Effect of the pH Grade of Silicates on Chemical Stability of Coground Amorphous Quinapril Hydrochloride and its Stabilization Using pH-Modifiers

SHUMET A. HAILU, ROBIN H. BOGNER

Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, U-3092, Storrs, Connecticut 06269

Received 10 October 2008; revised 29 January 2009; accepted 26 February 2009

Published online 10 April 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21767

ABSTRACT: The effect of pH grade of silicates on chemical stability of amorphous drugs coground with silicates (Neusilin and Aerosil) was investigated using quinapril HCl (QHCl) as a model drug. The ability of pH-modifiers (ascorbic acid and MgO) to improve chemical stability was explored. PXRD and polarized light microscopy indicated complete amorphization of all samples by cryo-grinding. All samples remained amorphous during stability study at 40°C and 48% RH. In general, drug degradation was greater in the QHCl/silicate (1:3) coground amorphous samples than the neat amorphous QHCl. The rate of diketopiperazine formation by cyclization of QHCl was higher in the presence of lower pH grades than higher pH grades of silicates. However, the pH-stability profile of coground amorphous systems prepared with different pH grades of silicates was not consistent with the pH-stability profile of the drug in solution. A basic pH-modifier (MgO) in a lower pH grade silicate (Neusilin US2) stabilized coground amorphous QHCl. Also, an acidic pH-modifier (ascorbic acid) in a higher pH grade silicate (Neusilin FL2) suppressed QHCl hydrolysis. The pH grade of silicates is a major factor affecting the chemical stability of a coground amorphous drug and pH-modifiers are useful for chemical stabilization without compromising physical stability. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:3358–3372, 2009

Keywords: quinapril hydrochloride; amorphous; stability; chemical stability; stabilization; cogrinding; milling; silicates; pH grade of silicates; pH modifiers

INTRODUCTION

Amorphous systems of active pharmaceutical ingredients have been used to enhance dissolution rate and bioavailability of poorly soluble drugs.[1–4] However, in general, amorphous forms are chemically less stable than their corresponding crystalline forms.[5–8] For instance, completely amorphous aspirin in controlled pore glass solid dispersions resulted in a higher drug degradation than partially amorphous aspirin.[8] Studies on oxidation and UV-induced degradation of amorphous and crystalline peptides indicated faster degradation of the amorphous forms.[5] Also, higher drug degradation through a cyclization reaction is reported in amorphous quinapril hydrochloride (QHCl) and spirapril hydrochloride than in the corresponding crystalline forms.[5,7] These differences in chemical stability are attributed to the higher molecular mobility and reactivity of the amorphous states compared to the crystalline forms. As a result, the practical application of amorphous drugs for improved dissolution rate and bioavailability may be limited due in part to lower chemical stability.

Correspondence to: Robin H. Bogner (Telephone: 860-486-2136; Fax: 860-486-2076; E-mail: robin.bogner@uconn.edu)

Journal of Pharmaceutical Sciences, Vol. 98, 3358–3372 (2009)

Silicates are used in pharmaceutical formulations as glidants, anticaking agents, adsorbents, disintegrants, and suspending agents.[9] In addition, various silicates have been investigated for their ability to improve the physical stability of amorphous drugs, and hence to enhance dissolution rate and bioavailability of poorly soluble drugs.[2–4,10–14] Formation of physically stable amorphous drugs by cogrinding with silicates has been attributed to drug–silicate interactions, for instance, through hydrogen bonding.[12,13] It is reasonable to expect that the interaction of drug molecules with silicates would also result in lower molecular mobility of drugs as compared to the neat amorphous drug alone, thereby improving the chemical stability of coground amorphous drug as well. However, silicates are catalysts known to facilitate hydrolysis, oxidation, and other reactions commonly involved in drug degradation.[8,15–17] Therefore, it is unclear whether drug amorphization with silicates improves chemical stability due to lower molecular mobility and/or facilitates degradation due to the catalytic effect of silicates.

Reports in the pharmaceutical literature show inconsistent effects of silicates on chemical stability of drugs.[8,15–18] Daniels et al.[16,17] reported the hydrolysis of acetylsalicylic acid and propantheline bromide adsorbates on silica surfaces. The authors indicated that drug degradation was influenced by surface impurities of silica, adsorbed water and pH of drug–silica suspensions. However, in those studies, the pH was adjusted by addition of NaOH or phosphate buffer during the preparation of drug–silica adsorbates rather than using different pH grades of silicates. Therefore, their studies may not reflect the effect of inherent pH of silica. Lau-Cam et al.[15] reported hydrolytic degradation of amorphous digoxin in silicon dioxide and ascribed the results to the acidity, surface area, and pore size of silica. On the other hand, Gore et al.[18] showed stabilization of aspirin in tablets containing 1–15% colloidal silica as compared to the control aspirin tablets at 82% RH and 40°C. The authors proposed that in addition to other factors, the stabilization was due to internal moisture scavenging by silica. However, samples were open to the humidity and moisture scavenging may not provide a suitable explanation, since there would be equilibration of humidity. Yonemochi et al.[8] reported an inverse relationship between %RH and degradation rate constants for the hydrolysis of 1% amorphous aspirin in controlled pore glass at 50°C. The results were attributed to the competitive adsorption of aspirin and water molecules on silica surface, which would result in reduced catalytic effect of silica surface at higher %RH.

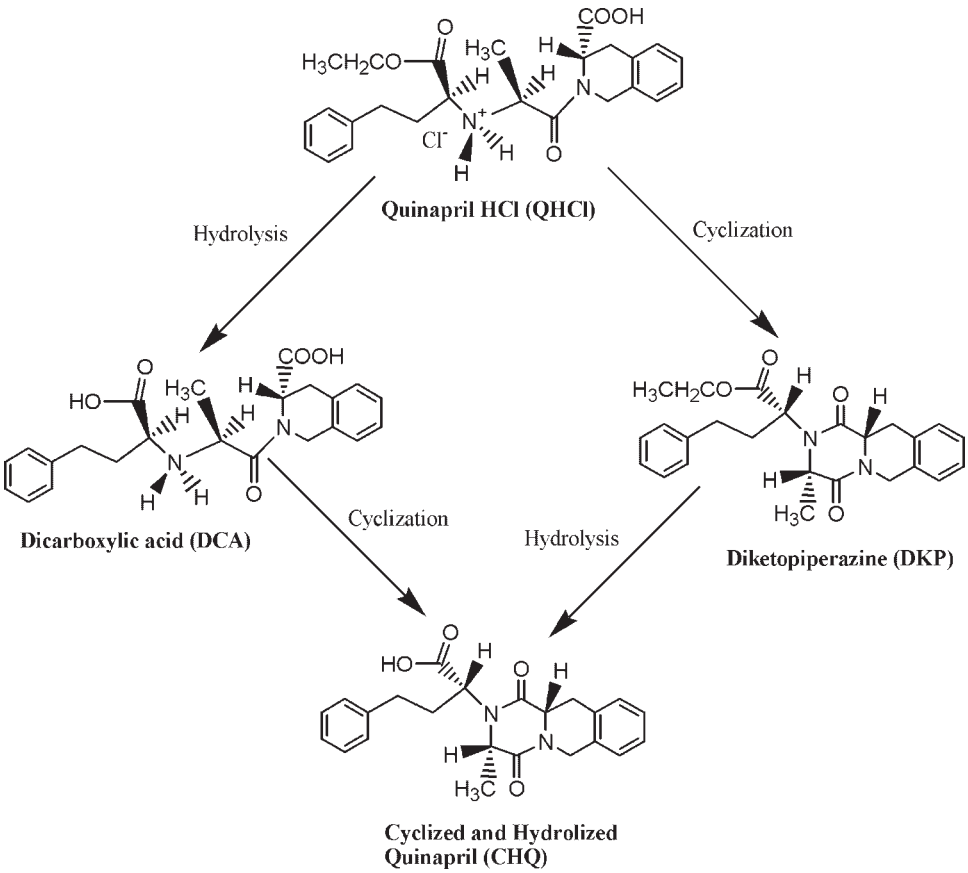
In the present study, the effect of pH grade of silicates on chemical stability of a drug amorphized by cogrinding with silicates was investigated. The potential use of acidic and basic pH-modifiers to improve the chemical stability of amorphous drugs coground with silicates was also explored. QHCl, an ACE-inhibitor, which is prone to degradation involving cyclization and hydrolysis reactions (Scheme 1) was selected as the model drug. Since both cyclization and hydrolysis of QHCl are pH-sensitive, QHCl is a useful probe to investigate the effect of pH grade of silicates on chemical stability and to study the potential use of pH-modifiers for stabilization. Most ACE-inhibitors are structurally related and known to degrade by cyclization and hydrolysis. Therefore, QHCl is a representative drug for structurally related ACE-inhibitors and other drugs, which degrade by similar mechanisms. QHCl was received as a partially amorphous drug. Neat amorphous QHCl, which has a glass transition temperature (T_g) of 91°C,[19] remained amorphous for the entire period of stability study. Therefore, neat amorphous QHCl prepared by grinding of the drug alone was used as a control during the chemical stability study of QHCl/silicate coground amorphous samples.

