

Modelling of Selective Separation of Mixture Obtained by Penicillin G Enzymatic Hydrolysis

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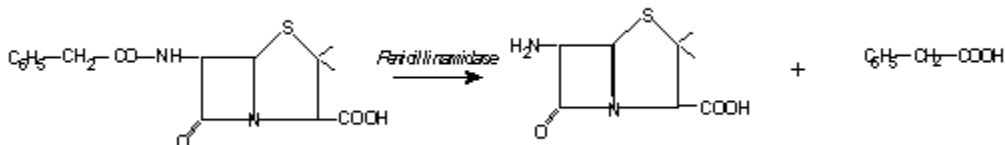
Abstract

The study on the selective separation of components from the mixture obtained by PG enzymatic hydrolysis to 6APA acid indicated that the selectivity factor is strongly influenced by pH-value for 6APA extraction systems and by pH-value and extractant concentration for PG separation.

By means of the cumulated influence of the pH-value of the aqueous solution and the Amberlite LA-2 concentration in the organic phase, some correlations between the selectivity factors for 6APA, on the one hand, and PG separation, on the other hand, and the mentioned parameters were established. The proposed mathematical models are of polynomial regression equation type of six orders and offer a good concordance with the experimental results, the correlation factors being of.

Keywords: Reactive extraction, selective extraction, 6-Aminopenicillanic acid, Penicillin G, Phenylacetic acid, Amberlite LA-2.

Introduction



6-Aminopenicillanic acid (6APA) is the main component of the semisynthetic Penicillins, antibiotics that are obtained by 6APA acylation and formation of amidic bonds differing from the natural ones. 6APA could be obtained by fungus grown on nutritive media

without precursors, but the biosynthesis of this compound is economically inefficient. For these reasons, some chemical or enzymatic methods for Penicillin G hydrolysis to 6APA are applied at industrial scale [1-4]. Among them, the enzymatic hydrolysis is more advantageous and is catalyzed by *penicillinamidase* free or immobilized on different polymeric supports [1-3]. The Penicillin G enzymatic hydrolysis is described by the following reaction:



The most used *penicillinamidase* at industrial scale is produced by *E. coli*, but *penicillinamidases* biosynthesized by *Bacillus megaterium*, *Streptomyces lavendulae*, *Achromobacter sp.*, *Proteus rettgeri*, *Actinoplanes sp.*, *Bovista plumbea*, *Kluyvera atrophila*, *Pseudomonas melanogenum*, *Fusarium sp.*, *Chainia* have been recently isolated and purified, being used for Penicillin G or V hydrolysis [4,5].

The solution obtained by Penicillin G enzymatic hydrolysis contains about 4 – 5% 6APA, 1.8 – 3% phenylacetic acid (PAA) and 0.8 – 1% unhydrolyzed Penicillin G (PG). The industrial separation of 6APA is achieved by acidification at pH=2.5, extraction of PG and PAA with butyl acetate, concentration and acidification with hydrochloric acid for 6APA precipitation [1,2]. This separation technology needs high materials and energy consumption, thus increasing the 6APA cost.

In the previous experiments the conditions for reactive extraction of 6APA and selective separation of the mixture obtained by Penicillin G enzymatic hydrolysis were settled [6,7]. For selecting the most efficient extraction system, the individual extraction of 6APA with extracting agents of organophosphoric acids (di-(2-ethylhexyl) phosphoric acid, D2EHPA) and of high molecular weight amines type (lauryl-trialkyl-methylamine, Amberlite LA-2) was studied. The results indicated that extraction with Amberlite LA-2 dissolved in 1,2-dichloroethane leads to the highest 6APA separation yields [6].

Using the influences of the aqueous phase pH-value and the Amberlite LA-2 concentration in 1,2-dichloroethane on the selective separation of 6APA, PG and PAA from a mixture, a process flow sheet for the fractionation of solution obtained by PG enzymatic hydrolysis has been elaborated and applied [7]. Thus, at pH=10, 6APA was selectively separated by four extraction stages, the overall extraction degree being of 98.8%. PG was selectively extracted from raffinate at pH=6 by three extraction stages, adjusting the molar concentration of Amberlite LA-2 to the value of PG molar concentration for each extraction stage. The antibiotic extraction degree was of 99.6%. The final aqueous

phase contains only PAA that can be used as precursor for PG biosynthesis. By means of the experimental data, a process flow sheet for the selective separation of 6APA, PG and PAA from the mixture obtained by Penicillin G enzymatic hydrolysis has been elaborated and applied.

