapril (1.5:1) solution mixture; a 1D ^{1}H NMR spectrum of β-CD alone is shown for comparison. Because the ^{1}H NMR spectrum for quinapril overlapped with that for β-CD in the region above 3.40 ppm, the region below 3.40 ppm was selected for studying any chemical shift change for quinapril as a function of β-CD concentration. The chemical shifts at 2.75 ppm can be assigned to the internal standard, trimethylamine hydrochloride (TMA), and the chemical shifts at, 2.6, 3.1, and 3.2 ppm to Hs-1 ($-CH_2-$), Hs-23 ($-CH_2-$), and Hs-21 ($-CH_2-$) based on the 2D HMBC-NMR spectrum discussed below. Hs-1, Hs-23, and Hs-21 represent the protons attached to carbons 1, 23, and 21, respectively. Figure 4 demonstrates that the chemical shifts at 3.1 ppm and 3.2 ppm were shifted downfield after the addition of β-CD due to deshielding that resulted from the inductive effect of a hydrogen bond interaction between $-CH_2-$ groups and internal –OH groups of β-CD (H-3 and H-5).[23] More specifically, a part of Hs-21 was significantly shifted downfield because a Hs-21 proton likely interacts strongly with a –OH group inside β-CD. In contrast the Hs-1 chemical shift (2.6 ppm) is only shifted downfield slightly. To identify the chemical shifts observed in the ^{1}H NMR spectrum for QHCl, a 2D HMBC ^{1}H-^{13}C NMR spectrum for QHCl was obtained using a HMBC pulse program (see Figure 5). Figure 5 shows the 2D HMBC ^{1}H-^{13}C correlation spectrum for QHCl, while the 1D ^{1}H NMR spectrum is shown at the top. In this spectrum, the coupling interactions between protons and close carbons are shown, from which up to a four-bond connection can be observed, and that for most protons, 1–4 bond correlations are observed.[24] From Table 2 and Figure 4, it can be seen that the protons attached to carbon 21 and 23 are apparently affected due to the formation of the β-CD/quinapril complex, although Hs-1 is also affected slightly. However, probably only one of the protons attached to carbon 21 was strongly influenced by such complexation, indicating that encapsulation of the isoquinoline ring by β-CD appears predominant, although there is still the possibility of involvement of the phenyl ring in the complex. The involvement of the isoquinoline

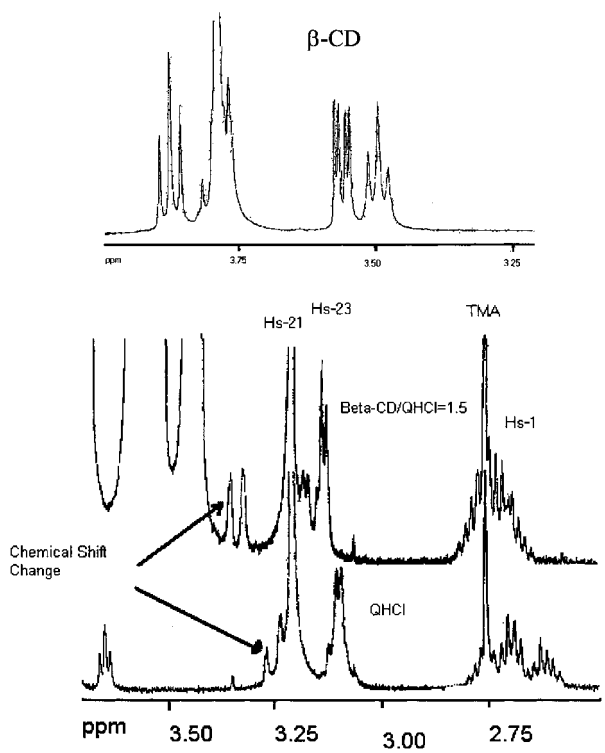


Figure 4. 1D ^{1}H NMR spectra for quinapril, β-CD/quinapril (1.5:1), and β-CD solutions at pH 1.0 (TMA: trimethylamine).

