Investigation of the Solid State Properties of Amoxicillin Trihydrate and the Effect of Powder pH

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ABSTRACT

The purpose of this research was to investigate some physicochemical and solid-state properties of amoxicillin trihydrate (AMT) with different powder pH within the pharmacopoeia-specified range. AMT batches prepared using Dane salt method with the pH values from 4.39 to 4.97 were subjected to further characterization studies. Optical and scanning electron microscopy showed that different batches of AMT powders were similar in crystal habit, but the length of the crystals increased as the pH increased. Further solid-state investigations using powder x-ray diffraction (PXRD) demonstrated the same PXRD pattern, but the intensity of the peaks raised by the powder pH, indicated increased crystallinity. Differential scanning calorimetry (DSC) studies further confirmed that as the powder pH increased, the crystallinity and, hence, thermal stability of AMT powders increased. Searching for the possible cause of the variations in the solid state properties, HPLC analysis showed that despite possessing the requirements of the United States Pharmacopoeia (USP) for purity/impurity profile, there was a direct relationship between the increase of the powder pH and the purity of AMT, and also decrease in the impurity I (α-Hydroxyphenylglycine) concentration in AMT powder. Recrystallization studies confirmed that the powder pH could be controlled by adjusting the pH of the crystallization.

KEYWORDS: Amoxicillin trihydrate, impurity profile, degree of crystallinity, DSC, PXRD, HPLC.

INTRODUCTION

Amoxicillin trihydrate or AMT (Figure 1) is a commonly used β-lactam antibiotic, which is highly active against a broad spectrum of bacteria. AMT had sales of $1300 million in the United States market as a bulk-formulated drug and in combination with clavulanic acid in 2004. High solubility, high rate of absorption, and stability of AMT under acid conditions are among the most important advantages of this antibiotic.[1-3] AMT, like the other β-lactam antibiotics, is usually produced by a semisynthetic route[4,5] using reactive groups of 6-aminopenicillanic acid (6-APA). Also, various hydrated forms of amoxicillin, including monohydrate, dihydrate, and trihydrate, have been reported, among which, the trihydrate is the most stable hydrated form.[6-11] Crystallization of AMT, like the other crystalline drugs, plays a critical role in controlling the crystal form, shape, size, and size distribution. Moreover, this process influences the solid state properties and the type of presented impurity in AMT powder.[12] Solid state properties of pharmaceutical compounds have been increasingly reported using experimental techniques, such as powder x-ray diffraction (PXRD), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and thermogravimetry analysis (TGA).[13-17] Different solid forms of the same chemical compound can exhibit different physical and chemical properties including different solubility and dissolution profiles, which in turn affect the bioavailability and stability of the drug substance.

In line with previous studies of the crystalline structure and the stability profile of ampicillin as a function of the residual solvents,[18] the relationship between the powder pH and the crystal structure of AMT was investigated. Powder pH is among the important characteristics of AMT, which should be monitored carefully based on the United States Pharmacopeia (USP) specifications. Despite the fact that USP 25 monograph broadens the AMT powder pH range from 3.5 to 6.0, AMT powder subjected to the present study had a narrower pH range of 4.39 to 4.97. However, these powders showed a significant variation in physicochemical characteristics. In this work, both optical and scanning electron microscopy were used to investigate the crystal habit of AMT batches with different powder pH. Further solid state studies, including PXRD and DSC were performed to investigate different solid properties of AMT powders. The high-performance liquid chromatography (HPLC) analysis was then used to

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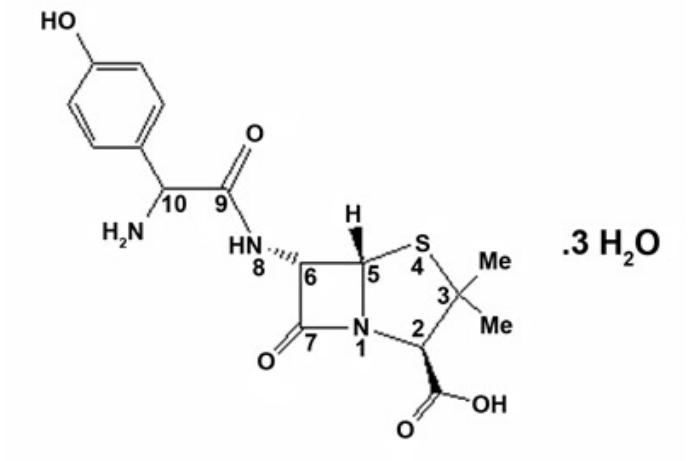

Figure 1. Molecular structure of amoxicillin trihydrate.

search for any possible correlation between the solid state properties and the purity/impurity profile of AMT batches with various powder pH.

