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Solution-mediated crystallization of amorphous azithromycin

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Water and water vapor are the bane of amorphous drug stability, both in storage and after administration. As is to be expected, crystallization of amorphous azithromycin did occur when exposed to water as dissolution medium. However, experimental results showed that, although solution-mediated phase transformation had occurred, it was not a rapid process for this drug. It is considered to be an advantageous characteristic of amorphous azithromycin and likely due to the high molecular mass (748.984 g/mol) and complex structure necessitating more energy for transformations to occur. A high apparent solubility is maintained for a considerable period of time, potentially rendering a higher percentage of this BCS Class II drug available for absorption when administered orally.

1. Introduction

Amorphous drugs are of great interest to pharmaceutical researchers, mainly due to the dissolution and apparent solubility advantages they offer. Much literature is available which discusses the pros and cons associated with pharmaceutical amorphous solid-state forms. The greatest advantage, namely improved apparent aqueous solubility of a given drug, is usually outweighed by the disadvantage of crystallization of the amorphous form to the most stable crystalline form upon exposure to moisture or dissolution media. During this study the crystallization of amorphous azithromycin was investigated during dissolution testing in water at different temperatures. Water was considered to be the single most relevant medium in demonstrating solution-mediated transformation, as all gastric and intestinal fluids are aqueous in nature.

2. Investigations, results and discussion

Figure 1 depicts the dissolution profiles obtained for crystalline azithromycin dihydrate and amorphous azithromycin in distilled water at (a) 25 °C, (b) 30 °C and (c) 35 °C. From the dissolution profiles it is evident that amorphous azithromycin exhibits typical dissolution behavior of a metastable solid-state form (Carstensen 1990; Guszmán et al. 2007; Babu and Nangia 2011; Hancock and Parks 1999). Solution-mediated phase transformation occurred at all three temperatures and the rate of the transformation process increased with an increase in temperature, with a logical opposite effect on maximum apparent solubility. The dissolution profiles show the typical "spring and parachute" effect as first described by Guszmán et al. 2007. This phenomenon is typically observed in instances where the bioavailability of a drug is limited by the dissolution rate thereof. Azithromycin, being classified as a BCS Class II drug with low solubility and high permeability, fits that description (Adebayo and McFarlane 2014). Amorphous azithromycin, being a higher energy state of the drug provides the "spring", which is signified by a significant increase in dissolution. However, the resulting solution is supersaturated with regards to the stable crystalline dihydrate and crystallization to the thermodynamically stable solid-state form of the drug will limit this "spring" effect, which would typically be signified by a sharp decrease (within minutes) of the dissolved concentration of the drug (Guszmán et al. 2007; Babu and Nangia 2011). This is the "parachute" phase, characterized by precipitation or sudden "crashing out" of the drug.

Interestingly, amorphous azithromycin displayed an unusually long "parachute" phase *in vitro*, without the addition of any stabilizing polymers or additive excipients. From Fig. 1 it is evident

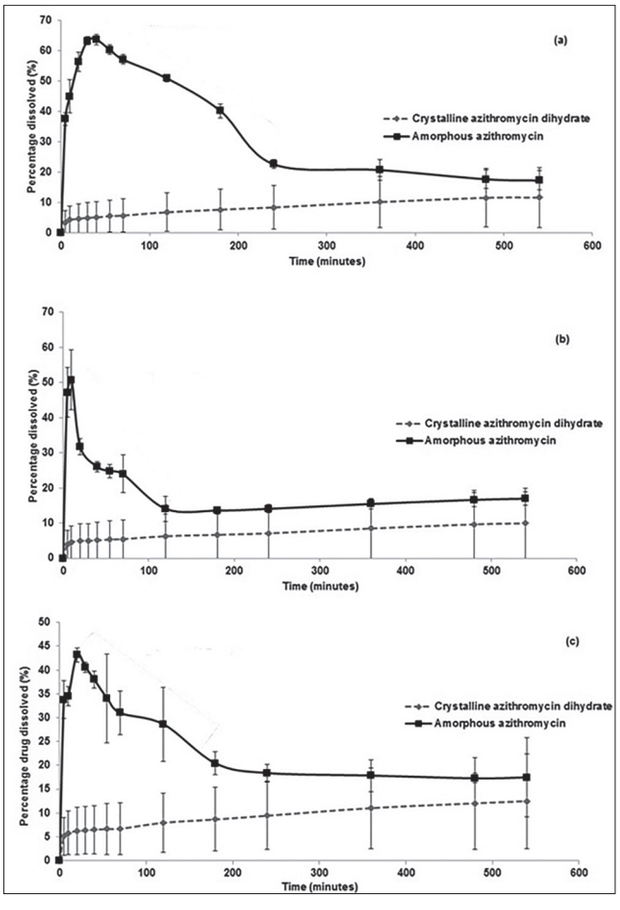

Fig. 1: Dissolution profiles of crystalline azithromycin dihydrate and amorphous azithromycin at dissolution medium temperatures of (a) 25 (b) 30 and (c) 35 °C.

that a significant improvement in solubility is still evident after 2 h for even the worst case (Fig. 1b – 30 °C). Clearly there is an interplay between temperature's effect on 1) increasing the rate of dissolution to create a supersaturated state; and 2) increasing the rate of transformation. The shape of the curves in Fig. 1 suggest a two-step transformation which could mean that the monohydrate precedes the dihydrate. Supersaturation is required for crystallization to occur, therefore the *in vivo* effect may be even more pronounced if the drug's absorption rate is sufficiently fast to avoid that state from being reached.