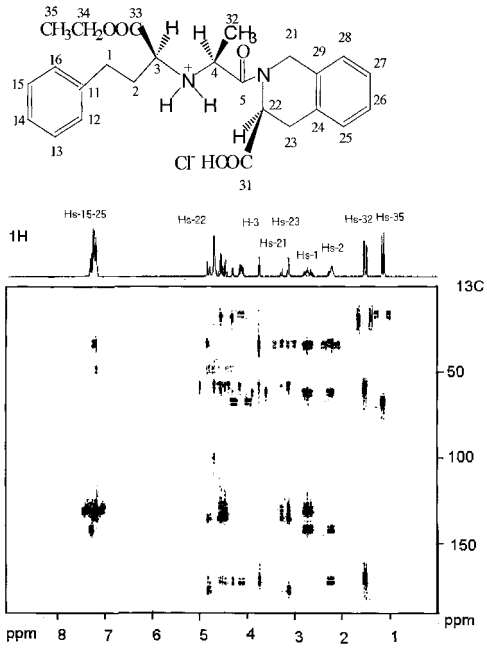


Figure 5. 2D HMBC ^{1}H-^{13}C NMR spectrum of quinapril solution at pH 2.40.

group has two implications. First, the isoquinoline ring of quinapril is probably completely inserted into the cavity of β-CD because $-CH_2$ groups of Hs-21 were significantly affected considering the ring size. Second, one proton from Hs-21 appears to have exhibited a hydrogen bond interaction with the −OH group belonging to H-3 inside the βCD cavity. Because this proton interacted strongly with β-CD, it will be chosen to follow the complexation process in the rest of this study.

To quantitatively measure the extent of complex formation, the concentration of β-CDs was systematically increased while that of quinapril was kept constant. For each such solution the chemical shift due to Hs-21 on quinapril was measured and plotted as a function of β-CD or HP−β-CD concentration, as shown in Figure 6 for solutions at pH 1 and in Figure 7 for β-CD at pH 1.0 and pH 3.0. Figure 6 shows an initially rapid increase in chemical shift with β-CD concentration followed by an approach to a plateau. The overall change for HP−β-CD is less than that for β-CD, indicating that interaction of quinapril with β-CD should be greater than with HP−β-CD (see Discussion section for a quantitative analysis). Figure 7 shows that for β-CD/quinapril the chemical shift changes at pH 1.0 and 3.0 are quite similar (see the Discussion section for a quantitative analysis).

Infrared Spectroscopy

Four samples including quinapril alone and colyophilized β-CD/quinapril mixtures lyophilized from a pH 3 solution with molar ratios of 0.5, 1.0, and 2.0 have been studied using FTIR to assess possible complex formation in the solid sample.[25,26] Figure 8 displays the region of the FTIR spectrum (650–800 cm^{-1}) where C−H from the benzene ring (748 cm^{-1}) and the benzene ring itself (700 cm^{-1}) have strong absorption.[27] The absorption band attributed to the −CH (out of plane bending) can be observed to systematically shift to higher frequency with increasing molar ratio. Similarly, the absorption band attributed to the benzene ring bending is also shifted to higher frequency although the magnitude of the shift is smaller. Figure 9 shows another region of the FTIR spectra (1600–1800 cm^{-1}) for β-CD/quinapril samples where −CONR (1650 cm^{-1}), −COOH and −COOEt (1740 cm^{-1}) exhibited strong absorption. The absorption bands at 1650 and

Table 2. Chemical Shift and Correlation Assignment for the ^{1}H Solution NMR Spectrum of Quinapril