The conversion of crystalline drugs to amorphous states in the presence of silicates is known to be a spontaneous process.[10–13] Therefore, this research may be useful for crystalline drug formulations containing pharmaceutical silicates. However, the focus in the present study is on amorphous systems prepared by grinding, so that the chemical degradation kinetics will not be confounded by the crystalline form of the drug that would have been present during the spontaneous conversion to the amorphous form.

MATERIALS AND METHODS

Materials

Quinapril hydrochloride (3-isoquinolinecarboxylic acid, 2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, [3*S*-[2[*R**(*R**)],3*R**]]), QHCl, was purchased from Farmhispania, S.A. (Barcelona, Spain). Reference standards of the two major



Scheme 1. Degradation pathways of quinapril hydrochloride (QHCl).

degradation products of QHCl, quinapril diketopiperizine (ethyl[3*S*-[2(*R**),3α,11αβ]]-1,3,4,6,11, 11α-hexahydro-3-methyl-1,4-dioxo-α-(2-phenylethyl)-2*H*-pyrazino[1,2-*b*]isoquinoline-2-acetate) and quinapril dicarboxylic acid (3-isoquinolinecarboxylic acid, 2-[2-[(1-carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-,[3*S*-[2[*R**(*R**)],3*R**]]), were obtained from USP (Rockville, MD). Neusilin (magnesium aluminometasilicate) and Aerosil (silicon dioxide) were obtained from Fuji Chemicals (Inglewood, NJ) and Degussa Corporation (Parsippanny, NJ), respectively. The pH grade of silicates along with surface area specifications are given in Table 1. Acetonitrile (Optima LC/MS grade), methanol (HPLC grade), $Mg(NO_3)_2{\cdot}6H_2O$, HCl (1N), NaOH (1N), formic acid (88%), ascorbic acid (Certified ACS), and MgO (Certified ACS) were obtained from Fisher Scientific (Atlanta, GA).

Preparation of Amorphous Samples

Neat amorphous QHCl and QHCl/silicate co-ground amorphous samples with or without a pH-modifier (MgO or ascorbic acid) were prepared by cryo-grinding using a cryo-mill (6750 Freezer/Mill®, SPEX SamplePrep, Metuchen, NJ) equipped with polycarbonate sample tubes (2 cm diameter and 10 cm length) and a stainless steel impactor (0.9 cm diameter and 6 cm length). A sample size of 2 g material was used in each cryo-grinding batch. Samples were first precooled for 15 min in the cryo-mill filled with liquid nitrogen, then ground for a total of 30 min with alternating cycles of 2 min grinding and cooling at an impactor frequency of 10 cycles/s.

Neat amorphous quinapril samples were also prepared by lyophilization from aqueous QHCl solutions (5 mg/mL) adjusted with 1N HCl or NaOH to pH values of 1–7. The solutions (1 mL) were filled into 5 mL serum tubing vials and loaded onto a Dura-Stop Freeze Dryer (FTS Systems, Stone Ridge, NY). The shelf temperature was first held at −5°C for 30 min. Then the temperature was lowered to −40°C at 1°C/min and held for 6 h for freezing. Primary drying was carried out at −25°C with a chamber pressure of 100 mTorr for 24 h. For secondary drying, the

Table 1. Types and Properties of Silicates Used in This Study

Silicate Type	Abbreviations[a]	Specifications[b] Nominal pH[c]	Specifications[b] Surface Area (m^2/g)	Experimental pH[c,d] Silicate as Received	Experimental pH[c,d] QHCl/Silicate (1:3) 30 min Coground
Neusilin: magnesium aluminometasilicate					
Neusilin US2	NUS2	6.0–8.0	300	7.13	5.68
Neusilin NFL2N	NNFL2N	6.5–8.0	250	7.34	5.46
Neusilin FL2	NFL2	8.5–10.0	150	9.21	7.60
Neusilin FH2	NFH2	8.5–10.0	110	9.26	7.01
Aerosil: silicon dioxide					
Aerosil 200	A200	3.7–4.7	200	4.33	2.03
Aerosil R812S	AR812S	5.5–7.5	220	6.23	2.02
Aerosil 90	A90	3.7–4.7	90	4.49	2.04
Aerosil 380	A380	3.7–4.7	380	4.06	2.01

[a]Used in this article for simplicity.
[b]Product specifications from suppliers.
[c]pH of 4% (w/v) suspension in water.
[d]SD < 0.13 for $n = 3$.

temperature was increased to 25°C at 0.2°C/min and held at 25°C and 100 mTorr for 15 h. Lyophilized samples were sealed in the vial under vacuum and stored at −20°C until analyzed or placed on stability. A change in reconstituted pH of lyophilized quinapril as compared to the initial solution pH was observed (up to 1 pH unit) in some samples, which is expected due to evaporation of HCl during freeze drying process. Therefore, the reconstituted pH, which represents the pH of lyophilized samples, was used to determine the chemical stability profiles of lyophilized samples.

Characterization of Amorphous Samples

Neat amorphous QHCl and QHCl/silicate coground amorphous samples were characterized using X-ray diffractometry and polarized light microscopy to qualitatively evaluate the amorphous state of systems. Powder X-ray diffraction (PXRD) was performed using Cu Kα radiation at 45 kV and 40 mA. PXRD data was collected at an interval of 0.02° and a scanning rate of 2°/min over a 2θ range of 5–40°. Both fresh samples prepared by cryo-grinding and samples stored at specified conditions for 6 months were characterized to monitor any reversion to crystalline form on storage. The absence of birefringence under the polarized light microscope was considered as confirmation of the amorphous state.

The T_g of neat amorphous QHCl lyophilized from solutions of different pH were determined using differential scanning calorimetry (DSC Q1000, TA Instruments, New Castle, DE) in modulated mode using amplitude ±1°C, frequency 150 s and ramp rate 2°C/min. About 10 mg of the lyophilized samples were transferred into aluminum pans and hermetically sealed in a glove bag purged with dry nitrogen. T_g measurements were conducted under a dry nitrogen purge in the DSC.

Surface Area Measurement

Surface areas of QHCl and silicate samples (before and after grinding) and QHCl/silicate (1:3) coground samples were obtained using a surface area analyzer (FlowSorb II 2300, Micromeritics, Norcross, GA). A mixture of 30% nitrogen and 70% helium was used for surface area measurement. Sample sizes ranging from 50 to 300 mg were used, depending on the density of the powder. All samples were degassed at least for 8 h at 25°C prior to surface area measurement.

Chemical Stability of Amorphous Systems and Assay Using LC/MS and HPLC/UV

Neat amorphous QHCl and QHCl/silicate coground amorphous samples were stored at elevated temperature, below T_g of the drug, to study the chemical stability in the absence of reversion to the crystalline form. The T_g of neat amorphous QHCl equilibrated at 40°C and

different %RH was determined. The T_g values at 0, 6.3, 31.6, 48, and 53% RH were 91.4, 65.3, 60.9, 51.5, and 44.7°C, respectively. Therefore, both neat amorphous QHCl and QHCl/silicate coground amorphous samples were stored at 40°C and 48% RH ($Mg(NO_3)_2 \cdot 6H_2O$ saturated solution) in open vials. Stored samples were periodically assayed for the drug and major degradation products using LC/MS (Navigator® MS, Thermo/Finnigan, Waltham, MA) and HPLC/UV (HP Series 1100, Agilent Technologies, Santa Clara, CA).

The mass spectrometry method was electrospray ionization in positive ion mode (ESI^+) using a single quadrupole mass analyzer. The mobile phase was methanol/0.1% formic acid/acetonitrile (60/30/10) at a flow rate of 1 mL/min with a split of 1/3 of the flow directed into the mass spectrometer. For both LC/MS and HPLC/UV a C18 column (5 μm, 3.9 mm × 150 mm, Symmetry®, Waters Corporation, Milford, MA) was used. Sample solutions were prepared in methanol/water (50/50) to make a concentration equivalent to 20 μg/mL QHCl for LC/MS and 50 μg/mL QHCl for HPLC/UV, based on the initial amount of drug present in the sample. The injection volume was 10 μL. The mobile phase for HPLC/UV was 50 mM potassium phosphate buffer (pH 4)/methanol/acetonitrile (50/25/25) at a flow rate of 1 mL/min. A diode array detector monitored peaks at 215 nm. During sample preparation, drug solutions and drug/silicate suspensions were sonicated for 15 min and filtered through 0.2 μm regenerated cellulose filters (Corning® syringe filters) before injection.

