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Data Article

Data of clavulanic acid and clavulanate-imidazole stability at low temperatures

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ABSTRACT

Clavulanic acid (CA) is a β -lactam antibiotic with a strong inhibitory effect on β -lactamase enzymes. CA is produced in submerged cultures by the filamentous Gram-positive bacterium *Streptomyces clavuligerus* (*S. clavuligerus*). CA is an unstable molecule in aqueous solution and its stability depends strongly on temperature and concentration. In this contribution, the experimental data of CA stability, produced in chemically defined media and exposed to temperatures between -80 and 25 °C, are presented. The chromophore clavulanate-imidazole (CAI) is commonly used for analysis and quantification of CA samples by High Performance Liquid Chromatography (HPLC); nevertheless, this molecule is also susceptible to suffer degradation in aqueous solution, potentially affecting the quantification of CA. Data of CAI concentration for samples conserved at 4 °C and 25 °C are also presented. A reversible-irreversible kinetic model was applied to estimate the degradation rate of CA. Data from numerical simulations of CA degradation using the proposed kinetic model are also graphically presented. The data show the clavulanic acid instability in fermentation broths, in a range of temperatures of interest for

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bioprocess operation, downstream processing, samples quantification, conservation and storage.

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Specifications table

Subject area	Biotechnology
More specific subject area	Antibiotics stability and Antibiotics analysis
Type of data	Table, equation, figure
How data was acquired	Analysis of clavulanic acid concentration by HPLC with a DAD detector (1200 Series, Agilent Technologies, Waldbronn, Germany) and a Zorbax Eclipse XDB-C-18 chromatographic column with a C-18 guard column [1]
Data format	Processed, simulated
Experimental factors	Supernatant samples (undiluted, diluted 1:2 and 1:5) from <i>S. clavuligerus</i> batch fermentations were stored at -80°C , -20°C , 4°C and 25°C for analysis of clavulanic acid at different times in a time span of 43 h. Additionally, chromophore clavulanate-imidazole samples (0.636 mM and 0.310 mM) were stored at 4°C and 25°C and their concentration were measured at different times, up to 46 h.
Experimental features	Kinetics of degradation of clavulanic acid from fermentation broths and at different initial concentrations was determined at low temperatures of storage and pH 6.8, using a factorial experimental design. Kinetics of degradation of the chromophore clavulanate-imidazole with different initial concentrations was calculated with a 2-squared factorial design. Linear regression was applied to the data for the determination of kinetic parameters.
Data source location	Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Berlin, Germany.
Data accessibility	Data are presented in this article only
Related research article	D. Gómez-Ríos, H. Ramírez-Malule, P. Neubauer, S. Junne, R. Ríos-Esteva, Degradation Kinetics of Clavulanic Acid in Fermentation Broths at Low Temperatures, Antibiotics. 8 (2019) 6. https://doi.org/10.3390/antibiotics8010006 .

Value of the data

- Data show the clavulanic acid instability in fermentation broths, in a range of temperatures of interest for bioprocess operation, downstream processing, samples quantification, conservation and storage.
- Data of instability of the chromophore clavulanate-imidazole are of utmost importance in the analytical field, since quantification of clavulanic acid must consider the time elapsed between sample collection, sample derivatization and quantification.
- The basic information about thermal and concentration effects on clavulanic acid degradation kinetics are required to understand the instability of the clavulanic acid molecule in aqueous solutions and their potential reaction mechanisms.
- The applied kinetic model of degradation and simulation allow to predict the degradation of clavulanic acid at different concentrations and temperatures.
- The obtained data can be useful for further comparative initiatives of exploring clavulanic acid stability under different conditions.

1. Data

The mean concentrations of clavulanic acid (CA) and standard deviations (SD) for processed samples with initial concentrations (CA_0) of 0.636 mmol/L (Table 1), 0.329 mmol/L (Table 2), 0.127 mmol/L (Table 3), and 0.082 mmol/L (Table 4) stored at -80 , -20 , 4 and 25°C , are presented. Concentration data of the chromophore clavulanate-imidazole (CAI) with initial concentrations (CAI_0) of 0.310 mmol/L and 0.636 mmol/L, conserved at 4°C and 25°C , are presented in Tables 5 and 6, respectively. Figs. 1–4 show numerical simulation data of the equilibrium-irreversible first-order kinetic model used to describe the degradation of CA samples.

Table 1

Mean CA concentrations for samples of $CA_0 = 0.636$ mmol/L (126.7 mg/L) conserved at -80 , -20 , 4 and 25 °C, and quantified at different times.

