Physical Chemical Stability of Warfarin Sodium

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ABSTRACT Crystalline warfarin sodium is an isopropanol clathrate containing 8.3% isopropyl alcohol (IPA) and 0.57% water upon receipt. The hygroscopicity and impact of moisture on IPA status as well as on the stability of the clathrate was studied at different relative humidities. The IPA loss and water uptake were simultaneous but they did not exchange at 1:1 molar ratio. At 58% relative humidity (RH) or below, the exchange process was insignificant. At 68% RH or above, the clathrate tended to lose IPA while absorbing water and reverting to the amorphous state. The rate of IPA loss and moisture uptake was a function of RH. The thermal stability of the crystalline warfarin sodium was also examined. Physical change occurred after isothermal storage for 24 hours at 80°C and 11 hours at 120°C. The rate of IPA loss was temperature dependent.

KeyWords: warfarin sodium; isopropyl alcohol; clathrate; amorphous; crystallinity; physical stability; thermal stability.

Figure 1. Chemical structure of warfarin sodium.

INTRODUCTION

Warfarin sodium is the sodium salt of 3-(α -acetonylbenzyl)-4-hydroxycoumarin (Figure 1) with an apparent solubility larger than 7 g/mL. The compound was first synthesized by Schroeder and Link (1) with two solid forms available-amorphous and crystalline clathrate. The amorphous form is stable in ambient conditions. The crystalline clathrate form is warfarin sodium-isopropyl alcohol complex, which is prepared either from warfarin or amorphous warfarin sodium to eliminate impurities in warfarin sodium (2). The pharmacologic function of the compound is an anticoagulant that inhibits the synthesis of vitamin K-dependent coagulation factors. The treatment aims at preventing further extension of the formed clots and secondary thromboembolic complications that may result in serious and possible fatal sequelae (3).

In the past 30 years, very few people have studied the nature of the complex. Hiskey and Melnitchenko (4) focused on the continuous series of compositions that occur and only briefly mentioned that the clathrate would become less and less crystalline with an increase in water content. Gao and Rytting (5) developed a solution calorimetry method to determine the crystallinity of drugs and used warfarin sodium as one of the model compounds to study the relationship between enthalpy and isopropyl alcohol (IPA) loss and crystallinity. There have been no reports of work to quantitatively study the physical chemical stability of the warfarin sodium clathrate system, such as hygroscopicity, solid state properties, and so on.

As introduced by Hiskey and Melnitchenko, clathrate warfarin sodium is composed of warfarin sodium as the host molecule and IPA and water as the guest molecules. The warfarin sodium:IPA:water ratio may vary from 8:4:0 to 8:2:2 and still possess the monoclinic space lattice. Since the guest molecules

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play a critical role in the formation and stability of the clathrate, it is important to understand the hygroscopicity and its impact on this clathrate. In this study, both IPA and water content were monitored at various relative humidities. The solid state properties were studied using polarized microscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and X-ray powder diffraction patterns. A possible explanation for losing crystallinity after losing IPA is described. The thermal stability of the clathrate of warfarin sodium is presented. In general, the objective of this work is to more fully understand the physical chemical stability of warfarin sodium.

MATERIALS AND METHODS

Both amorphous and crystalline warfarin sodium were prepared by Chemoswed (Jane, Sweded) and were used as received. All solvents were high-performance liquid chromatography (HPLC) grade and other reagents were analytical grade. All solutions were prepared in Milli-Q (Millipore Corporation, Bedford, MA) distilled water that had a resistance of greater than 17 M Ω -cm.

Humidity chambers of 75%, 68%, 58%, 33%, and 20% were prepared using sodium chloride, cupric chloride, sodium bromide, magnesium chloride, and potassium acetate, respectively (6). Saturated salt solutions remained in contact with excess solid salt in a sealed desiccator. The chamber was equilibrated at room temperature for at least 24 hours before use, and a relative humidity (RH) gauge remained in the chamber during the sample incubation period for RH monitoring.

