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Research article

Dehydration effect on the stability of ampicillin trihydrate

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ABSTRACT

In this study, the effect of dehydration conditions (air temperature and relative humidity) on the stability of ampicillin trihydrate was investigated. Firstly, the material was characterized. The results of gravimetric experiments provided the conditions where the trihydrate transforms into the anhydrate. The analysis of X-ray diffractometry patterns, Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Scanning Electron Microscopy (SEM) results allowed to confirm that the material used in this study is the ampicillin trihydrate. Secondly, the dehydration rate was found to increase strongly with ambient air temperature and to decrease with air relative humidity. The degradation of the trihydrate ampicillin (quantified by the HPLC method) during storage was activated by losing water molecules. This phenomenon has been explained by the enthalpy-entropy compensation phenomena. The measured evolutions of ampicillin trihydrate amount with time during dehydration for all considered ambient conditions were found to be well represented by the First order chemical kinetic model. The parameters of this equation were correlated to temperature and relative humidity by a simple expression which could be a possible mathematical representation of the ampicillin's degradation curves. This kind of simple degradation curve fitting equation can be very helpful for the modelling and optimal control of pharmaceutically active components drying processes.

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INTRODUCTION

Differences in crystal packing play a major role in drug stability, especially as temperature and humidity effects are concerned. Thermal stability is an important physical parameter in the design and selection of novel drug candidates. Certainly, thermal instability can pose major problems in the synthesis, processing, formulation, and shelf-life of new drug molecules. This can lead to significant delays in development and regulatory approval. Many medicaments are known to crystallize with water molecules as an integral part of their crystal structure. It is a matter of great importance that one should accept in mind the fact that the pharmaceutical hydrates display marked alterations in several physico-chemical properties, such as chemical stability or dissolution rate on being processed to formulation or on exposure to relative humidities of varying degrees (Fukumori et al., 1983; Ohsawa et al., 1988). Many investigators have described the influence of sorbed moisture on the solid-state decomposition. For these reasons, it is necessary to obtain information on the thermal stability of promising drug candidates.

Ampicillin or aminobenzylpenicillin or 6-[d(-)-aminophenylacetamido] penicillanic acid (Fig. 1) is a penicillin class antibiotic with a beta-lactam structure. Literature reports that ampicillin may exist in two anhydrous polymorphic forms (form I or form B; form II) and hydrate forms (trihydrate; monohydrate or form A) (Shefter et al., 1973; James and Hall, 1968; Ivashkiv, 1973). The trihydrate and anhydrous form I have been the most studied forms, while the monohydrate and anhydrous form II did not receive analogous attention. In the different commercial pharmaceutical formulations like injectable or oral preparations, capsules or tablets, the active pharmaceutical ingredient is present in the anhydrous form or as sodium salt or as the trihydrate form. Alburn et al. (1967) report that the anhydrous form I has the advantage of high storage stability characteristics, related to its negligible water content. Moreover, anhydrous ampicillin exhibits slower absorption in the gut and prolonged blood levels with more effective action, relatable to its lower solubility in water.

The objective of this paper is to study the influence of the dehydration operating conditions (Temperature and Relative Humidity) on the stability of ampicillin and to correlate the dehydration kinetic parameters to the thermodynamic proprieties in a simple mathematical representation of the ampicillin's degradation curves. These equations can be very helpful for the modelling and optimization of pharmaceutically active component drying processes.

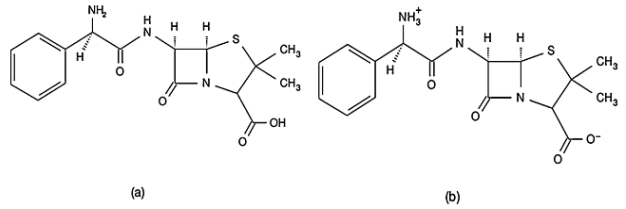

Fig. 1. (a) Structure of anhydrous ampicillin and (b) ampicillin zwitterionic form (Baraldi et al., 2014).