Both LC/MS and HPLC/UV methods yielded well-resolved peaks for QHCl and its degradation products (dicarboxylic acid (DCA), cyclized and hydrolyzed quinapril (CHQ), and Diketopiperazine (DKP), Scheme 1). For instance, by using the HPLC/UV method, retention times for DCA, CHQ, QHCl, and DKP were 1.9, 4.9, 8.2, and 15.0 min, respectively. The peaks in the LC/MS chromatogram were identified by the masses of molecular ions ($M+H$) of 393, 411, 421, and 439 corresponding to CHQ, DCA, DKP, and QHCl, respectively. HPLC/UV calibration curves were developed using pure QHCl and USP reference standards of the two degradation products (DKP and DCA) for quantification of the drug remaining and major degradation products formed. The calibration curves were linear ($r^2 \geq 0.999$) in the concentration range of 0.5–250 μg/mL.

Determination of pH-Stability Profile of QHCl in Aqueous Buffer Solutions

Aqueous buffer solutions (pH 1–10) with total buffer concentration of 10 mM were prepared according to the USP.[20] QHCl solutions of 100 μg/mL were prepared in the buffer solutions at each pH. The pH of the buffer solutions was adjusted, if necessary, after addition of the drug. Drug solutions were stored at 40°C in closed vials and assayed periodically by HPLC/UV.

RESULTS

Characterization of Amorphous Samples by PXRD and Polarized Light Microscopy

PXRD and polarized light microscopy were used to verify drug amorphization by cryo-grinding. Figure 1 shows sample PXRD patterns of QHCl, silicate and QHCl/silicate systems after different cryo-grinding times. All silicates (Tab. 1) used in this study were confirmed amorphous by their initial PXRD pattern (at 0 min grinding) in Figure 1, as indicated by the absence of diffraction peaks and a broad halo at 20–25° 2θ. The PXRD pattern of QHCl before grinding shows two diffraction peaks at 7° and 9° 2θ, and a broad halo at 16–22° 2θ indicating the partially amorphous nature of QHCl as received (Fig. 1). Cryo-grinding of QHCl alone for 10 min resulted in a decrease but not an elimination of the two diffraction peaks at 7° and 9°, and therefore, the drug was not completely amorphous. However,

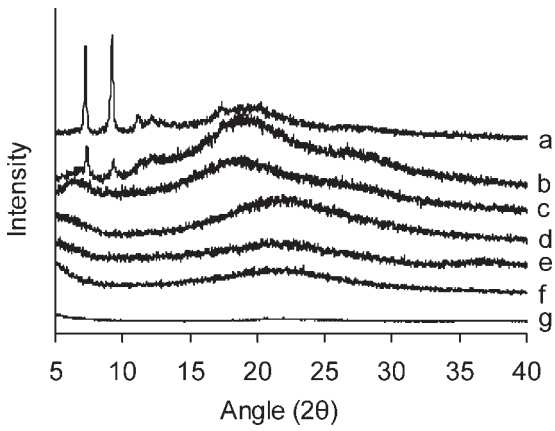


Figure 1. PXRD patterns of QHCl and silicate samples at different time of cryo-grinding; (a) QHCl-0 min, (b) QHCl-10 min, (c) QHCl-30 min, (d) QHCl:A200 (1:3)-30 min, (e) QHCl:NUS2 (1:3)-30 min, (f) AR812S-0 min, and (g) NUS2-0 min ground.

upon grinding for 30 min the diffraction peaks were completely eliminated, indicating that the drug was X-ray amorphous. In addition, a physical mixture of QHCl and silicate coground for 30 min resulted in an X-ray amorphous powder (Fig. 1). Polarized light microscopy confirmed the PXRD results as indicated by the absence of birefringence for X-ray amorphous samples (data not shown). The preparation of amorphous QHCl by grinding with a heavy-duty electric motor grinder and by solvent evaporation techniques has been reported,[19,21] which agrees with the results obtained in this study by using the cryo-grinding.

Neat amorphous QHCl and coground amorphous QHCl/silicate samples stored at 48% RH and 40°C were also analyzed by PXRD and polarized light microscopy to examine recrystallization upon storage. Neither neat amorphous QHCl (Fig. 2, diffractogram b) nor coground amorphous samples (Fig. 2, diffractogram d) showed diffraction peaks after storage for 6 months at 48% RH and 40°C, indicating that there was no recrystallization of the drug upon storage to confound the interpretation of chemical stability in the amorphous state. The absence of reversion of amorphous QHCl stored at 80°C and 0% RH was also reported,[19] which is consistent with our observation. The absence of recrystallization of amorphous QHCl on storage is not surprising, since crystalline QHCl is known to exist only as solvated crystals.[7,19,22]

Effect of the pH Grade of Silicates on Chemical Stability of Coground QHCl

The effect of silicate pH on chemical stability of coground amorphous QHCl was investigated by using several pH grades of Neusilin and Aerosil (Tab. 1). In general, for both Neusilin and Aerosil, the coground amorphous samples were found to be less stable than the neat amorphous QHCl (Figs. 3 and 4). More degradation was observed for QHCl coground with the lower pH grades of Neusilin (NUS2 and NNFL2N) than the higher pH grades

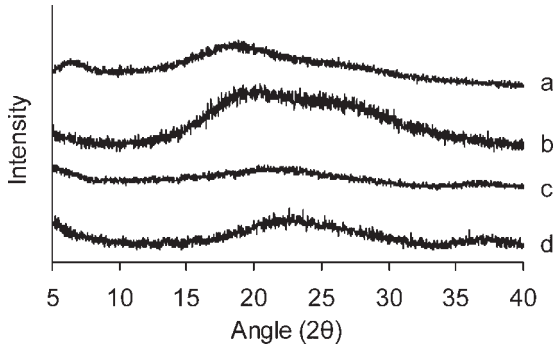


Figure 2. PXRD patterns of neat amorphous QHCl: (a) before and (b) after, and QHCl:NUS2 (1:3) coground amorphous samples: (c) before and (d) after, 6 months of storage at 48% RH and 40°C.

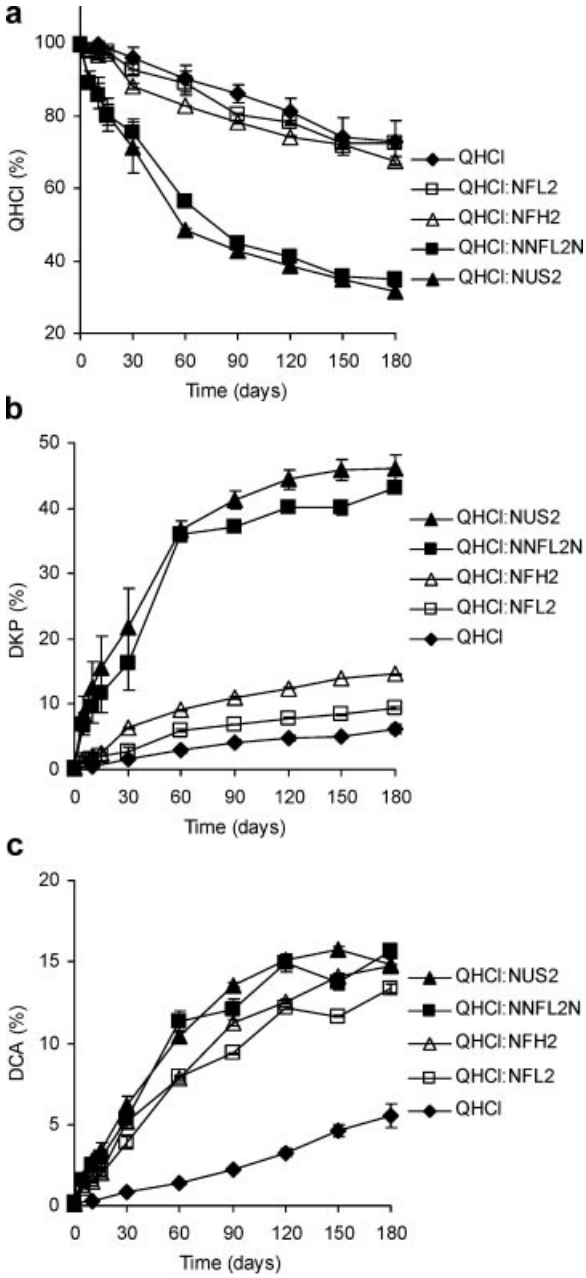


Figure 3. Chemical stability of neat amorphous QHCl and QHCl:Neusilin (1:3) coground amorphous samples at 48% RH and 40°C; (a) QHCl remaining, and (b) Diketopiperazine (DKP), and (c) Dicarboxylic acid (DCA) products formed.