Time (h)	T = -80 °C		T = -20 °C		T = 4 °C		T = 25 °C	
	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)
0.0	0.636	0.001	0.636	0.002	0.636	0.006	0.636	0.006
3.1	0.632	0.006	0.603	0.003	0.594	0.009	0.574	0.006
5.4	0.630	0.008	0.584	0.003	0.573	0.009	0.551	0.007
18.3	0.625	0.002	0.545	0.006	0.514	0.007	0.478	0.009
31.0	0.623	0.008	0.527	0.005	0.484	0.005	0.430	0.010
42.1	0.623	0.008	0.513	0.005	0.461	0.009	0.410	0.007

Table 2

Mean CA concentrations for samples of $CA_0 = 0.329$ mmol/L (65.5 mg/L) conserved at -80 , -20 , 4 and 25 °C, and quantified at different times.

Time (h)	T = -80 °C		T = -20 °C		T = 4 °C		T = 25 °C	
	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)
0.0	0.329	0.003	0.329	0.003	0.329	0.003	0.329	0.003
3.1	0.327	0.004	0.314	0.003	0.303	0.004	0.295	0.002
5.4	0.328	0.004	0.306	0.003	0.289	0.004	0.278	0.002
18.3	0.323	0.003	0.290	0.004	0.276	0.003	0.259	0.004
31.0	0.322	0.004	0.279	0.004	0.264	0.003	0.242	0.003
42.1	0.322	0.005	0.270	0.003	0.254	0.005	0.233	0.002

Table 3

Mean CA concentrations for samples of $CA_0 = 0.127$ mmol/L (25.3 mg/L) conserved at -80 , -20 , 4 and 25 °C, and quantified at different times.

Time (h)	T = -80 °C		T = -20 °C		T = 4 °C		T = 25 °C	
	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)
0.0	0.127	0.002	0.127	0.006	0.127	0.006	0.127	0.006
3.1	0.126	0.007	0.120	0.008	0.118	0.008	0.116	0.006
5.4	0.126	0.006	0.118	0.008	0.115	0.004	0.111	0.005
18.3	0.125	0.005	0.110	0.009	0.106	0.006	0.100	0.007
31.0	0.125	0.007	0.108	0.007	0.103	0.009	0.097	0.005
42.1	0.124	0.010	0.107	0.010	0.102	0.010	0.095	0.007

Table 4

Mean CA concentrations for samples of $CA_0 = 0.082$ mmol/L (16.3 mg/L) conserved at -80 , -20 , 4 and 25 °C, and quantified at different times.

Time (h)	T = -80 °C		T = -20 °C		T = 4 °C		T = 25 °C	
	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)	[CA] (mmol/L)	SD (mmol/L)
0.0	0.082	0.008	0.082	0.006	0.082	0.006	0.082	0.006
3.1	0.082	0.003	0.078	0.006	0.077	0.001	0.075	0.008
5.4	0.081	0.008	0.076	0.008	0.074	0.007	0.072	0.004
18.3	0.081	0.002	0.071	0.009	0.069	0.010	0.065	0.004
31.0	0.080	0.005	0.070	0.009	0.067	0.002	0.063	0.007
42.1	0.080	0.008	0.070	0.008	0.066	0.005	0.062	0.010

Table 5

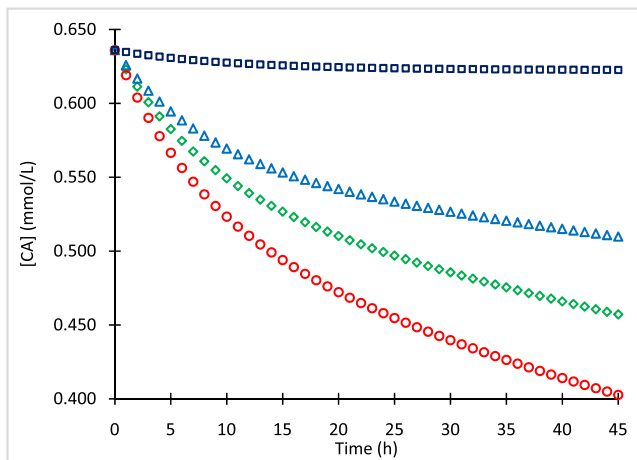
Mean CAI concentrations of samples conserved at 4 °C and quantified at different time points.

[CAI ₀] = 0.310 mmol/L			[CAI ₀] = 0.636 mmol/L		
Time (h)	[CAI] (mmol/L)	SD (mmol/L)	Time (h)	[CAI] (mmol/L)	SD (mmol/L)
0.0	0.307	0.004	0.000	0.636	0.002
21.0	0.186	0.004	24.200	0.386	0.007
30.3	0.162	0.010	34.600	0.343	0.009
41.0	0.135	0.009	46.000	0.254	0.002

Table 6

Mean CAI concentrations of samples conserved at 25 °C and quantified at different timepoints.