Atomic Position	Chemical Shift (ppm)	Correlation
Hs-35	1.1	H—C_{35}—C_{34} (14, 62 ppm)
Hs-32	1.5	H—C_{32}—C_4—C_5 (15, 57, 169 ppm)
Hs-2	2.2	H—C_2—C_3—C_{33} (32, 60, 167 ppm), H—C_2—C_1—C_{11} (32, 34, 142 ppm)
Hs-1	2.7	H—C_1—C_2—C_3 (34, 32, 60 ppm), H—C_1—C_{11}—$C_{\text{phenyl 2}}$ (34, 142, 128 ppm)
Hs-23	3.1	H—C_{23}—C_{22}—C_{31} (36, 57, 177 ppm), H—C_{23}—C_{24}—$C_{\text{phenyl 1}}$ (36, 134, 128 ppm)
Hs-21	3.2	H—C_{21}—C_{29}–$C_{\text{phenyl 1}}$(47, 134, 128 ppm)
Hs-3	3.7	H—C_3—C_{33} (60, 167 ppm), H—C_3—C_2—C_1(60, 32, 34 ppm),
Hs-22	4.8	H—C_{22}—C_{31} (57, 176 ppm), H—C_{22}—C_{23}—$C_{\text{phenyl 1}}$(57, 36 128 ppm)
Hs-phenyl ring	7.0	

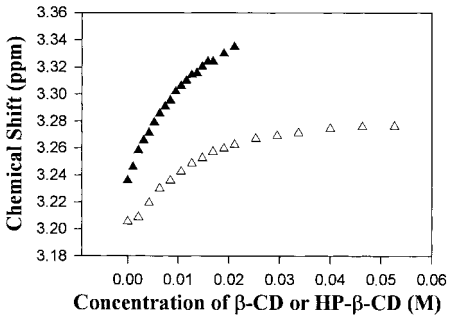


Figure 6. Chemical shifts of quinapril versus β-CD (▲) and HP−β-CD (△) concentration at pH 1.

1740 cm^{-1} are attributed to the stretching vibration of the amide carbonyl and the stretching vibration of the carbonyl group in the carboxylic acid or ester. Similar to what was observed with C−H and the benzene ring, the absorption band for the carbonyl group of the amide was slightly shifted to a higher frequency with an increase in molar ratio, while the absorption band due to the carbonyl group of −COOH or −COOEt was shifted to lower frequency. The above results show that possibly the benzene ring in either the phenyl or isoquinoline groups, or both, are affected by potential β-CD and quinapril interaction. However, because the carbonyl group of amide, close to the quinoline ring, is also affected by such interaction, the isoquinoline ring is most likely encapsulated by β-CD to form a complex (see Discussion section), although encapsulation of the phenyl ring by β-CD is possible. This is also supported by our solution NMR data.

Thermal/Dielectric Analysis

Table 3 lists values of the glass transition temperatures, T_g, for various amorphous forms utilized in this study. For those systems containing β-CD and HP−β-CD, it was not possible to observe sufficient heat capacity change to detect a T_g by DSC. In such cases, TSC appeared to detect the glass transition, so the values listed represent those obtained by either techniques. As seen in Table 3, the value of quinapril (lyophilized from a pH 3 solution) is 70°C, reflective of a T_g value for a mixture of QHCl and Q^{+-}. Note also that the T_g values for β-CD and HP−β-CD are both 50°C. As might be expected the T_g values for a 1:1 physical mixture of β-CD and quinapril yields their individual T_g values of 50 and 70°C, respectively. Both trehalose and dextran appear to have phase separated from quinapril upon lyophilization because the T_g values represent those of the individual components 70, 119, 70, and 225°C, respectively. In contrast, the colyophilized sample with 1 mol of quinapril to 1.2 mol of β-CD shows one major TSC transition at 64°C, indicative of a miscible mixture of the two components (T_g values of 50 and 70°C). In the case of 0.4 parts β-CD to 1 part quinapril, a transition at 64°C is observed, but DSC also shows a T_g at 70°C. This was not clearly seen with TSC because of the closeness of the temperatures (64 and 70°C); however, in the DSC only the peak at 70°C was measurable. Thus, we can conclude that the β-CDs with a T_g value of around 50°C form miscible systems with very little change in the T_g of quinapril. Hence, the effects of β-CDs on chemical instability do not appear to be related to any effects on molecular mobility due to effects on structural relaxation. It is interesting that quinapril is not able to form an amorphous molecular dispersion with trehalose and dextran despite a chemical composition similar to that of the β-CD. This further supports the hypothesis that stabilization of quinapril by β-CD is due to a molecular complex specific to the cyclodextrins.