Materials and Methods

Materials

AMT standards were obtained from Sigma (St Louis, MO). NH_4OH, HCl and isopropyl alcohol were obtained from Merck (Darmstadt, Germany).

Methods

Dane Salt Method

Amoxicillin trihydrate was prepared from the reaction of 6-aminopenicillanic acid (6-APA) with *p*-hydroxy phenyl glycine, using an enamine intermediate compound (Dane salt method).[19]

Powder pH Measurement

The pH of a solution of 2 mg/mL AMT in distilled water (prepared using an ultrasonic bath) was recorded by a Schott pH meter (model CG840, Schott Instruments, Mainez, Germany). The measured values using a calibrated pH meter could be reproduced to 0.02 pH units, as described in the USP monograph.

Optical and Scanning Electron Microscopy

An Olympus optical microscope (BX51TF, Tokyo, Japan) equipped with a digital camera along with MpegTV software were used to record the particle images. Scanning electron microscopy (SEM) was performed by gently distributing the powder sample onto the stainless steel stubs using SEM. Philips (XL30, Almelo, The Netherlands) instrument.

Powder X-ray Diffraction

The PXRD patterns of the AMT solid phase were determined using an x-ray generator (PW 1130/00) and goniometer (PW 1050, Philips) with Cu Kα radiation (wavelength: 1.541 Å), and counts were accumulated for 1 second at each step. Fifteen milligram samples were weighed accurately and packed in aluminum holders, and the instrument was operated between an initial and final 2θ angle of 4° and 40°, respectively, in 2θ increments of 0.05.

Differential Scanning Calorimetry

The thermal properties of samples were determined using a Shimadzu DSC-60 (Shimadzu, Kyoto, Japan) with heating and cooling rates at 10°C min^{-1} and using a condenser as coolant. The samples were weighed and carefully packed into a clean aluminum pan (5-5.5 mg) and sealed by crimping as aluminum lid onto the pan (Shimadzu crimper). An empty pan sealed with a cover pan was used as a reference sample. A scanning range of 10°C to 200°C was used for DSC analysis of samples, with increment rate at 10°C min^{-1} in nitrogen atmosphere.

High-Performance Liquid Chromatography Method

The HPLC system consisted of a 616E pump, a 996 photodiode array (PDA) detector, and a degasser module; data were acquired and processed using a Millennium software Version 2.1 (all from Waters, Milford, MA). The chromatographic separations were performed on Spherisorb (Waters) C-18 columns (250 mm × 4.6 mm, with a particle size of 5 μm). The mobile phase composition, column temperature, and detector wavelength for determination of assay and related substance were determined using the USP 25 method.

Recrystallization

Five grams of pure AMT was weighed, transferred to an Erlenmeyer flask, and dissolved in 25 mL of diluted HCl in water (pH 2) by soaking and agitating with magnetic stirrer at ambient temperature. The recrystallization of AMT was accomplished at pH 7 to 9, by adding NH_4OH gently to the solution. The resulting precipitate was filtered by sinter glass and then amoxicillin trihydrate crystals were washed with 50 mL of water/isopropyl alcohol (15:85) and were dried at room temperature. The crystallization pH should be carefully adjusted by adding NH_4OH step by step.

