Drug Design, Development and Therapy

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ORIGINAL RESEARCH

An Evaluation of Amoxicillin/Clavulanate Stability in Aqueous Systems, Including Its Suitability for Outpatient Parenteral Antimicrobial Therapy (OPAT)

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Purpose: Amoxicillin/clavulanate antibiotic combination is suitable for treating a range of infections, including some suited for Outpatient Parenteral Antimicrobial Therapy (OPAT). The aim of the study was to evaluate shelf-life values of amoxicillin at clinical concentrations in the presence of clavulanate for use in OPAT.

Methods: A stability-indicating HPLC assay was developed and validated. Kinetic studies were performed at 1 mg/mL and 15 mg/mL amoxicillin at 40–60 °C. Studies in elastomeric infusers included the pH lowered from 8.73 to 6.52 for 1 mg/mL; 8.85 to 7.69 for 7.5 mg/mL and 8.68 to 8.40 for 15 mg/mL amoxicillin plus clavulanate and stored at 2.9 °C.

Results: Amoxicillin and clavulanate eluted at 5.2 and 3.0 minutes, respectively, with linear concentration relationships. Forced degradation retained base-line separation of each component in the presence of degradation products. Amoxicillin 1 mg/mL had a shelf-life of 4.85 hours at pH 6.53 and 40 °C which on extrapolation to 25 °C was 22.8 h. Clavulanate was 1.38 h at 40 °C and 4.0 h at 25 °C. Amoxicillin 15 mg/mL at pH 8.34 gave a shelf-life of 0.11 h at 40 °C and clavulanate 0.41 h. In elastomeric infusers, amoxicillin 1 mg/mL, with lowering pH from 8.73 to 6.52, improved the shelf-life at 2.9 °C from 72 to >263.8 h and similarly for clavulanate. At 7.5 mg/mL amoxicillin, lowering pH from 8.85 to 7.69 improved the shelf-life from 4.2 to 51.8 h and clavulanate from 4.2 to 48.0 h. At 15 mg/mL amoxicillin, the shelf-life values at pH 8.68 or 8.40 were 3.8 h and 1.6 h and similarly for clavulanate.

Conclusion: Amoxicillin and clavulanate showed adequate stability at 2.9 °C for OPAT storage at 1 mg/mL and possibly 7.5 mg/mL, but not 15 mg/mL. Low shelf-life values at 25 °C also limit administration times.

Keywords: amoxicillin, clavulanate, stability, shelf-life, OPAT, infusers

Introduction

Amoxicillin is a moderate spectrum penicillin with greater antimicrobial activity than narrow spectrum penicillins against selected Gram-negative bacteria such as *Escherichia coli* and *Haemophilus influenzae*.[1] It is commonly prescribed for respiratory infections including bronchitis, community-acquired pneumonia, urinary tract infections, acute prostatitis and for endocarditis prophylaxis in high-risk patients.[2] Amoxicillin is inactivated by bacterial strains that produce beta-lactamase enzymes, which hydrolyse the beta-lactam ring, rendering amoxicillin inactive. The combination of amoxicillin with clavulanate, a beta-lactamase inhibitor with little innate antibacterial activity, broadens the spectrum of its activity to include *Staphylococcus aureus and Bacteroides fragilis*.[3] In addition, it provides activity against some beta-lactamase enzymes produced by *Escherichia coli* and *Klebsiella* species.[2,3]

Amoxicillin is a time-dependent antibiotic and its efficacy during the dosing interval is dependent on the time the free-serum concentration is maintained above the minimum inhibitory concentration (MIC).[1] Concentrations can be maintained above the MIC for longer periods of time when administered by continuous infusion.[4]

The stability of amoxicillin has been examined in a range of kinetic and clinical studies. It is an amphoteric substance with the protonated amino species (pKa 2.63) powerfully attracting electrons providing higher acidic stability than penicillin G.[5] At

Received: 21 April 2024
Accepted: 10 September 2024
Published: 24 September 2024

low concentrations, amoxicillin undergoes hydrolytic degradation exhibiting pseudo first-order kinetics over the pH range 1 −10 with a pH minimum value at pH 6, with little change in reaction rates between pH 5–7. The deprotonated carboxylic acid form exists primarily above pH range 7 (pKa 7.16) and the deprotonation of the phenolic moiety (pKa 9.55) has little underlying effect on beta-lactam ring cleavage.[5–8] Both citrate and phosphate buffers have marked catalytic effects.[6] Amoxicillin also undergoes an autocatalytic reaction (self-ammonolysis) through a nucleophilic attack of the amino group on its side chain on the β-lactam ring leading to dimerization and subsequent polymerisation.[8] The rate of this reaction is dependent on the initial concentration of amoxicillin and becomes clinically important when high initial concentrations of amoxicillin are prepared. It has been shown to exhibit second- and third-order kinetics.[8] Binson has also reported increased degradation rates of amoxicillin over the concentration range 25–250 mg/mL.[4] As a result of the autocatalytic reaction amoxicillin is also less stable in frozen solutions from −5 °C to −20 °C, compared with room temperature storage, owing to increasing amoxicillin concentrations occurring in partially frozen solutions, above its eutectic temperature. This has the effect of increasing the reaction rate to a greater extent, than the effect of temperature lowering.[9]

