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Modeling solubility, acid–base properties and activity coefficients of amoxicillin, ampicillin and (+)6-aminopenicillanic acid, in $NaCl_{(aq)}$ at different ionic strengths and temperatures

Francesco Crea *, Daniela Cucinotta, Concetta De Stefano, Demetrio Milea, Silvio Sammartano, Giuseppina Vianelli

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, Viale Ferdinando Stagno d'Alcontres, 31, I-98166 Messina (Vill. S. Agata), Italy

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ABSTRACT

The total solubility of three penicillin derivatives was determined, in pure water and NaCl aqueous solutions at different salt concentrations (from ~0.15 to 1.0 mol L^{-1} for ampicillin and amoxicillin, and from ~0.05 to 2.0 mol L^{-1} for (+)6-aminopenicillanic acid), using the shake-flask method for generating the saturated solutions, followed by potentiometric analysis. The knowledge of the pH of solubilization and of the protonation constants determined in the same experimental conditions, allowed us to calculate, by means of the mass balance equations, the solubility of the neutral species at different ionic strength values, to model its dependence on the salt concentration and to determine the corresponding values at infinite dilution. The salting parameter and the activity coefficients of the neutral species were calculated by the Setschenow equation.

The protonation constants of ampicillin and amoxicillin, determined at different temperatures (from T = 288.15 to 318.15 K), from potentiometric and spectrophotometric measurements, were used to calculate, by means of the Van't Hoff equation, the temperature coefficients at different ionic strength values and the corresponding protonation entropies. The protonation enthalpies of the (+)6-aminopenicillanic acid were determined by isoperibol calorimetric titrations at T = 298.15 K and up to I = 2.0 mol L^{-1}. The dependence of the protonation constants on ionic strength was modeled by means of the Debye–Hückel and SIT (Specific ion Interaction Theory) approaches, and the specific interaction parameters of the ionic species were determined. The hydrolysis of the β-lactam ring was studied by spectrophotometric and H NMR investigations as a function of pH, ionic strength and time.

Potentiometric measurements carried out on the hydrolyzed (+)6-aminopenicillanic acid allowed us to highlight that the opened and the closed β-lactam forms of the (+)6-aminopenicillanic acid have quite different acid–base properties. An analysis of literature solubility, protonation constants, enthalpies and activity coefficients is reported too.

1. Introduction

The penicillins are naturally occurring compounds produced by different microorganisms and the first class of antibiotic available for humans. These molecules have a selective anti-bacterial toxicity and perform their activity interfering on the growth processes and on the proliferation of the microorganisms. Some penicillins behave inhibiting the cellular wall growth of bacteria, others interfering with their mechanism of the proteic synthesis (Miller, 2002; Oshiro, 1999; Raynor, 1997).

The fundamental part of penicillin derivatives is represented by the β-lactam ring which is associated to a thiazolidine molecule [6-aminopenicillanic acid (6-APA), Fig. 1a]. The (+)6-aminopenicillanic acid is an intermediate product with special importance for the pharmaceutical industry, since is the main starting block for the preparation of numerous semisynthetic penicillins (β-lactam antibiotics) by means of acylation, esterification, amidation and hydroxyamidation reactions, etc.; this allows to obtain new derivatives with enlarged spectrum of biological activity.

Among the different penicillin types, the amino-penicillins are undoubtedly the most important, since the primary amino-group is bound to a large radical that avoid the attack of the penicillinase to the β-lactam ring; the amino-penicillins have also a fairly large anti-bacteria activity (Archer et al., 1970; Gower et al., 1983; Gutiérez Navarro et al., 1998; Krasnikova and Iozep, 2003; Kupka, 1997; Lepidi and Nuti, 1971; Miller, 2002; Oshiro, 1999; Pratt et al., 1996; Proctor et al., 1982; Raynor, 1997).

* Corresponding author. Tel.: +39 0906765761; fax: +39 090392827.
E-mail address: fcrea@unime.it (F. Crea).

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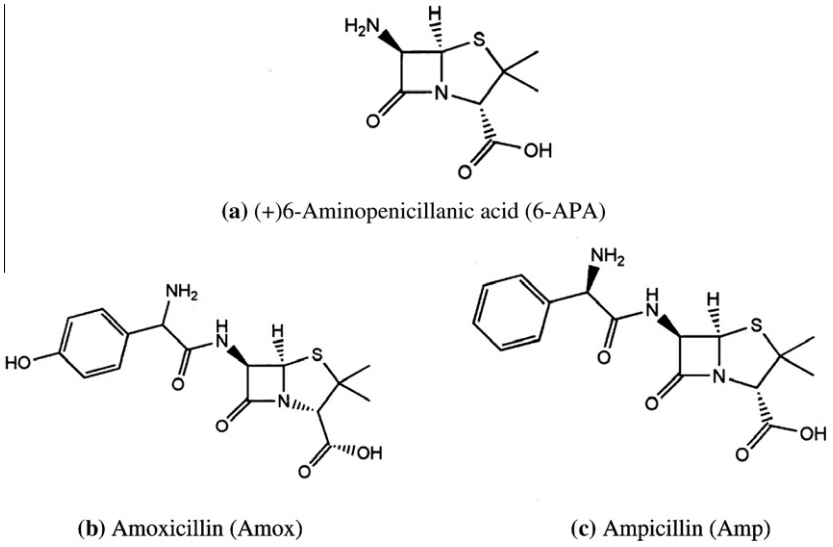

(a) (+)6-Aminopenicillanic acid (6-APA)

(b) Amoxicillin (Amox)

(c) Ampicillin (Amp)

Fig. 1. Structural formula of the ligands.

