Solid-State Surface Acidity and pH-Stability Profiles of Amorphous Quinapril Hydrochloride and Silicate Formulations

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ABSTRACT: To determine the surface acidity and stability profiles of quinapril hydrochloride (QHCl) coground with silicates, solid-state equivalent pH (pHeq) of amorphous samples was measured by diffuse reflectance spectroscopy using pH indicator probes. Calibration curves for pH indicators were developed in buffer solutions. Amorphous samples with and without pH indicators were prepared by cryo-grinding. Different pH grades of silicates and various QHCl/silicate ratios were used to make amorphous samples over a range of surface acidity. Diffuse reflectance spectra of amorphous samples were measured and pHeq was determined using the calibration curves of pH indicators developed in solution. Suspension pH of amorphous samples was also measured. The chemical stability of coground amorphous samples was assessed at 40°C and 0% or 48% RH. The chemical stability of neat amorphous quinapril lyophilized from solutions over a range of pH was also assessed at 40°C/0% RH and the reconstituted pH-stability profile of lyophiles was determined. For all silicate and QHCl/silicate amorphous samples, the same pH rank order was obtained based on pHeq and suspension pH. However, the pHeq was significantly lower than the corresponding suspension pH. Discrepancies between pH-stability profiles based on the pHeq and the suspension pH were observed. In general, the pHeq- and reconstituted pH-stability profiles were essentially identical, but the suspension pH-stability profile deviated from the reconstituted pH-stability profile by 2–3 pH units. The results indicate that solid-state surface acidity measurement provides a more accurate prediction of the effective surface acidity of amorphous formulations than the suspension pH. In conclusion, solid-state surface acidity measurement of excipients and solid formulations using pH indicator probes as surrogates can be used to determine the ionization state of the drug and to predict the chemical stability profile of the drug in actual solid formulations. J Pharm Sci99:2786–2799, 2010

Keywords: quinapril hydrochloride; amorphous; stability; chemical stability; cogrinding; milling; silicates; Aerosil; Neusilin; pH grade of silicates; surface acidity; surface pH; equivalent pH; acidity function; pH-stability profile

INTRODUCTION

Chemical stability is a critical quality attribute of pharmaceutical products. Although the rate of drug degradation is faster in solution, significant degradation also occurs in the solid state, particularly in amorphous systems.[1–3] In general, amorphous forms are chemically less stable than the corresponding crystalline forms due to higher molecular mobility and reactivity of the amorphous systems.[1,4–6] Recent studies showed that annealing of amorphous formulations below the glass transition temperature resulted in slower molecular mobility and improved chemical stability.[7,8] For example, stabilization of aspartame lyophilized with sucrose or trehalose by annealing below the glass transition temperature has been reported.[7] Despite the benefits of amorphous systems to improve dissolution rate and bioavailability of poorly soluble drugs,[9–12] their practical application in formulation development may be limited due in part to lower chemical stability. In addition to molecular mobility, the chemical stability of amorphous drugs is also influenced by other

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factors, such as moisture content and acidity of the formulation.[3,13–18] For instance, a correlation between pH of solutions prior to freeze drying and degradation rate of lyophilized solids suggests that chemical stability is influenced by the acidity of lyophilized solids.[3,14–18]

Solid-state surface acidity is described in the literature as "microenvironmental pH," "surface pH," "equivalent pH (pHeq)," or using Hammett acidity function. In general, solid-state acidity refers to the effect of formulation environment (matrix) on the ionization state of the drug, which may influence the chemical stability. Unlike the pH in dilute solutions, it is not an absolute measure of proton activity. Rather, it is a prediction of the pHeq that would result in the same extent of ionization of the drug in the solid matrix, which is different from the solution environment. For example, differences in polarity and dielectric constant between solution and solid states due to the difference in water content may influence drug ionization and stability. Depending on the chemistry of the drug and the acidity of formulations, studies reported both stabilizing and destabilizing effects of surface acidity on chemical stability of drugs in the formulation.[19,20] Microcrystalline cellulose granulated with buffer solutions of different pH was used to investigate the influence of surface acidity on chemical stability of pirenzepine dihydrochloride.[20] The "pHeq" was determined by using diffuse reflectance spectroscopy of solid formulations. Significantly higher degradation rates of the drug were observed in buffered granules of lower pH. Similarly, the chemical stability of acetylsalicylic acid was influenced by the acidity of dicalcium phosphate granulated with buffer solutions of different pH.[19] The degradation rate was minimum around pH 3, and greater at lower and higher pH values.

Authors have reported the use of acidic or basic components (i.e., pH-modifiers) in the formulation to control surface acidity of the formulation at an optimum pH and improve drug stability.[2,21–26] Badawy et al.[22,23] used acids in solid formulations, added as dry blends or as solutions during wet granulation, to reduce the hydrolysis of ester prodrugs. The stabilization was attributed to a decrease in microenvironmental pH of the formulation, which was approximated by the pH of saturated solutions of the acids. Other studies reported that the addition of sodium carbonate as a basic pH-modifier into solid formulations (using dry blending or wet granulation process) reduced acid-catalyzed degradation of drugs, presumably due to increased microenvironmental pH of the formulation.[21,25] Hailu and Bogner[26] investigated the ability of acidic and basic pH-modifiers to improve the chemical stability of amorphous quinapril hydrochloride (QHCl) prepared by cryo-grinding with different pH grades of silicates. A basic pH-modifier, magnesium oxide, reduced the cyclization of quinapril coground with a lower pH grade silicate. Also, an acidic pH-modifier, ascorbic acid (AA), reduced the hydrolysis of quinapril coground with a higher pH grade silicate. These results were consistent with a change in surface acidity of the formulation by the pH-modifiers.

The relationship between solution pH and drug stability, the pH-stability profile, can be used directly to predict stability in solution formulations. A similar approach has been attempted to relate drug degradation to the pH of solid formulations. However, the solid-state surface pH is not as well established as the solution pH. Several authors demonstrated that some solid state pH-stability profiles are qualitatively similar to the solution pH-stability profiles.[19,20] In addition, the surface acidity and stability of lyophilized products was shown to be influenced by the initial pH of the solution before lyophilization.[3,14–16] However, other studies reported that the pH-dependence of stability in solid state was not related to the pH-stability profile of the drug in solution.[17,18,26] These findings suggested that the pH-stability profile of the drug in solution is not always a reliable predictor of drug stability in solid state. A direct measurement of surface acidity of solid formulations as well as an understanding of reaction mechanisms and kinetics in the solid state are required for more accurate prediction of solid-state drug stability.

Solid-state pH cannot be defined in the same way that it is defined in solution and there is no universally accepted standard method for its measurement. However, several techniques have been used as indirect measures of surface acidity and the results depend on the basic principles and assumptions of the method used. Suspension pH measurement has been used to approximate solid-state surface acidity.[22,26–28] However, the suspension pH depends on the concentration of the solid and the solubility of each component in the formulation. Moreover, pH measurement in the suspension is affected by the presence of particles, also called the "suspension effect."[29–31] Electron paramagnetic resonance (EPR) spectroscopy and confocal microscopy have been used to characterize the pH within polymeric microspheres,[32–35] but they are used for highly hydrated solids, such as eroding microspheres and other hydrogels. Similarly, a large amount of water is used for suspension method. Therefore, these techniques are not suitable for measurement of the solid-state surface acidity of considerably dry pharmaceutical formulations and excipients.