MATERIALS AND METHODS

Materials

Ampicillin anhydrous and ampicillin trihydrate were obtained from Sigma (St. Louis, MO). All compounds were of the highest available quality and were used as received. CsF, LiCl, $MgCl_2.6H_2O$, $Mg(NO_3)_2$, K_2CO_3, and NaBr salts, used for hygrothermal conditioning of ampicillin trihydrate samples, were supplied by Sigma-Aldrich at Analar grade.

Methods

Differential scanning calorimetry (DSC)

A differential scanning calorimeter (Model Q200, Thermal Analysis Instruments) was used. The instrument was calibrated with pure indium. About 4-5 mg ampicillin sample was packed in aluminum pans, crimped with lids having several pinholes. Tests were run from room temperature to 200°C, at a heating rate of 5°C/min under dry nitrogen purge (50ml/min). For the determination of heat capacity, a typical calibration run in modulated mode was performed with 20 mg of sapphire. Apparent heat capacity measurements were carried out for a dried product, with 10 mg samples placed in sealed aluminium pans. The scan was run between 10°C and 130°C for the dried samples with an amplitude of modulation equal to ±1°C every 60 s.

Thermogravimetric analysis (TGA)

The thermogravimetric analyser (TGA NETZSCH TG 209 F1 Iris®ASC) was used. The sample was heated in an open aluminum pan from room temperature to 200°C, under nitrogen purge (50ml/min), at a rate of 1°C/min.

X-ray diffractometry

X-ray powder (XRPD) theta-theta diffractometer (Bruker axs D8, Bruker AXC GmbH, Karlsruhe, Germany) was used. The XRPD experiments were performed in symmetrical reflection mode with CuK_α radiation (λ = 1.54 Å) using Göbel mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. The angular range was 5-30° with a scan speed of 5° 2θ min^{-1}.

Scanning Electron Microscopy (SEM)

Hitachi S-800 Field Emission Gun (FEG) Scanning Electron Microscope (SEM) was used. All samples were coated with gold for an increased signal/noise ratio.

High-performance liquid chromatography (HPLC)

HPLC experiments were performed on Agilent Technologies (Palo Alto, CA) HPLC 1100 equipped with a photodiode array detector and ChemStation for LC 3D software. Ampicillin forms were assayed using a 250 mm×4.6 mm Phenomenex (Torrance, CA) Luna C18 column with a 5µm particle size. The detection wavelength was 260 nm. The mobile phase consisted of water–acetonitrile–KH_2PO_4 (1.0 M)–acetic acid (1.0 M) (909:80:10:1, v/v/v/v) and was pumped at a flow rate of 1.5 ml/min. The typical sample size was about 1 mg/ml and the injection volume was 20µl.

Isothermal gravimetric method

Saturated solutions of CsF, LiCl, $MgCl_2$.6H2O, $Mg(NO_3)_2$, K2CO3 and NaBr were prepared and stored at 20, 30, 40, 50 and 80°C, producing relative humidity conditions detailed in Table 1. The samples of ampicillin trihydrate were placed in closed containers filled with different solutions. Samples were weighed periodically and analyzed by the HPLC until a constant weight was obtained.

Table 1. Temperature and relative humidity conditions used in the dehydration experiments

Salt	Temperature (°C) and corresponding relative humidity (%)				
	20°C	**30°C**	**40°C**	**50°C**	**80°C**
CsF	03.8	03.0	02.4	02.1	02.6
LiCl	11.3	11.3	11.2	11.1	10.5
K_2CO_3	43.2	43.2	42.3	45.6	45.0
$Mg(NO_3)_2$	54.4	51.4	48.4	45.4	42.4
$MgCl_2$	90.1	90.0	89.2	88.9	85

RESULTS AND DISCUSSION

Identification of starting materials

DSC and TGA curves for the anhydrate and trihydrate ampicillin are shown in Fig. 2 and 3. For all forms, TGA data show a weight loss onset just above 200 °C. The trihydrate form exhibits a large endothermic step at 118 ◦C (78.85 J/g) due to the dehydration and vaporization process. The TGA confirms a significant weight loss before 130 °C (close to 14%, theoretical weight loss of 13,4%), a temperature indicative of the presence of strongly bonded crystallization water due to the presence of a strong H-bond. This process is immediately followed by an exothermic peak, which should be related to the partial crystallization of the anhydrous form I. Actually, after melting the trihydrate crystals, experimental conditions, such as slow heating rate and the presence of residual water molecules from the trihydrate, favour the formation of anhydrous form I crystals. The formation of amorphous monohydrate cannot be excluded too, depending on the effect of the purge gas flow on the conditions in the DSC pan.