[CAI ₀] = 0.310 mmol/L			[CAI ₀] = 0.636 mmol/L		
Time (h)	[CAI] (mmol/L)	SD (mmol/L)	Time (h)	[CAI] (mmol/L)	SD (mmol/L)
0.0	0.310	0.008	0.000	0.636	0.005
3.0	0.279	0.005	4.900	0.527	0.003
15.3	0.174	0.008	21.700	0.280	0.002
23.7	0.141	0.005	25.700	0.235	0.004
33.6	0.088	0.006	41.500	0.133	0.008
40.1	0.066	0.007	45.400	0.113	0.003

**Fig. 1.** Simulation of CA degradation of CA₀ = 0.636 mmol/L at -80 °C (squares), -20 °C (triangles), 4 °C (diamonds) and 25 °C (circles).

2. Experimental design, materials, and methods

Batch fermentations of *S. clavuligerus* DSM 41826 were carried out in a 15 L stirred tank bioreactor (Techfors S, Infors AG, Bottmingen, Switzerland) operated at 5 L filling volume using chemically defined media, pH 6.8, 0.6 vvm and 28 °C [2,3]. Samples (50 mL) of fermentation broths were withdrawn at 36 h of cultivation coinciding with phosphate limitation and onset of the exponential growth phase. Biomass was separated by centrifugation at 12000 rpm and filtration using 0.2 μm pore size filters. Supernatants containing CA were adjusted to pH 6.8 and then vortexed and divided into 2 mL aliquots in Eppendorf tubes, according to the number of treatments. Dilutions (1:2 and 1:5) were also prepared. Finally, samples were divided into four groups and stored at the corresponding exposition temperatures (-80 °C, -20 °C, 4 °C and -25 °C).

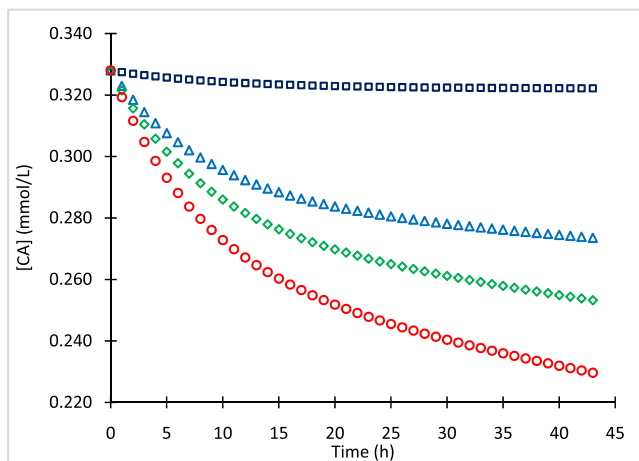


Fig. 2. Simulation of CA degradation of $CA_0 = 0.329$ mmol/L at -80 °C (squares), -20 °C (triangles), 4 °C (diamonds) and 25 °C (circles).

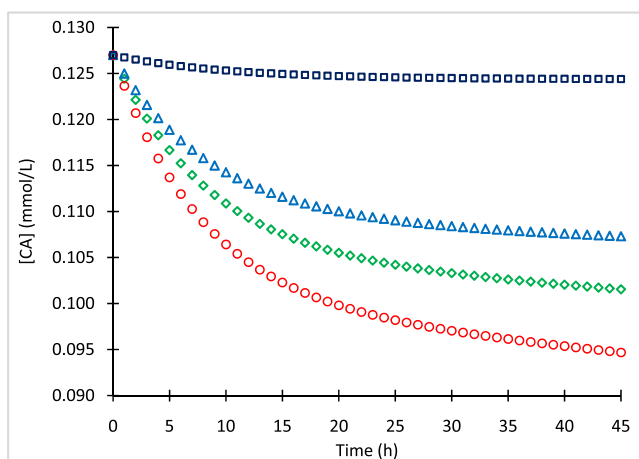


Fig. 3. Simulation of CA degradation of $CA_0 = 0.127$ mmol/L at -80 °C (squares), -20 °C (triangles), 4 °C (diamonds) and 25 °C (circles).