Diffuse reflectance spectroscopy, using pH indicators, has been recently used to determine the surface acidity of essentially dry solids.[27,28,36–39] The pH-dependent ionization of indicator probes was used to

construct calibration curves in solutions of different pH. The diffuse reflectance spectra of solid samples containing pH indicators were then measured and the pHeq, which results the same extent of ionization of the given indicator as the solution, was determined using the calibration curve developed in solution. pHeq measurement of pharmaceutical excipients using diffuse reflectance spectroscopy showed that pHeq was consistently lower than the corresponding suspension pH.[27,28] Thus, the use of suspension pH to predict drug stability in solids could be misleading.

In our previous study, the nominal pH grade of silicates was shown to be a major factor affecting the chemical stability of coground amorphous QHCl; pH-modifiers were used to improve the chemical stability.[26] However, a discrepancy between the suspension pH-stability profile of QHCl/silicate amorphous samples prepared with different pH grades of silicates and the reconstituted pH-stability profile of lyophilized neat amorphous quinapril was observed. It was proposed that the discrepancy in the pH-stability profiles was due to the difference between solid-state pHeq and suspension pH of amorphous formulations. Therefore, in this study the solid-state pHeq of neat amorphous QHCl and QHCl/silicate coground amorphous samples were measured using diffuse reflectance spectroscopy. The pHeq-stability and the suspension pH-stability profiles were determined and compared to the reconstituted pH-stability profile of lyophilized quinapril to evaluate the discrepancy in stability profiles. In addition, the solid-state pHeq of amorphous formulations with and without pH-modifiers was measured to quantify the adjustment of surface acidity by the pH-modifiers.

MATERIALS AND METHODS

Materials

Quinapril hydrochloride (3-isoquinolinecarboxylic acid, 2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, [3S-[2[R^*(R^*)],3R^*]]), QHCl, was purchased from Farmhispania, S.A. (Barcelona, Spain). Reference standards of the two major degradation products of QHCl, quinapril diketopiperazine (ethyl[3S-[2(R^*), 3α,11$\alpha\beta$]]-1,3,4,6,11,11α-hexahydro-3-methyl-1,4-dioxo-α-(2-phenylethyl)-2H-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-[3S-[2[R^* (R^*)],3R^*]]), were obtained from USP (Rockville, MD). The chemical structures of QHCl along with its degradation products due to cyclization (diketopiperazine, DKP) and hydrolysis (dicarboxylic acid, DCA) are shown in Figure 1. Neusilin (magnesium aluminometasilicate) and Aerosil (silicon dioxide) were obtained from Fuji Chemicals (Inglewood, NJ) and Degussa Corporation (Parsipanny, NJ), respectively. The pH grade of silicates along with surface area specifications are given in Table 1. The pH indicators listed in Figure 2, thymol blue, bromophenol blue, bromocresol green, bromocresol purple, and phenol red, received as monosodium salts, and neutral red received as a chloride salt, were obtained from Sigma–Aldrich (St. Louis, MO). Acetonitrile (Optima LC/MS grade), methanol (HPLC grade), sodium hydroxide solution (1 N), hydrochloric acid (1 N), acetic acid (2 N), and formic acid (88%) were obtained from Fisher Scientific (Atlanta, GA). Phosphorus

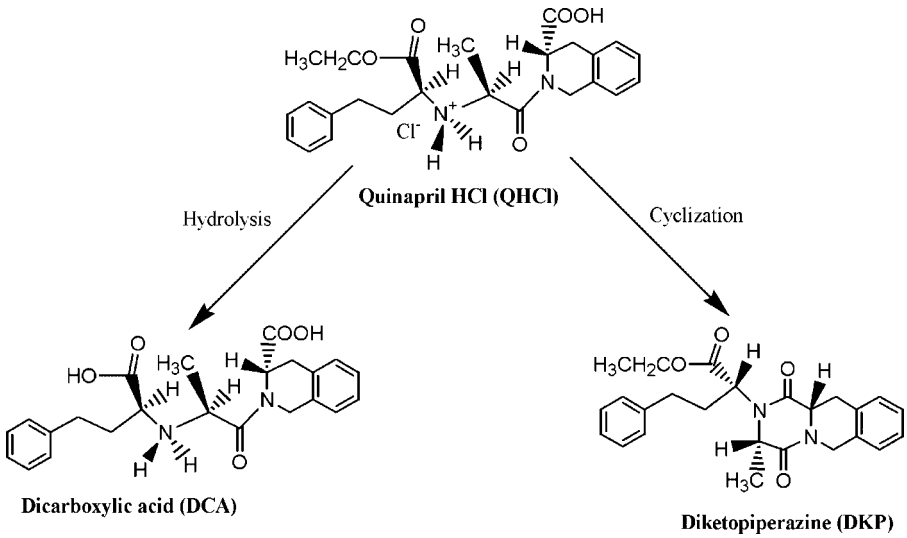

Figure 1. Major degradation pathways and products of quinapril hydrochloride (QHCl).

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Table 1. Specifications of Different Types of Silicates Used in This Study

Silicate Type	Abbreviations[a]	Product Specifications[b] Nominal pH[c]	Surface Area (m^2/g)
Neusilin: magnesium aluminometasilicate			
Neusilin US2	NUS2	6.0–8.0	300
Neusilin FL2	NFL2	8.5–10.0	150
Aerosil: silicon dioxide			
Aerosil 200	A200	3.7–4.7	200
Aerosil R812S	AR812S	5.5–7.5	220

[a]Used in this paper for convenience.
[b]From suppliers.
[c]pH of 4% (w/v) suspension in water.

pentoxide, magnesium nitrate hexahydrate, sodium acetate trihydrate, monobasic potassium phosphate, potassium chloride, potassium biphthalate, boric acid, assorbic acid, and magnesium oxide, all Certified ACS grades, were also obtained from Fisher Scientific.

Diffuse Reflectance Spectroscopy

The application of UV–Vis diffuse reflectance spectroscopy to measure surface acidity of pharmaceutical formulations and excipients using pH indicator probes has been used recently with great success.[27,28,36–39] The absorbance (A) peak ratio (Eq. 1) of deprotonated to protonated forms of the indicator provides a good correlation against solution pH (Eq. 2) in the pH range around the pK_a of the indicator[27,28,39]

$$\frac{A_{In-}}{A_{InH}} = \frac{(\varepsilon_{In-})(c_{In-})}{(\varepsilon_{InH})(c_{InH})} \tag{1}$$

where ε is the extinction coefficient and C is the concentration of the indicator in solution, and subscripts refer to the deprotonated ($In-$) and protonated (InH) forms of the indictor.

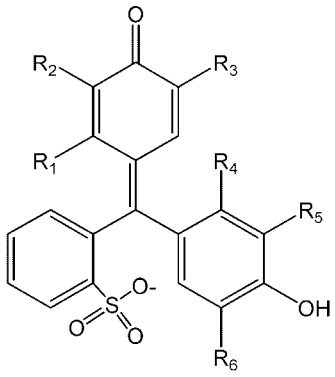
$$\log\left(\frac{A_{In-}}{A_{InH}}\right) = a(\mathrm{pH}) + b \tag{2}$$

where a is the slope and b is the y-intercept of the plot of $\log(A_{In-}/A_{InH})$ versus pH.