In clinical studies, the solubility of amoxicillin becomes important when high concentrations of the antibiotic are required. Solubility has been reported to be constant over the pH range 3.5–6.5 in 0.5 M KCl at 37 °C as 0.015 M (5.8 mg/mL of amoxicillin sodium).[5] Other data have reported approximately 7 mmolal (2.6 mg/mL) at 25 °C over the same pH range, rising to 24 mmolal (9.1 mg/mL) at pH 7.6.[10]

Hydrolytic degradation of clavulanate has been reported in aqueous systems to follow pseudo first-order kinetics with a pH minimum at pH 6, similar to that of amoxicillin.[11] When evaluated separately, clavulanate overall is two to three-fold less stable in dilute solutions than amoxicillin, dependent on pH and temperature. In combination with amoxicillin in acetate and phosphate buffers, clavulanate also acted as an additional catalytic species which further destabilised amoxicillin if formulated in these combinations.[12]

Outpatient Parenteral Antimicrobial Therapy (OPAT) allows patients to receive intravenous (IV) treatment at home improving their quality of life, reducing the risk of nosocomial infections, and freeing hospitals beds.[13–15] Infusers for OPAT administration include electrical, spring and elastomeric devices.[16] However, despite the potential benefits of OPAT, its use for continuous infusion may be limited by the stability of amoxicillin and clavulanate.

With the shelf-life of amoxicillin being temperature, pH and concentration dependent, this study aimed to evaluate its shelf-life under clinical concentrations including pH control to evaluate its stability in the presence of clavulanate for use in OPAT.

Methods

Analytical grade amoxicillin and clavulanate [Sigma-Aldrich (batch 077M4892V)]; commercial grade amoxicillin and clavulanate [Amoxiclav 1000/200mg (Juno®) (batch 9K51VL, expiry 04/2023)] were purchased commercially. Phosphate buffers used potassium dihydrogen orthophosphate, [Univar (batch 1809274114)] and di-potassium hydrogen orthophosphate [Univar (batch 1807268388)]; all chemicals were used prior to their expiry dates. Reconstitution of amoxicillin and clavulanate as well as potassium dihydrogen orthophosphate and di-potassium hydrogen orthophosphate used Milli-Q water [Milli-Q Ultrapure Water System (Merck, VIC, Australia)]. Orthophosphoric acid (85%) [Thermo Fisher Scientific (WA, Australia)] was used to achieve a pH of 4.4 ±0.1 and pH was monitored using a Hanna HI8519N pH meter (Perth Scientific).

Phosphate buffers contained sodium chloride [Sigma-Aldrich USA (batch SLBK9112V)] to maintain a constant ionic strength (μ = 0.5). Potassium hydroxide pellets [AnalaR (batch B921055 702)] were dissolved in Milli-Q water and added dropwise to reach the desired pH values of 6.5, 7.0 and 8.0.

Forced degradation studies used commercial amoxicillin and clavulanate. Base hydrolysis used 0.1M sodium hydroxide [Univar (batch 1309149539)] at 40.0 ±0.1 °C. Oxidative degradation used 3% hydrogen peroxide [Univar (batch 1608225558)] at 40 ±0.1 °C. Acid hydrolysis used 0.1M hydrochloric acid [Univar (batch 1404100006)] carried out at 60 ±0.1 °C in a water bath (Fisher Biotech). Each study was performed over 4 hours and samples were taken at T=0, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 hours.

Assays of amoxicillin and clavulanate used high performance liquid chromatography (HPLC), which consisted of a HPLC- DGU-20A5R degasser unit, LC20AT – liquid chromatographer, SIL20ACT HT auto sampler and SPD-20 UV/Vis

detector, Lab Solutions, Version 5.85 software (Shimadzu Corp, Kyoto, Japan). HPLC assays were conducted using a C18 column [Agilent Technologies, Zorbax Eclipse plus 5µ, 4.6×150 mm diameter (serial no. 605120344.1)]. Another C18 column [Agilent Technologies, Zorbax, Eclipse plus, 5µ 4.6×150 mm diameter internal (serial no. USUXB19205)] was also used. Ultra-violet (UV) detection was at 228 nm using a SPD-20 UV/Vis detector (Shimadzu Corp, Kyoto, Japan).

The flow rate was set to 1.0 mL/min and a run time of 10 minutes. Low pressure gradient phase was used throughout the study and all samples were analysed in triplicate. The mobile phase (95:5) consisted of phosphate buffer at (pH 4.4 ±0.1) and methanol [Honeywell Burdick and Jackson B&J ACS/HPLC (lot number T8TG1H, Batch no 100233)].

Analytical grade amoxicillin and clavulanate, as amoxicillin trihydrate and potassium clavulanate [Sigma-Aldrich (batch LRAB3723, PHR 1127–1G)], were used to develop a calibration curve from a stock solution of 1.20 mg/mL amoxicillin and 0.3 mg/mL clavulanate. Intra and inter-day precision was based on the United States Pharmacopeia's guideline that a relative standard deviation (RSD) of less than 2% was required for replicated assays of an analyte.[17]

Stability Studies

Stability studies were carried out at 1.0 mg/mL/0.2 mg/mL using commercial amoxicillin and clavulanate powder for injection, at pH 6.5, 7.0 and 8.0 and 40, 45, 52 and 60 ±0.1 °C. The 15.0 mg/mL/3.0 mg/mL combination was only investigated at pH 8.0. The temperature range selected was to minimise any possibility of amoxicillin precipitation influencing the findings. These solutions were prepared separately as double strength in two different volumetric flasks, equilibrated simultaneously in the water bath for 5 minutes, then mixed together and shaken thoroughly. An approximate 2 mL aliquot of the sample was transferred to a glass container, sealed by a plastic stopper, and left to equilibrate to room temperature. Accurate 1 mL samples were then diluted ten-fold using Milli-Q water, for assay. The first sample was designated time zero. The pH was checked at time zero and the last testing period. Testing was ceased when approximately 50% degradation had occurred.