Results and Discussion

Optical microscopy (Figure 2) demonstrated crystalline structures for AMT samples in the investigated powder pH range (powder pH 4.39 [a] and 4.97 [b]). It could be seen that the

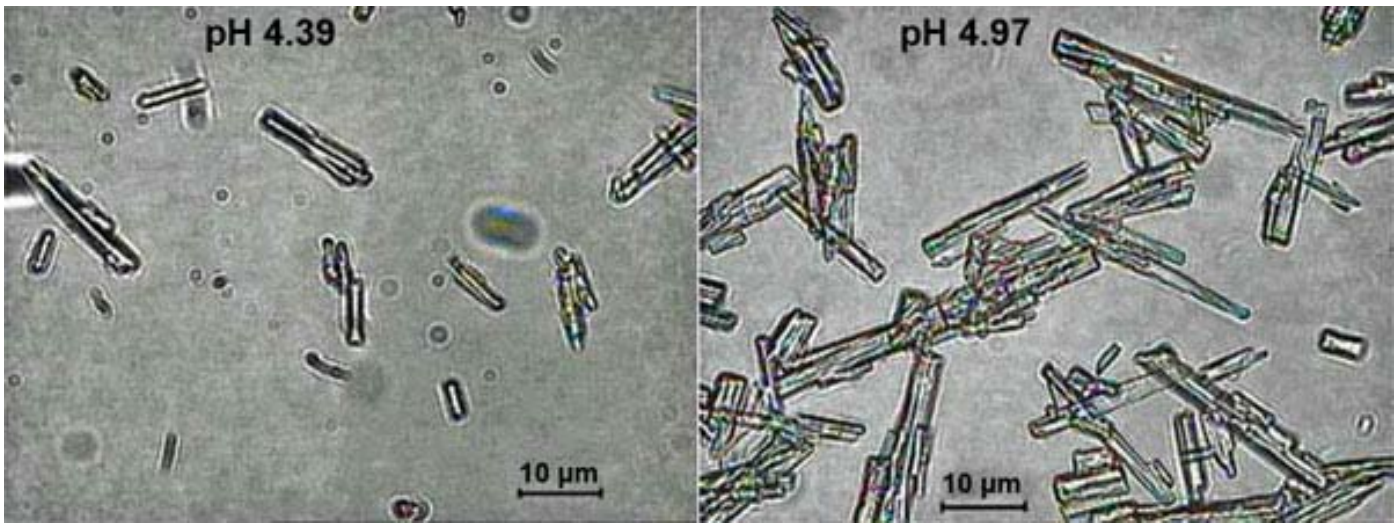

Figure 2. Optical images of 2 solid forms of amoxicillin trihydrate with pH 4.39 and 4.97.

crystals with pH 4.97 were elongated in 1 dimension compared with the crystals with pH 4.39, and it might be generally suggested that, as the powder pH increased the crystal length also increased. The results of SEM confirmed the columnar structure of the crystals, and an overall increase in the particle size with increase in the powder pH (Figure 3). Of interest, Brittain and his colleagues comparing the anhydrous and trihydrated forms of ampicillin showed a similar