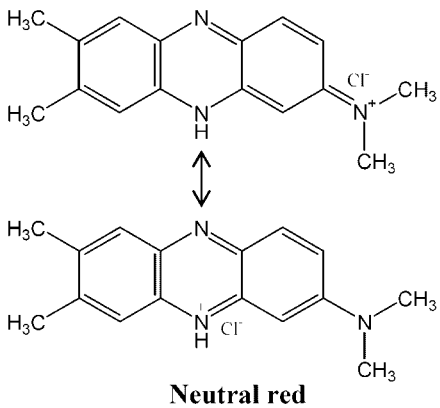
For solid samples containing pH indicators, the proportionality between indicator concentration (C') and diffuse reflectance (R) is given by the Kubelka–Munk transform (Eq. 3), which is applied in diffuse reflectance measurement of solid samples.[28,39–41] R is the fraction of incident light diffusively reflected by the powder bed.

$$F(R) = \frac{(1-R)^2}{2R} = \frac{\varepsilon' c'}{S} \tag{3}$$

where $F(R)$ is the Kubelka–Munk transformed reflectance, ε' is the extinction coefficient of the



Sulfonephthalein indicators



Neutral red

Name of Indicator	R_1	R_2	R_3	R_4	R_5	R_6
Thymol blue	CH_3	H	$CH(CH_3)_2$	CH_3	H	$CH(CH_3)_2$
Bromophenol blue	H	Br	Br	H	Br	Br
Bromocresol green	CH_3	Br	Br	CH_3	Br	Br
Bromocresol purple	H	Br	CH_3	H	CH_3	Br
Phenol red	H	H	H	H	H	H

Figure 2. Chemical structures of pH indicators used for surface acidity measurement. Sulfonephthalein indicators are shown as anions of monosodium salts and neutral red is a chloride salt.

indicator in the solid sample and S is the scattering coefficient of the powder bed.

When both $In-$ and InH are present in the solid sample, the $F(R)$ peak ratio for the two species, assuming S is independent of wavelength, is[28,40]

$$\frac{F(R)_{In-}}{F(R)_{InH}} = \frac{(\varepsilon'_{In-})(c'_{In-})}{(\varepsilon'_{InH})(c'_{InH})} \quad (4)$$

The ratio given by Eq. (4) is a measure of the concentration ratio of deprotonated to protonated forms (i.e., extent of ionization) of the indicator in the solid sample, which is equivalent to the ratio of absorbances in solution (Eq. 1) at a particular pH. Thus, the absorbance ratio in Eq. (2) can be substituted by the $F(R)$ ratio (Eq. 4) to calculate the pHeq for the solid sample (Eq. 5). Therefore, the solid-state pHeq is the pH of a solution that gives the same extent of ionization of a given indicator as the solid sample.[27,28,39]

$$\text{pHeq} = \frac{1}{a}\left[\log\left(\frac{F(R)_{In-}}{F(R)_{InH}}\right) - b\right] \quad (5)$$

pHeq is a measure of the extent of ionization of the indicator probe in the solid environment and unlike the pH in solution, it is not meant to be an exact measure of proton activity. For the pHeq to be exactly the same as the pH in solution, the pK_a of the indicator and the ratio of extinction coefficients of $In-$ and InH must be the same in solution and solid states. However, the solid state is different from the solution environment in its properties (such as, polarity and dielectric constant) and the pK_a of the indicator may not be the same in the two states. Therefore, in this study, pHeq is used only to express the solid-state surface acidity as a measure of the extent of ionization of the indicator probe so as to predict the ionization state and stability profile of the drug in solid formulations.

Preparation of Calibration Curves for pH Indicators in Solution

Calibration curves were developed for each indicator dissolved in buffer solutions of pH range within about 2 pH units of the pK_a of the indicator. Aqueous buffer solutions (50 mM) of different pH were prepared according to the USP.[42] The type of buffer used for each indicator and the pH range within which the calibration curve was constructed are given in Table 2. To determine the optimum concentration of the indicator used to develop the calibration curve, the absorbances of different concentrations of the indicator in a solution of constant pH (close to the pK_a of the indicator so that it is partially ionized) were measured. The absorbance peak ratios for deprotonated to protonated forms of the indicators (A_{In-}/A_{InH}) were independent of the concentration in the range of 5–50 μg/mL for all the pH indicators used (data not shown). This concentration range also resulted in a linear correlation of absorbance versus concentration (Beer–Lambert's law) for both deprotonated and protonated forms of the indicators. Therefore, since there is no significant effect of concentration on the absorbance peak ratio (A_{In-}/A_{InH}) between 5 and 50 μg/mL, a pH indicator concentration of 20 μg/mL in buffer solutions was used to develop calibration curves. The pH of solutions was measured using a pH electrode calibrated with standard buffer solutions of pH 1, 2, 4, 7, and 10. The absorbances of pH indicator solutions were measured using a 10 mm pathlength disposable methacrylate cuvettes and a UV–Vis spectrophotometer (Cary 100 Bio, Varian, Palo Alto, CA). The spectra were recorded in the wavelength range of 200–800 nm in double beam mode with 1 nm data collection interval, 600 nm/min scan rate and 4 nm slit width. Buffer solutions without pH indicators were used as blanks for baseline correction.

Preparation of Amorphous Samples

Amorphous samples of neat QHCl and QHCl/silicate with and without pH indicators were prepared by cryo-grinding using a cryo-mill (6750 Freezer/Mill®, SPEX SamplePrep, Metuchen, NJ). Details of the cryo-grinding procedure have been reported previously.[26] QHCl (25% w/w) was coground with silicates (75% w/w) of different pH grades (Tab. 1) to produce amorphous samples of different surface

Table 2. Calibration Curves for pH Indicators in Buffer Solutions of Specified pH Range

pH Indicator	pK_a	Buffer	Calibration Curve[a]	R^2	pH Range
Thymol blue-acidic	1.6[b]	HCl	$Y = 0.708X - 1.321$	0.996	1.2–3.2
Bromophenol blue	4.1[c]	Acetate	$Y = 0.974X - 3.549$	0.998	2.1–4.9
Bromocresol green	4.7[c]	Phthalate	$Y = 0.899X - 3.976$	0.996	3.3–5.4
Bromocresol purple	6.3[c]	Phosphate	$Y = 0.872X - 5.222$	0.999	4.0–6.5
Neutral red	6.8[b]	Phosphate	$Y = 0.256X - 1.749$	0.994	5.7–8.1
Phenol red	7.9[c]	Phosphate	$Y = 0.970X - 7.061$	0.999	5.8–8.0
Thymol blue-basic	9.0[c]	Alkaline borate	$Y = 0.908X - 7.715$	0.997	7.1–9.5

[a] $Y = \log(A_{In-}/A_{InH})$ and $X = \text{pH}$ (Eq. 2).
[b] First pK_a.
[c] Second pK_a.

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acidity. In addition, QHCl was coground with different percentages of a single pH grade of silicate (Neusilin FL2, NFL2) to investigate changes in surface acidity of amorphous samples with changes in the QHCl/Neusilin ratio. For this purpose, QHCl/NFL2 samples containing 0, 5, 25, 50, 75, and 95% (w/w) NFL2 were cryo-ground. Silicate samples with and without pH indicators were also prepared by cryo-grinding for surface acidity measurement of different pH grades of silicates in the absence of the drug. For all samples prepared with pH indicators, 1 mg of a pH indicator was mixed with 2 g of a solid sample for a 0.05% (w/w) indicator concentration. The optimum concentration of 0.05% w/w was selected, since the diffuse reflectance peak ratios for deprotonated and protonated forms of the indicators, $F(R)_{In-}/F(R)_{InH}$, were independent of the concentrations of the pH indicators in the range of 0.025–0.1% w/w (data not shown). All amorphous samples were equilibrated at 40°C and 48% RH ($Mg(NO_3)_2{\cdot}6H_2O$ saturated solution) in open vials for 24 h prior to diffuse reflectance measurement.