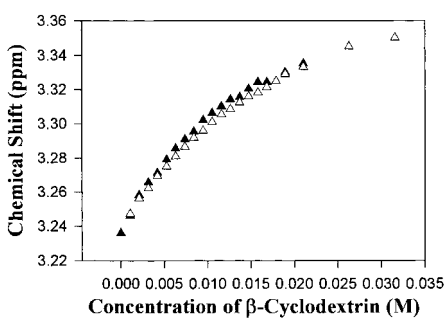


Figure 7. Chemical shifts of quinapril vs. β-CD concentration at pH 1 (▲) and 3 (△).

DISCUSSION

It is generally recognized that all solid-state chemical reactions require sufficient molecular mobility to occur over a practical timescale. Therefore, it is not surprising that most chemical reactions have rates that increase as one increases the degree of disorder, i.e., crystal-to-amorphous-to-liquid states.[3] Previous studies of the chemical instability of quinapril in the amorphous state indicate that significant intramolecular cyclization occurs over the temperature range of

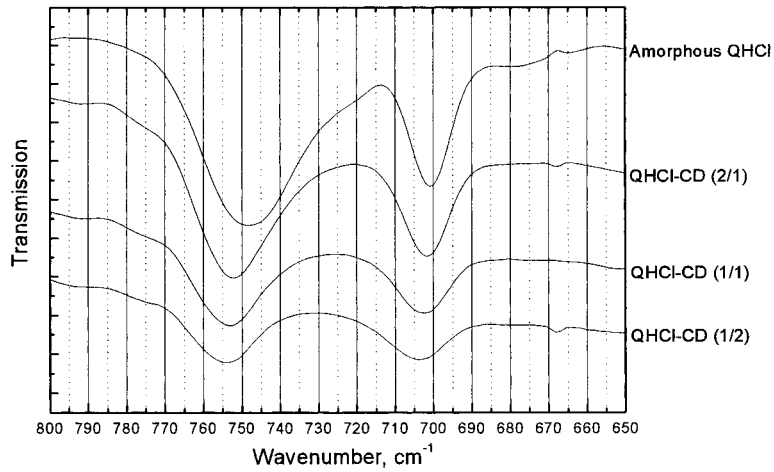


Figure 8. Infrared spectra of quinapril and colyophilized β-CD/quinapril mixtures in the region of 650 to 800 cm^{-1}.

70–100°C with quinapril samples exhibiting glass transition temperatures ranging from 51 to 91°C, depending on the composition of QHCl and Q^{+-}.[10,11] In the present study, samples lyophilized from solutions of pH 3 produce amorphous materials with a T_g of 70°C and, hence, reactivity of this sample without β-CDs present at 80°C is occurring 10 degrees above T_g, in a region where the time scale for molecular mobility (structural relaxation) is less than roughly 100 s (relaxation time at T_g).[28] Consequently, to reduce the rate of this reaction through an effect solely on molecular mobility, either the temperatures would have to be reduced well below T_g, or an excipient with a very high T_g and with the ability to form a miscible molecular dispersion must be added. From the results of this study, it is clear that excipients forming a miscible molecular dispersion, i.e., the β-CDs, are capable of inhibiting the chemical reactivity of quinapril in the amorphous state. Because the β-CDs both have T_g values equal to 50°C, however, we can conclude that the significant inhibition of chemical reactivity is not the result of a greatly reduced molecular mobility at 80°C through an antiplasticizing effect caused by the excipient. This would suggest, rather, that the inhibition of chemical reactivity by the β-CDs is related more to some type of specific interaction between quinapril and the β-CDs. From the results shown in Figure 3 and Table 1, it furthermore would appear that there is a definite stoichiometric relationship between β-CD concentration and the rate of reactivity. Thus, it appears that the effects of the β-CDs might be related directly to their ability to form a molecular complex with quinapril. The lack of any tendency of quinapril to form molecular dispersions with trehalose and dextran would also support the likelihood of a more specific molecular complex forming between quinapril and the β-CDs. Indeed, the plateau in the reaction at and above a β-CD/quinapril molar ratio of 1:1 (Figure 3) strongly suggests that the complex being formed has a stoichiometry of 1:1.