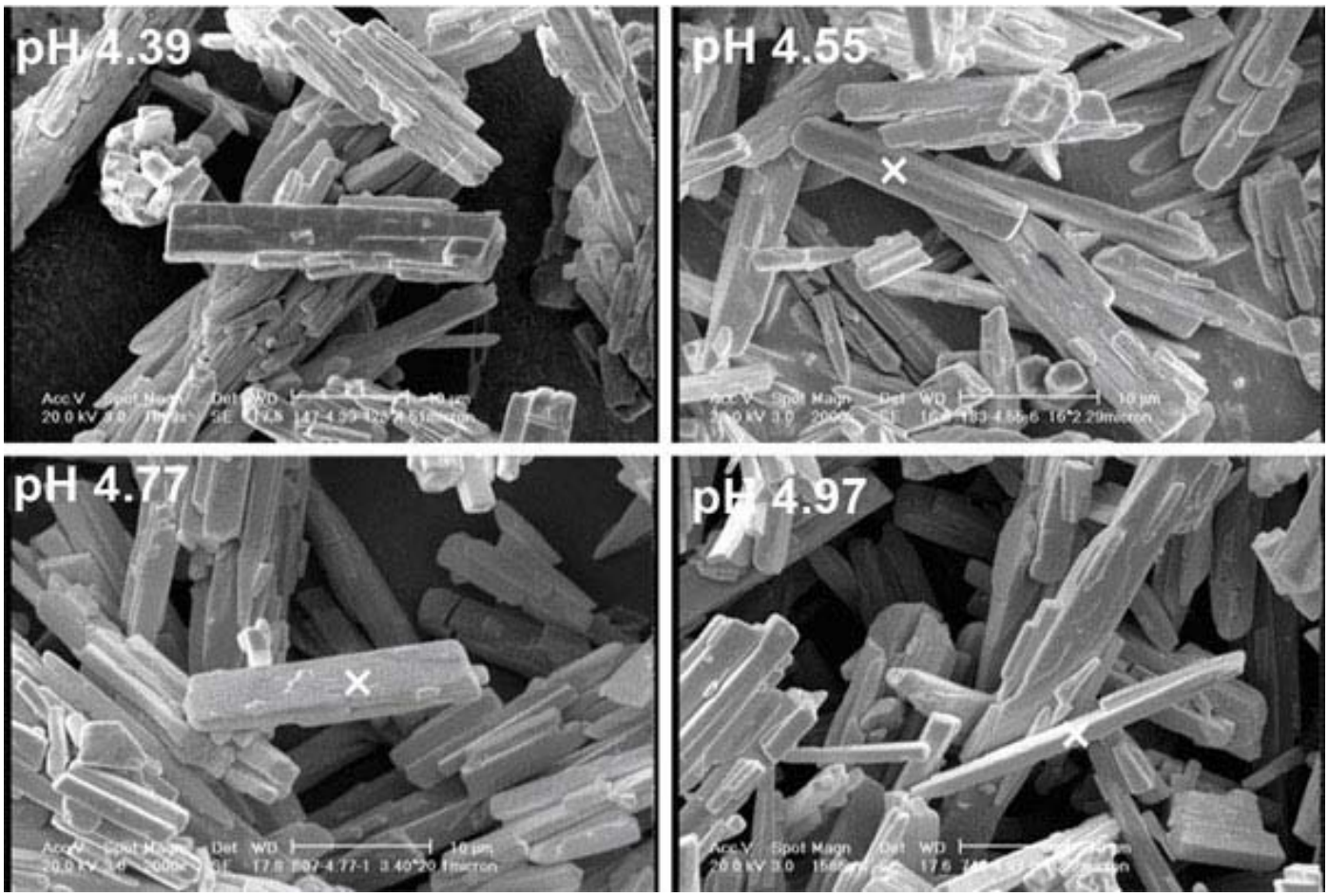

Figure 3. Scanning electron photomicrographs of amoxicillin trihydrate with powder pH 4.39, 4.55, 4.7, and 4.97. × indicates crystal used in study to measure increase in length.

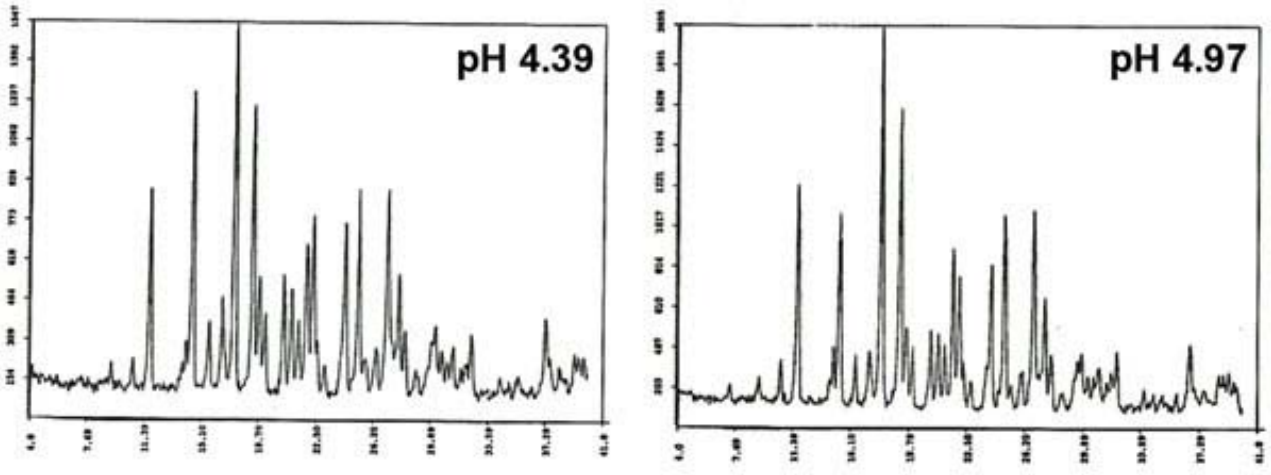

Figure 4. Powder diffraction pattern of amoxicillin trihydrate with powder pH of 4.39 and 4.97.