Diffuse Reflectance Measurement of Amorphous Samples

Amorphous powder samples were packed into a sample holder with a quartz window (PCH-010-UV–Vis, Labsphere, North Sutton, NH). For each sample, 3 g powder was loaded into the sample holder to ensure identical packing density. Diffuse reflectance spectra of amorphous powders were recorded at room temperature (~23°C) using a UV–Vis spectrophotometer (Cary 100 Bio, Varian) equipped with integrating sphere diffuse reflectance accessory (DRA-CA-30I, Labsphere). A zero degree wedge was used at the reflectance port to keep the powder sample surface perpendicular to the incident light. The diffuse reflectance spectra of samples were recorded relative to the spectralon® standard (Labsphere) in the wavelength range of 200–800 nm in double beam mode with 1 nm data collection interval, 600 nm/min scan rate and 4 nm slit width. Amorphous samples without pH indicators prepared and stored under the same conditions were used as blanks for baseline correction. The spectra were recorded as the Kubelka–Munk transformed reflectance, $F(R)$, versus wavelength and analyzed to determine solid-state pHeq using Eq. (5).

Selection of pH Indicators

The pH indicator used for a given solid sample was selected so that the indicator was partially deprotonated on the solid surface. Ideally, a pH indicator with pK_a similar to the surface pH of the solid sample would be appropriate. However, since the surface pH of solid samples was unknown, the suspension pH of solid samples was used as a first approximation. Then pH indicators with higher or lower pK_a values were evaluated until suitable indicators, which resulted in pH values within the calibration range, were identified. Using the diffuse reflectance spectra of amorphous samples, a pH indicator with reflectance peak heights as similar as possible for deprotonated and protonated forms of the indicator (peak ratio close to 1) was selected for determination of pHeq. In addition, the transition color of the indicator in solution was used as a visual means of indictor selection for a solid sample, since an appropriate indictor coground with a solid sample had a color similar to the transition color of the indicator in solution.

Chemical Stability of Amorphous Samples

The chemical stability of neat amorphous QHCl and QHCl (25% w/w) coground with silicates (75% w/w) of four different pH grades (Tab. 1) was investigated at 40°C and 0% or 48% RH in open vials. The amounts of drug remaining and major degradation products formed (Fig. 1) during storage were assayed using HPLC/UV (HP Series 1100, Agilent Technologies, Santa Clara, CA) as reported previously.[26] Additionally, the chemical stability of amorphous QHCl coground with different percentages of NFL2 (0, 5, 25, 50, 75, and 95% w/w) was studied at 40°C and 0% RH. For comparison, the chemical stability of neat amorphous quinapril lyophilized from solutions of different pH (adjusted with NaOH or HCl) was investigated at 40°C and 0% RH, as previously described.[26]

pH-Stability Profiles of Amorphous Samples

The solid-state pHeq and the 4% (w/v) suspension pH of amorphous QHCl coground with silicates, measured at room temperature (~23°), were used to determine pHeq-stability and suspension pH-stability profiles, respectively. In addition, the reconstituted pH-stability profile of lyophilized neat amorphous quinapril was determined using reconstituted solution pH of the lyophiles, also measured at room temperature (~23°). Both pHeq-stability and suspension pH-stability profiles of amorphous QHCl coground with silicates were compared with the reconstituted pH-stability profile of lyophilized neat amorphous quinapril to evaluate deviations between the different pH-stability profiles.

RESULTS AND DISCUSSION

The chemical stability of solid drug formulations can be affected by the acidity of the formulation.[17–20,26] Since discrepancies in pH-stability profiles between solution and solid states have been reported,[17,18,26]

solid state drug stability profile may not always be predicted from solution stability profile. Therefore, a more accurate prediction of solid-state pH-stability profile is required for better understanding of drug stability in solid formulations. In this paper, the solid-state pHeq of silicates and QHCl/silicate amorphous formulations determined using pH indicator probes is presented. The reliability of pHeq measurement is demonstrated using several structurally different pH indicators. The stability of amorphous formulations as a function of pHeq, suspension pH and reconstituted pH values are presented, and discrepancies in pH-stability profiles are discussed.

Absorption Spectra and Calibration Curves of pH Indicators in Solution

In solution, the extent of ionization of pH indicators is dependent on solution pH. As an example, Figure 3a shows absorption spectra of bromocresol green in phthalate buffer solutions of different pH. The absorption peaks at 443 and 615 nm correspond to the protonated (*InH*) and deprotonated (*In*−) forms of the indicator, respectively. The ratio of absorbance

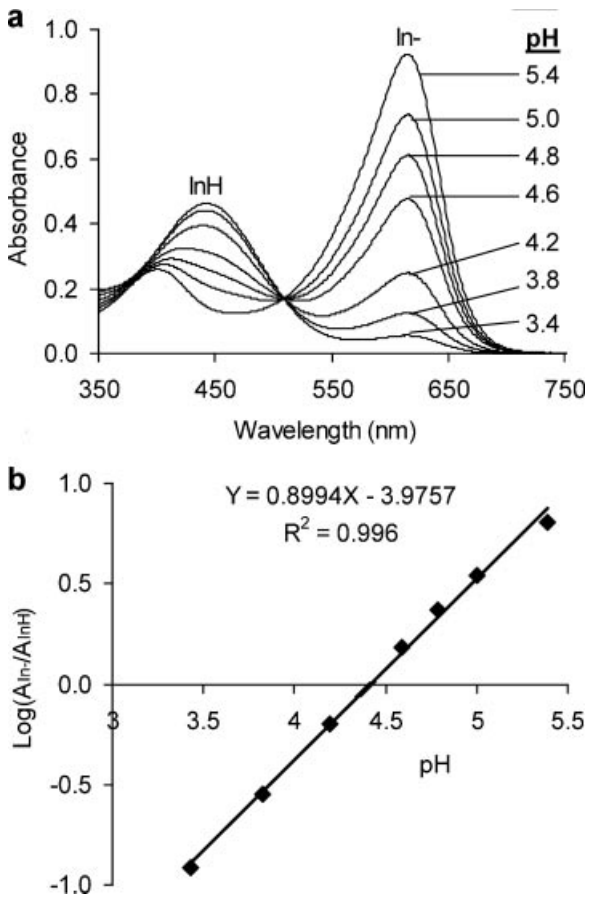

Figure 3. Absorption spectra (a) and calibration curve (b) for bromocresol green in phthalate buffer solutions of different pH.

peaks of *In*− and *InH* forms (A_{In-}/A_{InH}) for each spectrum was used to construct the calibration curve against solution pH (Eq. 2, Fig. 3b). The absorption spectra (data not shown) and calibration curves for all other pH indicators were determined similarly and the results are provided in Table 2.