A final endothermic peak is detected at 171.46 °C (117.9 J/g) caused by melting with decomposition.

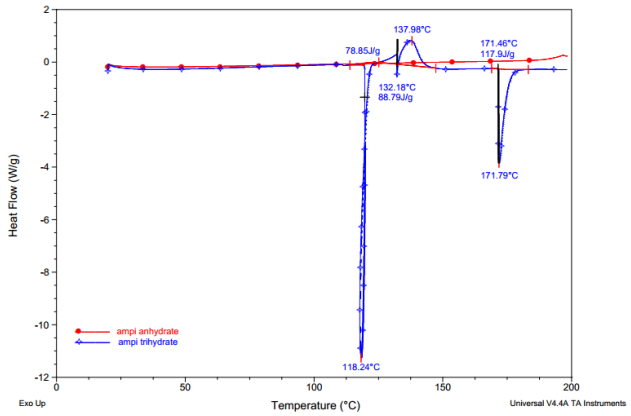

Fig. 2. DSC profiles of ampicillin forms

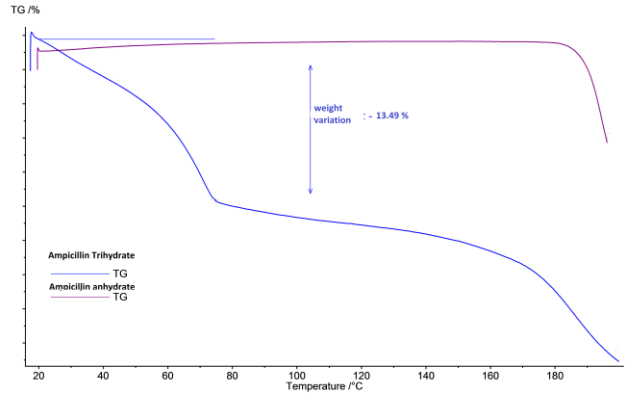

Fig. 3. TGA profiles of ampicillin forms.

The heat and mass transfer analysis from the free water surface plays an important role in determining the rate of drying. The shape of the heat capacity profiles contains thermodynamic information on the modes of transfer, drying temperature and purity of the product.

The heat capacity values of ampicillin forms (these data are average values from three experiments with a mean error of approximately 3%) are shown in Fig. 4. Heat capacity measurements were obtained from 0 to 120°C.

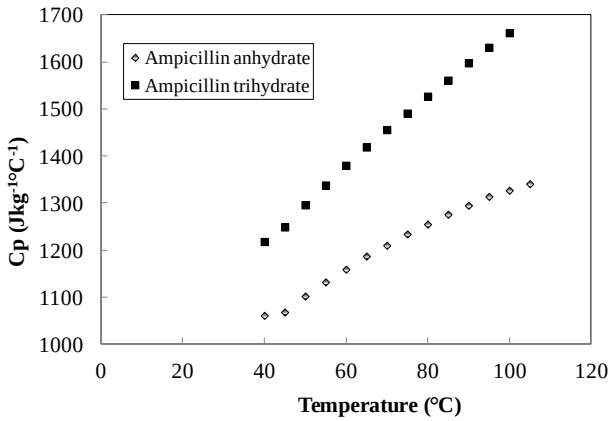

Fig. 4. Ampicillin's heat capacity versus temperature

This Figure shows that heat capacity increase with temperature. For the totally dehydrated ampicillin, between 0◦C and 120◦C, the following relationship resulting from average experimental values was obtained:

For the Trihydrate Form:
Cp (kJ/kg K) = 923.77 + 7.4912 * T (R^2 = 0.9979) (**eq. 1**)

For the Anhydrate Form:
Cp (kJ/kg K) = 881.63 + 4.5499 * T (R^2 = 0.9889) (**eq. 2**)

Where T is the temperature of the sample (°C).