crystalline structure for trihydrate crystals using SEM.[18] From the SEM results, an increase in the particle length could also be observed for the cross-signed crystals (as indicated by × on photomicrograph, Figure 3). This increase was evident for AMT batches with powder pH 4.39, 4.55, 4.77, and 4.97 from 9.3 to 16.0, 20.1, and 32.2 μm, respectively (Figure 3).

PXRD is a well-known technique in the pharmaceutical industries for identification of different crystalline structures and various degrees of crystallinity. The PXRD spectra of AMT samples with 2 different powder pH (pH 4.39 and pH 4.97) are presented in Figure 4a and b, respectively. In the powder diffractograms, sharp peaks at diffraction angles (2θ) 12.11, 14.73, 17.57, 18.43, 25.48, and 27.38 were repeated for both samples, indicating the same unit cell structures for different batches of AMT. Similar PXRD profile was reported by Han and Suryanarayanan for AMT, where, upon heating, the degree of crystallinity diminished stepwise to an amorphous structure as a result of dehydration.[20] This may highlight the importance of the hydrated crystal structure in physical stability of AMT powder, which is similar to the report by Brittain and his colleagues for ampicillin trihydare.[17] However, the intensity of the peaks for the same quantity of AMT with pH 4.97 was noticeably higher than that for AMT powder with pH 4.39, which has not been reported previously. From this finding it can be concluded that a direct relationship between the powder pH and the degree of crystallinity in the above mentioned samples exists, probably because of different crystallization conditions and the status of crystallization for AMT molecules in the pH 4.97 samples.

The results of DSC analysis (Table 1) showed that the onset temperature (as well as the peak temperature) increased from 119.51°C (130.43°C for the peak temperature) to 126.01°C (134.99°C for the peak temperature), as the powder pH increased from 4.39 to 4.97. The enthalpy of fusion, also demonstrated the same profile of increase from −77.67 J/g to −109.32 J/g, as the powder pH increased from 4.39 to 4.97. DSC results further confirmed the results of PXRD technique, and again hinted to an increase in the thermal stability of AMT powder parallel to the increase in the powder pH. The possible explanation for these observations can be the relatively weaker intermolecular forces, lower energy for breaking, and decreased enthalpy of fusion, which results in quicker decomposition for the samples with the lower powder pH. These phenomena may also be related to the induction of higher numbers of bonds in AMT molecules[21] due to the crystallization conditions in powders with higher pH value. In theory, a melting transition of a pure crystalline drug should occur within an infinitely narrow temperature range. A broadening of the melting range provides a sensitive criterion of impurity.[22] In practice, following the chemical reactions in synthetic processes, the crystallization process takes place in reactors where the reactants, by-products, and intermediate chemicals (or impurities) are present. These impurities can influence the crystal structure and crystal habit of the final product by attaching to a

Table 1. Results of Differential Scanning Calorimetry for Amoxicillin Trihydrate Powder With Different Powder pHs

pH	Peak (°C)*	Onset (°C)†	Heat (J/g)
4.39	130.43	119.51	–77.67
4.42	131.86	118.51	–92.68
4.45	131.95	118.92	–96.47
4.47	134.05	124.19	–99.50
4.53	131.85	120.23	–99.82
4.59	132.79	118.86	–92.68
4.6	133.52	121.8	–101.43
4.66	133.19	119.76	–111.72
4.71	133.63	120.96	–103.73
4.77	134.37	119.46	–102.12
4.97	134.99	126.01	–109.32

*The center of differential scanning calorimetry peak.
†The starting point of peak.