The IPA concentrations were measured with a gas chromatographic method that was based in part on the analytical method in USP 24 (USP 24, 2000). n-Propanol was employed as an internal standard with detection by flame ionization (HP 5890A, Hewlett Packard, Palo Alto, CA). The hydrogen and air flow rates were 30 mL/min and 240 mL/min, respectively. Separation was performed on a Chromosorb 101 80-100 mesh (Celite Corporation, Lompac, CA), 122 cm x 4 mm i.d.-packed glass column with helium flow rate of 11 mL/min and oven temperature of 120°C. The injector and detector temperatures were 200°C. Data acquisition was completed with a VAX-based program that calculated the sample concentrations

from a standard curve based on the IPA peak areas (Multichrom Software, Fisons Instruments, Beverly, MA). Fresh standards were prepared for each analysis. The approximate retention time was 5.5 minutes for IPA and 8.4 minutes for n-propanol. The resolution was 2.1 for the separation.

The warfarin sodium concentrations were measured with an isocratic reverse-phase HPLC method. Separation was performed on a 4.6 mm x 15 cm Novapak C₈ column (Waters Chromatography, Milford, MA) with an eluant consisting of methanol:water:glacial acetic acid (64:35:1). A flow rate of 1.4 mL/min was employed. An ultraviolet detector was used at 280 nm. Data acquisition was completed with a VAX-based program that calculated the sample concentrations from a standard curve based on the warfarin sodium peak areas (Multichrom Software, Fisons Instruments, Beverly, MA). The retention time for warfarin sodium was approximately 8 minutes.

Samples were examined periodically by X-ray powder diffraction. Two different models of a Philips instrument were used in these studies. One was a Philips model APD 3720 (Natick, MA) automated powder diffractometer equipped with a variable slit (Q-compensating slit), a scintillation counter, and a graphite monochromator. CuK α radiation (40 kV, 30 mA) was employed. The intensity of the diffracted radiation was detected automatically every 0.5 seconds by the scintillation detector. The other one was a Philips electronic type 42266 (Natick, MA) wide-angle goniometer with graphite monochromator. A Philips model 3100 XRG with a long, fine-focus copper tube (50 kV, 40 mA) was used. Data were recorded by a Philips 3000 data measuring system (Natick, MA) with a Radix Databox (Materials Data Inc., Livermore, CA). The samples were scanned from an angle (2 θ) of 2° to 60°; the step size was 0.02 degrees; and the count time was 0.5 sec/step under either dry condition or the corresponding RHs.

Differential scanning calorimetry (DSC, Model 910, TA Instruments, New Castle, DE) and thermogravimetric analysis (TGA, Model 2950, TA Instruments) were performed for warfarin sodium by placing the drug substance into an open sample pan and heating at 10°C/min under a 50 mL/min stream

of dry nitrogen. Sample weights of 2 mg to 5 mg were used.

A hot-stage microscope (Mettler FP82, Highstown, NJ) and polarized-light microscopy (Leitz Aristomet microscope, Leica, Allendale, NJ) were used in addition to X-ray powder diffraction and DSC to determine the relative degree of crystallinity and changes in physical properties during the heating process. Pictures were taken at points of interest, such as the melting temperature and the temperature of solvent release. The warfarin sodium sample was prepared for microscopy by suspending it in light mineral oil before covering it with microscope glass.

Water content was analyzed by Karl Fischer coulometric determination (KF684 Coulometer, Brinkmann, Westbury, NY). Sample sizes of 2 mg to 10 mg were used depending on the water content of the sample. The drift of the instrument was approximately 10 and the absolute water weight was controlled in the range of 100 μ g to 500 μ g for the best accuracy of the measurements.

RESULTS

IPA Loss and Water Uptake at Different Relative Humidities

Warfarin sodium crystalline used in this study was a clathrate with 8.3% IPA and 0.57% water upon receipt. At 33% RH or below, the IPA content remained virtually unchanged during storage. At 58% RH, the IPA loss rate was not obvious. At 68% RH, the compound lost IPA rapidly during the first 11 days (Figure 2). Simultaneously, the water uptake behavior at 20%, 33%, 58%, and 68% RH was consistent with the IPA loss profile (Figure 3). At 75% RH or above, the clathrate deliquesced within 72 hours.