The powder X-ray diffraction pattern of the Trihydrate Ampicillin is shown in Fig. 5. This pattern was in excellent agreement with those reported in the Powder Diffraction Files (International Centre for Diffraction Data) and by Nojavan et al. (2005).

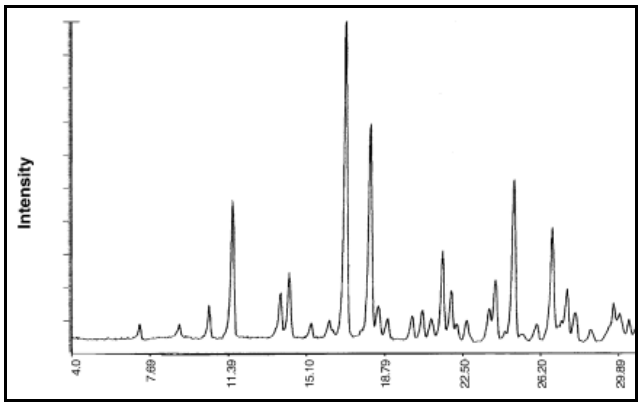

Fig. 5. XRD pattern of Ampicillin Trihydrate

A scanning electron microscope was used to observe the surfaces of ampicillin anhydrous and trihydrate forms. The SEM image showed that ampicillin trihydrate crystals were in a plate shape with a size of around 10µm (Fig. 6).

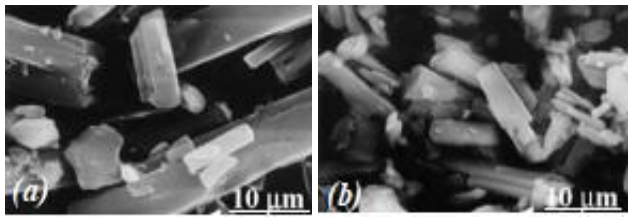

Fig. 6. Scanning electron microscope (SEM) image of ampicillin trihydrate (a) and anhydrous form (b)

Desorption isotherms

The final equilibrium contents obtained from the isothermal and isohumid gravimetric experiments were used to plot the desorption isotherms of ampicillin (Fig. 7). The plot of desorption equilibriums presents one plateau for high humidities which corresponds to the trihydrated form and a plateau for low humidities which corresponds to the anhydrous form.

The hygroscopic threshold was found at moisture content X = 0.13% which is stoichiometrically equivalent to the trihydrate form. These results showed the trihydrate to be present at relative humidities higher than 43 % and 54% at temperatures of 25°C and 80°C, respectively. The increasing relative humidity required to form a hydrate at higher temperatures can be explained by the weakening of H-bonds between the water and cristal (Touil et al., 2013).

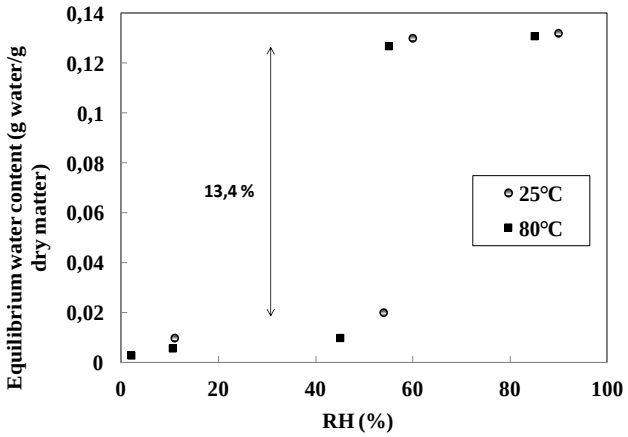

Fig. 7. Desorption isotherms of the ampicillin

Dehydration and degradation of ampicillin trihydrate

The water content versus time plots for the dehydration of ampicillin trihydrate at 11 % and 54 % relative humidity and at different temperatures (25°C and 80°C) are shown in Fig.8. The dehydration rate increased strongly with temperature and decreased considerably with relative humidity.