Degree of crystallinity was determined and results showed the following percentages of amorphous azithromycin had converted to the crystalline dihydrate form after 120 min: 99.5 % at 35 °C (n = 1); 76.9 % at 30 °C (n = 1); and 63.7 % at 25 °C (n = 1); respectively. The heat of solution data obtained during the study was used to construct a plot of degree of conversion (a) *versus* time (Fig. 2). From the plotted graph one can observe the difference in conversion rate as a function of dissolution test temperature, with complete conversion of amorphous azithromycin to the stable azithromycin dihydrate in a period of 120 min at 35 °C. The conversion took substantially longer at 25 and 30 °C, with complete transformation of the amorphous form to the dihydrate at approximately 480 min. The conversion at 30 °C is 4 times slower than at 35 °C.

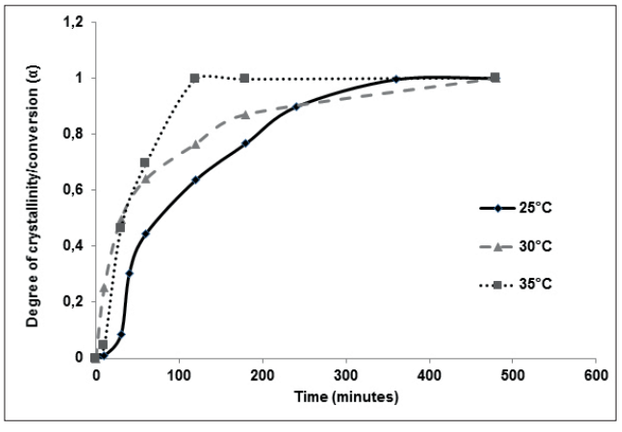

Fig. 2: Graph depicting the degree of conversion of amorphous azithromycin to azithromycin dihydrate over a time range of 0 to 480 minutes, in distilled water at temperatures of 25, 30 and 35 °C.

3. Experimental

3.1. Determination of degree of crystallinity

The degree of crystallinity of one of the (n = 1) solid samples, remaining after each of the dissolution experiments, was determined by means of heat of solution. For this a TAM III (TA Instruments, USA) was used. Just one sample could be tested for each dissolution experiment, because heat of solution experiments are time-consuming. Testing the remaining solids from the other five dissolution vessels in sequence would yield false data, as the conversion process would continue to progress. The heat of solution data was collected at 25 °C. Approximately 10 mg of the undissolved powder was weighed into glass crushing ampoules. The crushing ampoules were sealed with wax plugs. 20 mL of absolute ethanol was pipetted into the reaction vessel of the solution calorimeter. The crushing ampoule containing the sample was affixed to the gold stirrer of the calorimeter, followed by attaching the reaction vessel containing the solvent. The stirring speed was set to 300 rpm. The complete reaction unit was lowered into the calorimeter. The experiment was started by two electrical calibration steps followed by crushing of the ampoule. The degree of crystallinity was determined by assuming 100% crystallinity for commercial azithromycin dihydrate and 0% crystallinity for amorphous azithromycin prepared *via* quench cooling of the melt.

3.2. Powder dissolution testing

A VanKel700 dissolution bath was used for dissolution testing. USP apparatus 2 (paddle) was set up at 25, 30 or 35 °C with a rotational speed of 100 rpm, 900 mL distilled water was added to each of the six (n = 6) dissolution vessels. Approximately 600 mg of powder was weighed into 10 mL test tubes, to which 300 mg glass beads, ≤ 106 µm (Sigma-Aldrich, South Africa) was added. 5 mL of dissolution medium (distilled water maintained at 25, 30 or 35 °C) was added to each test tube. The mixtures were agitated for a period of 120 s, using a vortex mixer. The resulting mixtures were transferred to each dissolution vessel. Solution (5 mL) was withdrawn from each dissolution vessel at predetermined time intervals. The dissolution medium was not replaced after each withdrawal since a supersaturated solution is required to observe solution-mediated transformations. After withdrawal, the samples were filtered through 0.45 µm PVDF filters into HPLC vials. The filtered solutions were analyzed by HPLC.

3.3. High-performance liquid chromatography (HPLC)

The samples obtained from the powder dissolution studies, were assayed by means of HPLC. A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system was used. The system consisted of a SIL-20AC auto-sampler fitted with a sample temperature controller, a UV/VIS Photodiode Array detector (SPD-M20A) and a LC-20AD solvent delivery module. The mobile phase consisted of 0.06 M potassium orthophosphate buffer, pH adjusted to 6.0 with 1.0 M sodium hydroxide solution. The buffer was mixed with acetonitrile in the ratio 700:300. A Luna C_{18} 150 × 4.6 mm column was used at a flow rate of 1.0 mL/min. A wavelength of 205 nm was used for detection (Odendaal et al. 2012). Both the column and sample tray temperature were set to control the temperature at 25, 30 or 35 °C. This was done in order to prevent any crystallization from solution due to a possible decrease in sample solution temperature.

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