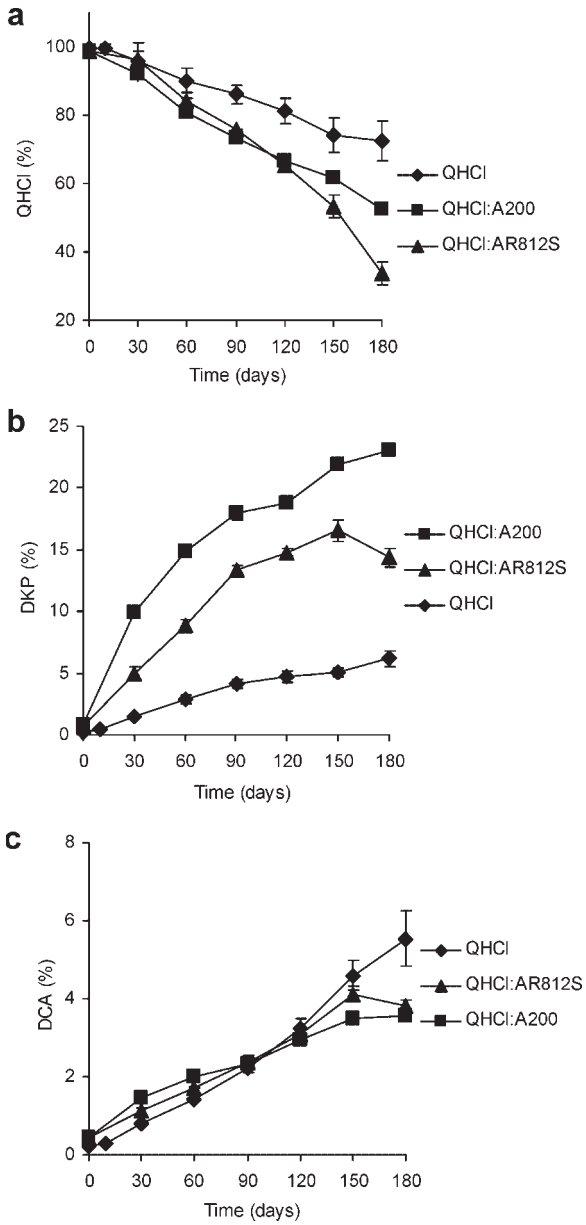


Figure 4. Chemical stability of neat amorphous QHCl and QHCl:Aerosil (1:3) coground amorphous samples at 48% RH and 40°C; (a) QHCl remaining, and (b) Diketopiperazine (DKP) and (c) Dicarboxylic acid (DCA) products formed.

(NFL2 and NFH2), Figure 3. The cyclization reaction product (DKP) was more dependent on the pH of Neusilin than was the hydrolysis product (DCA), Figure 3b and c.

For all QHCl:Neusilin coground samples, about 95% of the total mass was accounted for by QHCl remaining and the two major degradation products (DKP and DCA) formed. Incomplete mass balance on storage, particularly for QHCl alone, was due to other degradation products of QHCl and further degradation of DKP and DCA as indicated by additional peaks in LC/MS and HPLC/UV chromatograms (data not shown). For example, the peak for CHQ resulted from the hydrolysis of DKP and cyclization of DCA (Scheme 1) was observed and identified by its molecular ion peak ($M + H = 393$) in the LC/MS analysis. However, CHQ was not quantified due to the lack of a reference standard.

Similar to the results for Neusilin, higher production of DKP was observed for the more acidic Aerosil (A200) than the less acidic Aerosil (AR812S), Figure 4b. However, the amount of QHCl remaining was lower for AR812S than for A200 coground sample, particularly at 6 months of storage (Fig. 4a). This was due to other degradation products formed, as indicated by additional peaks in the chromatogram (data not shown), which were higher in the presence of AR812S than A200. As a result, in the presence of Aerosil, the total mass balance accounted for by QHCl remaining and the two degradation products (DKP and DCA) was low. At 6 months storage, the mass balance was 52.0 ± 4.1 and $79.1 \pm 0.9\%$ for QHCl coground with AR812S and A200, respectively (Fig. 4). The presence of several unknown degradation products in QHCl:Aerosil coground amorphous samples and the resulting low mass balance indicated the possibility of a change in reaction pathway or further degradation of DKP and DCA. In either case, without better mass balance, a mechanistic understanding of the effect of Aerosil pH on the chemical degradation of coground amorphous QHCl could not be gained with any degree of confidence.

Effect of Surface Area of Silicates on Chemical Stability of Coground QHCl

The surface areas of silicates used to investigate the effect of the pH grade on chemical stability of coground amorphous QHCl differ (Tab. 1) and therefore, had the potential to skew the effect of pH. For instance, as seen in Table 1, the lower pH grades of Neusilin (NUS2 and NNFL2N) have higher surface areas than the higher pH grades (NFL2 and NFH2). So, the greater drug degradation in the presence of lower pH grades could be attributed to the higher surface area. However, comparison of the results for the same pH grades of Neusilin with different surface areas showed no significant differences in chemical stability of the

drug (Fig. 3). For example, the difference in chemical stability of QHCl coground with NUS2 and NNFL2N, which have the same pH grade but different surface areas, was insignificant. In addition, QHCl coground with NFL2 and NFH2 showed no significant difference in chemical stability, indicating that surface area was not a major factor, at least at the 1:3 drug/silicate ratio used in this study.

Surface area changes upon grinding of silicates and cogrinding of drug/silicate samples and therefore, the absence of a clear surface area effect on chemical stability could be due to changes in surface area during sample preparation. To investigate this possibility, the surface areas of silicates (before and after grinding) and QHCl/silicate (1:3) coground samples were measured. Grinding of silicates alone and cogrinding with QHCl resulted in a decrease in surface area (Tab. 2). The surface area values before grinding obtained in this study (Tab. 2) are different from product specifications (Tab. 1). This is not unexpected, since surface area measurements may not have been carried out under the same conditions. In this study, the surface area measurements of all samples were conducted under the same conditions so that relative comparison of the results can be made.

From the results of surface area measurement, it can be argued that the lack of surface area effect on chemical stability of coground amorphous QHCl for NFL2 and NFH2 (and perhaps for NUS2 and NNFL2N) could be due to the collapse of surface area differences after cogrinding with the drug (Tab. 2 and Fig. 3). It is also possible that the surface area differences, even before grinding, are not large enough to show the effect on chemical stability of the coground amorphous drug. To further investigate these possibilities two silicates of the same pH grade with a large difference in surface area (A90 and A380, Tab. 1) were studied. The surface areas of A90 and A380 alone (before and after grinding) and coground with QHCl were determined (Tab. 2). Only 3–10% greater degradation was observed for QHCl coground with A380 than A90 (Fig. 5), despite a large difference in surface area between the two Aerosils before and after grinding or cogrinding (Tab. 2). Therefore, at the drug/silicate ratio (1:3) investigated here, surface area was not a significant factor affecting the chemical stability of the coground drug.

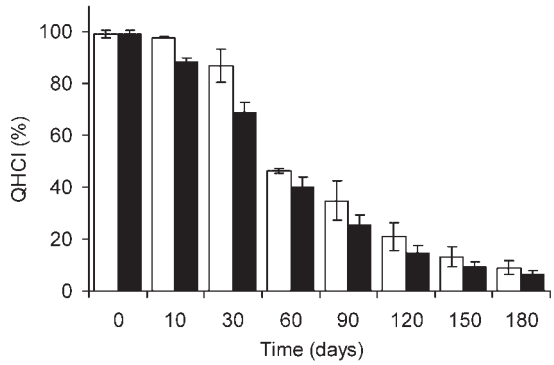


Figure 5. Chemical stability of QHCl:Aerosil (1:3) coground amorphous samples at 48% RH and 40°C; □ QHC1:A90 and ■ QHC1:A380.