These experiments showed that both temperature and humidity affected the dehydration rate of ampicillin trihydrate. The same kinds of curves were also obtained for all temperatures and relative humidities considered, but these results are not shown here.

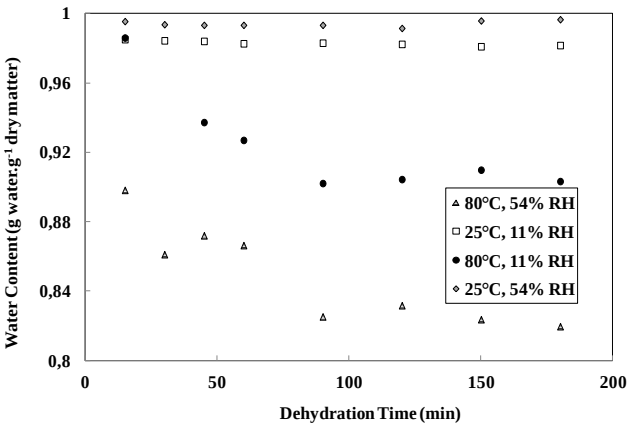

Fig. 8. Ampicillin trihydrate's dehydration profiles at different temperatures and different relative humidities

When samples were kept at different temperatures and different relative humidities (water activities at the equilibrium), some of them showed a colour change from white to yellow. These samples could have high contents of methylene chloride. The degradation of the trihydrate ampicillin during storage was activated by losing water molecules under the effect of the operating conditions.

Thus, the temperature effect on the stability of the trihydrate ampicillin was investigated and the product was quantified by the HPLC method during and after storage (Fig. 9 and 10).

The obtained results in Fig. 9 show that the intensity decreases during dehydration and degradation is complete after 48 h.

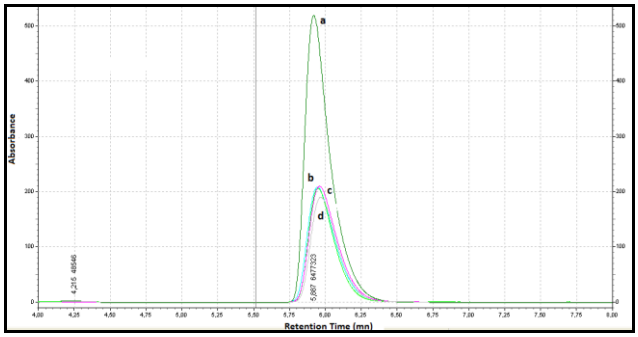

Fig. 9. Comparison of ampicillin trihydrate intensity in different times of dehydration test [(a) 0, (b) 3, (c) 6, (d) 25 and (e) 28 h at 80°C and 11% RH].

The rate constant can be a function of water activity (Relative Humidity of the air storage) if one views the absolute rate as being influenced by the solution enthalpy/entropy and the chemical reaction enthalpy/ entropy (Lumry and Rajender, 1970). Labuza (1980) has explained the influence of water activity on reaction rates by this enthalpy-entropy compensation phenomenon.

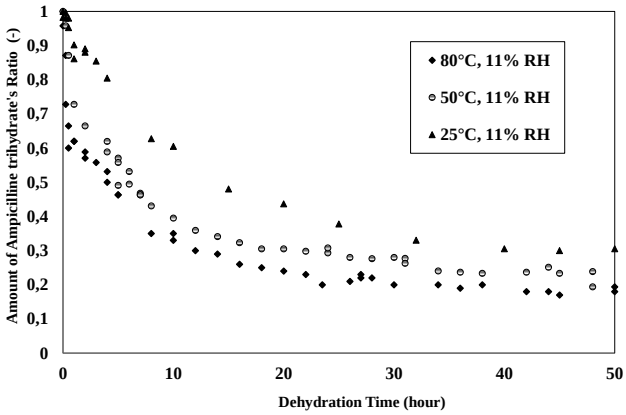

Fig. 10. Degradation curves of the ampicillin trihydrate during dehydration at different temperatures and relative humidities