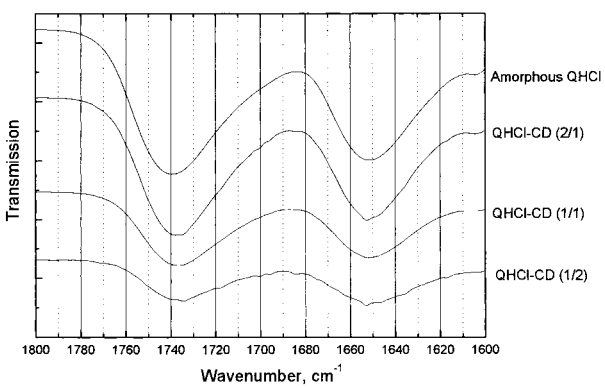


Figure 9. Infrared spectra of quinapril and colyophilized β-CD/quinapril mixtures in the region of 1600 to 1800 cm^{-1}.

To further analyze the possibility of a distinct 1:1 complex in the amorphous state, solution state samples were examined using NMR, as shown in Figures 6 and 7. The data can be interpreted with the initial assumptions that: (1) β-CDs and quinapril form a 1:1 complex, and (2) quinapril exists as QHCl at pH 1 and as a mixture of QHCl and Q^{+-} at pH 3.

Table 3. Glass Transition Temperatures for Quinapril Lyophilized From a pH 3.0 Solution and When Mixed With Various Substances

Sample Name	T_{g1} (°C)[a]	T_{g2} (°C)[a]	Method
Quinapril	70	—	DSC, TSC
β-CD	50	—	TSC
HP−β-CD	50	—	TSC
Physical mixture (1:1)	50	70	DSC, TSC
β-CD/quinapril (0.4:1)	64	70	DSC, TSC
β-CD/quinapril (1.2:1)	50	64	TSC
Trehalose/quinapril (1:1)	70	119	DSC
Dextran/quinapril (1:1)	70	225	DSC

[a] T_{g1} and T_{g2} are the first and second glass transition temperatures observed when phase separation occurred.

Consequently, we can express the equilibrium for such a complex with a stoichiometry of 1:1 as:

$$\beta - \text{CDs} + \text{quinapril} \rightleftharpoons \beta - \text{CDs quinapril} \quad (1)$$

and

$$\text{K}_{11} = \frac{[\beta - \text{CDs quinapril}]}{[\beta - \text{CDs}]\,[\text{quinapril}]} \quad (2)$$

Because the kinetics for the formation of a complex in solution is rapid relative to the NMR measurement time scale, the chemical shift observed for β-CD/quinapril solution (see Figures 4 and 5) is an average of chemical shifts for all species. The observed chemical shift δ is a weighted average of the chemical shifts in the free and complexed states as shown below:

$$\delta = f_{10}\delta_{\text{free}} + f_{11}\delta_{\text{complex}} \quad (3)$$

where the weighting factors f_{10} and f_{11} are the fractions of free and complexed quinapril. Because $f_{10} + f_{11} = 1$, rearranging above equation leads to $\Delta = f_{11}\Delta_{11}$. Thus, based on the definition of K_{11} (eq. 2), the following equation can be obtained:

$$\Delta = \frac{\Delta_{11}K_{11}[\beta - \text{CDs}]}{1 + K_{11}[\beta - \text{CDs}]} \quad (4)$$

where $\Delta = \delta - \delta_{\text{free}}$ and $\Delta_{11} = \delta_{\text{complex}} - \delta_{\text{free}}$. Recognizing that the concentration of β-CDs is comparable to that of drug, quinapril, the following analysis can be carried out. Based on mass balance [β-CDs] = [β-CDs]$_t$ − [β-CDs quinapril] and assuming [β-CDs quinapril] = [quinapril]$_t$(Δ/Δ_{11}), we can rearrange eq 4 to give:[29]

$$\frac{[\beta - \text{CDs}]_t}{\Delta} = [\beta - \text{CDs}]_t + [\text{quinapril}]_t \times \frac{-[\beta - \text{CDs quinapril}]}{\Delta_{11}} + \frac{1}{\Delta_{11}K_{11}} \quad (5)$$