The aim of this study is to establish the mathematical models that describe the selective extraction of 6APA from 6APA, PG and PAA mixture, and the selective separation of PG from the resulted raffinate. For this purpose, by means of the cumulated influence of the aqueous phase pH-value and extracting agent concentration in organic phase, some correlations between the selectivity factors and the considered parameters have been developed using Microcal Origin 6.0 software.

Materials and Methods

The experiments have been carried out using an extraction column with vibratory mixing described in previous papers [6-9].

The aqueous phase consisted on a mixture of 6APA, PG and PAA, these components concentrations varying as follows:

- 6APA: 4.32 - 45 g.l⁻¹
- PG: 7.49 - 10 g.l⁻¹
- PAA: 2.72 - 24 g.l⁻¹.

The maximum concentration values correspond to the composition of the solution obtained by Penicillin G enzymatic hydrolysis.

The solvent was 1,2-dichloroethane used or as a solution Amberlite LA-2. The extractant concentration in organic phase varied between 0 and 80 g.l⁻¹. The volume ratio between the aqueous solution and organic solvent was of 1, each phase volume being of 50 ml.

The initial aqueous solution pH adjustment has been made with a solution of 0.1N hydrochloric acid or 0.1N sodium hydroxide, depending on the desired pH value (pH values were determined using a digital pH-meter of Consort C832 type). The pH values have been recorded throughout each experiment and any pH change was noted.

The extraction degree has been calculated by means of the mentioned components concentrations in the initial solution and in the raffinate:

$$Y = \left(1 - \frac{C_R}{C_0} \right) \cdot 100, \%$$

6APA, PG and PAA concentrations have been measured using high performance liquid chromatography technique (HPLC) with a Lichrospher 100 RP - 18 column (5 μm) and an

UV detector at 225 nm. The mobile phase was a mixture of phosphate buffer (pH = 6) and acetonitrile in a volume ratio of 4 : 1.

Results and Discussions

1. Selective extraction of 6APA from 6APA, PG and PAA mixture

The experimental data of the previous studies on reactive extraction of 6APA showed that the highest extraction degrees are obtained using Amberlite LA-2 as extracting agent (at pH=10, the extraction yield with Amberlite LA-2 is over 4.7 times greater compared with physical extraction or reactive extraction with D2EHPA). Moreover, the literature indicated that the maximum extraction efficiency of PG and PAA with Amberlite LA-2 is reached for pH<5, decreasing for higher pH-values and becoming 0 at pH=9 for PG, respectively pH=8 for PAA [10].

By means of these assumptions, the reactive extraction of 6APA, PG and PAA from mixtures at different pH-values was studied. The experimental results are depicted in **Figure 1** and suggest that the separation of PG and PAA is more effective for a pH domain below 7, over this pH-value only the 6APA extraction being possible [7].

The separation selectivity can be described using the selectivity factor. For the studied extraction system, the selectivity factor, S_1 , is defined as the ratio between the 6APA extraction degree and overall extraction degree of PG and PAA:

$$S_1 = \frac{Y_{A6AP}}{Y_{(PG + PAA)}}$$

Analyzing the above results through the selectivity factor, it was observed that for pH < 7 S_1 is lower than 1, increasing then significantly and tending to ∞ for pH > 9 (**Figure 2**). This evolution suggests the possibility of 6APA selective separation by reactive extraction with Amberlite LA-2 at pH=10. Because the extraction degree of 6APA with 80 g.l⁻¹ Amberlite LA-2 at pH=10 is about 67%, for its total recovery from aqueous solution four extraction stages are required. In these circumstances, the final raffinate will contain only PG and PAA.

Owing to the less significant influence of the Amberlite LA-2 concentration on 6APA separation selectivity compared with pH effect, for mathematical describing of the 6APA selective extraction only the influence of the pH-value was taken into account. Thus, the proposed mathematical model is of polynomial regression equation type of six orders:

$$S_1 = a_0 + a_1 \cdot x + a_2 \cdot x^2 + a_3 \cdot x^3 + a_4 \cdot x^4 + a_5 \cdot x^5 + a_6 \cdot x^6$$

where: x - pH-value of aqueous phase

a_0, \dots, a_6 - regression coefficients.