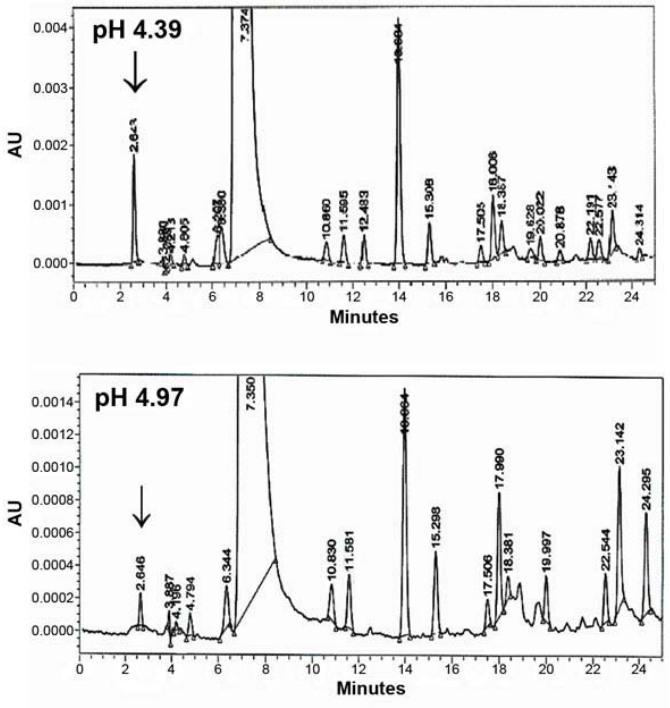

Figure 5. High-performance liquid chromatogram of amoxicillin trihydrate powder with pH 4.39 and 4.97.

particular face of the crystal and inhibit the growth of that face.[23] In this context, the changes in crystal habit as shown before (Figure 2 and Figure 3) can be justified.

To further investigate the possible reasons for these differences, HPLC analysis was used to study the profile of the purity/impurities within different samples. The profiles of the purity/impurities of different AMT batches were investigated using standard USP 25 method. USP has introduced several important impurities of AMT: A (6-aminopenicillanic acid), B (L-amoxicillin), C (amoxicillin diketopiperazines), D (penicilloic acids of amoxicillin), E (penilloic acids of amoxicillin), F (3-(4-hydroxyphenyl) pyrazin-2-ol), and I (α-hydroxyphenylglycine). The results of HPLC analysis (Figures 5a and 5b) for AMT samples with different powder pH showed that the samples had acceptable purity/impurity profile (in the USP range), and the total percentage of the impurities in each sample was less than 1%, which was comparable to the standard AMT. The results showed that A, B, C, D, E, and F impurities had no significant variations within different samples (with powder pH from 4.39 to 4.97). However, different samples showed various concentrations of I (α-Hydroxyphenylglycine) impurity. Of interest, as the powder pH increased, the resulting chromatograph showed a noticeable decrease (Figures 5a and 5b) in the impurity I peak intensity (as demonstrated by arrows). A linear relationship (Figure 6a) could be drawn between the powder pH and the purity, with regression coefficient more than 0.9. Moreover, investigation of a correlation between the impurity I concentration and the powder pH showed the same linear correlation (Figure 6b), which further highlighted the importance of the crystallization conditions on the existence of impurity I in the crystal structure and, hence, solid state properties of AMT powder.

Recrystallization studies have been performed to investigate the possible effect of the crystallization pH on the powder's pH. The results showed that as the pH of the crystallization increased by adding NH_4OH, the pH of the resulting powder moved toward the upper limit of the specified pH range, and also the purity profile improved. The possible explanation for this observation could be the increase of α-hydroxyphenylglycine solubility in water by increasing the pH. Therefore, increasing the crystallization pH resulted in the presence of more α-hydroxyphenylglycine in the ionic form and less α-hydroxyphenylglycine coprecipitating with amoxicillin molecules. This phenomenon could lead to more favorable crystallization conditions, and hence increasing the quality