The pH indicators used in this study are monosodium salts of diprotic acids (InH_2) except neutral red, which was monoprotic acid (*InH*), Figure 2. For all pH indicators, except thymol blue and neutral red, calibration curves were developed in the pH range around the second pK_a (Tab. 2). The ionization equilibrium for the second acid dissociation constant (Ka_2) is between InH^- and In^{2-}, as shown below (Eq. 6).

$$InH_2 \overset{Ka_1}{\rightleftarrows} InH^- + H^+ \overset{Ka_2}{\rightleftarrows} In^{2-} + H^+ \qquad (6)$$

In this paper, the terms protonated (*InH*) and deprotonated (*In*−) forms of the indicator are used for convenience. However, it should be noted that for all pH indicators with calibration curves developed in the pH range around the second pK_a (pK_{a2}), the protonated (*InH*) and deprotonated (*In*−) forms refer to InH^- and In^{2-}, respectively (Eq. 6). On the other hand, when the calibration curve is constructed in the pH range around the first pK_a (pK_{a1}), for example, thymol blue (p$K_{a1} = 1.6$, Tab. 2), the protonated (*InH*) and deprotonated (*In*−) forms refer to InH_2 and InH^-, respectively (Eq. 6). For monoprotic acids with one pK_a, for example, neutral red, the protonated (*InH*) and deprotonated (*In*−) forms refer to *InH* and In^-, respectively. For pH indicators with two pK_a values, the contribution of one pK_a when the calibration curve is developed in the pH range of the other pK_a is negligible, since the two pK_a values are far apart for the indicators used in this study. For instance, for thymol blue with p$K_{a1} = 1.6$ and p$K_{a2} = 9.0$, the contribution of pK_{a1} when the calibration curve is developed around pK_{a2} would be negligible, since only InH^- and In^{2-} species, but not InH_2, (Eq. 6) would be present in solutions of pH around p$K_{a2} = 9.0$.

Diffuse Reflectance Spectra and Equivalent pH (pHeq) of Amorphous Samples

The diffuse reflectance spectra of silicates coground with pH indicators were measured to determine the solid-state surface acidity of different pH grades of silicates. Figure 4 shows the diffuse reflectance spectra of thymol blue and phenol red coground with NFL2 and Neusilin US2 (NUS2), respectively. For both spectra, the peaks at the lower and higher wavelengths are for protonated (*InH*) and deprotonated (In^-) forms of the indicators, respectively (Fig. 4). The ratio of $F(R)$ peaks for deprotonated and protonated species of the indicator was calculated for each spectrum in Figure 4 and the solid-state

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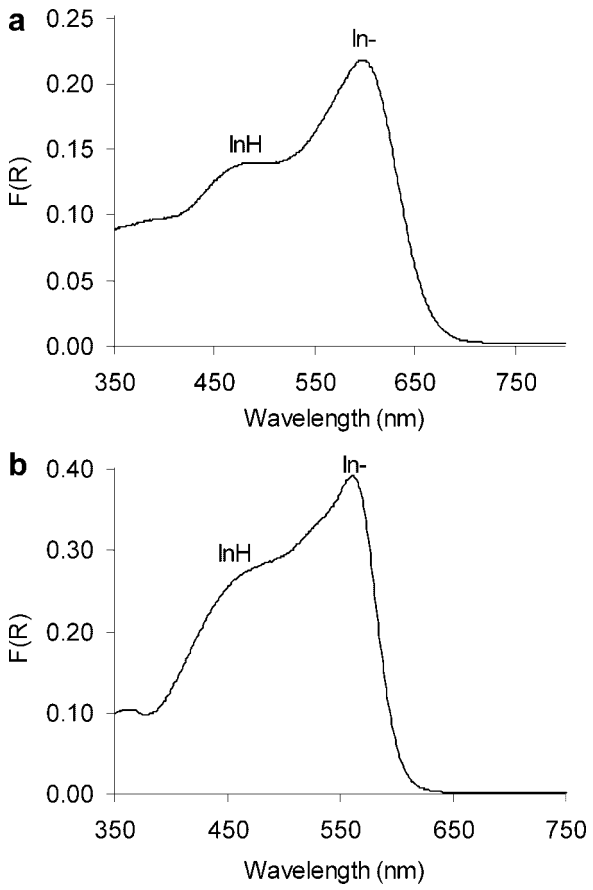

Figure 4. Kubelka–Munk transformed diffuse reflectance, $F(R)$, spectra of silicates coground with pH indicators; (a) Neusilin FL2 coground with thymol blue and (b) Neusilin US2 coground with phenol red.

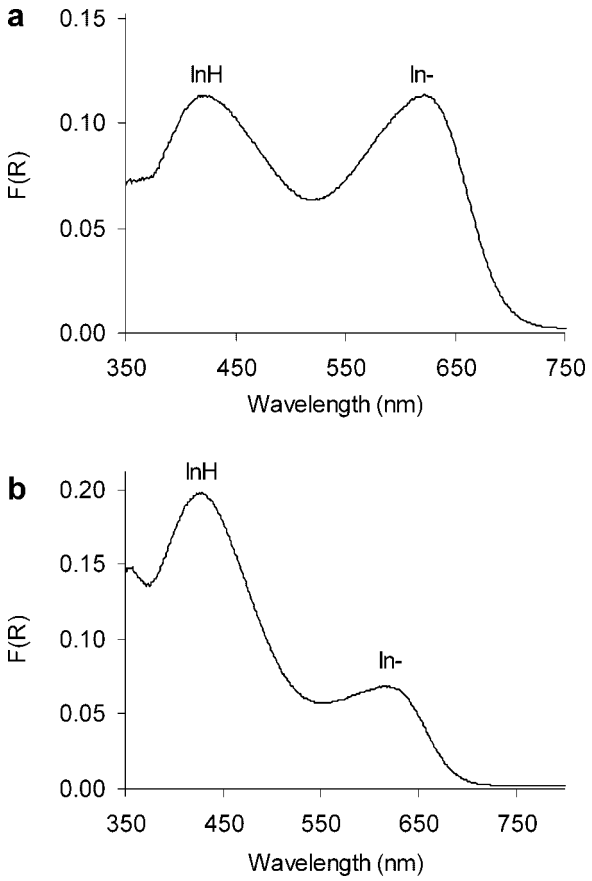

Figure 5. Kubelka–Munk transformed diffuse reflectance, $F(R)$, spectra of amorphous QHCl (25% w/w) coground with (a) Neusilin FL2 (75% w/w) and (b) Neusilin US2 (75% w/w) using bromocresol green pH indicator.

pHeq of silicates (Eq. 5) was determined using the calibration curve developed in solution for the corresponding indicator (Tab. 2). The pHeq values of Aerosil 200 and Aerosil R812S were also determined similarly, and the results are presented in Table 3. In addition, the 4% (w/v) suspension pH of silicates in water was also measured after grinding of the silicates (Tab. 3). The suspension pH of silicates measured after grinding with and without pH indicators were similar, since the concentration of pH indicators used was negligible (0.05% w/w). As shown in Table 3, about the same pH rank order was obtained using pHeq and suspension pH of silicates. However, pHeq was consistently 1–2 pH units lower than the corresponding suspension pH value for all the silicates.