The values of the regression coefficients were calculated using Microcal Origin 6.0 software, the following correlation for selective extraction of 6APA being obtained:

$$S_1 = -0.97 + 3.50 \cdot \text{pH} - 2.88 \cdot \text{pH}^2 + 1.12 \cdot \text{pH}^3 - 0.22 \cdot \text{pH}^4 + 2.02 \cdot 10^{-2} \cdot \text{pH}^5 - 6 \cdot 10^{-4} \cdot \text{pH}^6$$

The correlation factor R^2 for the proposed equation is of 0.9997, thus indicating a very good concordance with the experimental values of selectivity factor S_1 .

2. Selective extraction PG from PG and PAA mixture

The supplementary fractionation of raffinate is based on the pH effect on the reactive extraction of PG and PAA with Amberlite LA-2. From Figure 1 it can be seen that both components are extracted in the acidic pH domain, the PG extraction degree being superior. For this extraction system, the selectivity factor, S_2 , has been calculated as the ratio between the reactive extraction yields of PG and PAA:

$$S_2 = \frac{Y_{\text{PG}}}{Y_{\text{PAA}}}$$

The selectivity factor S_2 is maintained at a value close to 1 for $\text{pH} \leq 4$, then strongly increasing and tending to ∞ for $\text{pH}=8$ (Figure 2). Indifferent of the pH domain the PG reactive extraction occurs simultaneously with PAA reactive extraction, therefore the consideration of pH-value as the unique parameter which controls the extraction selectivity is not sufficiently. Owing to the higher acidity of PG ($k_a = 1.74 \cdot 10^{-3}$ for PG, respectively $k_a = 4.88 \cdot 10^{-5}$ for PAA [10]), Amberlite LA-2 will react mainly with PG. For this reason, the limitation of PAA coextraction is possible by using an extracting agent concentration inferior than the stoichiometric need for the chemical reaction with both components of aqueous phase [7].

By plotting the cumulated effect of pH-value and Amberlite LA-2 concentration on selectivity factor S_2 two variation regions can be observed (Figure 3). Thus, indifferent of the considered factors levels, for PG and PAA concentrations in aqueous phase corresponding to those obtained by PG enzymatic hydrolysis (9.81 g.l⁻¹ PG, 24 g.l⁻¹ PAA), the selectivity factor S_2 increases with extractant concentration increase, reaches a maximum value for 10 g.l⁻¹ Amberlite LA-2, decreasing then for higher values of extractant concentration. Therefore, as it was established in the previous papers, the highest selectivity of PG separation from PAA is reached at a molar concentration of Amberlite LA-2 in organic phase equal to that of PG in the aqueous phase [7].

By means of the cumulated influence of the aqueous phase pH-value and extractant concentration on separation selectivity, a mathematical correlation between the selectivity factor S_2 and the mentioned parameters was developed using Microcal Origin 6.0 software. The correlation can be described by a polynomial regression equation of six orders having the general expression:

$$S_2 = a_0 + a_1 \cdot x + a_2 \cdot x^2 + a_3 \cdot x^3 + a_4 \cdot x^4 + a_5 \cdot x^5 + a_6 \cdot x^6$$

where: x – Amberlite LA-2 concentration

a_0, \dots, a_6 - regression coefficients, which are function of pH-value.

For an extractant concentration varied between 0 and 40 g.l⁻¹ and a pH-domain of 2-6, the following equation has been obtained:

$$S_2 = 1.75 + 0.308 \cdot \text{pH} - (5.85 \cdot \text{pH} - 6.61) \cdot C_A + (7.95 \cdot \text{pH} - 10.78) \cdot C_A^2 - (3.19 \cdot \text{pH} - 4.49) \cdot C_A^3 + (0.58 \cdot \text{pH} - 0.83) \cdot C_A^4 - (5 \cdot 10^{-2} \cdot \text{pH} - 7 \cdot 10^{-2}) \cdot C_A^5 + (1.7 \cdot 10^{-3} \cdot \text{pH} - 2.4 \cdot 10^{-3}) \cdot C_A^6$$

the correlation factor being $R^2 = 0.8913$. The corresponding surface is given in (Figure 4).

