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pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin

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HIGHLIGHTS

- Three β-lactam antibiotics were studied: ampicillin, cefalotin and cefoxitin.
- Antibiotic hydrolysis was investigated under different pH and temperature regimes.
- Half-lives were 5.3–27days at pH7 & 25°C; increased 2.5–3.9 fold for each 10°C rise.
- Arrehenius coefficients were calculated.
- Degradation products showed hydrolysis of lactam, ester, carbamate and amide moieties.

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ABSTRACT

An understanding of antibiotic hydrolysis rates is important for predicting their environmental persistence. Hydrolysis rates and Arrhenius constants were determined as a function of pH and temperature for three common β-lactam antibiotics, ampicillin, cefalotin, and cefoxitin. Antibiotic hydrolysis rates at pH 4–9 at 25 °C, 50 °C, and 60 °C were quantified, and degradation products were identified. The three antibiotics hydrolyzed under ambient conditions (pH 7 and 25 °C); half-lives ranged from 5.3 to 27 d. Base-catalyzed hydrolysis rates were significantly greater than acid-catalyzed and neutral pH hydrolysis rates. Hydrolysis rates increased 2.5- to 3.9-fold for a 10 °C increase in temperature. Based on the degradation product masses found, the likely functional groups that underwent hydrolysis were lactam, ester, carbamate, and amide moieties. Many of the proposed products resulting from the hydrolysis of ampicillin, cefalotin, and cefoxitin likely have reduced antimicrobial activity because many products contained a hydrated lactam ring. The results of this research demonstrate that β-lactam antibiotics hydrolyze under ambient pH and temperature conditions. Degradation of β-lactam antibiotics will likely occur over several weeks in most surface waters and over several days in more alkaline systems.

1. Introduction

Contamination of surface and ground waters by synthetic antibiotic compounds is a potential threat to human and ecological health. The increase in bacterial antibiotic resistance observed over the past three decades (Knapp et al., 2010) has resulted in a rise in life threatening infections such as methicillin-resistant *Staphylococcus aureus* (MRSA). Antibiotic contamination in cattle feedlots can drive selection for antibiotic resistance in *E. coli* (Subbiah et al., 2012), and antibiotic contamination of surface waters has been shown to impart sub-lethal effects to aquatic biota (Park and Choi, 2008).

As a result of antibiotic-resistant pathogens, new antibiotics have been developed and antibiotic usage has increased (Zinner, 2005). Wise (2002) reported that 100,000 to 200,000 metric tons of antibiotics are consumed annually worldwide; the most common class, β-lactam antibiotics, makes up 50–70% of sales (Kummerer, 2009). Two of the most common antibiotic groups within the β-lactam class are penicillin and cephalosporin antibiotics; they make up 44% and 15% of the antibacterial products sold in the U.S. from 2010 to 2011 for use in human medicine (US FDA, 2012). Sulfonamides and trimethoprim make up 15%, quinolones 9%, macrolides 5%, tetracyclines and nitroimidazoles 4% each, and lincosamides 2%; other

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antibiotic classes make up less than 1% (US FDA, 2012). Penicillins and cephalosporins comprise approximately 4.7% and 0.3% of the antimicrobial sales in animal husbandry (US FDA, 2009).

Antibiotic contamination of surface and ground water can occur through municipal and agricultural waste liquid and solid effluents. Antibiotics have been quantified in municipal effluent receiving streams (Massey et al., 2010) and in soil leachate from contaminated manure, biosolids, and reclaimed water land application (Dolliver and Gupta, 2008; Kinney et al., 2006; Hamscher et al., 2005). The releases of antibiotics to the environment are significant because a large percentage of antibiotic doses are excreted unchanged, and can remain bioactive in soil-water systems (Subbiah et al., 2011). For example, approximately 30% of ampicillin is excreted when administered orally, and 75% is excreted after intravenous administration (Clarke and Moffat, 1986). About 90% of cefoxitin doses are excreted in urine when administered intravenously (Schrogie et al., 1978), and about 85% of cefalotin doses are excreted (Gambertoglio et al., 1983). β-Lactam antibiotics are removed by 95% after 10 h during conventional municipal wastewater treatment (Li and Zhang, 2010); they are not frequently detected in surface water, most likely due to their higher removal efficiencies during wastewater treatment and also relatively high method reporting limits (Massey et al., 2010). The concentration of one β-lactam antibiotic was found to be 2.1–3.5 μg/L in a swine lagoon, which was near the detection limit of 2 μg/L (Campagnolo et al., 2002).