The catalytic effect of silicates on acid–base, oxidation–reduction, polymerization, and other reactions is ascribed to their ability to donate/accept protons (Bronsted acid/base) and electrons (Lewis base/acid).[23] For silicates of the same surface chemistry (i.e., pH grade), the number of reactive sites per unit surface area is considered to be the same. For instance, the number of silanol (Si-OH) groups on silica surface per nm^2 is about 5, which is independent of the specific surface area, porosity, pore size distribution, etc.[24] So, the total number of reactive sites increases proportionately with increasing the total surface

Table 2. Surface Area of QHCl, Silicate and QHCl/Silicate (1:3) Coground Amorphous Samples Measured Experimentally

	Surface Area ± SD (m^2/g), $n = 3$		
Sample	Sample Alone Not Ground	Sample Alone 30 min Ground	QHCl/Silicate (1:3) 30 min Coground
QHCl	1.3 ± 0.1	4.6 ± 0.1	—
Neusilin FH2	83.7 ± 1.3	8.6 ± 0.1	3.2 ± 0.1
Neusilin FL2	111.3 ± 0.9	10.3 ± 0.6	5.3 ± 0.3
Aerosil 90	109.7 ± 2.6	70.8 ± 0.9	34.5 ± 1.8
Aerosil 380	348.0 ± 4.0	143.4 ± 1.8	75.3 ± 4.8

area of silicate in the formulation. Therefore, any difference in total surface area is also expected to affect the chemical stability of amorphous drugs prepared with silicates of the same pH grade proportionately. The absence of a significant effect of surface area in this study could be due to the presence of excess silicate surface at the 1:3 drug/silicate ratio. To more definitively determine the effect of surface area, an investigation of chemical stability at both low and high drug/silicate ratios is required, which is underway. However, to a first approximation, the absence of surface area effect in this study at 1:3 drug/silicate ratio indicates that the effect of the pH grade of silicates on chemical stability of coground amorphous QHCl was not confounded by the effect of surface area.

Effect of pH-Modifiers on Chemical Stability of QHCl Coground With Silicates

The pH grade of silicates is a major factor affecting the chemical stability of coground amorphous QHCl (Fig. 3). Thus, it is reasonable to use pH-modifiers to adjust the surface pH of silicates for improved drug stability. To better understand the effect of the pH grade of silicates on chemical stability of the coground amorphous QHCl, the pH-stability profile of the drug in aqueous buffer solutions was determined. The results indicate that cyclization of QHCl to form DKP occurs predominantly at lower pH and hydrolysis to form DCA at higher pH (Fig. 6), which is similar to the reported pH-stability profile of moexipril.[25–27] Therefore, QHCl should be useful as a model to investigate the ability of basic pH-modifiers to reduce cyclization and acidic pH-modifiers to reduce hydrolysis of amorphous drugs coground with silicates.

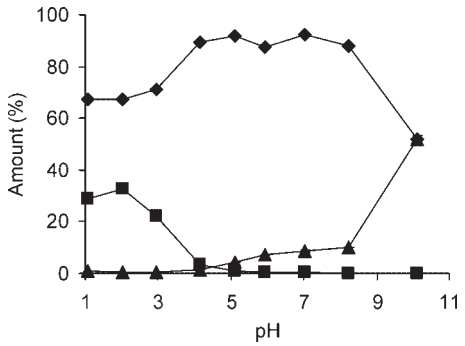


Figure 6. pH-stability profile of QHCl in buffer solutions at 40°C for 90 h; ◆ QHCl remaining and degradation products, ■ DKP and ▲ DCA, formed.

QHCl coground with the lower pH grade Neusilin (NUS2) resulted in higher DKP formation due to cyclization of quinapril. Therefore, a basic pH-modifier (MgO) was used to potentially increase the surface pH and reduce the cyclization of quinapril in QHCl:NUS2:MgO (1:2.6:0.4) coground amorphous formulations. On the other hand, since hydrolysis of quinapril occurs at higher pH, an acidic pH-modifier (AA) was used with a higher pH grade Neusilin (NFL2) to potentially lower the surface pH and reduce the hydrolysis of quinapril in QHCl:NFL2:AA (1:2.6:0.4) coground amorphous samples. The level of the pH-modifier was set at 10% of the total formulation.

The use of MgO as a basic pH-modifier in QHCl:NUS2:MgO coground amorphous samples greatly improved QHCl stability (Fig. 7). In the presence of MgO, only 2% DKP was observed at 6 months as compared to 45% in the absence of MgO (Fig. 7b). MgO also reduced the hydrolysis of QHCl in QHCl:NUS2:MgO coground amorphous samples (Fig. 7c). Acid catalyzed hydrolysis of ACE inhibitors at lower pH has been suggested.[25,28] The hydrolysis product formed in QHCl:NUS2 (1:3) coground amorphous samples could be from the acid catalyzed hydrolysis. Therefore, in the presence of a basic pH-modifier, the surface pH would be modified to a higher pH resulting in reduced DKP and DCA formation. Indeed, the 4% (w/v) suspension pH of QHCl:NUS2:MgO coground sample is higher (9.0) than that of the QHCl:NUS2 coground sample (5.7, Tab. 1). However, it should be noted that the suspension pH may not represent the actual surface pH of the formulation. For example, solid state surface acidity measurement of pharmaceutical excipients by diffuse reflectance spectroscopy using pH indicators showed that surface pH was consistently lower than suspension pH.[29,30] Measurement of surface pH of QHCl/silicate coground amorphous systems in solid state, which is under investigation, will provide more information on the actual surface acidity and surface pH-stability profile of the QHCl/silicate amorphous formulations.

The addition of ascorbic acid (AA) as an acidic pH-modifier in QHCl:NFL2:AA coground amorphous samples reduced QHCl hydrolysis (Fig. 8c), which is consistent with a reduction in the surface pH of the coground system. Moreover, the 4% (w/v) suspension pH of QHCl:NFL2:AA coground sample is 5.8, which is lower than that of the QHCl:NFL2 coground sample (7.6, Tab. 1). The decrease in surface pH by the acidic pH-

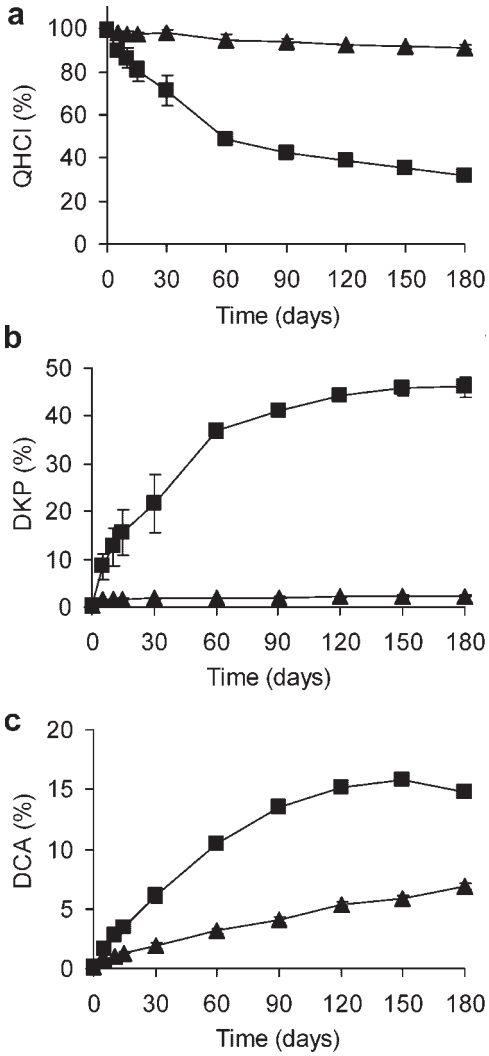


Figure 7. Chemical stability of QHCl:NUS2 (1:3), ■, and QHCl:NUS2:MgO (1:2.6:0.4), ▲, cogrounded amorphous samples at 48% RH and 40°C; (a) QHCl remaining, and degradation products, (b) DKP and (c) DCA, formed.

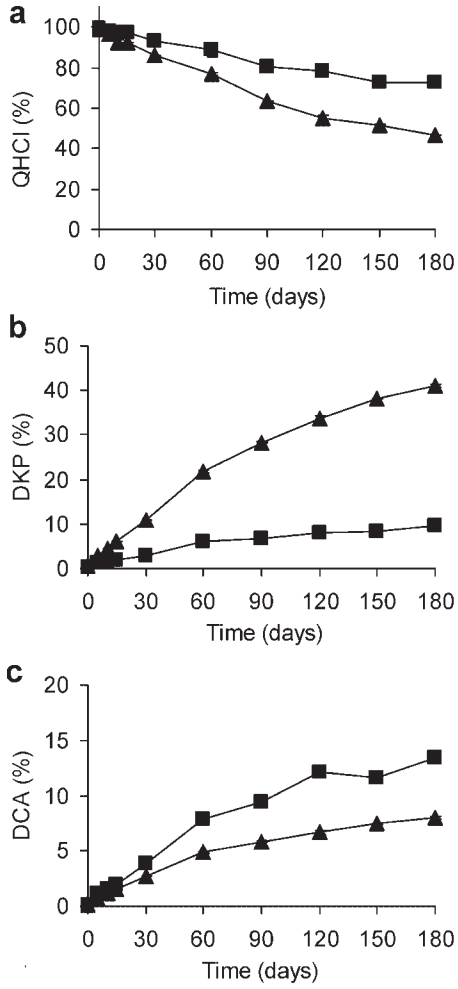


Figure 8. Chemical stability of QHCl:NFL2 (1:3), ■, and QHCl:NFL2:AA (1:2.6:0.4), ▲, cogrounded amorphous samples at 48% RH and 40°C; AA = ascorbic acid; (a) QHCl remaining, and degradation products, (b) DKP and (c) DCA, formed.

modifier is also indicated by an increased DKP formation as compared to QHCl:NFL2 (1:3) cogrounded amorphous samples (Fig. 8b). As a result, the stability of QHCl decreased (Fig. 8a) due to increased DKP formation in the presence of AA. However, this result shows the potential use of acidic pH-modifiers to prevent alkaline hydrolysis of amorphous drugs cogrounded with high pH grade silicates.