Here, the chemical shift difference between the plateau region and free drug is taken as Δ_{11} and [β-CDs quinapril] is calculated from the total concentration of quinapril and Δ/Δ_{11}(see ref. 29 for details). Based on eq 5 and the data shown in Figures 6 and 7, [β-CD]$_t/\Delta$, [HP−β-CD]$_t/\Delta$, ([β-CD]$_t$ + [quinapril]$_t$-[β-CDquinapril])$/\Delta_{11}$ and ([HP−β-CD]$_t$ + [quinapril]$_t$ − [HP−β-CDquinapril])$/\Delta_{11}$ can be calculated. Then, [β-CD]$_t/\Delta$ is plotted as a function of ([β-CD]$_t$ + [quinapril]$_t$ − [β-CDquinapril])$/\Delta_{11}$ (see Figures 10 and 11). Similarly [HP−β-CD]$_t/\Delta$ is plotted against ([HP−β-CD]$_t$ + [quinapril]$_t$ − [HP−β-CDquinapril])$/\Delta_{11}$ (see Figure 10). From the intercepts of the plots in Figures 10 and 11 ($1/\Delta_{11}K_{11}$), the complexation constant (K_{11}) can be calculated (see Table 4). As seen in Table 4, we report K_{11} values for quinapril complexation with β-CD and HP−β-CD at pH 1 and pH 3. Table 4 shows that HP−β-CD has less tendency to form a complex with quinapril than does β-CD, most likely due to its greater hydrophilicity.[30] For both β-CD and HP−β-CD, the complexation constant is higher at low pH (pH = 1) presumably because increasing pH proportionally increases the amount of zwitterionic form of QHCl in the solution, which might be less favorable in forming the complex because of the

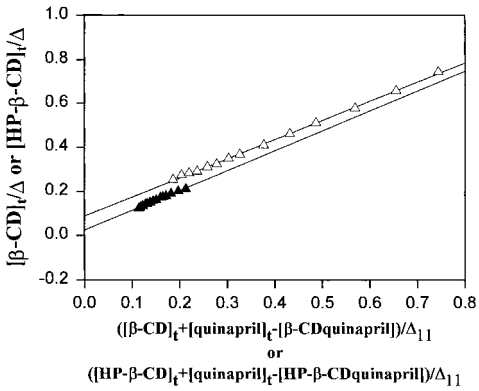


Figure 10. $[\beta\text{-CD}]_t/\Delta$ or $[\text{HP}-\beta\text{-CD}]_t/\Delta$ versus $([\beta\text{-CD}]_t + [\text{quinapril}]_t - [\beta\text{-CD quinapril}])/\Delta_{11}$ (▲) or $([\text{HP}-\beta\text{-CD}]_t + [\text{quinapril}]_t - [\text{HP}-\beta\text{-CD quinapril}])/\Delta_{11}$ (△) at pH 1.

hydrophilic nature of the carboxylate ion.[30] The values of K_{11} measured against such values for many drug complexes with β-CDs indicate that the tendency for quinapril to complex is moderate but significant.[31] Typically, the complexation constant values can range from less than 10 M^{-1} to more than 1000 M^{-1}, with most of them centering around a few hundred, such as 94 M^{-1} for nitrazepam.[32]

Although the solution-state NMR data support the formation of a complex, it is reasonable to question whether the complexing tendencies of quinapril carry over to the amorphous state. As discussed in the Materials and Methods section, it was not possible to observe chemical shifts using ^{13}C solid-state NMR, due to the broad peaks observed for amorphous quinapril. However, distinct changes in the FTIR solid-state spectrum of quinapril were observed in the presence of β-CD (Figures 8 and 9). In the solid state, functional groups (carbonyl of amide and C−H) close to and in the isoquinoline ring of quinapril are most affected by colyophilization with β-CD relative to other regions. This is consistent with the solution NMR results, in which Hs-21 and Hs-23 of the isoquinoline ring were observed to show the greatest chemical shift change. To analyze this further, the wavenumber, ν, for the C−H vibration is plotted as a function of the β-CD/quinapril molar ratio in Figure 12. Note that ν increases with the β-CD/quinapril molar ratio, eventually reaching a plateau, indicating a 1:1 relationship between β-CD and quinapril. Although these results cannot be quantified in the context of a molecular complexation constant, it seems clear that some type of interaction between β-CD and quinapril occurs in the solid state, and that functional groups expected to be involved in the reaction are involved in the spectral change. If, indeed, quinapril exists in a lyophilized solid form as a 1:1 molecular complex with the β-CDs, we can expect two general forms: (1) free QHCl and Q^{+-}, the amount depending on the initial pH; and (2) 1:1 complexed forms. To a first approximation we can then conceptualize two environments within which chemical reactivity is possible: free quinapril and bound quinapril. If we assume that the reaction rate constants in each environment are k_{free} and $k_{complex}$, we can write that