Figure 5 shows the diffuse reflectance spectra of QHCl (25% w/w) and NUS2 or NFL2 (75% w/w)

Table 3. Solid-State Equivalent pH (pHeq) of Different pH Grades of Silicates Determined by Diffuse Reflectance Spectroscopy Using pH Indicators

Silicate	pH Indicator	pK_a of Indicator	pHeq[a]	Suspension pH[b,c]
Neusilin FL2	Thymol blue-basic	9.0[d]	8.8	9.6
Neusilin US2	Phenol red	7.9[d]	7.5	8.8
Neusilin US2	Neutral red	6.8[e]	7.1	8.8
Aerosil R812S	Bromophenol blue	4.1[d]	2.3	3.5
Aerosil 200	Bromophenol blue	4.1[d]	2.2	4.2

[a] Standard deviation <0.15 for $n = 3$.
[b] pH of 4% (w/v) suspension of silicates (after grinding) in water.
[c] Standard deviation <0.20 for $n = 3$.
[d] Second pK_a.
[e] First pK_a.

amorphous samples coground with bromocresol green. The peaks at 443 and 615 nm wavelengths correspond to the protonated (*InH*) and deprotonated (*In*−) forms of bromocresol green, respectively. For the QHCl/NFL2 coground amorphous sample, the peak heights for both protonated and deprotonated forms of the indicator are close (Fig. 5a). On the other hand, for the QHCl/NUS2 coground amorphous sample, the peak for the protonated form is significantly higher than the peak for the deprotonated form of the indicator (Fig. 5b), suggesting that the QHCl/NUS2 coground amorphous sample is more acidic than the QHCl/NFL2 coground amorphous sample. This was qualitatively expected, since NUS2 is a lower pH grade Neusilin than NFL2 (Tab. 1). As shown in Table 4, the pHeq of QHCl/NUS2 sample was 3.9, which was indeed more acidic than the QHCl/NFL2 sample (pHeq 4.5). The solid-state pHeq and the suspension pH values of different amorphous QHCl formulations are presented in Table 4. Similar to the results observed in Table 3, pHeq of all amorphous QHCl formulations was significantly lower than the corresponding suspension pH (Tab. 4).

pHeq of Amorphous Samples Measured Using Structurally Different Indicators

To examine the dependence of surface acidity measurement on the pH indicator used, structurally different pH indicators were used for pHeq determination of the same sample (Tab. 4). For example, the pHeq of QHCl/NUS2 coground amorphous sample was determined using bromophenol blue, bromocresol green, and bromocresol purple. The pHeq values obtained were within a narrow pH range (3.8–4.2), Table 4. Moreover, the pHeq of QHCl/NFL2 coground amorphous sample determined by using bromocresol green and bromocresol purple were essentially the same, 4.5 and 4.4, respectively. These results indicate that the solid-state pHeq measurement is not significantly dependent on the type of pH indicator used, provided that an appropriate indicator is selected to ensure partial ionization when incorporated into the solid sample.

pH indicator probe molecules have been used as surrogates to determine the ionization state of drug molecules in solid formulations.[28,36,37] Similarly, in this study, pH indicators were used to probe the acidity of silicates, and to understand the ionization state and stability of amorphous QHCl coground with silicates. However, the ionizable functional groups of pH indicators are different from the ionizable groups of the drug. For instance, for all pH indicators (Fig. 2), except neutral red, the ionization involves the phenolic hydroxyl (−OH) group. On the other hand, the ionization of QHCl involves the carboxylic acid group (−COOH) for the first pK_a (3.0) and the ammonium group ($=NH_2^+$) for the second pK_a (5.4). To examine the influence of different ionizable groups on solid-state acidity measurement, the pHeq of NUS2 was determined using phenol red and neutral red, which have phenolic hydroxyl (−OH) and ammonium ($=NH^+$) ionizable groups, respectively, (Fig. 2). Very close pHeq values (7.5 and 7.1) were obtained for NUS2 by using the two very different probes (Tab. 3), suggesting that even pH indicators with ionizable groups different from the ionizable group of the drug are useful as surrogates for approximate prediction of the ionization state of the drug in solid formulations.

The reproducibility in pHeq values (within ±0.5) demonstrated using two or more indicator probes with the same and different ionizable groups indicates that pHeq measurement is reasonably precise. The extent of ionization of pH indicators in solution (before lyophilization) and in amorphous samples

Table 4. Solid-State pHeq of Neat Amorphous QHCl and QHCl Coground With Different pH Grades of Silicates at 25% (w/w) QHCl in the Total Formulation

Amorphous Samples	pH Indicator	pK_a of Indicator	pHeq[a]	Suspension pH[b,c]
QHCl	Thymol blue-acidic	1.6[d]	1.3	1.9
QHCl/NUS2	Bromophenol blue	4.1[e]	3.8	5.7
QHCl/NUS2	Bromocresol green	4.7[e]	3.9	5.7
QHCl/NUS2	Bromocresol purple	6.3[e]	4.2	5.7
QHCl/NFL2	Bromocresol green	4.7[e]	4.5	7.6
QHCl/NFL2	Bromocresol purple	6.3[e]	4.4	7.6
QHCl/A200	Thymol blue-acidic	1.6[d]	1.4	2.0
QHCl/AR812S	Thymol blue-acidic	1.6[d]	1.2	2.0
QHCl/NFL2/AA	Bromocresol green	4.7[e]	3.9	5.8
QHCl/NUS2/MgO	Bromocresol purple	6.3[e]	4.7	9.0

Ascorbic acid (AA) and MgO were used as pH-modifiers at 10% (w/w) in the total formulation.
[a]Standard deviation <0.15 for $n = 3$.
[b]pH of 4% (w/v) suspension of amorphous samples in water.
[c]Standard deviation <0.20 for $n = 3$.
[d]First pK_a.
[e]Second pK_a.

(after lyophilization) has been determined for trehalose–citrate systems.[36] The ionization extent of the indicators was shown to be lower in the lyophile than in the corresponding prelyophilization solution, suggesting a lower pH for the lyophile than the solution. However, calculation of pHeq of the lyophiles from the ionization extent of the indicators showed that the lyophiles were only about 0.1–0.5 pH units lower than the corresponding solution pH. The authors attributed the difference in the ionization extent to the difference in water content and polarity between the lyophile and solution systems. Nevertheless, the difference in pH was within the uncertainty of the pHeq measurements observed in this study when different indicator probes were employed, that is, within 0.5 pH units. Therefore, determination of pHeq using indicator probes can be useful to predict the ionization state and stability profile of drugs in solid formulations with an uncertainty of ± 0.5 pH units. This could provide more accurate prediction of pH-stability profiles of solid drug formulations than the suspension pH, for example, which will be shown later.

Grinding as a Method to Incorporate pH Indicators into Solid Samples

Previous studies used solvent deposition to incorporate pH indicators to solid samples for surface acidity measurement.[27,28,38,39] In the solvent deposition method, aqueous or alcoholic solutions of pH indicators were mixed with solid excipients or formulations using a mortar and pestle or high shear mixer. In contrast, here, cryo-grinding was used to incorporate solid pH indicators into solid samples. Cryo-grinding eliminates the possibility of dissolution of formulation components and the resulting complications in surface properties (acidity), for instance, due to ionization and chemical reaction of the dissolved components on the solid surface. Moreover, it seems prudent to use the same processing conditions to incorporate the drug and the pH indicator to avoid any effect of different processing variables. In this study QHCl/silicate coground amorphous formulations were prepared by cryo-grinding without using any solvent. Therefore, to use the pH indicators as surrogates to determine the ionization state of QHCl, the same processing (i.e., cryo-grinding) conditions were applied to incorporate the pH indicators into silicates and QHCl/silicate amorphous formulations. The cryo-grinding method was effective not only to make amorphous samples, but also to get homogenous distribution of the pH indictors into solid formulations. The homogeneity of the pH indicators was confirmed by the reproducibility of the intensity of diffuse reflectance spectra peaks obtained from triplicate measurements, which resulted in pHeq values with standard deviations <0.15. This study demonstrated that grinding can be used effectively to incorporate pH indicators into solid formulations and has the advantage of simulating formulation processes involving essentially dry conditions.