Elastomeric Infuser Studies

Commercial amoxicillin and clavulanate at 1.0 mg/mL/0.2 mg/mL, 7.5 mg/mL/ 1.5mg/mL and 15.0 mg/mL/3.0 mg/mL were used for the stability studies in the elastomeric infusers. Elastomeric infusers 250 mL (Slade Health, batch 0200628231) were provided by Sir Charles Gairdner Hospital (Pharmacy Department, Perth, Western Australia). Amoxicillin and clavulanate were reconstituted in refrigerated 0.9% sodium chloride solution to 400 mL. For pH adjustment, 10M hydrochloric acid was added (dropwise) to 375 mL of refrigerated 0.9% sodium chloride solution containing amoxicillin and clavulanate, until the required pH was reached and then made up to 400 mL using 0.9% sodium chloride solution. The reconstituted solutions were injected into duplicate elastomeric infusers (200 mL in each) and placed in a water bath (Heterofrig, Radiometer Pacific, Perth) at 2.9 ±0.1 °C. The infusers were equilibrated for 5 minutes. Triplicate 1.0 mL aliquots were taken from each, equilibrated to room temperature in glass volumetric flasks sealed by a plastic stopper. Each 1.0 mL sample was then diluted appropriately in Milli-Q water and submitted to HPLC analysis in triplicate. The first sample was designated time zero, and at each subsequent time, triplicate samples were taken from each elastomeric infuser. The HPLC analysis was, with respect to an analytical sample of amoxicillin and clavulanate, freshly made each day and stored in a refrigerator when not in use. In addition, samples were visually checked for clarity, colour and pH daily.

Data Analysis

Amoxicillin and clavulanate concentrations were evaluated from peak height or peak areas of the respective HPLC traces. Concentrations (as peak height or area) were converted to percentages based on the areas or heights of the analytical peaks. Percentage concentrations for the kinetic studies were fitted to the first-order kinetic relationship and evaluated for linearity. When linearity was not achieved then the second-order relationship was used. The zero time sample was designated as 100%. Shelf-life was defined as ≥90% (90–110%) or ≥95% (95–105%) of the initial concentration being retained in accordance with the USP specifications.[17] All kinetics analyses used least square

regression analysis and all results are reported as mean ± standard deviation. Temperature dependence used the Arrhenius relationship.[18]

Kinetics Relationships

The following equations were used for determining the stability-related data.[18]

Half-Life for a First Order Reaction

$$t_{1/2} = \frac{0.693}{k_1} \tag{1}$$

First Half-Life for a Second-Order Reaction

$$t_{1/2} = \frac{1}{k_2 A_0} \tag{2}$$

Shelf-Life for First-Order Reaction

$$t_{90} = \frac{0.105}{k_1} \; or \; t_{95} = \frac{0.051}{k_1} \tag{3}$$

First Shelf-Life for Second-Order Reaction

$$t_{90} \; or \; t_{95} = \left(\frac{1}{A} - \frac{1}{A_0}\right)/k_2 \tag{4}$$

Where k_1 is the first-order rate constant; k_2 the second-order rate constant, A_0 is the initial concentration and A is the concentration at other time intervals.

First-Order Reaction Relationship

$$\frac{-d[A]}{dt} = k_1[A] \tag{5}$$

Where: [A] is the drug concentration at time t and k_1 is the first-order rate constant.

Second-Order Reactions Involving Equal Concentrations

$$-\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k_2[A][B] or [A^2] \tag{6}$$

Where: A and B are the initial concentrations of reactants A and B and k_2 the second-order rate constant.

The Arrhenius Equation

$$k = Ae^{-E_a/RT} \tag{7}$$

Extrapolating of Data

$$log\left(\frac{k_2}{k_1}\right) = \frac{E_a}{2.303R} \times \left(\frac{T_2 - T_1}{T_2.T_1}\right) \tag{8}$$

Where: k is the rate constant, A is the Arrhenius factor, e the exponential number, E_a the activation energy, R the gas constant and T temperature in Kelvin.

Results

The HPLC chromatogram indicated that amoxicillin had a retention time of 5.2 minutes and clavulanate 3.0 minutes with baseline separation. Peak height was used for all calculations as it was less affected by any minor baseline changes. The standard curve demonstrated a linear relationship between amoxicillin peak height and concentration over the range 0 to 120 µg/mL (y=2479.28x +0, R^2=0.999) and clavulanate peak height and concentration over the range 0 to 30 µg/mL (y=1642.42x +0, R^2=0.999).