Amoxicillin and ampicillin are the most important amino-penicillins; these antibiotics have similar mechanisms of action, since they do not kill bacteria, but they stop bacteria from multiplying, by preventing bacteria from forming the walls that surround them. This wall is necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together. Amino-penicillins are effective against many different bacteria including *Haemophilus influenzae*, *Neisseria gonorrhoea*, *Escherichia coli*, pneumococci, streptococci, and some staphylococci (Miller, 2002; Oshiro, 1999; Raynor, 1997).

Amoxicillin, is usually the drug of choice within the class, because it is better absorbed than other β-lactam antibiotics.

In different industrial fields, the knowledge of the drug solubility is a very important task for pharmaceutical product design, because it affects the drug efficacy, its future development and formulation efforts, and also influences the pharmacokinetics, such as the release, transport and the degree of absorption in the organism (Bard et al., 2008; Belal et al., 2000; Domanska et al., 2011; Hamelink et al., 2002; Mota et al., 2009; Ruckenstein and Shulgin, 2004; Rudolph et al., 2001; Su et al., 2007; Tavare and Jadhav, 1996).

Solubility data involving new drugs are frequently not available in the literature, and in some cases thermodynamic models can be used to predict drug solubility (Bard et al., 2008; Domanska et al., 2011; Ni and Yalkowsky, 2003; Xu et al., 2011); the predicted solubility values have some time low accuracy, and for this reason, the availability of experimental data is still fundamental for an appropriate model development and evaluation.

Since many years, our research group has undertaken a systematic study of the modeling of the acid–base properties and solubility of different class of ligands (Battaglia et al., 2008; Bretti et al., 2008a, 2008b, 2005; Bretti et al., 2006) and of some molecules of great interest from a biological point of view (Bretti et al., 2012b; Cataldo et al., 2009; Cigala et al., 2012, 2010). The information achieved from these investigations allowed us to determine the total solubility of the ligands and of the neutral species, and to calculate the corresponding activity coefficients using the Setschenow equation (Setschenow, 1889).

The literature reports many information about the biological activity and the application of such molecules in medicine (Miller, 2002; Oshiro, 1999; Raynor, 1997), whilst data regarding their thermodynamic properties appear till now few and confusing. Any significant modeling study regarding the dependence of such parameters on ionic medium, ionic strength and temperature is reported, despite this kind of investigation allows researchers to propose simple semi-empirical equations able to model the behavior of the solution thermodynamic parameters over a wide range of experimental conditions.

In this order, the aim of this paper is to give an important contribution to the knowledge of the thermodynamic properties (protonation constants, solubility, formation enthalpies and entropies) and behavior of three different biological active molecules [namely: (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or (+)-6-aminopenicillanic acid [6APA] (Fig. 1a); (2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or amoxicillin [Amox] and (2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or ampicillin [Amp] (Fig. 1b and c, respectively)) in NaCl aqueous solutions, the main component of natural and biological fluids, at different temperatures and over a wide ionic strength range.

Penicillin derivatives tend to quickly degrade in aqueous solution, especially in the acidic or basic region (Archer et al., 1970; Frau et al., 1997; Gensmantel et al., 1978, 1980; Gutiérez Navarro et al., 1998; Kheirolomoom et al., 1999; Krasnikova and Iozep, 2003; Lepidi and Nuti, 1971; Pirt, 1990; Pratt et al., 1996; Proctor et al., 1982; Shamsipur et al., 2002); the presence of a lateral chain allows to protect these molecules from the hydrolysis/opening of the β-lactam ring (Shamsipur et al., 2002). The resulting product has different chemical behavior, and from a biological point of view, the anti-bacterial activity is lost. For this reason, the hydrolysis process of the β-lactam ring was investigated for the (+)6-aminopenicillanic acid, the less resistant β-lactam derivatives to the opening of the four terms ring, as a function of the pH, ionic strength and time, by means of spectrophotometric and H NMR measurements. The acid–base properties of the hydrolyzed 6-aminopenicillanic acid (penicillonic acid) were studied at different ionic strength values too.

2. Materials and methods

2.1. Chemicals

Amoxicillin, ampicillin and (+)-6-aminopenicillanic acid (Fluka products) were used without further purification. The ligand purity was checked by alkalimetric titration and resulted to be >99%. Sodium chloride solutions were prepared by weighing pure salt