In true first-order reactions, water, hydronium ions, or hydroxyl ions may be necessary to promote an activated state before conversion to the product. The rate constant of the initial forward step is k and is the rate-limiting step of the reaction. The free energy change for the formation of the intermediate activated complex can be expressed as:

$$\Delta G=\Delta H- T.\Delta S \quad \textbf{(eq. 3)}$$

Where ΔH is the enthalpy change, T is the absolute temperature, and ΔS is the entropy change. The common assumption is that ΔG remains constant for any condition, such that a change in ΔS must be compensated for by a change in ΔH (Lumry and Rajender, 1970). However, as moisture is removed, the system properties change. Duckworth (1981) and Simatos et al. (198l) proved that as water activity is lowered, the aqueous environment becomes more ordered (less entropic). In addition, the reactant concentration increases which together with the decreased entropy results in the equilibrium constant for product formation, k, being less.

Thus, ΔG does not remain constant but increases as K decreases as shown in eq. 4.

$$\Delta G = -RT \ln(K) \quad \textbf{(eq. 4)}$$

Therefore, if the change in entropy decreases and the change in free energy increases as the water activity decreases, then the enthalpy of formation of the activated state increases resulting in an increase in the activation energy as is generally observed. The changes in enthalpy and activation energy, Ea, for the formation of the intermediate are related by:

$$Ea = \Delta H + RT \quad \textbf{(eq. 5)}$$

A hypothetical example of how water activity influences reaction rate constants as explained by the modified enthalpy-entropy compensation theory is shown in Table 2. As seen in this table, the rate constant decreases with decreasing water activity over the entire relative humidity range.

Table 2. Variation of thermodynamic parameters with temperature and relative humidity

RH (%)	T (K)	ΔG (kJ/mole)	T.ΔS (kJ/mole)	ΔH (kJ/mole)	K. 10^{-2}
11	298,15	6,326	15,988	22,315	7,79
45	298,15	6,667	12,821	19,489	6,79
55	298,15	7,062	7,618	14,680	5,79
85	298,15	8,112	5,209	13,322	3,79

Mizrahi et al. (1970) showed that the activation energy for non-enzymatic browning decreased as water activity increased. Labuza (1980) has also shown in a review of ascorbic acid degradation data that as the water activity was reduced, the measured activation energy increased. Kamman et al. (1981) also showed this phenomenon for thiamin degradation. Bell and Labuza (1991) found the same trend for aspartame degradation.

In order to interpret and fit the experimental degradation curves, several equations were proposed in the literature. The model of the classical first-order chemical kinetics was applied to our experimental data. First, the data were plotted according to the linearised equations. From the slopes of the fitted straight lines, the values of dehydration rate parameter k were evaluated.

The *k* parameter was considered as a function of air temperature and relative humidity and was represented by the classical Arrhenius equation:

$$lnk = lnk_0 - E_a/RT \quad \textbf{(eq. 6)}$$

According to the above equation, *lnk* was plotted as a function of the inverse of the temperature. From the linear fits (Fig. 11), the activation energy (E_a) and the pre-exponential coefficient k_0 were calculated as a function of relative humidity (Table 3).

Table 3. The activation energies for the first order model

RH (%)	Ea (KJ/mol)	Ln (K_0)	R^2
11	24.794	7.49	0.9908
45	21.968	5.21	0.9896
55	17.159	4.04	0.9880
85	15.801	0.61	0.8947

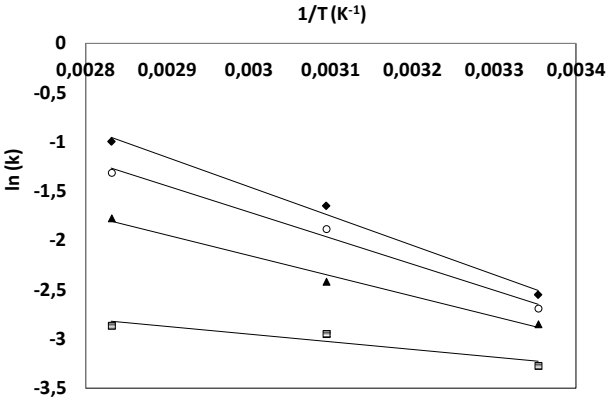

Fig. 11. Rate constant as a function of temperature fitted by the Arrhenius equation for dehydration of ampicillin trihydrate at different relative humidity

The activation energy appeared to be varying very little with relative humidity and was approximated by its average value. On the contrary, the logarithm of the pre-exponential factor k_0 was found to increase linearly with the relative humidity (Fig. 12).