Conclusions

The study on selective separation of components from mixture obtained by PG enzymatic hydrolysis to 6APA acid indicated that the selectivity factor is strongly influenced by pH-value for 6APA extraction systems and by pH-value and extractant concentration for PG separation.

By means of the cumulated influence of pH-value of aqueous solution and Amberlite LA-2 concentration in organic phase, some correlations between the selectivity factors for 6APA, on the one hand, and PG separation, on the other hand, and the mentioned parameters were established. The proposed mathematical models offer a good concordance with the experimental results, the correlation factors being of 0.8913 – 0.9997.

Notations

CA	-	Amberlite LA-2 concentration in organic phase,	g.l-1
C6APA0	-	initial concentration of 6APA in aqueous phase,	g.l-1
CPAA0	-	initial concentration of PAA in aqueous phase,	g.l-1
CPG0	-	initial concentration of PG in aqueous phase,	g.l-1
ka	-	acidity	index
S1	-	selectivity factor for 6APA separation from PG and PAA	

S2 - selectivity factor for PG separation from PAA
Y - extraction degree, %

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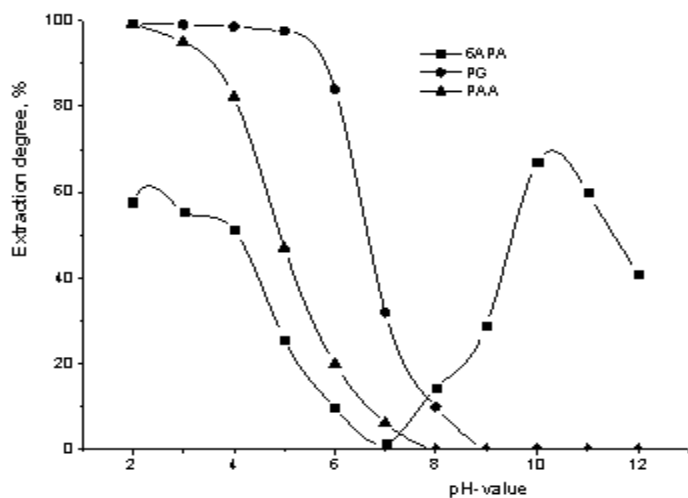


Figure 1. Influence of aqueous phase pH-value on extraction degree of 6APA, PG and PAA from a mixture using Amberlite LA-2 ($C_{6APA0} = 5.04 \text{ g.l}^{-1}$, $C_{PG0} = 8.70 \text{ g/l}$, $C_{PAA0} = 3.13 \text{ g.l}^{-1}$, $C_A = 80 \text{ g.l}^{-1}$).

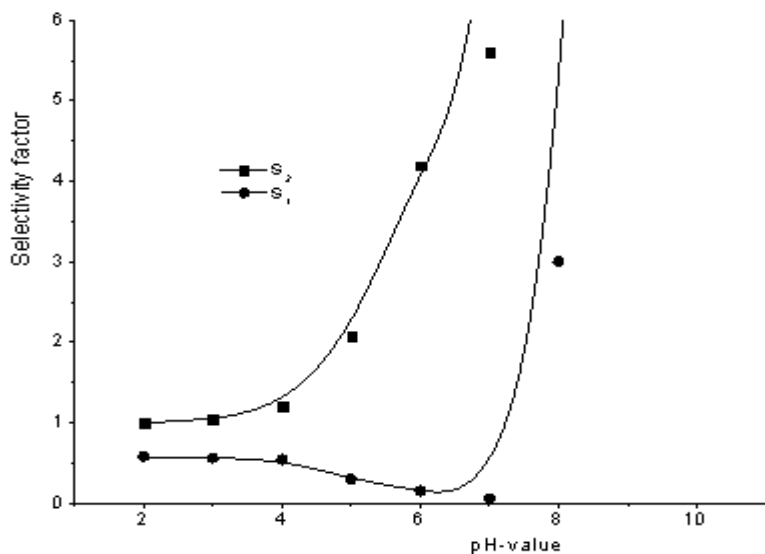


Figure 2. Influence of aqueous phase pH-value on selectivity of 6APA, PG and PAA extraction from a mixture with Amberlite LA-2 ($C_{6APA0} = 5.04 \text{ g.l}^{-1}$, $C_{PG0} = 8.70 \text{ g/l}$, $C_{PAA0} = 3.13 \text{ g.l}^{-1}$, $C_A = 80 \text{ g.l}^{-1}$).