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula of the line based on the calibration curve. Amoxicillin had a LOD of 0.029 µg/mL and LOQ of 0.098 µg/mL and clavulanate LOD was 0.078 µg/mL and LOQ 0.26 µg/mL. The intra-day and inter-day precision RSD values for amoxicillin's peak areas were 0.29% and 0.55% and for clavulanate 0.27% and 0.43%, respectively, demonstrating adequate precision.[17]

Stress testing studies for both amoxicillin and clavulanate samples showed significant degradation for each stress condition. When exposed to 0.1M sodium hydroxide rapid degradation occurred with additional peaks in the chromatograms and by 4 hours amoxicillin and clavulanate distinct peaks were absent, leaving no peaks at their retention times. Exposure to 0.1M hydrochloric acid resulted in rapid degradation over the testing period, no amoxicillin or clavulanate peaks were evident at the end of the test period. Thermal degradation in water occurred, illustrated by additional peaks, but smaller amoxicillin and clavulanate peaks were present at the end of the 4-hour period. Upon addition of 3% hydrogen peroxide to amoxicillin and clavulanate, almost instant degradation occurred at zero time, with a number of additional peaks indicating degradation. No amoxicillin and clavulanate peaks remained. Under all conditions investigated the retention times for amoxicillin and clavulanate were not compromised by the presence of any new peaks occurring during the degradation reactions.

Stability Studies

Data in Table 1 shows the degradation profiles for low clinical concentrations of 1 mg/mL amoxicillin and 0.2 mg/mL clavulanate. Amoxicillin and clavulanate followed first-order kinetics, as shown by linear relationships in Figure 1. Table 1 indicates the superior shelf-life of amoxicillin over clavulanate at all temperatures and pH values studied. It is notable that the shelf-life of amoxicillin at 40 °C and near pH 6.5 was almost double that at pH values near 7 and 8 and the marked effect of temperature on shelf-life. These data were collected around the pH minimum at pH 6.5 and 7 but are above the pH minimum at pH 8. Employing Equation 8, data for amoxicillin and clavulanate were extrapolated to 32 °C and 25 °C giving shelf-life values (t_{90}) for amoxicillin of 10.6 h and 22.8 h, respectively, and for clavulanate, 2.4 h and 4.0 h, respectively.

At higher clinical concentrations of 15 mg/mL amoxicillin and 3 mg/mL clavulanate, markedly different findings were evident as seen in Table 2. Higher pH values were used compared with 1 mg/mL amoxicillin owing to precipitation occurring

Table 1 The Effect of pH and Temperature on the Degradation Rates of Amoxicillin and Clavulanate in a Solution Initially Containing 1 mg/mL Amoxicillin and 0.2 mg/mL Clavulanate, Where k_1 is the First-Order Rate Constant, $t_{½}$ the Half-Life and t_{90} and t_{95} Shelf-Life Values

Temperature (°C)	pH		Amoxicillin				Clavulanate			
	Start	End	k_1 (h^{-1}) ±2s	$t_{1/2}$ (h)	t_{90} (h)	t_{95} (h)	k_1 (h^{-1}) ±2s	$t_{1/2}$ (h)	t_{90} (h)	t_{95} (h)
40	6.53	6.48	$2.16 \times 10^{-2} \pm 3.53 \times 10^{-4}$	32.00	4.85	2.36	$7.60 \times 10^{-2} \pm 3.21 \times 10^{-4}$	9.12	1.38	0.67
	6.96	6.96	$3.82 \times 10^{-2} \pm 3.18 \times 10^{-4}$	18.12	2.74	1.34	$1.02 \times 10^{-1} \pm 6.22 \times 10^{-4}$	6.79	1.03	0.50
	7.89	7.85	$3.78 \times 10^{-2} \pm 1.57 \times 10^{-3}$	18.34	2.78	1.35	$1.21 \times 10^{-1} \pm 1.41 \times 10^{-3}$	5.71	0.87	0.42
45	6.56	6.89	$2.80 \times 10^{-2} \pm 3.85 \times 10^{-4}$	24.66	3.73	1.82	$9.80 \times 10^{-2} \pm 3.51 \times 10^{-4}$	7.29	1.07	0.52
	6.96	6.99	$4.26 \times 10^{-2} \pm 8.65 \times 10^{-4}$	16.27	2.46	1.20	$1.42 \times 10^{-1} \pm 4.44 \times 10^{-4}$	4.88	0.74	0.35
	7.79	7.65	$4.77 \times 10^{-2} \pm 1.41 \times 10^{-3}$	14.54	2.20	1.06	$1.71 \times 10^{-1} \pm 1.11 \times 10^{-3}$	4.05	0.61	0.30
52	6.61	6.55	$5.64 \times 10^{-2} \pm 2.59 \times 10^{-3}$	12.28	1.86	0.90	$1.38 \times 10^{-1} \pm 6.99 \times 10^{-3}$	4.99	0.75	0.37
	7.05	7.02	$7.07 \times 10^{-2} \pm 1.41 \times 10^{-3}$	9.80	1.49	0.72	$1.51 \times 10^{-1} \pm 5.44 \times 10^{-3}$	4.59	0.70	0.34
	8.02	7.97	$1.00 \times 10^{-1} \pm 1.06 \times 10^{-3}$	7.00	1.06	0.51	$3.34 \times 10^{-1} \pm 7.72 \times 10^{-4}$	2.07	0.31	0.15
60	6.60	6.40	$1.31 \times 10^{-1} \pm 2.25 \times 10^{-3}$	5.28	0.80	0.39	$4.00 \times 10^{-1} \pm 4.90 \times 10^{-3}$	1.80	0.27	0.13
	7.05	7.08	$1.12 \times 10^{-1} \pm 5.26 \times 10^{-3}$	5.90	0.89	0.46	$4.57 \times 10^{-1} \pm 1.10 \times 10^{-2}$	1.51	0.23	0.11
	8.03	7.96	$2.10 \times 10^{-1} \pm 6.31 \times 10^{-3}$	3.37	0.51	0.24	$8.82 \times 10^{-1} \pm 5.37 \times 10^{-2}$	0.79	0.12	0.06