$$k_{app} = f_{free}k_{free} + f_{complex}k_{complex} \qquad (6)$$

where f is the molar fraction of quinapril in each environment and k_{app} is the overall rate constant at 80°C. If we presume, as is often true in solution,[33] that $k_{free} >>> k_{complex}$, we could then explain the tendency of the cyclodextrins in general to reduce chemical reactivity in solution and in the solid state. Furthermore, rearranging eq. 2 provides an estimate of free fraction of quinapril in solution at a given composition of β-CD at pH 3.0 where K_{11} is 190 M^{-1} (Table 4):

$$[\text{quinapril}] = \frac{[\beta - \text{CD quinapril}]}{K_{11}[\beta - \text{CD}]} \qquad (7)$$

In Table 5 we provide estimates of free fraction of quinapril for a variety of molar ratios at pH 3.0 (25°C), and it is clear that even when the molar ratios of β-CD to quinapril are 1:1 or greater, there is a significant amount of free quinapril present. Thus, although at any time some quinapril is complexed with β-CD, significant amounts of quinapril are still available for reactivity in solution. In contrast, for the colyophilized samples Figure 3 shows that above a 1:1 molar ratio, there appears to be insufficient free quinapril

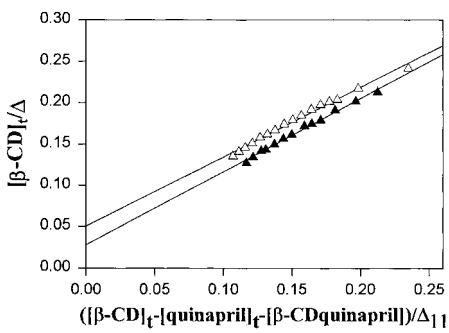


Figure 11. $[\beta\text{-CD}]_t/\Delta$ versus $([\beta\text{-CD}]_t + [\text{quinapril}]_t - [\beta\text{-CD quinapril}])/\Delta_{11}$ at pH 1 (▲) and pH 3 (△).

Table 4. Complexation Constants in Solution at Different pH Values for β-CD/Quinapril and HP−β-CD/Quinapril

Complexing Agent	pH	Complexation Constant K_{11} (M^{-1}) (±10%)
β-CD	1.0	380
β-CD	3.0	190
HP−β-CD	1.0	160
HP−β-CD	3.0	110

The number in parenthesis represents one average standard deviation.

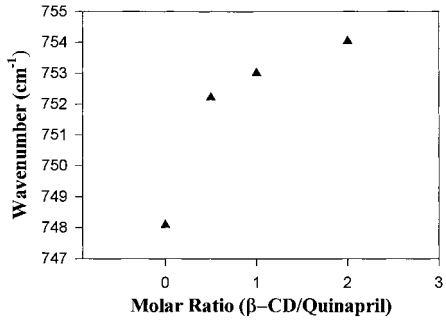


Figure 12. FTIR wavenumber shift for ν(C−H) as a function of β-CD/quinapril molar ratio.