DISCUSSION

Previous studies reported both stabilizing and destabilizing effects of silicates on chemical stability of drugs.[8,15–18] However, the effects of characteristics such as silicate properties (pH grade and surface area), drug/silicate ratio and relative humidity were not always provided in detail. Therefore, the mechanisms involved in stabilization and/or destabilization of drugs in the presence of silicates are not unambiguously understood. Since preparation of physically stable amorphous drugs using silicates has been established,[2–4,10–14] the current studies on chemical stability provide useful data to those formulators interested to use amorphization with silicates as an approach to improve dissolution rate and bioavailability.

The results of this study show that pH grade of silicates is a major factor affecting the chemical

stability of coground amorphous QHCl. Although the silicates used to investigate the effect of the pH grade have different surface areas, the effect of surface area on chemical stability at 1:3 drug/silicate ratio was not significant. Therefore, differences in stability profiles of coground amorphous systems prepared with various silicates were primarily due to differences in the pH grade of silicates.

The rate of DCA product formation by hydrolysis of QHCl was not significantly different in the presence of either higher or lower pH grades of Neusilin (Fig. 3c). Based on the suspension pH of Neusilin (Tab. 1), greater formation of DCA would be expected in the presence of NFL2 and NFH2 (i.e., higher pH grades of Neusilin), since hydrolysis of the drug predominantly occurs at higher pH (Fig. 6). The insignificant effect of Neusilin pH on DCA formation could be due to lower surface pH of QHCl:Neusilin coground systems than that of Neusilin alone. Indeed, as shown in Table 1, the suspension pH of QHCl:Neusilin (1:3) coground systems is lower than that of the corresponding Neusilin, due to the presence of QHCl in the coground systems. In the lower pH range, the rate of hydrolysis would be slower and less sensitive to pH, as observed in Figure 6. However, it should be noted that the solubility of the coground drug in the suspension varies with pH and the suspension pH of the coground system may not represent the surface pH in solid state. A more direct measure of surface acidity of silicates and drug/silicate coground amorphous systems is preferred.

Another possible explanation for the lack of significant effect of silicate pH grade on DCA formation is the limited moisture (48% RH) during the stability study of the coground amorphous samples. At the limited moisture level, water can be a rate limiting reactant for the hydrolysis process and the rate of DCA formation would be suppressed so that any effect of pH would not be seen clearly. The water content of drug:Neusilin formulations could not be determined by Karl Fischer (KF) titration, because of the interference of structural water and silanols of Neusilin, which react with KF-reagents. The water content of Neusilin includes adsorbed water and structural water. Adsorbed water can be released by heating at 70–150°C. Removal of structural water, mainly by condensation of silanols, requires heating at 150–800°C. Since these high temperatures are not applicable for drug:Neusilin formulations, water content of the formulation could not also be determined by TGA.

However, in this study, the water content of QHCl:Neusilin coground samples was determined relative to the water content at 0% RH using dynamic vapor sorption (DVS, Q5000, TA Instruments). QHCl:Neusilin amorphous samples were first equilibrated at 40°C/0% RH for 6 h and then at 40°C/48% RH for another 6 h. The weight gain at 48% RH over the weight at 0% RH was 4.4% and 3.4% for QHCl coground with Neusilin US2 and FL2, respectively. The lack of significant effect of Neusilin pH on DCA formation could be due in part to the small difference in water content (1%) between the two formulations. To further understand the effect of water content, the chemical stability of QHCl:Neusilin amorphous samples at different %RH is under investigation.

The degradation of QHCl in drug/silicate coground amorphous samples by cyclization to form DKP was significantly dependent on the pH grade of silicates. In general, greater formation of DKP, which is the primary degradation product of QHCl, was observed in lower pH grades of silicates than the higher pH grades. For example, lower pH grades of Neusilin (NUS2 and NNFL2N) resulted in greater DKP formation than the higher pH grades of Neusilin (NFL2 and NFH2), Figure 3b. These results are in general agreement with the pH-stability profile of QHCl in solution, which also shows predominantly higher DKP formation at lower pH (Fig. 6). However, the pH-stability profile of QHCl in solution is not in complete agreement with the stability profile of drug/silicate coground amorphous samples of different pH grade silicates. For example, QHCl coground with lower pH grade Neusilin (NUS2) has a suspension pH of 5.7 (Tab. 1) and resulted in a very high rate of DKP formation (Fig. 3b), whereas in solution DKP formation primarily occurs below pH 4 (Fig. 6).

To further evaluate the discrepancy in pH-stability profiles, the pH-rate profiles of QHCl in solution and coground amorphous systems were determined using first order rate equation. First order kinetics has been used to describe the degradation of moexipril and quinapril in solution and amorphous systems.[19,21,25–27,31] Figure 9 shows first order kinetic plots of QHCl degradation in solution and amorphous systems. For the QHCl/silicate coground amorphous samples, data over the initial 30–40% degradation were used to determine the observed rate constant (k_{obs}), since the kinetics were not well described by the first order beyond that.

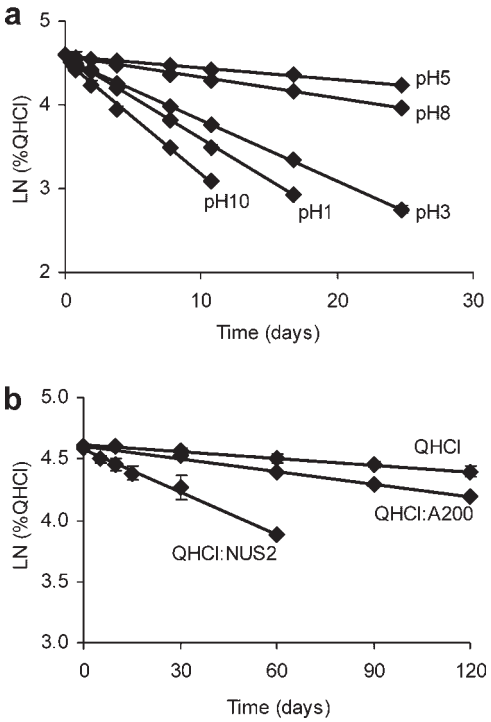


Figure 9. First order kinetic plots of QHCl degradation in (a) solutions of different pH, and (b) neat amorphous QHCl and QHCl/silicate (1:3) coground amorphous samples.

In solution, quinapril exists as a cation (Q+), zwitterion (Q±), or anion (Q−) depending on the pH (Scheme 2). The pH-rate profile of QHCl (Fig. 10) indicated that the degradation of QHCl in solution involved spontaneous degradation of Q+ and Q±, and spontaneous and specific base catalyzed degradation of Q−. The k_{obs} can be expressed in terms of the fractions of quinapril species and apparent rate constants of the degradation processes involved, as follows.

$$\text{Rate} = k_{obs}[\text{QHCl}]_t$$

where $[\text{QHCl}]_t$ is the total concentration of QHCl at time t.