Figure 4 shows the mole percentage of water, IPA, and the total solvent at 68% RH for up to 35 days. The IPA loss and moisture uptake rate slowed down after 11 days of storage and effectively reached equilibrium. The final IPA content and water content were approximately 3.3% (wt/wt) and 11% (wt/wt), respectively. The total solvent mole percentage increased with the storage. Thus, the IPA loss and water uptake in clathrate warfarin sodium was not a 1:1 molar exchange. This result is in good agreement with what was observed by Gao and Rytting (5).

Figure 2. IPA content in crystalline warfarin sodium as a function of time at 68% relative humidity (RH) (—○—), 58% RH (—●—), 33% RH (—□—), and 20% RH (—■—). Data points are the mean of 3 replicates with standard deviation values contained within the data points.

Figure 3. Moisture uptake in crystalline warfarin sodium as a function of time at 68% relative humidity (RH) (—○—), 58% RH (—●—), 33% RH (—□—), and 20% RH (—■—). Data points are mean $\pm$ standard deviation of 3 replicates.

Figure 4. Mole percentage of IPA (—○—), water (—●—), and total solvent (—■—) as a function of time at 68% relative humidity. Total solvent is the sum of water and IPA. Data points are the mean of 3

Figure 5. Comparison of amorphous warfarin sodium (-□-) and crystalline warfarin sodium (-●-) moisture uptake rate at 68% relative humidity. Data points are the mean $\pm$ standard deviation of 3 replicates.

Figure 5 shows the water uptake rate of amorphous warfarin sodium at 68% RH. The amorphous material contained initially 3.8% water, 7 times the water content in crystalline warfarin sodium. The amorphous warfarin sodium sorbed water faster than the crystalline material at 68% RH, and the rate of water sorption slowed down after 13 days. This finding may explain the results in Figure 3. Because the clathrate of warfarin sodium decreased gradually in crystallinity while losing IPA, the clathrate began to decrease in crystallinity and convert to the amorphous state. The amorphous state continuously took up water at a faster rate than did the crystalline state. Therefore, the total solvent mole percentage increased with storage and eventually reached equilibrium when the amorphous material water uptake reached saturation.

X-ray powder diffraction revealed that the drug substance became much less crystalline upon storage at 68% RH and reverted potentially to its amorphous state. Figures 6 and 7 show the X-ray diffraction pattern of clathrate warfarin sodium initially and after storage at 68% RH for 35 days, respectively. The diffraction patterns were similar, but the peak intensities reduced after the uptake of water.

The relative crystallinity results were confirmed by polarized-light microscopy. The amount of crystalline substance observed under polarized light became much lower after 36 days storage at 68% RH. These findings are also in qualitative agreement with the findings of Hiskey and Melnitchenko, who

Figure 6. X-ray powder diffraction pattern of clathrate warfarin sodium.

Figure 7. X-ray powder diffraction pattern of clathrate warfarin sodium after storing at 68% relative humidity for 35 days.

reported that as the percentage of water increased in the crystallizing medium, the size of the crystals diminished and their appearance as crystals became less and less crystalline (4).

Warfarin sodium content was monitored periodically by HPLC during storage at 68% RH and during the thermal stress studies. No degradation was detected at any occasion, which suggests that warfarin sodium was chemically stable under the conditions tested.

Thermal Analysis

The tendency to lose crystallinity was also noted by thermal analysis. A typical DSC thermogram of clathrate warfarin sodium produced a broad-shoulder endotherm with a peak temperature at 186°C, which

corresponded to the evolution of IPA and its subsequent melting (Figure 8). The behavior was confirmed by hot-stage microscopy with the evolution of IPA seen as a streaming from the crystal of a miscible swirl into the immersion oil followed by subsequent melting. The observations are also supported by modulated differential scanning calorimetry studies (7). However, after a 35-day storage at 68% RH, a sharp endotherm was observed at approximately 115°C, which was confirmed to be the result of dehydration. The dehydration was based on the observation of the release of immiscible bubbles into immersion oil by hot stage microscopy. In addition, the endotherm at 186°C became much less pronounced and lacked the shoulder peak. This not only suggested the presence of a certain amount of water but also some physical changes in the sample. X-ray powder diffraction results for crystalline material after 35 days of storage at 68% RH confirmed a loss of crystallinity.