pH-Stability Profiles of QHCl Coground With Different pH Grades of Silicates

The chemical stability of neat amorphous QHCl and QHCl coground with different pH grades of silicates at 25% (w/w) QHCl in the total formulation (Tab. 4) was assessed at 40°C and 48% RH. As shown in Table 4, AA and MgO were used as pH-modifiers at 10% (w/w) of the total formulation. The solid-state pHeq and suspension pH values of the amorphous samples (Tab. 4) were used to determine the pH-stability profiles (Fig. 6). A wide discrepancy in pH-stability profiles was observed when stability was expressed as a function of the pHeq versus the suspension pH. For instance, for the QHCl/NUS2 sample the pHeq-stability profiles show the least

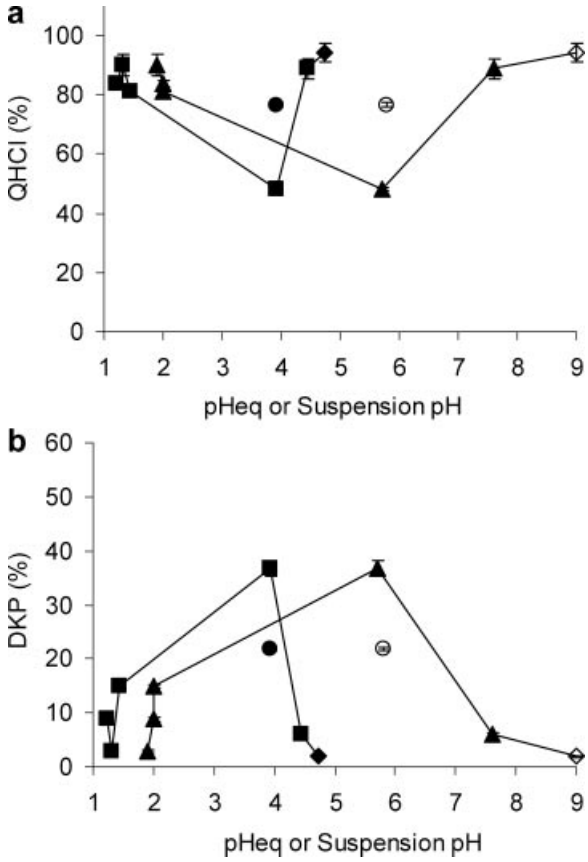

Figure 6. pH-stability profiles of different amorphous QHCl formulations (Tab. 4), at 40°C and 48% RH for 60 days, based on pHeq and suspension pH values. Formulations: QHCl/silicate (■: pHeq, ▲: suspension pH); QHCl/NUS2/MgO (◆: pHeq, ◇: suspension pH); and QHCl/NFL2/AA (●: pHeq, ○: suspension pH). (a) QHCl remaining and (b) diketopiperazine (DKP) formed. See the text for discussion of deviations in stability.

amount of QHCl remaining (Fig. 6a) and the correspondingly highest amount of DKP formed (Fig. 6b) at the pHeq of 4. On the other hand, the suspension pH-stability profiles show the least amount of QHCl remaining (Fig. 6a) and highest amount of DKP formed (Fig. 6b) at the suspension pH of 6 for the same sample.

The use of pH-modifiers to reduce pH-sensitive degradation of drugs in solid formulations has been reported.[21–23,26] In our previous study, the use of MgO as a basic pH-modifier in QHCl/NUS2 amorphous samples reduced DKP formation.[26] On the other hand, the addition of AA as an acidic pH-modifier in QHCl/NFL2 samples increased DKP formation. These observations were consistent with a proposed change in surface acidity of the formulation by the pH-modifiers. In this study, the pHeq of QHCl/Neusilin samples with and without pH-modifiers was measured to quantify the change in surface acidity. As shown in Table 4, the addition of AA in QHCl/NFL2 samples reduced the pHeq from 4.5 to 3.9, while the use of MgO in QHC/NUS2 samples increased the pHeq from about 4.0 to 4.7. The stability data in the presence of pH-modifiers are included in Figure 6 using the pHeq and suspension pH values (Tab. 4). The stability data for QHCl/NFL2/AA sample deviated from the stability profile of other QHCl/silicate formulations. For example, the pHeq of QHCl/NFL2/AA and QHCl/NUS2 are the same, 3.9 as measured by diffuse reflectance of bromocresol green (Tab. 4). Similarly, the suspension pH of the two samples is also the same, that is, 5.7–5.8 (Tab. 4). However, a much lower QHCl remaining (Fig. 6a) and higher DKP formed (Fig. 6b) were observed in the QHCl/NUS2 sample than the QHCl/NFL2/AA sample. AA has been reported to reduce DKP formation due to its interference in quinapril cyclization bond formation.[43] This is consistent with the lower DKP observed in the QHCl/NFL2/AA than QHCl/NUS2 samples, despite the same pH values. As shown in Figure 6, the stability data for QHCl/NUS2/MgO sample concurred with the stability profile of other QHCl/silicate samples. Therefore, it seems evident that the primary mechanism of pH-modifiers to improve chemical stability is through alteration of the surface acidity of the formulation to the optimum pH for drug stability.

To further evaluate the discrepancy between pHeq-stability and suspension pH-stability profiles observed in Figure 6, the pH-stability profile of neat amorphous quinapril lyophilized from solutions of different pH was determined and compared with the pHeq and suspension pH profiles of coground amorphous samples. The chemical stability of lyophilized neat amorphous quinapril was assessed at 40°C and 0% RH, since the glass transition temperatures (T_g) of lyophiles in the higher pH range (above 3) were low.[26] The chemical stability of neat amorphous QHCl and QHCl (25% w/w) with NUS2 or NFL2 (75% w/w) prepared by cryo-grinding was also studied at 40°C and 0% RH to compare the pH-stability profiles at the same conditions. However, it has to be noted that there was no clearly detectable thermal event for QHCl/silicate amorphous samples to determine the T_g, perhaps due to the high T_g of silicates (above 1100°C). The stability of coground amorphous samples as a function of pHeq agreed very well with the reconstituted pH-stability profile of lyophilized quinapril samples (Fig. 7). For example, in both lyophilized and coground samples, QHCl was the least stable (Fig. 7a) with the highest amount of DKP formed (Fig. 7b) at pHeq and reconstituted pH values of 4–5. The stability profile of the drug is very sensitive in this pH range. Therefore, the minor deviations observed between pHeq- and reconstituted pH-stability profiles could be due to the fact that the data points for the pHeq of coground samples and the reconstitute pH of lyophilized samples were not