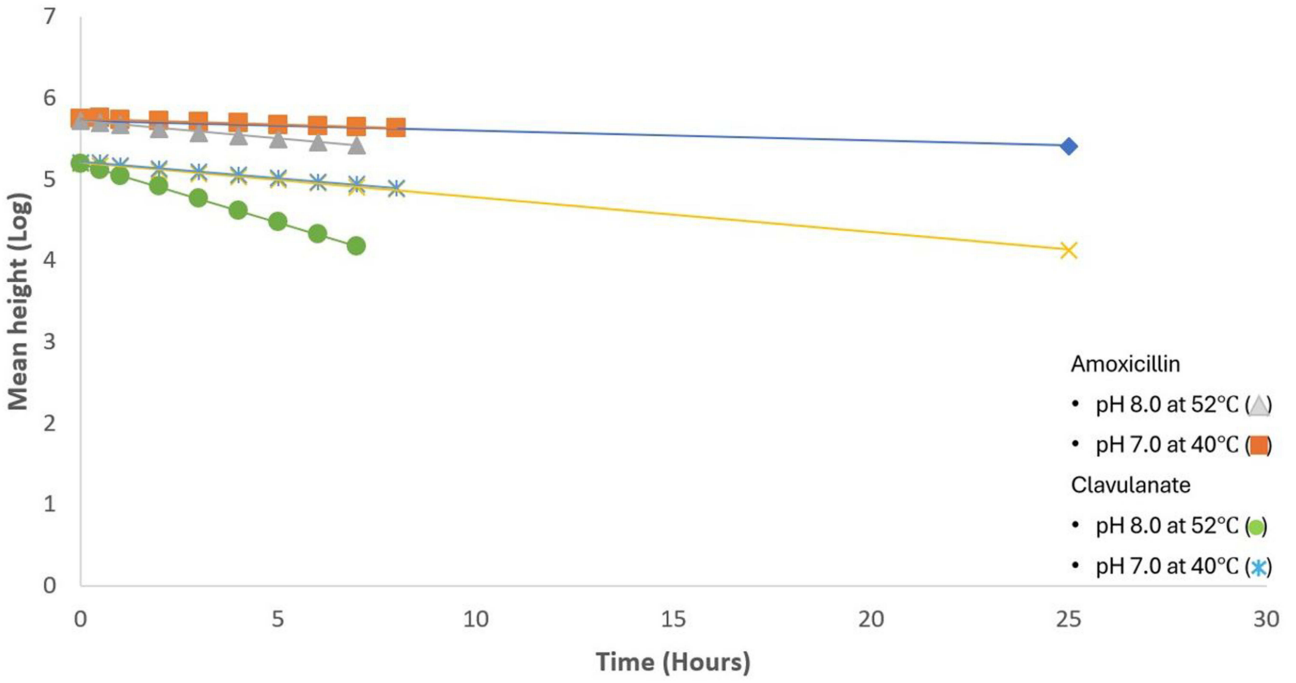

Figure 1 First-order degradation plots of 1.0 mg/mL amoxicillin and 0.2 mg/mL clavulanate.

in solutions of 15 mg/mL when lowered below pH 7.7 at room temperature. Amoxicillin degradation no longer followed pseudo first-order kinetics. It is notable that the large amount of acid degradation products exceeded the buffer capacity of the buffer employed, causing notable reductions in pH during the course of the stability run. Most data for amoxicillin followed second-order kinetics and rate constants and their half-life and shelf-life values relate to the initial values, as these best reflect shelf-life. Some curvature flattening occurred with some second-order plots, however the initial part was linear and that was analysed as it best reflected the degradation rate that occurred for the first 10% loss. It is notable the marked reduction in the shelf-life of amoxicillin when the concentration was increased to 15 mg/mL. The shelf-life of clavulanate is greater than that of amoxicillin under these conditions and now is not limiting the overall shelf-life (Table 2).

Temperature Effects

Data in Table 3 shows the temperature dependency of amoxicillin and clavulanate at each pH studied over the temperature range 40 °C to 60 °C. As the pH rises, more of the deprotonated carboxylic acid species will be present, which could influence the activation energy. The activation energy values for the 15 mg/mL amoxicillin are lower than the 1 mg/mL which would indicate a lower temperature dependency for the autocatalytic reaction.