Hydrolysis can be a significant degradation pathway in the environment for some organic compounds, especially esters and amides. Products of ester and amide hydrolysis may be less bioaccumulative than the parent compound because they are more polar, resulting in higher water solubility and lower octanol water partition coefficients (Lin et al., 2007). Common hydrolysis reaction sites on antibiotics include labile carbonyl moieties such as esters, lactones, and lactams (Waterman et al., 2002). Hydrolysis half-lives of organic contaminants vary from minutes to years. In general, esters are more reactive than amides; ester-like compounds, such as carbamate esters, also rapidly undergo hydrolysis (Watts, 1998).

Rates of hydrolysis are a function of environmental conditions, with temperature and pH the most important parameters. For example, hydrolysis rates typically increase as temperature increases. Base-catalyzed hydrolysis predominates over acid-catalyzed and neutral hydrolysis for many organic compounds (Mabey and Mill, 1978); although water and hydroxide anion are both attracted to electron-deficient atoms, hydroxide anion is a stronger nucleophile than water. Alternatively, hydrolysis may be catalyzed in acidic solutions when protons shift the electron density of a molecule, so that a susceptible site can be attacked by water (Larson and Weber, 1994). Hydrolysis rates are also affected by ionic strength (Mabey and Mill, 1978) as well as the presence of divalent metal ions (Gensmantel et al., 1980), oxide surfaces (Torrents and Stone, 1994), and clays (Pusino et al., 1996). Hydrolysis of labile functional groups is a major degradation pathway in systems without a large microorganism population, such as streams, rivers, and ground waters; however, biodegradation pathways are usually predominant in wastewater. For example, Li et al. (2011) calculated a biodegradation half-life of 6.6 d in 5% cattle manure flush water for a cephalosporin antibiotic compared to a hydrolysis half-life of 96 d in sterilized flush water.

An understanding of antibiotic hydrolysis rates is important for predicting their environmental persistence, especially as new antibiotics are developed and subsequently released to the environment. Hydrolysis rates have not yet been determined for the β-lactam antibiotics ampicillin, cefalotin, and cefoxitin, and hydrolysis degradation products have not been shown for these antibiotics. The objectives of this research were to (1) determine second-order acid and base hydrolysis rate constants for three β-lactam antibiotics (ampicillin, cefalotin, and cefoxitin) over a range of temperature and pH regimes, (2) calculate Arrhenius coefficients for hydrolysis rates of the antibiotics, and (3) display hydrolysis degradation product masses and proposed structures for the three β-lactam antibiotics.

2. Materials and methods

2.1. Materials

The three β-lactam antibiotics, ampicillin, cefalotin, and cefoxitin, were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). All three of the compounds have one or more hydrolytically reactive moiety. Each has a lactam ring and secondary amide; in addition, cefalotin has a side chain with a carboxylic acid ester and cefoxitin has a side chain with a carbamate group. The antibiotic physical properties are listed in Table 1.

Methanol, sodium acetate, sodium borate, boric acid, and formic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetic acid was obtained from Fisher Scientific (Hampton, NH, USA). Double-deionized water was purified to >18 MΩ•cm using a Barnstead E-pure system (Dubuque, IA, USA).

2.2. Experimental conditions

Acetate and borate buffers (10 mM) at pH 4 to 9 were prepared daily. These buffers are commonly used in hydrolysis studies to minimize effects caused by nucleophilic salts that can promote acid- or base-catalyzed hydrolysis (Mabey and Mill, 1978).

Reactions consisted of 5 mL of buffered solutions adjusted to pH 4, 5, 6, 7, 8, or 9 in 20 mL glass volatile organic analysis vials. The solutions were incubated for at least 6 h prior to antibiotic addition; a 25 μL aliquot of the antibiotic stock solution (1 g L^{-1} in methanol) was then added to the incubated buffer and mixed for a final antibiotic concentration of 5 mg L^{-1}. The final organic solvent content of the reaction was 0.5% by volume. Control reactions were conducted in parallel with deionized water (pH 6.5) used in place of buffer solution to observe any variations in hydrolysis rate caused by the buffer.

The vials were then mixed and incubated in the dark at a constant temperature in an MIR-154 Sanyo incubator. The temperatures used

Table 1
β-Lactam antibiotic properties.

Compound	MW (g mol^{-1})	Water solubility (mg L^{-1})	Log K_{ow}	pK_a	Henry's law constant (atm m^3 mol^{-1})
Ampicillin sodium salt	371.4	10,100 (21 °C)[a]	1.35[a]	2.5, 7.3 (23 °C)[c]	2.4×10^{-17}[b]
Cefalotin sodium salt	418.4	158 [b]	−0.41 [a]	2.2 (35 °C)[c]; 3.8[d]	1.7×10^{-17}[b]
Cefoxitin sodium salt	449.4	105[b]	−0.02[a]	3.5[e]	6.5×10^{-21}[b]

MW — Molecular weight.
[a] (US EPA, 2008).
[b] (US NLM, 2011).
[c] (Clarke and Moffat, 1986).
[d] (SRC, 2009).
[e] (El-Shaboury et al., 2007).