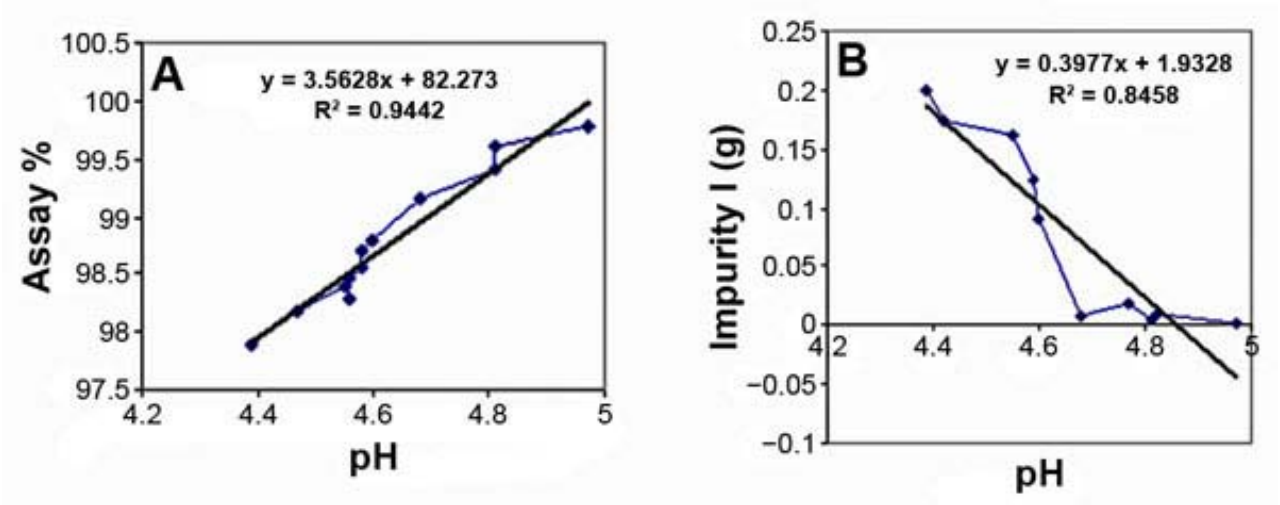

Figure 6. Linear correlation between the powder pH and amoxicillin trihydrate (a) or impurity I (b) concentrations.

of the crystallized AMT. Incorporation of structurally related impurities into the host crystal lattice has been already reported in the literature.[24] The possible mechanism behind this phenomenon is that the crystal faces are sometimes unable to discriminate between the host and impurity molecule. This can lead to various consequences as incorporated impurities can change the physical and chemical properties of the crystals. Of interest, in a recent research publication, the effect of 0.1% wt/wt structurally related impurities of an active pharmaceutical ingredient (API) on the phase transformation of the solid crystal was investigated. The selectivity of impurities to suppress or accelerate the polymorphic transformation was also highlighted in the above mentioned work.[25]

CONCLUSION

It can be concluded that the crystallization pH has a major effect on the AMT crystal shape, degree of crystallinity, thermal stability, and impurity profile. The crystals showed significant changes in the length, PXRD intensity, DSC profile, and impurity I content. These findings suggest that using Dane-salt technique to prepare AMT, accurate control of crystallization pH, can lead to more crystalline and thermally more stable API with improved purity profile. A linear relationship can be drawn to calculate the purity/impurity profile of AMT batches using the crystallization pH.

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REFERENCES

1. Zayed MA, Abdallah SM. Synthesis and structure investigation of the antibiotic amoxicillin complexes of d-block elements. *Spectrochim Acta A Mol Biomol Spectrosc.* 2005;61:2231–2238.

2. Dousa M, Hosmanova R. Rapid determination of amoxicillin in premixes by HPLC. *J Pharm Biomed Anal.* 2005;37:373–377.

3. Michael IP. *The Chemistry of β-Lactams*. Glasgow, UK: Chapman & Hall; 1992.

4. Youshko MI, Langen LMV, Vroom ED, Rantwijk FV, Sheldon RA, Svedas VK. Penicillin acylase-catalyzed ampicillin synthesis using a pH gradient: a new approach to optimization. *Biotechnol Bioeng.* 2002;78:589–593.

5. Giron D, Goldbronn Ch, Mutz M, Pfeffer S, Piechon PH, Schwab PH. Solid state characterizations of pharmaceutical hydrates. *J Therm Anal Calorim.* 2002;68:453–465.

