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

Jinjiang Li,1 Yushen Guo,1,2 and George Zografi1,3

Received October 3, 2001; accepted October 8, 2001

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

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

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

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

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

INTRODUCTION

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

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

In view of these earlier observations, we chose to form amorphous molecular dispersions of QHCl and citric acid by lyophilization from aqueous solution of known initial pH. From the acid-base equilibria shown for QHCl (QHCl → Q−− + Cl−, pKa = 3.05) and citric acid (citric acid → monosodium citrate, pKa1 = 3.12) we were able to know the composition of QHCl, Q−−, citric acid, and monosodium citrate in solution at each initial pH chosen. Based on previous studies with lyophilized protein solutions that seem to retain their initial state of ionization when lyophilized to an amorphous solid, i.e., “pH memory” (12,13), we began our studies by varying the initial solution pH of citric acid-QHCl combinations and estimated the composition of various species in the solid state from their solution equilibria based on the pKa values. In the case of quinapril, however, we could not be sure whether the zwitterionic form was retained or whether the removal of water had produced the neutral form of quinapril, as shown in Scheme 1 (10). Because we knew the Tg values for the various species (QHCl, 91°C; Q−−, 51°C; citric acid, 11°C; and monosodium citrate, 69°C), we also were in a position to attempt to account for the changes in Tg of the lyophilized solid, and therefore, the possible role of any changes in molecular mobility due to various components. In the first series of experiments, we have maintained the pH constant for various systems while systematically altering the Tg of the lyophilized solid. In the second series of experiments, we systematically titrated the pH of our solutions in a certain range of pH, which maintained the system Tg essentially constant.
MATERIALS AND METHODS

Materials

QHCl [3S-[2[R*(R*)], 3R*]-2-[2-[[1-ethoxycarbonyl]-3-phenylpropyl]-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid, hydrochloride was received from the Chemical Processing Division of the Warner-Lambert Co. (Holland, MI) as a gift. The zwitterionic form of quinapril, Q⁺⁻, was prepared according to the following procedure. A combination of 0.1 N NaOH and 0.1 N sodium bicarbonate solutions was added to an aqueous solution of QHCl. The precipitate was first filtered and then washed more than three times with deionized water followed by drying in a desiccator containing P₂O₅ under vacuum. There was no Cl⁻ present in the product based on ion chromatographic analysis. High-performance liquid chromatography (HPLC) measurement showed a single peak corresponding to the retention time of quinapril, and no degradation product was detected. Both citric acid and monosodium citrate were purchased from Mallinckrodt Chemical Co. (Paris, KY). HPLC-grade acetonitrile and methanol were purchased from EM Scientific Co. (Gibbstown, NJ). Other chemicals used including sodium hydroxide and hydrochloric acid were all analytical grade. Deionized water was obtained using a SYBRON/Barnstead pressure cartridge purification system (Pressurized Cartridge System, Boston, MA).

Methods

Lyophilization

All solution samples with quinapril concentration of 1.05 × 10⁻² M were lyophilized using a commercial tray dryer (Dura-Stop, FTS Systems, Stone Ridge, NY) in combination with a condenser module (Dura-Dry-MP, FTS Systems). The vials used were liquid scintillation vials from Research Products International Co. (Mount Prospect, IL) with a volume of 20 ml (diameter, 27–28 mm; and height, 57.5 ± 0.1 mm). First, solution samples were transferred into scintillation vials, about 8 ml for each vial, followed by transferring the sample containing vials to a freeze-dryer, which was then frozen to −40°C and kept at this temperature for more than 24 h before applying a vacuum. After 24 h under vacuum, the temperature was raised to −30°C, −20°C, −10°C, and 0°C, respectively every 12 h, and secondary drying was performed at 25°C for 24 h. After lyophilization, samples were pulverized in a glovebox under N₂ atmosphere followed by vacuum-oven drying for 24 h. The water content of all samples was found to be <0.2% by Karl Fischer titration.

pH Measurement

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

Ion Chromatography

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

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