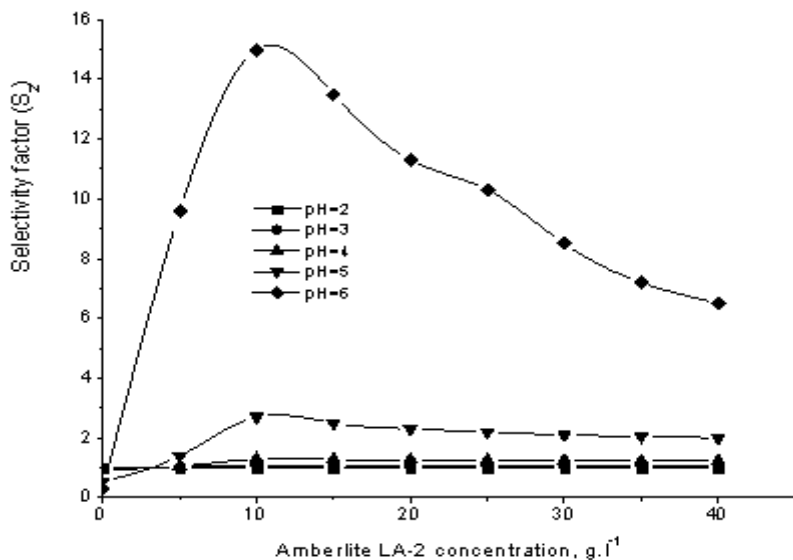


Figure 3. Cumulated influence of Amberlite LA-2 concentration in 1,2-dichloroethane and pH-value on extraction selectivity for PG and PAA separation from a mixture ($C_{PG0} = 9.81 \text{ g.l}^{-1}$, $C_{PAA0} = 24 \text{ g.l}^{-1}$).

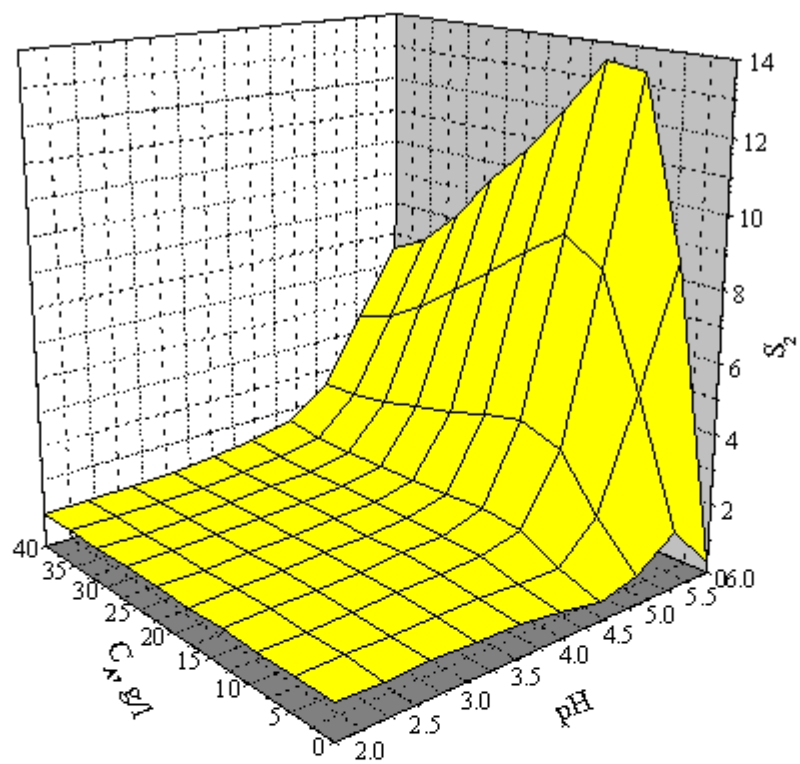


Figure 4. Surface plotted for cumulated influence of aqueous phase pH-value and Amberlite LA-2 concentration on selectivity of PG extraction.