6. Goncalves LRB, Jr, Sousa R, Jr, Fernandez-Lafuente R, Jr, et al. Enzymatic synthesis of amoxicillin: avoiding limitations of the mechanistic approach for reaction kinetics. *Biotechnol Bioeng.* 2002; 80:622–631.

7. Dolezalova M, Kunteova B, Jobanek R. Determination of the purity of ampicillin by micellar electrokinetic chromatography and reversed phase liquid chromatography on a monolithic silica column. *J Sep Sci.* 2004;27:560–568.

8. Kolar P, Shen W, Tsuboi A, Ishikawa T. Solvent selection for pharmaceuticals. *Fluid Phase Equilib.* 2002;194-197:771–782.

9. Wei DZ, Yang L. Effects of ethylene glycol on the synthesis of ampicillin using immobilized penicillin G acylase. *J Chem Technol Biotechnol.* 2003;78:431–436.

10. Tung JC, Gonzales AJ, Sadowsky JD, Leary DJ. On the ^{1}H NMR chemical shift assignments for ampicillin. *Magn Reson Chem.* 2000;38:126–128.

11. Li Y, Tang Y, Yao HV, Fu J. Determination of ampicillin and amoxycillin by flow injection chemiluminescence method based on their enhancing effects on the luminol-periodate reactin. *Luminescence.* 2003;18:313–317.

12. Vippagunta SR, Brittain HG, Grant DJW. Crystalline solids. *Adv Drug Deliv Rev.* 2001;48:3–26.

13. Cetina-Cizmek B, Tudja M, Mestrovic E, Zovko M, Zorc B, Tudja P. Solid-state investigation of piroxicam benzoate. *Acta Pharm.* 2003;53:165–173.

14. Shefter E, Fung H, Mok O. Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate. *J Pharm Sci.* 1973;62: 791–794.

15. Han J, Gupte S, Suryanarayanan R. Applications of pressure differential scanning calorimetry in the study of pharmaceutical hydrates: ampicillin trihydrate. *Int J Pharm.* 1998;170:63–72.

16. Salari A, Young R. Application of attenuated total reflectance FTIR spectroscopy to the analysis of mixtures of pharmaceutical polymorphs. *Int J Pharm.* 1998;163:157–166.

17. Brittain H, Bugay D, Boghanovich S, DeVincentis J. Spectral methods for determination of water. *Drug Dev Ind Pharm.* 1988;14: 2029–2048.

18. Nojavan S, Ghassempour A, Bashour Y, Khalilian M, Ahmadi SH. Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure. *J Pharm Biomed Anal.* 2005;36:983–988.

19. Henniger PW, Van Der Drift JK, Van Veen GJ, inventors. Gist Brocades, assignee. Process for the preparation of 6-D-alpha-amino-(p-hydroxyphenyl)-acetamido penicillanic acid. EP patent 0001133. March 21, 1979.

20. Han J, Suryanarayanan R. A method for the rapid evaluation of the physical stability of pharmaceutical hydrates. *Thermochim Acta.* 1999;329:163–170.

21. Boles M, Girven R, Gane A. The structure of amoxycillin trihydrate and a comparison with the structure of ampicillin. *Acta Crystallogr.* 1978;B34:461–466.

22. Van Dooren A, Muller BW. Purity determination of drugs with differential scanning calorimetry (DSC): a critical review. *Int J Pharm.* 1984;20:217–233.

23. Mirmehrabi M, Rohani S, Keshava Murthy KS, Radatus B. Polymorphic behavior and crystal habit of an anti-viral/HIV drug: Stauvidine. *Cryst Growth Des.* 2006;6:141–149.

24. Meenan PA, Anderson SR, Klug DA. The influence of impurities and solvents on crystallization. In: Myerson AS, ed. *Handbook of Industrial Crystallization.* Boston, MA: Butterworth-Heinemann; 2002:67–97.

25. Mukuta T, Lee AY, Kawakami T, Myerson AS. Influence of impurities on the solution-mediated transformation of active pharmaceutical ingredient. *Cryst Growth Des.* 2005;5:1429–1436.