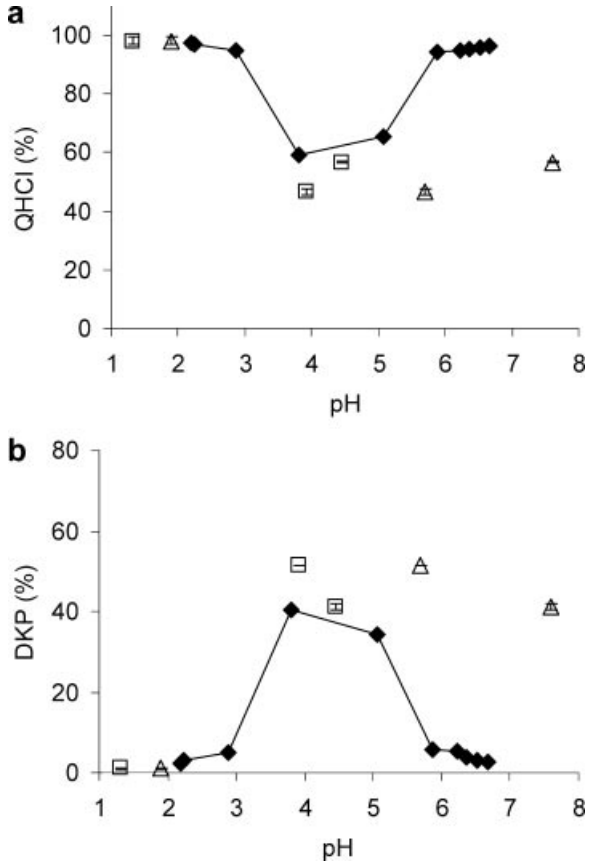

Figure 7. pH-stability profiles of amorphous QHCl at 40°C and 0% RH for 60 days. ◆: reconstituted pH-stability profile of lyophilized neat amorphous quinapril; and □: pHeq-stability and △: suspension pH-stability profiles of neat amorphous QHCl and QHCl (25% w/w) with Neusilin US2 or FL2 prepared by cryo-grinding (Tab. 4). (a) QHCl remaining and (b) diketopiperazine (DKP) formed.

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Table 5. Solid-State Equivalent pH (pHeq) of Amorphous QHCl Coground With Different Percentages of Neusilin FL2 (NFL2) in the Total Formulation

NFL2 (% w/w)	pH Indicator	pK_a of Indicator	pHeq[a]	Suspension pH[b,c]
0	Thymol blue-acidic	1.6[d]	1.3	1.9
5	Thymol blue-acidic	1.6[d]	1.6	2.7
25	Thymol blue-acidic	1.6[d]	2.3	3.7
50	Bromophenol blue	4.1[e]	3.7	5.4
75	Bromocresol green	4.7[e]	4.5	7.6
95	Phenol red	7.9[e]	7.5	8.9

[a]Standard deviation <0.15 for $n = 3$.
[b]pH of 4% suspension of amorphous samples in water.
[c]Standard deviation <0.20 for $n = 3$.
[d]First pK_a.
[e]Second pK_a.

exactly the same pH values. Moreover, the uncertainty in the pHeq measurement (i.e., as much as ±0.5) could cause the minor deviation observed in pH-stability profiles, particularly in the pH range (4–5) where stability is highly sensitive to pH. As shown in Figure 7, the suspension pH-stability profile did not match the reconstituted pH-stability profile nearly as well. For instance, the least stable coground amorphous samples had suspension pH values of 5.7 and 7.6, where QHCl was shown to be stable, based on the reconstituted pH of the lyophiles (Fig. 7). These results indicate that solid-state pHeq better predicts the effective surface acidity of the formulation than does the suspension pH.

pH-Stability Profiles of Amorphous QHCl Coground With Different Percentages of Neusilin FL2

The chemical stability of amorphous QHCl coground with different percentages of NFL2 was assessed at 40°C and 0% RH. The solid-state pHeq and suspension pH of each sample were also measured. As shown in Table 5, both the pHeq and suspension pH of the QHCl/NFL2 amorphous samples increased with increasing percentages of NFL2, which is expected, since NFL2 is a higher pH grade silicate and QHCl is relatively acidic. However, the pHeq was lower than the suspension pH for a given percentage of NFL2 (Tab. 5), which is consistent with the results observed in Tables 3 and 4. The largest deviation is at 75% NFL2, where the difference between pHeq and suspension pH was 3.1.

Figure 8 shows the pHeq-stability and suspension pH-stability profiles of amorphous QHCl coground with different percentages of NFL2 plotted using the pH values in Table 5. For comparison, the reconstituted pH-stability profile of lyophilized neat amorphous quinapril is included in Figure 8. A far superior agreement between the pHeq and reconstituted pH profiles than between the suspension pH and reconstituted pH profiles (Fig. 8) indicates that, again, the solid-state pHeq provides a more accurate prediction of the effective surface acidity of amorphous samples than the suspension pH. Moreover, the agreement between the pHeq-stability profile of coground amorphous QHCl and the reconstituted pH-stability profile of neat lyophilized quinapril suggests that pH of the coground formulation is the major factor affecting the stability of QHCl coground with NFL2.

In this study, the solid-state pHeq of all silicates and QHCl/silicate amorphous formulations investigated was found to be lower than the corresponding suspension pH value (Tabs. 3–5). This observation is consistent with previous studies.[27,28,36] Lower pHeq than suspension pH was reported for microcrystalline cellulose, dicalcium phosphate, lactose, calcium carbonate and magnesium stearate.[27,28] For solid drug formulations, the state of ionization and stability profile of the drug is influenced by the microenvironmental properties (such as, micropolarity and surface acidity) of the actual formulation in the solid state, not in solution or suspension. Therefore, the use of drug stability profiles obtained in

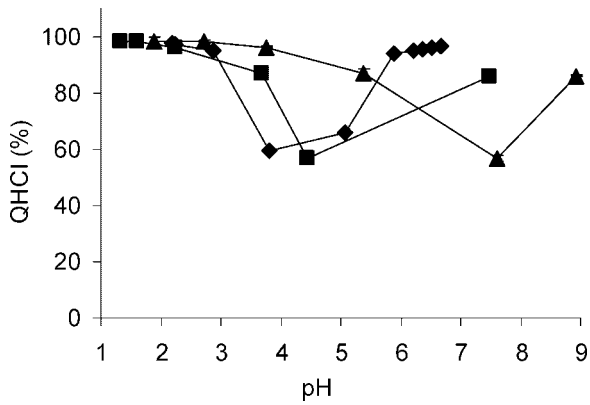

Figure 8. pH-stability profiles of amorphous QHCl at 40°C and 0% RH for 60 days. ◆: reconstituted pH-stability profile of lyophilized neat amorphous quinapril; and ■: pHeq-stability and ▲: suspension pH-stability profiles of amorphous QHCl coground with different percentages of Neusilin FL2 (Tab. 5).

solution or suspension to predict the stability profile of the drug in solid formulations could be misleading. Indeed, discrepancies between pH-stability profiles of drugs in solution and solid states have been observed.[17,26] Our previous study showed that the pH-stability profile of amorphous QHCl coground with different pH grades of silicates is quite different from the pH-stability profile of the drug in solution.[26] Therefore, measurement of solid-state surface acidity and determination of pHeq-stability profile of the drug in the solid formulation leads to a better understanding of the ionization state and chemical stability of the drug than does solution or suspension based pH-stability profile.

CONCLUSIONS

Solid-state surface acidity measurement of excipients and solid formulations using pH indicator probes as surrogates can be used to determine the ionization state of the drug and to predict the chemical stability profile of the drug in solid formulations. Solid-state surface acidity measurement using diffuse reflectance spectroscopy is reliable as demonstrated by using two or more structurally different pH indicator probes for a given sample. Discrepancies in pH-stability profiles of drugs between solid and solution or suspension systems have been reported.[17,26] Therefore, the application of solid-state surface acidity measurement can provide a better understanding of drug stability profile in the actual solid formulation and eliminate prediction from solution or suspension stability profile. For amorphous QHCl coground with Neusilin, the surface acidity of amorphous formulations was found to be the major factor affecting the chemical stability of the drug.

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