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Effect of ethanol on the solubility of ampicillin and phenylglycine in aqueous media

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ABSTRACT

Ampicillin is one of the most consumed antibiotics. Due to environmental issues the production of ampicillin has been forbidden by the conventional chemical route. An alternative is the enzymatic route followed by crystallization of the antibiotic. Therefore, this work aims the determination of a series of ampicillin and phenylglycine solubility data in aqueous media using an analytical method. The measurements were carried out at 283.15 and 298.15 K, varying the pH between 3 and 8, and ethanol composition up to 70 wt%. Dissociation constants (pKa's) have also been measured at the studied temperatures and ethanol compositions. It is demonstrated that ideal thermodynamic model with the predetermined pKa's is able to describe satisfactorily solubility profiles. Solubility measurements of ampicillin and phenylglycine have been determined at different conditions of industrial interest. These data may be applied for the evaluation of the crystallization step of ampicillin in the enzymatic synthesis.

1. Introduction

The discovery of ampicillin represented a landmark in the era of antibiotics, being still the most commonly prescribed class of antibiotics in human and veterinary medicine. Amoxicillin and ampicillin are in the list of the most consumed antibiotics. Both are semi-synthetic penicillin belonging to the class of β-lactams, whose structure presents a β lactam heteroatomic ring responsible for the antibacterial activity. Brazil, for instance, in 2014 closed its trade balance of medicines containing ampicillin (AMP) and its salts in 3.99 million US$ FOB negative, corresponding to 80.8 tons. This year the importations already reached 2.24 million US$ FOB [1].

Traditionally these antibiotics are produced on industrial scale by chemical synthesis in a complex process requiring extreme conditions of temperature and the use of toxics solvents, generating non-biodegradable wastes [2,3]. Due to environmental laws increasingly restrict, this route is not allowed in many countries. The enzymatic synthesis appears as an alternative, where the antibiotic is produced at moderate temperatures and without the use toxic solvents. However, this process presents a series of side reactions. That is, beyond the antibiotic synthesis, there is hydrolysis of the substrate and antibiotic itself, yielding 6-aminopenicillanic acid (6-APA), phenylglycine (PG) and alcohol, as byproducts. These undesirable reactions promote low yield to the process, when compared to the conventional process.

When the antibiotic is removed from liquid phase, it becomes unavailable for hydrolysis, making the crystallization a promising technique. Several studies have been conducted to determine the most effective method for obtaining these antibiotics in its pure form right after production. Examples of these techniques are extraction involving volatile solvents or ionic liquids [4,5], liquid–liquid extraction [6], and introduction of cosolvents and polymeric resins [7–10]. However, the technique that stands out is the crystallization, as suggested by Youshko et al. [11] for the synthesis of ampicillin. Therefore, the knowledge of the antibiotic solubility is essential for the process.

Reports of ampicillin's solubility are scarce in the literature at different operating conditions. Santana et al. [12] obtained AMP and PG data in pure water varying pH and temperature, i.e., 5.5 to 7.5 and 283.15–298.15 K, respectively. Rudolph et al. [13] presented solubility data in pure water varying pH at 298.15 K. They also

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studied AMP and PG solubilities at the isoelectric point (pI) in pure water as function of the temperature and for water+1-butanol mixtures at 298.15 K. Youshko et al. [11] determined also the solubility of AMP and PG in water at 298.15 K varying pH.

Ampicillin and phenylglycine present in their structure both amino and carboxyl groups, i.e., acidic and basic characters coexist in the same molecule. As usual chemical groups have different affinities for protons. Thus, in solution these molecules can coexist in three different ionic forms, i.e., cationic, zwitterionic and anionic, depending on the pH of the solution. Different ionic forms present different solubilities, being the minimal when the molecules are in their *zwitterionic* form (no net charge), or so called isoelectric point.

The knowledge of the dissociation constant (pKa) is essential for predicting the ionization state of a molecule with respect to pH, since this parameter indicates the tendency of an acid donate proton to a solvent [14]. There are several available methods to determine pKa values, such as potentiometry, conductivity, calorimetry, UV–visible and NMR spectroscopy, mass spectroscopy, liquid chromatography, capillary electrophoresis, and predictions from computational tools [14–17].