Table 2 The Effect of pH and Temperature on the Degradation Rates of Amoxicillin and Clavulanate in a Solution Initially Containing 15 mg/mL Amoxicillin and 3 mg/mL Clavulanate, Where k_1 is the First-Order Rate Constant, k_2 is the Second-Order Rate Constant and $t_{½}$ and t_{90} and t_{95} Shelf-Life Values

Temperature (°C)	pH		Amoxicillin				Clavulanate			
	Start	End	k_2 (mL.mg.$^{-1}$h) ±2s	$t_{1/2}$ (h)	t_{90} (h)	t_{95} (h)	k_1 (h^{-1}) ±2s	$t_{1/2}$ (h)	t_{90} (h)	t_{95} (h)
40	8.34	7.92	$7.83 \times 10^{-2} \pm 2.80 \times 10^{-3}$	0.85	0.095	0.045	$2.55 \times 10^{-1} \pm 3.45 \times 10^{-3}$	2.72	0.41	0.20
45	8.35	7.95	$1.01 \times 10^{-1} \pm 4.17 \times 10^{-3}$	0.66	0.073	0.035	$3.32 \times 10^{-1} \pm 2.86 \times 10^{-3}$	2.09	0.32	0.15
52	8.53	7.86	$1.31 \times 10^{-1} \pm 6.38 \times 10^{-3}$	0.51	0.057	0.027	$5.13 \times 10^{-1} \pm 6.07 \times 10^{-3}$	1.35	0.21	0.10
60	8.47	7.77	$2.37 \times 10^{-1} \pm 9.00 \times 10^{-3}$	0.28	0.031	0.015	$9.61 \times 10^{-1} \pm 4.82 \times 10^{-3}$	0.72	0.11	0.05

Table 3 Activation Energy Data at Each Concentration (1 mg/mL and 15 mg/mL) of Amoxicillin and (0.2 mg/mL and 3 mg/mL) Clavulanate, at Selected pH Values

Concentration (mg/mL)	pH	Activation Energy (Ea) (kJ mol^{-1})
Amoxicillin 1mg/mL	6.5	79.76
	7.0	50.63
	8.0	75.84
Clavulanate 0.2mg/mL	6.5	55.46
	7.0	60.10
	8.0	86.50
Amoxicillin 15mg/mL	8.0	46.7
Clavulanate 3mg/mL	8.0	57.6

Temperature dependency was established using the Arrhenius Equation (Equation 7) for the various pH values, concentration and temperature conditions. The slopes of the lines enable the activation energies to be calculated and are reported in Table 3. It was found that at pH 7.0 the lowest activation energy occurred for amoxicillin 1 mg/mL and at pH 8.0 for amoxicillin 15 mg/mL. The activation energy values allow extrapolation of data at higher temperatures to those at lower temperatures which has relevance to the infuser studies, which were performed at 2.9 °C. Equation 8 can be used to determine these values. When higher activation energies occur, then greater benefit of increased shelf-life is derived when temperature is lowered, such as to refrigeration temperatures (2 to 8 °C).

Elastomeric Infuser Study

Tables 4–6 show the stability profiles of amoxicillin and clavulanate in 0.9% NaCl with and without pH adjustment when stored in elastomeric balloon infusers. It is notable with the 1 mg/mL (0.2 mg/mL clavulanate) samples (Table 4) that lowering the pH from 8.73 to 6.52 showed improved stability of both compounds. The shelf-life of amoxicillin improved

Table 4 Mean Percentage Concentrations of Amoxicillin (1 mg/mL) and Clavulanate (0.2 mg/mL) Remaining in Infusers and pH Values, When Stored at 2.9°C in a Refrigerated Water Bath

Time (h)	Amoxicillin in 0.9% NaCl		Clavulanate in 0.9% NaCl		Amoxicillin in 0.9% NaCl at pH 6.5		Clavulanate in 0.9% NaCl at pH 6.5	
	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH
0.0	100.0	8.73	100.0	8.73	100.0	6.52	100.0	6.52
0.9	102.0	8.70	102.0	8.70	102.2	6.40	102.7	6.40
3.6	100.0	8.60	100.2	8.60	101.2	6.35	101.1	6.35
23.3	96.0	8.52	96.2	8.52	98.8	6.42	98.1	6.42
25.2	96.2	8.54	97.2	8.54	94.6	6.45	94.3	6.45
72.0	90.1	8.28	91.7	8.28	100.3	6.52	98.6	6.52
100.4	87.1	8.24	88.4	8.24	102.6	6.51	99.6	6.51
173.3	79.9	8.25	80.9	8.25	100.4	6.51	97.4	6.51
192.3	78.0	8.21	79.2	8.21	98.7	6.65	95.0	6.65
218.6	78.7	8.16	77.8	8.16	99.6	6.69	95.6	6.69
241.3	77.6	8.10	77.7	8.10	97.7	6.55	93.6	6.55
263.8	76.1	8.12	75.2	8.12	98.6	6.57	93.9	6.57

Table 5 Mean Percentage Concentrations of Amoxicillin (7.5 mg/mL) and Clavulanate (1.5 mg/mL) Remaining in Infusers and pH Values, When Stored at 2.9°C in a Refrigerated Water Bath