The CA degradation was tested using a factorial experimental design, wherein concentration and temperature were defined as factors varying at three and four levels, respectively. CA_0 of the supernatant was set as the highest level, whereas dilutions 1:2 and 1:5 were set as the medium and low levels, respectively. Twelve experimental runs were performed by duplicate. Supernatant samples were stored at -80 °C, -20 °C, 4 °C and 25 °C, respectively, for 43 h. Samples were withdrawn at 3.1 h, 5.4 h, 18.3 h, 31.0 h and 42.1 h of storage, derivatized with imidazole solution 20% m/v during 30 min at 30 °C and 800 rpm in mixing block, and immediately analyzed in a High Performance Liquid Chromatography (HPLC) device (1200 Series, Agilent Technologies, Waldbronn, Germany). Additional runs of supernatant samples with higher CA_0 (0.636 mmol/L), from a different batch cultivation produced under identical conditions, were exposed to the above-mentioned temperatures and treated as previously indicated. In the study of stability of the chromophore CAI, a 2-squared factorial design with duplicates was applied. Derivatized CA samples with a CAI_0 of

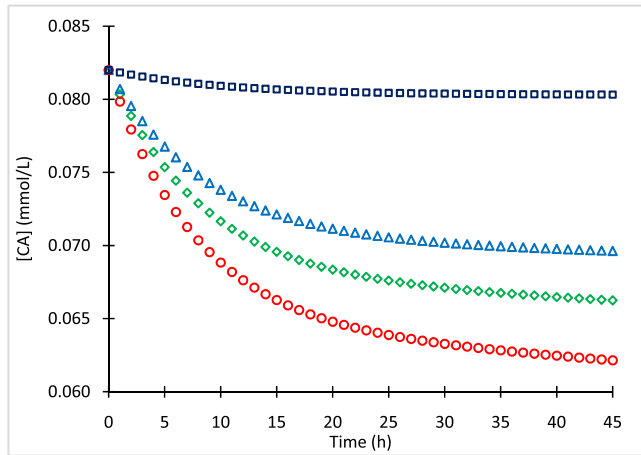


Fig. 4. Simulation of CA degradation of $CA_0 = 0.082$ mmol/L at -80 °C (squares), -20 °C (triangles), 4 °C (diamonds) and 25 °C (circles).

0.636 mmol/L and 0.310 mmol/L were stored at 4 °C and 25 °C. Aliquots were withdrawn at different intervals in a time span of 46 h.

CA is poorly retained in C-18 reverse phase columns, thus the preparation of the chromophore CAI is required for detection and quantification [1]. Derivatization reagent was prepared by dissolving 8.25 g of imidazole in 24 mL of distilled water; the pH of the derivatization reagent was adjusted to 6.8 by addition of HCl (25%v/v) and distilled water was added up to 40 mL. The derivatization of CA samples was performed by adding 100 μ L of imidazole reagent to 300 μ L of sample, followed by agitation in a mixing block at 800 rpm, at 30 °C during 30 min.

The derivatized samples were analyzed in an Agilent Technologies 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with DAD detector, using a Zorbax Eclipse XDB-C-18 chromatographic column (Agilent Technologies, Waldbronn, Germany) and a C-18 guard column (Phenomenex[®], Aschaffenburg, Germany). Quantifications were performed at 30 °C, flow rate of 1 mL/min and an injection volume of 25 μ L. The mobile phase consisted of KH_2PO_4 (pH 3.2; 50 mM) and methanol (HPLC grade) as the solvents A and B, respectively. For the analysis, a gradient method was used as described: linear gradient from 6% to 7.6% solvent B for 8 min, linear gradient to 95% solvent B for 2 min, 95% solvent B for 2 min and linear gradient to 6% solvent B for 2 min [1]. The chromophore CAI was detected at 311 nm wavelength. The calibration line used in the quantifications is presented in Eq. (1), where [CAI] is the concentration of the chromophore detected and A is the integration area [1]. The calibration was valid in the range 0.2–400 mg/L.

$$[\text{CAI}] = 86.73 A + 155.86 \quad (1)$$

The mechanistic approach of CA degradation included an equilibrium reaction, in which an active intermediate (I^*) is produced by a first-order reversible reaction (Eqs. (2) and (3)) with forward and backward constants k_1 and k_{-1} , respectively. This reaction is followed by a first-order irreversible reaction (Eq. (4)) with kinetic rate constant k_2 . In this irreversible step, the formed intermediate I^* reacts with a CA molecule to form the final degradation product (D), as follows [4–7]:



$$K_{eq} = \frac{k_1}{k_{-1}} \quad (3)$$



Numerical simulation of the reaction network was performed by solving the resulting differential equations according to the rate expressions presented in Eqs. (2) and (4) and the equilibrium constant defined by Eq. (3). The values of the kinetic rate constants are available in the literature [5]. The ordinary differential equation (ODE) system was solved by using the deterministic method LSODA for stiff and non-stiff ODEs [8].

Acknowledgments

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Transparency document

Transparency document associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2019.103775>.

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