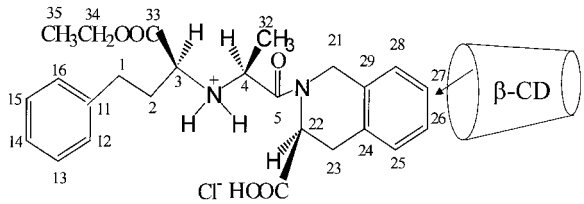
to support reactivity, and the rate of reactivity proportionally decreases as the amount of β-CD increases. Indeed, above a molar ratio of 1:1 the reaction rate has been reduced by about two orders of magnitude from that in the absence of β-CD. This strongly suggests that the amount of free quinapril present in these amorphous samples is extremely small and, therefore, not reflective quantitatively of the K_{11} estimated from solution studies. It is also interesting to note, that HP−β-CD, which exhibits smaller values of K_{11} than β-CD, also exhibits significant inhibition at a 1:1 molar ratio, but only about one-half of the inhibiting effect of β-CD (see Table 1).

It is not entirely clear why the β-CDs are so extremely effective in inhibiting the chemical reactivity of quinapril in the colyophilized amorphous state. We can assume first of all that the amount of quinapril that complexes as the initial solution is dried during lyophilization is very high, or in effect the apparent K_{11} in the amorphous state is much greater than that for a corresponding system in solution. We have no quantitative solid-state evidence that can support this, but we have observed a significant change in the solid-state FTIR spectrum of quinapril indicative of complexation. It is conceivable that even though water levels are reduced in these samples there is sufficient molecular mobility remaining to efficiently bring more quinapril in contact with β-CD in the proper orientation for efficient insertion into the β-CD cavity. It is also possible that the different solubility of complex and free quinapril at low temperature drive the complexation equilibrium to the right during freezing or with sublimation of water the environment around quinapril increasingly become hydrophobic, shifting the equilibrium to the right. There is, of course, the possibility that the β-CDs also provide a significant chemical medium effect not related to complexation or an effect on molecular mobility. Exactly how a complex with the β-CDs might mechanistically inhibit the reactivity of quinapril cannot be ascertained for amorphous structures, but our solution NMR and solid-state FTIR results do provide some clues. Both NMR and FTIR results show that the $-CH_2$ groups in the isoquinoline ring or isoquinoline ring itself are most affected by the presence of the β-CDs. Thus, we can suggest a two-fold inhibition mechanism

Table 5. Estimates of the Amount of Free Quinapril in Solution at Various β-CD Concentrations Obtained From the Solution Equilibrium Constant versus Corresponding Solid-State Reaction Rate Constant

Molar Ratio (β-CD/Quinapril)	Free Fraction of Quinapril in Solution Estimated From eq 7 (%)	Kinetic Constant in the Solid State, k_{app} (h^{-1}) (±5%)
0.2	87.3	0.011
0.4	75.9	0.0095
0.8	57.3	0.0027
1.0	50.0	0.00058
1.2	43.9	0.00046
1.4	38.8	0.00049
1.8	31.0	0.00030

The number in parenthesis represents one average standard deviation.



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Scheme 3.

as shown in Scheme 3. First, due to insertion of the isoquinoline ring into the β-CD cavity quinapril cannot undergo the necessary conformational change from trans to cis required for intramolecular cyclization and the formation of the corresponding diketopiperazine (see Scheme 1).[10] Second, it is possible that such an encapsulated complex can sterically prevent the −COOH or $-COO^-$ from approaching the −NH− group to complete the nucleophilic attack required for completion of the reaction.

CONCLUSIONS

Quinapril, colyophilized with β-CD and HP−β-CD forms miscible molecular dispersions in the amorphous state, whereas other carbohydrates such as trehalose and dextran phase separate into individual amorphous forms. Only in the case where such miscible molecular dispersions are formed does β-CD or HP−β-CD significantly inhibit the intramolecular cyclization of quinapril. TSC measurements show that β-CD and HP−β-CD have glass transition temperatures of about 50°C. Because the T_g of the quinapril samples prepared from an initial solution with pH 3 is about 70°C, we can conclude that the β-CDs are not acting as antiplasticizers to reduce molecular mobility and hence chemical reactivity. Solution NMR and solid-state FTIR support the presence of 1:1 molecular complexes between quinapril and the β-CDs in the amorphous state. Such molecular complexes appear to be able to efficiently interfere with the mechanism of the intramolecular cyclization, decreasing the reaction rate by two orders of magnitude.

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