TGA produced a total weight loss of 7.61% for clathrate warfarin sodium upon receipt and 9.05% after a 35-day storage at 68% RH on heating to 200°C (Figure 9). The results were consistent with the DSC thermograms shown in Figure 8. Initially, the weight loss was mainly due to the loss of IPA, whereas it was mainly water loss after 35 days storage at 68% RH. The water content obtained by Karl-Fisher method was higher than the TGA weight loss. This may indicate that some water is bound to the clathrate tighter and may be involved in the structure of the clathrate. After a 35-day storage at 68% RH, the sample weight loss began at a lower temperature than did the initial sample, suggesting that the clathrate warfarin sodium evolved IPA at a higher temperature than amorphous warfarin sodium-released water.

Thermal Stability

The loss of crystallinity after the release of IPA was supported by X-ray powder diffraction patterns observed after heating the clathrate to 180°C then cooling to 30°C (Figure 10a). The peak intensities were reduced. After reheating to 180°C, no increase in peak intensities occurred (Figure 10b). This suggested that IPA played an important role in the clathrate structure of warfarin sodium and the crystalline structure was not recovered by reheating.

These results were consistent with what was observed in the DSC thermograms (Figure 11).

Figure 8. DSC thermogram of clathrate warfarin (---) with an onset temperature, melting point, and desolvation/melting enthalpy at 170°C, 187°C, and 53.3 J/g respectively. DSC thermogram of clathrate warfarin sodium after 35-day storage at 68% relative humidity (solid line) with an onset temperature, melting point, and melting enthalpy of 178°C, 188°C, and 13.2 J/g, respectively. An additional endothermic peak with an onset temperature of 110°C and enthalpy of 53.3 J/g occurred at 115°C.

Figure 9. Thermogravimetric analysis of crystalline warfarin sodium (dashed line) and crystalline warfarin sodium after 35-day storage at 68% relative humidity (solid line).

Figure 10. X-ray powder diffraction pattern of clathrate warfarin sodium, a: after heating to 180°C and cooling to 30°C. b: reheating to 180°C.

Figure 11. DSC thermogram of clathrate warfarin sodium heating to 180°C (1→), cooling to 30°C (2←), and reheating to 180°C (3→).

Figure 12. IPA content as a function of time at 80°C (—■—) and 120°C (—○—). Data points are the mean and standard deviation of 3 replicates.

Figure 13. X-ray powder diffraction pattern of clathrate warfarin sodium held at 120°C for 23 hours.

Figure 14. X-ray powder diffraction pattern of clathrate warfarin sodium held at 80°C for a: 24 hours, b: 48 hours, c: 8 days.

Crystalline warfarin sodium was equilibrated rapidly to 80°C over 4.5 minutes or 120°C over 7.0 minutes and held isothermally by TGA. During the equilibration process, the weight loss was 0.29% and 0.92% at 80°C and 120°C, respectively. At 120°C, the weight loss reached equilibrium within 10 hours; the total weight loss was 8.2%. At 80°C, the total weight loss was 5.0% after 90 hours. The IPA content during the thermal treatment was quantified at various times (Figure 12). IPA loss in crystalline warfarin sodium increased with increased temperature. By X-ray powder diffraction, only an amorphous carbon halo band was observed after 23 hours at 120°C, which was indicative of conversion

to amorphous material (Figure 13). After this treatment, the IPA content was less than 0.8%. No degradation was detected by HPLC after heating to 120°C for 23 hours. The X-ray powder diffraction patterns for clathrate warfarin sodium after thermal treatment at 80°C for 24 hours, 48 hours, and 8 days were shown in Figure 14, respectively. Peak intensities decreased with increased storage time at 80°C and can be noted in the area of 20° 2 θ . Compared to the pattern at 120°C, the loss of crystallinity was accelerated at the higher temperature.

Attempts to Prepare Other Possible Warfarin Sodium Clathrates

Preparation of clathrates of methanol, ethanol, n-propanol, butanol, and IPA were attempted using amorphous warfarin sodium as the starting material. Only the IPA clathrate was obtained successfully. Amorphous warfarin sodium was regenerated in the other solvents. This is consistent with literature reports (1, 5).

DISCUSSION

Effect of Water on Physical Stability

Clathrate warfarin sodium was moderately hygroscopic. Based on the water uptake rate at different RHs, the critical RH was approximately 68%. It was chemically stable at different RHs and only physical changes were observed.