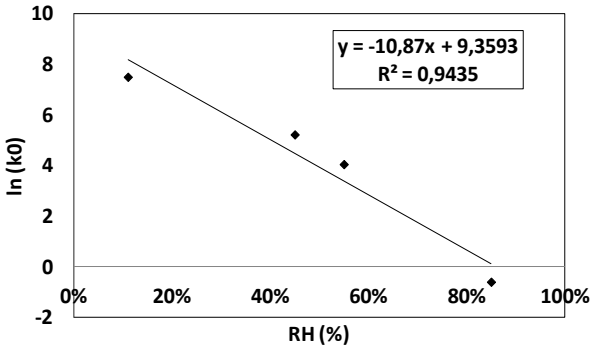

Fig. 12. Logarithm of the preexponential factor in the Arrhenius equation versus relative humidity for the dehydration of ampicillin trihydrate

The fitting coefficients *m* and *n* of the expression:

$$lnk_0 = mRH + n \quad \textbf{(eq. 7)}$$

These are given in Table 4. The best correlation factors R^2 were again obtained with the first order model.

Table 4. Values of constant parameters of first-order model

Ea (kJ/mol)	*m* (-)	*n* (-)	R^2 (-)
20,58	-10.87	9.35	0,943

Thus, the classical first-order chemical kinetics model coupled with the Arrhenius expression for *k* (with constant activation energy E_a) and a linear expression for lnk_0 could be a possible mathematical representation of the ampicillin's degradation curves. This representation would include the influence of both air temperature and relative humidity in a quite simple way. In order to check the validity of this approach, the ampicillin trihydrate's ratio values corresponding to experimental recording times were calculated according to First Order Model and using equations (eq. 5, 6 and 7), with the values of the parameters given in Table 4. Then, the calculated

ampicillin trihydrate ratio values were plotted versus the experimental ones in Fig. 13.

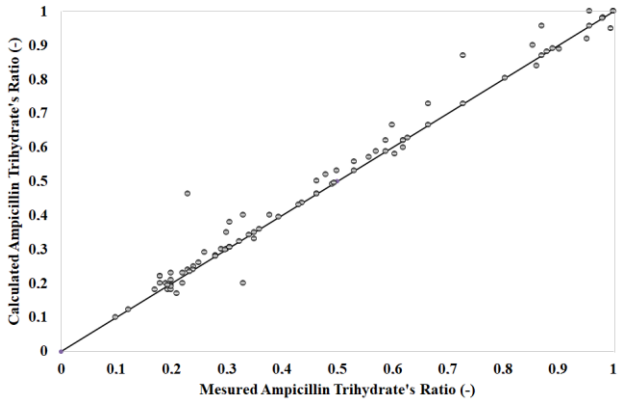

Fig. 13. Experimental versus predicted data (First Order model) for the degradation of the ampicillin trihydrate

The points are rather narrowly distributed along the bisecting line and thus the correlation between the two sets of data can be considered satisfactorily.

CONCLUSIONS

The effect of ambient conditions (air temperature and relative humidity) on the dehydration of ampicillin trihydrate was investigated. The results of gravimetric experiments provided the conditions where the trihydrate transforms into the anhydrate. The analysis of PXRD patterns enabled to confirm that the material used in this study was the ampicillin trihydrate. The dehydration rate was found to increase strongly with ambient air temperature and to decrease with air relative humidity. The measured evolutions of ampicillin trihydrate amount with time during dehydration for all considered ambient conditions were found to be well represented by the First order chemical kinetic model. The parameters of this equation were correlated to temperature and relative humidity by exponential expressions.

CONFLICTS OF INTEREST

The author(s) declare(s) no conflicts of interest.