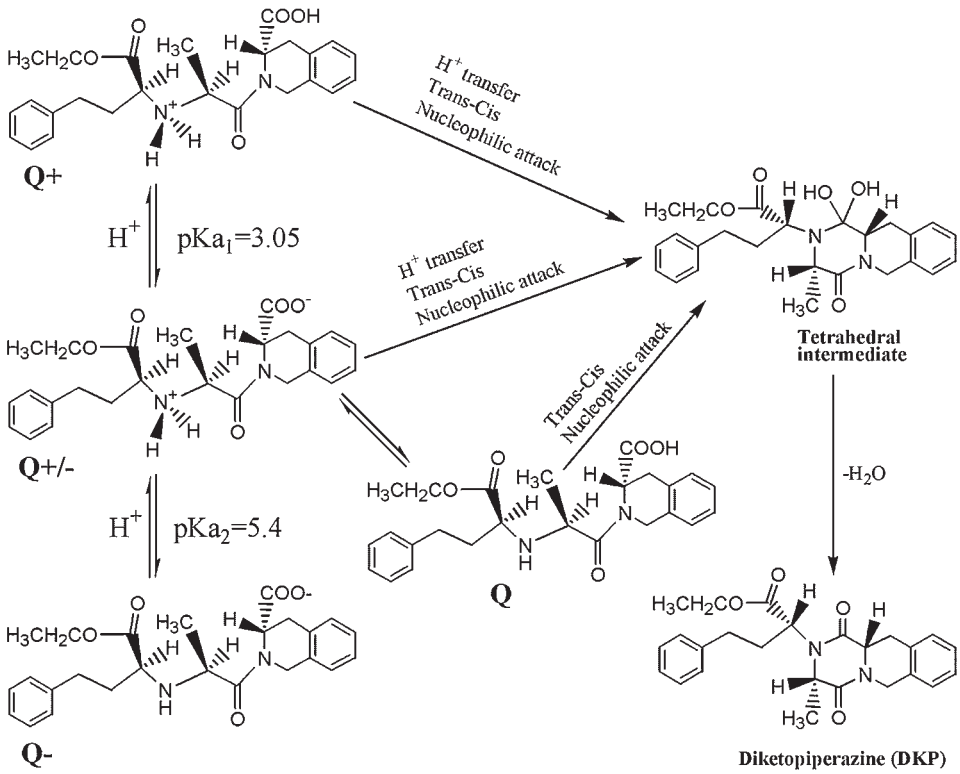
$$k_{obs} = k_1 f_{Q+} + k_2 f_{Q\pm} + (k_3 + k_4 a_{OH}) f_{Q-}$$

where f_{Q+}, $f_{Q\pm}$, and f_{Q-} are fractions of quinapril present as Q+, Q±, and Q−, respectively. The fractions are given by $f_{Q+} = a_H^2/x$, $f_{Q\pm} = a_H Ka_1/x$, and $f_{Q-} = Ka_1 Ka_2/x$, where $x = a_H^2 + a_H Ka_1 + Ka_1 Ka_2$, and Ka_1 and Ka_2 are dissociation constants of quinapril (Scheme 2). a_H and a_{OH} are activities of hydrogen and hydroxide ions, respectively. The parameters, k_1, k_2, k_3, and k_4 are apparent rate constants for the spontaneous degradation of Q+, Q±, and Q−, and specific base catalyzed degradation of Q−, respectively. The agreement between the experimental results of k_{obs} and the nonlinear curve fit (Fig. 10) along with a similar expression reported for moexipril[25–27] indicates that the expression given for k_{obs} is appropriate to describe the influence of solution pH on QHCl degradation.

As shown in Figure 10, the degradation rate constants for coground amorphous systems are significantly lower than that of the solutions, with the very notable exception of the lower pH grades of Neusilin (NUS2 and NNFL2N). In general, the slower reaction rate observed in the amorphous powders is expected due to limited moisture (48% RH) used for stability study, which would lower the apparent rate constants, as compared to the drug in solution. However, the reaction rates of QHCl coground with lower pH grades of Neusilin (NUS2 and NNFL2N) are comparable to the reaction rates in solution (Fig. 10). Moreover, the coground amorphous systems showed a maximum k_{obs} in the pH range where the k_{obs} in solution is a minimum. For example, the pH-rate profile has a maximum for QHCl coground with NUS2, which has a suspension pH of 5.7, whereas the pH-rate profile of the solution has a minimum at about the same pH (Fig. 10), clearly indicating the deviation in pH-stability profile of quinapril between coground amorphous and solution systems.

The pH-stability profile of neat amorphous quinapril lyophilized from solutions of different pH (adjusted with NaOH or HCl) was determined (Fig. 11) to compare with the pH-stability profile of QHCl/silicate coground amorphous samples (Fig. 10). The results indicate that the pH-stability profiles of lyophilized and coground amorphous samples are similar, both of which show high degradation rates in the pH range of about 3–6. However, the pH-stability profile of lyophilized quinapril (Fig. 11) is quite different from the pH-stability profile of the solution (Fig. 6). For lyophilized samples, a relatively high degradation rate is observed between pH 3–6, where the degradation rate in solution is relatively low.

The discrepancy in pH-stability profiles of QHCl between solution and amorphous states can be explained by the mechanisms and quinapril species involved in the degradation process. Other authors have proposed the cyclization pathways for ACE inhibitors including QHCl (Scheme 2).[7,19,21,22,25–27] Intramolecular



Scheme 2. Mechanisms of quinapril degradation by cyclization reaction to form diketopiperazine (DKP) product; Q+: cation, Q±: zwitterion, Q−: anion, and Q: neutral quinapril.

cyclization to form DKP involves the reaction between the amine and the carboxylic groups of quinapril. Since the carboxylate anion of Q− does not have a leaving group, no cyclization occurs from the Q−. The mechanisms involved in cyclization of quinapril (Scheme 2) include proton transfer or loss of HCl and *trans–cis* conformational change prior to nucleophilic attack to form

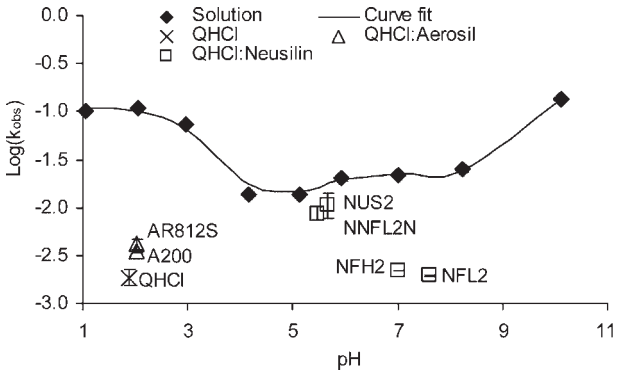


Figure 10. pH-rate profiles of QHCl in buffer solutions at 40°C, and neat amorphous QHCl and QHCl/silicate (1:3) coground amorphous powders at 40°C and 48% RH. Neat amorphous QHCl and QHCl/silicate (1:3) coground amorphous samples are plotted at the pH of 4% QHCl solution (pH 1.9) and 4% suspensions of coground systems (Tab. 1), respectively.

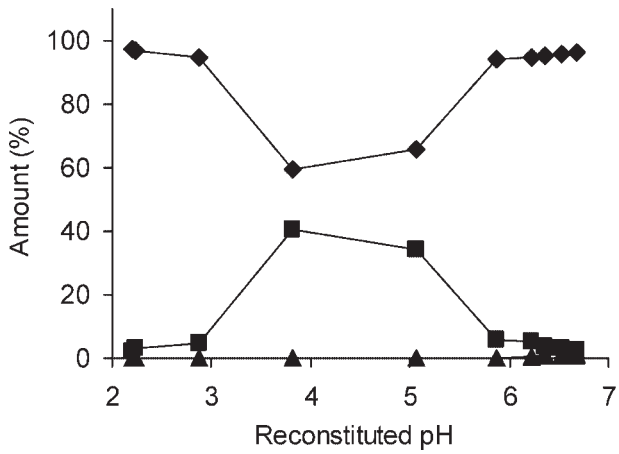


Figure 11. pH-stability profile of lyophilized amorphous quinapril at 40°C and 0% RH for 60 days; ◆ QHCl remaining and degradation products, ■ DKP and ▲ DCA, formed.