Besides the scarce aqueous solubility data involving semi-synthetics β-lactam antibiotics and their precursors, the available thermodynamic models are not able to describe properly the behavior found for these systems [13]. This is mainly due to the complexity of the mixtures containing charges, dissociation, organic chains, demanding a more rigorous thermodynamic approach. Nevertheless experimental database and reliable thermodynamic models are indispensable tools for optimizing separation of ampicillin processes.

Therefore, in the framework of enzymatic synthesis of β-lactam antibiotics, the aim of this work is to report ampicillin and phenylglycine solubility data in different values of pH (3–8), temperature (283.15 and 298.15 K) and ethanol composition (0–70 wt%). Furthermore, dissociation constants were also determined for each set of conditions, i.e., temperature and solvent composition. Finally, the ideal thermodynamic model was applied to describe the solubility profiles, demonstrating to be a tool for the estimation of the solubility behavior in the conditions of industrial interest.

2. Materials and methods

2.1. Chemicals

PG and AMP were obtained from Sigma–Aldrich. PG has 99% of purity grade. The purity of AMP was checked by Total Organic Carbon (TOC) analysis with a 100 ppm solution, prepared gravimetrically, estimating a value of 90%. Furthermore, a differential thermal analysis (DTG) of the AMP sample determined a melting point within 1% of the literature value. NaOH and HCl, both of analytical grade according ACS, were obtained from Neon and Vetec, respectively. Absolute ethanol 99% was provided from Merck. All the reagents were used without further purification. In all measurements deionized water milli-Q were used. Conductivity and pH measurements were used to check the quality of the water, and to avoid carbon dioxide interference nitrogen bubbling was provided during the potentiometric titration.

2.2. Experimental procedure for solubility

The jacketed glass cell was charged with a mixture of water and ethanol prepared gravimetrically in the desired concentration. The temperature of the cell was controlled by circulating thermostated water (Tecnal thermostated bath, model TE-184) in the jacket. The temperature stability was determined by the sensor connected to a Metrohm pHmeter, model 827 pH-lab, and is considered to be accurate within 0.1 K. Then, an excess of solute was added to ensure saturation, and the pH was adjusted to the desired value by adding 0.1 molal NaOH or 0.1 molal HCl aqueous solution. Under these conditions, the mixture was kept at constant magnetic stirring for at least three hours when PG was used as solute, and seven hours,

Table 1
Solubility data of phenylglycine in aqueous media at different solvent compositions of ethanol and 298.15 K.

pH	[EtOH]'[a] wt%	[PG] mmolal	SD mmolal	pH	[EtOH]'[a] wt%	[PG] mmolal	SD mmolal
3.07	0	33.72	0.08	6.16	30	13.68	0.16
3.52	0	32.59	0.08	6.68	30	13.69	0.04
4.22	0	32.14	0.04	7.02	30	13.71	0.09
4.52	0	31.95	0.12	7.33	30	13.88	0.07
5.04	0	31.82	0.08	7.86	30	14.61	0.15
5.61	0	31.92	0.08	3.12	50	16.26	0.12
6.05	0	31.79	0.14	3.54	50	12.39	0.10
6.49	0	32.02	0.02	4.08	50	11.10	0.09
7.00	0	32.27	0.05	4.49	50	10.93	0.04
7.39	0	32.88	0.12	4.94	50	10.62	0.04
7.94	0	34.05	0.06	5.36	50	10.54	0.07
3.12	10	23.66	0.23	5.92	50	10.34	0.08
3.69	10	23.16	0.20	6.55	50	10.47	0.05
4.12	10	23.04	0.10	7.07	50	10.54	0.14
4.56	10	22.95	0.08	7.47	50	10.78	0.09
5.00	10	22.99	0.10	7.90	50	11.12	0.11
5.59	10	23.06	0.02	3.21	70	12.31	0.09
5.88	10	23.12	0.13	3.61	70	7.62	0.06
6.52	10	23.26	0.19	4.09	70	6.11	0.04
6.93	10	23.35	0.04	4.63	70	5.56	0.04
7.34	10	23.52	0.10	4.93	70	5.27	0.01
7.91	10	24.94	0.07	5.57	70	5.20	0.06
3.13	30	15.94	0.12	6.20	70	5.23	0.01
3.53	30	14.51	0.16	7.05	70	5.24	0.07
4.06	30	13.88	0.10	7.25	70	5.27	0.05
4.51	30	13.77	0.10	7.56	70	5.26	0.03
4.87	30	13.71	0.07	8.08	70	5.20	0.02
5.54	30	13.69	0.07				

[a] [EtOH]' = solute free ethanol composition.