DECLARATION

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REFERENCES

Alburn HE, Chester W, Grant NH (1967). Process for the preparation of 6-(alpha-aminoacylamino) penicillanic acids, US Patent Office 3, 299, 046.

Baraldi C, Tinti A, Ottani S, Gamberini MC (2014). Characterization of polymorphic ampicillin forms. Journal of Pharmaceutical and Biomedical Analysis, 100, 329–340. https://doi.org/10.1016/j.jpba.2014.08.021

Bell LN, Labuza TP (1991). Aspartame degradation kinetics as affected by pH in intermediate and low moisture food systems. Journal of Food Science, 56, 17-20. https://doi.org/10.1111/j.1365-2621.1991.tb07964.x

Duckworth RB (1981). Solute mobility in relation to water content and water activity. In Water Activity: Znjluences on Food Quality, eds L. B. Rockland & G. F. Stewart. Academic Press, New York, USA. https://doi.org/10.1016/B978-0-12-591350-8.50018-8

Fukumori Y, Fukuda T, Yamamoto Y, Shigitani Y, Hanyu Y, Takeuchi Y, Sato N (1983). Physical characterization of erythromycin dihydrate, anhydrate and amorphous solid and their dissolution properties. Chemical and Pharmaceutical Bulletin, 31(11), 4029-4039. https://doi.org/10.1248/cpb.31.4029

Ivashkiv E (1973). Ampicillin in: Florey K (Ed.), Analytical profiles of drug substances, vol. 2, Academic Press, New York, pp. 1-61. https://doi.org/10.1016/S0099-5428(08)60035-0

James MNG, Hall D (1968). Crystalline modifications of ampicillin I: the trihydrate, Nature 220, 168–170. https://doi.org/10.1038/220168a0

Kamman JF, Labuza TP, Warthesen JJ (1981). Kinetics of thiamin and riboflavin loss in pasta as a function of constant and variable storage conditions. Journal of Food Science, 46, 1457-61. https://doi.org/10.1111/j.1365-2621.1981.tb04197.x

Labuza TP (1980). The effect of water activity on reaction kinetics of food deterioration. Food Technology, 34(4), 36-41.

Lumry R, Rajender S (1970). Enthalpy-entropy compensation phenomena in water solutions of proteins and small molecules: a ubiquitous property of water. Biopolymers, 9, 1125-1127. https://doi.org/10.1002/bip.1970.360091002

Mizrahi S, Labuza TP, Karel M (I970). Feasibility of accelerated tests for browning in dehydrated cabbage. Journal of Food Science, 35(6), 804-807. https://doi.org/10.1111/j.1365-2621.1970.tb01999.x

Nojavan S, Ghassempour A, Bashour Y, Khalilian M, Darbandi S, Ahmadi H (2005). Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure. Journal of Pharmaceutical and Biomedical Analysis, 36(5), 983-988. https://doi.org/10.1016/j.jpba.2004.08.031

Ohsawa M , Mogi T, Yamamoto H , Yamato I , Anraku Y (1988). Proline carrier mutant of Escherichia coli K-12 with altered cation sensitivity of substrate-binding activity: cloning, biochemical characterization, and identification of the mutation. Journal of Bacteriology, 170(11):5185-5191. https://doi.org/10.1128/jb.170.11.5185-5191.1988

Shefter E, Fung H, Mok O (1973). Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate, Journal of Pharmaceutical Sciences, 62(5), 791–794. https://doi.org/10.1002/jps.2600620518

Simatos D, Le Meste M, Petroff D, Halphen B (1981). Use of electron spin resonance for the study of solute mobility in relation to moisture content in model food systems. In Water Activity: Injluences on Food Quality, eds L. B. Rockland & G. F. Stewart. Academic Press, New York, USA. https://doi.org/10.1016/B978-0-12-591350-8.50019-X

Touil A, Peczalski R, Timoumi S, Zagrouba F (2013). Influence of air temperature and humidity on dehydration equilibriums and kinetics of theophylline. Journal of Pharmaceutics, 2013, 892632. https://doi.org/10.1155/2013/892632

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