Time (h)	Amoxicillin in 0.9% NaCl		Clavulanate in 0.9% NaCl		Amoxicillin in 0.9% NaCl at pH 7.7		Clavulanate in 0.9% NaCl at pH 7.7	
	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH
0.0	100.0	8.85	100.0	8.85	100.0	7.69	100.0	7.69
2.13	95.1	8.87	94.4	8.87	99.5	7.49	99.5	7.49
4.23	95.8	8.84	94.2	8.84	100.6	7.48	100.2	7.48
23.5	85.2	8.71	77.1	8.71	94.5	7.48	92.5	7.48
26.9	83.9	8.75	74.3	8.75	93.1	7.51	90.7	7.51
28.4	82.4	8.80	68.9	8.80	92.4	7.58	91.3	7.58
48	77.7	8.68	64.8	8.68	91.2	7.50	90.3	7.50
50.1	76.3	8.65	62.4	8.65	91.1	7.50	87.1	7.50
51.8	76.9	8.65	62.3	8.65	90.7	7.48	87.4	7.48
70.9	71.8	8.60	54.6	8.6	86.9	7.47	80.5	7.47
94.9	65.9	8.45	46.4	8.45	82.1	7.42	74.1	7.42

Table 6 Mean Percentage Concentrations of Amoxicillin (15mg/mL) and Clavulanate (3 mg/mL) Remaining in Infusers and pH Values, When Stored at 2.9°C in a Refrigerated Water Bath

Time (h)	Amoxicillin in 0.9% NaCl		Clavulanate in 0.9% NaCl		Amoxicillin in 0.9% NaCl at pH 8.4		Clavulanate in 0.9% NaCl at pH 8.4	
	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH	Amount Remaining (%)	pH
0.0	100.0	8.68	100.0	8.68	100.0	8.40	100.0	8.40
1.6	99.7	8.71	96.8	8.71	95.1	8.37	92.4	8.37
2.8	94.1	8.69	89.7	8.69	92.4	8.35	88.8	8.35
3.8	93.9	8.67	87.9	8.67	91.8	8.33	86.9	8.33
23.7	79.5	8.74	52.5	8.74	77.3	8.43	54.7	8.43
96.3	57.5	8.47	16.5	8.47	56.0	8.21	21.5	8.21

from 72 h to >263.8 h (1.57 weeks) and clavulanate from 72 h to also >263.8 h. This shows that lowering the pH by 2.21 units had a marked impact on degradation rates. All solutions remained clear and colourless throughout the study. The increased decomposition rate of clavulanate compared with amoxicillin is less marked when compared to the kinetic studies (Table 1).

In the case of 7.5 mg/mL amoxicillin (1.5 mg/mL clavulanate) (Table 5) owing to the limited solubility of amoxicillin, the initial pH could not be lowered below pH 7.7. However, pH lowering again resulted in notable improvements in shelf-life values, which were evident for both compounds. Both amoxicillin and clavulanate had shelf-life values of 4.23 h, which increased for amoxicillin to 51.8 h and clavulanate to 48.9 h, respectively. All solutions remained clear, however, from 48 h an initial feint yellow colouring occurred which increased to a clear yellow colour at 94.9 h. It would be expected that the concentration-dependent ammonolysis reaction would be contributing to the degradation rate of amoxicillin with both compounds also undergoing hydrolysis.

In Table 6 the data reported is when the amoxicillin concentration was increased to 15 mg/mL (clavulanate 3 mg/mL). However, lowering the pH to 7.5 caused cloudiness in the solution owing to the poor solubility of amoxicillin. To ensure this would not occur, the solution was adjusted to pH 8.4. This small reduction in pH of approximately 0.3 of a pH unit

from pH 8.68 to 8.40 had little effect on the shelf-life values of either amoxicillin or clavulanate. The shelf-life was defined by clavulanate as 1.6 h. The amoxicillin shelf-life was also short at 3.8 h. It would be expected that the major reaction pathway for amoxicillin would be the concentration-dependent ammonolysis reaction, whereas for clavulanate, it would be hydrolysis. From 23 h, the solutions began to turn pale yellow and by 96 h this became a dark yellow. All solutions remained clear.

Discussion

This study has evaluated the stability profiles of amoxicillin and clavulanate in the context of the suitability of the combination for patients using OPAT. It is evident from the kinetics data that the stability of amoxicillin (1 mg/mL) and clavulanate (0.2 mg/mL) are pH and temperature dependent. At temperatures of 40 °C the shelf-life values of amoxicillin are short and clavulanate even lower. Extrapolation of these values to 25 °C (room temperature) makes administering amoxicillin by OPAT feasible but not clavulanate. These systems followed pseudo first-order kinetics, enabling the use of the Ea values (Table 3) to estimate shelf-life values over any temperature range using Equation 8 where the same reaction mechanism occurs. Increasing the concentration of amoxicillin to 15 mg/mL changed the reaction mechanism to include the autocatalytic ammonolysis reaction, becoming increasingly dominant with increased amoxicillin concentration. This reaction pathway increased the rate of amoxicillin degradation markedly compared with the 1 mg/mL system. In addition, the reduced solubility of amoxicillin below pH 8 limited the ability to lower the pH and therefore any capability to improve stability. Lowering the pH may be less successful because the ammonolysis reaction will have a different rate-pH profile. These kinetic data are reflected in the elastomeric infuser study when using 1 mg/mL amoxicillin demonstrated the advantage of lowering the temperature to 2.9 °C and lowering the pH to improve storage shelf-life. Notably lowering the initial pH from 8.73 to 6.52 resulted in an almost 4-fold improvement in shelf-life for amoxicillin and clavulanate at 2.9 °C. This improved stability would also be reflected in improved stability when administering this combination at or slightly above room temperature, since amoxicillin and clavulanate had shelf-life values of 22.8 and 4.0 h, respectively. Increasing amoxicillin concentration to 7.5 mg/mL or 15 mg/mL resulted in marked reductions in amoxicillin and clavulanate shelf-life values, owing to the pH rise and the impact of the ammonolysis reaction, decreasing amoxicillin stability. Although amoxicillin 7.5 mg/mL retained a shelf-life after pH lowering of 51.8 h, clavulanate limited the overall value to 48.0 h. The 15 mg/mL amoxicillin concentration had shelf-life values of 3.8 h at 2.9 °C, and 1.6 h for clavulanate.