On storage at 68% RH, the intensity of the peaks in the X-ray powder diffraction pattern of the clathrate warfarin sodium decreased gradually and the peak resolution decreased. In addition, an amorphous carbon halo band appeared from 15-40° 2 θ as part of the baseline of the X-ray powder diffraction powder pattern. The changes in the diffraction pattern occurred in concert with the simultaneous loss of IPA and uptake of water. The changes reflected the conversion from an anhydrous IPA clathrate to a crystalline form containing both IPA and water prior to converting to the amorphous state and were consistent with earlier characterizations of this system (4).

A typical DSC thermogram for clathrate warfarin sodium showed a broad endotherm prior to the melting process. It was reduced and disappeared eventually with an increase in water content. The

release of liquid from the clathrate preceded the melting process as observed by hot-stage microscopy. Therefore, IPA content was analyzed by collecting samples after heating the crystalline material to 180°C in an open pan and cooling to 25°C. Since the melting point was not reached, the resultant material retained some crystallinity (Figure 10) but only 2.5% to 3.0% IPA remained. The IPA analysis and hot-stage microscopy behavior confirmed that the shoulder endotherm at approximately 180°C in the DSC thermogram was probably IPA release. This was consistent with a typical property of the clathrates (i.e., the guest molecules will be released prior to the melting process).

Amorphous warfarin sodium sorbs much more water than does the clathrate. This may be because of the lack of the periodic structure in the system; thus, it provides more opportunity to accommodate water (8). According to Franks (9) and Hancock, et al.(12), the process may also be considered as a plasticizing effect where water is a plasticizer. Generally, the effect is very strong at low water content because of the strong solid-water interaction, and decreases at higher water amounts while the plasticizing efficiency of the water is reduced. Eventually the affinity of the water for the solid decreases to the extent that water would preferentially associate with itself (10). The water content equilibrated after 35 days of storage at 68% RH. The plasticizing effect may stop, and an equilibrium between the crystalline and amorphous forms may be obtained. Hydrogen bonding may have played an important role in the structure of clathrate warfarin sodium. The fact that an IPA content of 2.5% to 3.0% was retained in the system during storage at 68% RH may indicate that the clathrate warfarin sodium was converted partially to the amorphous form. This may be similar to the situation found with proteins, where the plasticizing effect may stop when the hydrogen bonding capacity of the solid is exceeded (8, 9, 11, 12). At 75% RH or above, the clathrate deliquesced within 72 hours. The driving force for this process may be the partial vapor pressure differences between the atmosphere and the solid system, which made the equilibrium process found at 68% RH no longer possible; therefore, the nature and strength of the water-solid interaction is material-dependent.

Effect of Temperature on Physical Stability

The rate of IPA loss was faster with an increase in temperature, but no rate law was found that described the process. The IPA content decreased to 0.8% after 23 hours at 120°C; storage at 68% RH resulted in the clathrate warfarin sodium IPA content decreasing to 2.5% to 3.0%. This indicates that the thermal stress of storage at 120°C was greater than the stress of exposure at 68% RH and its associated plasticizing effect in breaking the hydrogen bonding and the clathrate structure. The conversion from the crystalline to the amorphous state took place after the loss of IPA. This result confirmed that IPA played an important role in the stability of the clathrate form of warfarin sodium. Also, it was consistent with literature reports suggesting that the instability of the guest molecule in a clathrate is accelerated with thermal stress (13).

Attempts to Prepare Other Possible Warfarin Sodium Clathrates

The uniqueness of the IPA clathrate warfarin sodium system suggested that both noncovalent bonding and steric hindrance were important in the formation of a clathrate. The warfarin sodium-isopropyl alcohol complex was the only warfarin sodium clathrate found.

CONCLUSIONS

The physical stability of clathrate warfarin sodium was evaluated, and the water uptake and IPA loss at various RHs were quantified. At 68% RH or above, the compound lost IPA, sorbed water, and reverted to the amorphous state. The rate of IPA loss and water uptake was a function of RH (i.e., the higher the RH, the faster the loss of IPA and crystallinity). The process reached fruition when the clathrate converted to the amorphous state. Thermal stress also caused the IPA loss from the clathrate warfarin sodium in a temperature-dependent fashion.

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