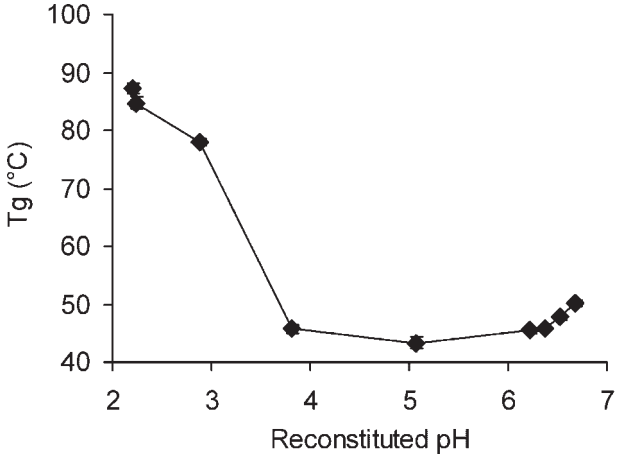


Figure 12. T_g of quinapril lyophilized from solutions of different pH.

the tetrahedral intermediate followed by dehydration forming DKP product.[7,19,21,22,25–27] In quinapril solutions of pH 3–5.4 (between pKa_1 and pKa_2), Q± is the major species. However, in amorphous solids of the same pH range, quinapril would be primarily found as the neutral form (Q), Scheme 2, since zwitterions are often less stable in solid state due to lack of hydration of ions. Since cyclization of neutral quinapril (Q) to form DKP in amorphous solids does not require the proton transfer step (Scheme 2), Q is expected to be more reactive contributing in part to the deviation of the amorphous pH-stability profile from the solution profile. Also, determination of the T_g of lyophilized quinapril against pH shows that the pH range where T_g is lower (Fig. 12) corresponds to the pH range of higher DKP formation (Fig. 11), indicating that Q is more reactive also due in part to its lower T_g. Therefore, the higher rate of DKP formation between pH 3–6 in the amorphous samples is due to the neutral quinapril (Q), which is a major species in this pH range. A higher degradation rate of Q than QHCl in the amorphous state and its correlation with T_g has been reported.[21,31] Furthermore, the pH-stability profile reported for lyophilized moexipril is consistent with our results.[27]

The pH-stability profile of lyophilized quinapril with high degradation rates between pH 3–6 (Fig. 11) and the high degradation rate observed for QHCl coground with lower pH grades of Neusilin (NUS2 and NNFL2N), Figure 10, suggests a surface pH of about 3–6 for the coground samples, which is significantly different from the Neusilin suspension pH.

CONCLUSIONS

The pH grade of silicates is a major factor affecting the chemical stability of amorphous drugs coground with silicates. The use of pH-modifiers is a useful approach for chemical stabilization without compromising physical stability. The pH-stability profile of quinapril in solution does not predict the pH-stability profile of quinapril in the amorphous state. Rather, the pH-stability profile in the amorphous solid along with an understanding of the mechanisms and kinetics of reactions involved in the solid state is necessary to select a stable formulation. Even with the understanding of pH in neat amorphous systems, an effective surface pH in coground or other composite systems remains more complex and cannot be approximated by suspension pH. Effective surface pH measurement of silicates and drug/silicate systems in solid state is required to determine surface pH-stability profile.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the Dane O. Kildsig Center for Pharmaceutical Processing Research. We are also grateful to USP for providing us reference standards and for granting a USP fellowship to Shumet Hailu. We are thankful to Dr. Steven Suib, Chemistry Department, University of Connecticut for access to the X-ray diffractometer.

REFERENCES

1. Hancock BC, Parks M. 2000. What is the true solubility advantage for amorphous pharmaceuticals? Pharm Res 17:397–404.
2. Kinoshita M, Baba K, Nagayasu A, Yamabe K, Shimooka T, Takeichi Y, Azuma M, Houchi H, Minakuchi K. 2002. Improvement of solubility and oral bioavailability of a poorly water-soluble drug, TAS-301, by its melt-adsorption on a porous calcium silicate. J Pharm Sci 91:362–370.
3. Bahl D, Bogner Robin H. 2008. Amorphization alone does not account for the enhancement of solubility of drug co-ground with silicate: The case of indomethacin. AAPS PharmSciTech 9:146–153.
4. Bahl D, Hudak J, Bogner RH. 2008. Comparison of the ability of various pharmaceutical silicates to amorphize and enhance dissolution of indomethacin upon co-grinding. Pharm Dev Technol 13:255–269.

5. Byrn SR, Xu W, Newman AW. 2001. Chemical reactivity in solid-state pharmaceuticals: Formulation implications. Adv Drug Deliv Rev 48:115–136.
6. Pikal MJ, Lukes AL, Lang JE, Gaines K. 1978. Quantitative crystallinity determinations for b-lactam antibiotics by solution calorimetry: Correlations with stability. J Pharm Sci 67:767–773.
7. Wei X. 1997. Investigation of solid-state stability of selected bioactive compounds. ed., West Lafayette, IN 47907: PhD Thesis, Purdue University.
8. Yonemochi E, Matsumura M, Oguchi T, Yamamoto K, Nakai Y. 1991. Stability of aspirin in controlled pore glass solid dispersion. Chem Pharm Bull 39:1027–1031.
9. Rowe RC, Sheskey P, Weller PJ. 2003. Handbook of pharmaceutical excipients. 4th edition. London: Pharmaceutical Press and: Washington, DC: American Pharmaceutical Association.
10. Konno T. 1986. Physical and chemical changes of medicinals in mixtures with adsorbents in the solid state. I. Effect of vapor pressure of the medicinals on changes in crystalline properties. Chem Pharm Bull 34:301–307.
11. Konno T. 1990. Physical and chemical changes of medicinals in mixtures with adsorbents in the solid state. IV. Study on reduced-pressure mixing for practical use of amorphous mixtures of flufenamic acid. Chem Pharm Bull 38:2003–2007.
12. Gupta MK, Vanwert A, Bogner RH. 2003. Formation of physically stable amorphous drugs by milling with Neusilin. J Pharm Sci 92:536–551.
13. Bahl D, Bogner RH. 2006. Amorphization of indomethacin by co-grinding with Neusilin U S2: Amorphization kinetics, physical stability and mechanism. Pharm Res 23:2317–2325.
14. Watanabe T, Wakiyama N, Usui F, Ikeda M, Isobe T, Senna M. 2001. Stability of amorphous indomethacin compounded with silica. Int J Pharm 226:81–91.
15. Lau-Cam CA, Pendharkar CM, Jarowski CI, Yang KY. 1991. Instability of digoxin-amorphous silicon dioxide triturates prepared by solvent deposition and ball milling. Pharmazie 46:522–525.
16. Daniels R, Kerstiens B, Tischinger-Wagner H, Rupprecht H. 1989. The stability of drug adsorbates on to silica. In: Rubinstein MH, editor. Pharmaceutical technology drug stability. Chichester, England: Ellis Horwood Limited. pp. 49–67.
17. Daniels R, Rupprecht H. 1985. Adsorption and chemical stability of a cationic aggregating esterpropantheline bromide-on silica surfaces in aqueous dispersions. Pharm Res 2:170–175.
18. Gore A, Banker G. 1979. Surface chemistry of colloidal silica and a possible application to stabilize aspirin in solid matrix. J Pharm Sci 68:197–202.
19. Guo Y, Byrn SR, Zografi G. 2000. Physical characteristics and chemical degradation of amorphous quinapril hydrochloride. J Pharm Sci 89:128–143.
20. USP. 2008. Reagents, indicators and solutions. USP, the official compendia of standards, USP 31. Vol. 1. Rockville, MD: United States Pharmacopeial Convention Inc. pp. 813–814.
21. Guo Y, Byrn SR, Zografi G. 2000. Effects of lyophilization on the physical characteristics and chemical stability of amorphous quinapril hydrochloride. Pharm Res 17:930–936.
22. Hausin RJ, Codding PW. 1991. Molecular and crystal structures of MDL27,467A hydrochloride and quinapril hydrochloride, two ester derivatives of potent angiotensin converting enzyme inhibitors. J Med Chem 34:511–517.
23. Theng G. 1981. Clay-activated organic reactions. In: Van-Olphen H, Veniale F, editors. International Clay Conference. Vol. 35. Bologna and Pavia, Italy: Elsevier. pp. 197–238.
24. Zhuravlev LT. 1987. Concentration of hydroxyl groups on the surface of amorphous silicas. Langmuir 3:316–318.
25. Gu L, Strickley RG. 1987. Diketopiperazine formation, hydrolysis, and epimerization of the new dipeptide angiotensin-converting enzyme inhibitor RS-10085. Pharm Res 4:392–397.
26. Gu L, Strickley RG. 1988. Preformulation stability studies of the new dipeptide angiotensin-converting enzyme inhibitor RS-10029. Pharm Res 5:765–771.
27. Strickley RG, Visor GC, Lin L-H, Gu L. 1989. An unexpected pH effect on the stability of moexipril lyophilized powder. Pharm Res 6:971–975.
28. Bundgaard H, Falch E. 1985. Allopurinol prodrugs. I. Synthesis, stability and physicochemical properties of various N1-acyl allopurinol derivatives. Int J Pharm 23:223–237.
29. Glombitza BW, Oelkrug D, Schmidt PC. 1994. Surface acidity of solid pharmaceutical excipients. Part 1. Determination of the surface acidity. Eur J Pharm Biopharm 40:289–293.
30. Govindarajan R, Zinchuk A, Hancock B, Shalaev E, Suryanarayanan R. 2006. Ionization states in the microenvironment of solid dosage forms: Effect of formulation variables and processing. Pharm Res 23:2454–2468.
31. Li J, Guo Y, Zografi G. 2002. Effects of a citrate buffer system on the solid-state chemical stability of lyophilized quinapril preparations. Pharm Res 19:20–26.