Several studies have reported data on the stability of amoxicillin alone or with clavulanate. At 22 °C, 10 mg/mL amoxicillin (2 mg/mL clavulanate) diluted using 0.9% sodium chloride, amoxicillin was reported as stable for 12 h; however, clavulanate retained its shelf-life for less than 3 h.[19] A recent study using 14.2 mg/mL amoxicillin and 2.86 mg/mL clavulanate prepared using Water for Injection were assessed at 4 °C, 25 °C and 37 °C. Amoxicillin at 4 °C had a shelf-life of 80.3 h and at 25 °C 24.8 h. Clavulanate values were 9.6 and 7.5 h, respectively.[20] These shelf-life values were higher than found in this study; neither study reported pH values. A recent study of amoxicillin alone, which used much higher concentrations of amoxicillin of 25 mg/mL to 250 mg/mL prepared in Water for Injection, reported amoxicillin solubility issues above 150 mg/mL. For 25 mg/mL the shelf-life at either 18–26 °C or 4–8 °C were 12 and 24 hours, respectively, and markedly higher than would have been expected based on this study.[4] No pH data are provided for that study. A further study has examined both amoxicillin stability and its plasma concentrations. They reported that when amoxicillin was prepared at 6 g per 240 mL (25 mg/mL) at 5 °C, the mean concentration of amoxicillin fell from 29.0 to 26.4 g/L (mg/mL) over 48 h. The plasma levels, however, provided an adequate therapeutic effect.[21] That study also reported that when the infusers were carried by volunteers for 24 h the mean concentration fell from 26.4 to 18.0 mg/mL (32%).[21] The need of exposing the infusers to ambient temperatures during administration has resulted in their exposure to lengthy periods above 25 °C. This was also dependent on whether they were retained on the patient's waist or kept outside the blankets at night, showing mean temperatures of either 30.9 °C or 26.2 °C.[22] Another study reported average kinetic temperatures over 24 hours of 17.4 °C in winter and 28.4 °C in summer; the latter achieved using ice-bricks changed at 8 h intervals.[23] Exposure to variable ambient temperatures can therefore impact on the feasibility of providing an OPAT service for antibiotics of limited stability.

This study has indicated that the combination of amoxicillin and clavulanate was not feasible for use in infusers for OPAT. Clinical concentrations required for OPAT would often require 15 mg/mL or higher. These findings indicate that would be unachievable in elastomeric infusers. The data reported in this study provided information that could be useable for preparing pre-loaded syringes or mini-bags enabling delivery over one to two hours.

A limitation of this study was the small number of time points in the 15 mg/mL study, where additional points could have better defined the shelf-life. The hydrochloric acid used was not sterile and some experimentation using lower strengths of acid, prepared sterile by filtration or autoclaved in ampoules, would be required. Measurement of pH in an aseptic area would be feasible by removing a small aliquot of the solution and measuring pH outside the laminar flow hood or enclosed hood system and then discarding it.

Conclusions

Amoxicillin prepared to be administered intravenously with clavulanate has a limited shelf-life, which is influenced by temperature, pH and amoxicillin concentration. Near pH 6 using low refrigeration temperatures stabilises low concentrations of amoxicillin and clavulanate. At clinical concentrations (15 mg/mL amoxicillin and 3 mg/mL clavulanate), neither component is satisfactorily stable for routine administration by OPAT. The limited shelf-life at 25 °C or higher also limits the time an infuser can be exposed to ambient temperatures. Amoxicillin at higher concentrations is also limited by its solubility, requiring even higher pH values to maintain adequate solubility. With the impact of higher concentrations further deceasing amoxicillin stability, it limits the options for stabilising these systems. It may be feasible to prepare pre-filled syringes at clinical concentrations which could be stored at low refrigeration temperature for approximately 2 hours but further studies would be necessary to develop a stable clinical delivery model. Formulating low concentrations, as close as possible to pH 7 and using the lowest possible temperature, without freezing, provides the optimum conditions for stability.

Data Sharing Statement

The datasets generated and used in this current study are available from the corresponding author on reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in conception, study design, execution, acquisition of data, analysis and interpretation, or in these areas; took part in drafting, revising or critical reviewing the article, gave final approval of the version to be published, have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Funds were provided from the Curtin Medical School to support the cost of consumables for this project. We would like to acknowledge Zach Nizich, Jason Seet and Sir Charles Gairdner Hospital for providing some infuser sets without charge.

Disclosure

The authors have no conflicts of interest with respect to this study.

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