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Mass Transfer Analysis of Penicillin Extraction

A. Kheirolomoom*, A.A. Sayfkordi[1], A. Kazemi-Vaysari[2], M. Ardjmand[3] and A. Baradar-Khoshfetrat[1]

In this paper, recovery of penicillin G from mycel-containing fermentation broth through physical extraction is investigated. The values of degree of extraction are evaluated as a function of media pH, partition coefficient between aqueous and organic phases for undissociated acid and the pK_a of penicillin. The pH variations were measured during extraction operation and the optimum pH value for penicillin G recovery considering its decomposition was obtained. The mass transfer analysis of the extraction system was carried out using two-resistance mass transfer theory. The overall as well as aqueous and organic phase mass transfer coefficients were determined. The investigated mass transfer resistances for both phases are in the same order of magnitude, demonstrating that the mass transfer resistances for both phases have to be considered in equipment design and operation of penicillin G extraction systems.

INTRODUCTION

Highly specialized strains of *Penicillium chrysogenum* are used for industrial production of penicillin G via fermentation technology. After isolation and purification, the manufactured penicillin G is either used in medicine, directly, as penicillin G salt or is cleaved to 6-aminopenicillanic acid (6-APA) which is the starting material for manufacturing semisynthetic penicillins. The third generation of penicillins are all derivatives of 6-APA.The isolation and purification of penicillin G from the culture broth is carried out through different processes: (a) Carbon process, (b) Solvent extraction process and (c) Penicillin acid process [1]. Due to some disadvantages of carbon process such as increased carbon requirements for production of higher quantities of penicillin and also the novelty of penicillin acid process, most of the penicillin G isolation and purification processes are carried out using solvent extraction process. In this process, the filtered broth is extracted by organic solvents at low pH values and short contact time. Solvents such as *n*-butyl acetate, *n*-amyl acetate, isobutyl-methyl ketone and butanol are usually used at a volume ratio of aqueous to organic phase from 4:1 to 8:1 in centrifugal extractors. After extraction, the penicillin is returned to the buffer and is again acidified and reextracted with a smaller volume. The concentrated penicillin is again brought into aqueous solution and crystallized.

Experimental aspects of the solvent extraction of penicillin G have been discussed by Herschloach et al. [1] and Souders et al. [2]. Operation of continuous, multistage, countercurrent centrifugal extractor is throughly discussed by Podbielniak et al. [3], Todd and Davies [4] and Swartz [5]. Recent reviews evaluate distribution coefficient for the reactive extraction of penicillin [6,7], the reactive extraction of penicillin G from mycel-containing broth [8] and also the extraction of penicillin G with neutral phosphorus esters [9]. Although, extensive research work has been carried out on extraction operation of penicillins, there is no published report on the mass transfer resistance analysis of the extraction system.

Since 10% to 15% loss in penicillin G occurs during its isolation and purification, mass transfer study of penicillin G recovery process is necessary for reduction of such losses. Therefore, values of mass transfer coefficients in organic and aqueous phases are investigated.

*. *Corresponding Author, Department of Chemical Engineering, Sharif University of Technology, Tehran, I.R. Iran.*

1. *Department of Chemical Engineering, Sharif University of Technology, Tehran, I.R. Iran.*
2. *The Biochemical and Bioenvironmental Research Center, Sharif University of Technology, Tehran, I.R. Iran.*
3. *Department of Chemical Engineering, Islamic Azad University, South Branch, Tehran, I.R. Iran.*

MASS TRANSFER ANALYSIS OF PENICILLIN G RECOVERY BY EXTRACTION

The physical extraction of penicillin G is carried out using different organic solvents that preserve penicillin G in a dissolved undissociated acid form (Equation 1). However, in an aqueous phase, it dissociates and shows the properties of a weak acid ($pK_a = 2.75$) according to Equation 3. The intrinsic partition coefficient of physical extraction, K_i, is equal to the ratio of free penicillin acid concentration in the organic phase, $C_{HP(org)}$, to that of the aqueous phase, $C_{HP(aq)}$; moreover, the association constant of penicillin acid, K_a, is defined as the ratio of the product of the concentration of proton, H^+, and penicillin acid anion, P^-, to that of the penicillin acid, HP, in the aqueous phase and is usually tabulated in units of kmol/m^3.

$$HP_{(aq)} \overset{K_i}{\rightleftarrows} HP_{(org)}, \tag{1}$$

$$K_i = \frac{C_{HP(org)}}{C_{HP(aq)}}, \tag{2}$$

$$HP_{(aq)} \overset{K_a}{\rightleftarrows} H^+_{(aq)} + P^-_{(aq)}, \tag{3}$$

$$K_a = \frac{C_{P^-(aq)} C_{H^+(aq)}}{C_{HP(aq)}}. \tag{4}$$

Only the undissociated acid form of penicillin G can be extracted by an organic solvent. The extraction of penicillin G is altered dramatically by changes in pH values. Although penicillin G can partly ionize in water, it will not ionize in organic solutes, significantly. The partition coefficient, K, is defined as:

$$K = x/y, \tag{5}$$

where x is the solute (penicillin G) concentration in the organic phase and y is the concentration of the same solute in the aqueous phase. Often the value of K will be constant, independent of the solute concentration for a given solvent pair. A constant value of K reflects the fact that most biochemical extractions take place in dilute solutions. The apparent partition coefficient is defined as the ratio of the penicillin acid concentration in the organic phase, $C_{HP(org)}$, to the sum of concentration of penicillin acid in the aqueous phase, $C_{HP(aq)}$, and that of the penicillin anion in the aqueous phase, $C_{P^-(aq)}$ (Equation 6). The apparent partition coefficient includes both concentrations of ionized and unionized forms of the solute. The concentrations of ionized and unionized solutes in water are subject to the equilibrium according to Equation 3.

$$K = \frac{C_{HP(org)}}{C_{HP(aq)} + C_{P^-(aq)}}. \tag{6}$$

Combining Equations 2, 4 and 6 yields:

$$K = \frac{K_i}{1 + K_a/C_{H^+(aq)}}. \tag{7}$$

Equation 7 can be written as:

$$\log_{10}(K_i/K - 1) = \mathrm{pH} - \mathrm{pK}_a, \tag{8}$$

or,

$$K = K_i \frac{1}{1 + 10^{\mathrm{pH}-\mathrm{pK}_a}}. \tag{9}$$

The degree of extraction, E, i.e., the fraction of the extracted penicillin with respect to its overall concentration, is defined by the following equation:

$$E = \frac{C_{HP(org)}}{C_{HP(org)} + C_{HP(aq)} + C_{P^-(aq)}}\%, \tag{10}$$

or,

$$E = \frac{E_f}{1 + E_f}. \tag{11}$$

The extraction factor, E_f, is given by:

$$E_f = \frac{KL}{H}, \tag{12}$$

where L and H represent the volume of organic and aqueous phases, respectively.

For purposes of establishing the nature of two-film theory and the overall coefficient of mass transfer, the two-phase extraction system is shown in Figure 1. The mass balance on penicillin G leads to:

(solute into the extraction system)

= (solute out of the extraction system), (13a)

$$Hy_0 + Lx_0 = Hy + Lx, \tag{13b}$$

where $x_0 = 0$, or:

$$x = \frac{H}{L}(y_0 - y). \tag{14}$$

y_0 and x_0 are the initial concentrations of the solute in the aqueous and organic phases, respectively. Also, the quantity y is the aqueous phase concentration of the solute in the bulk of the aqueous phase.

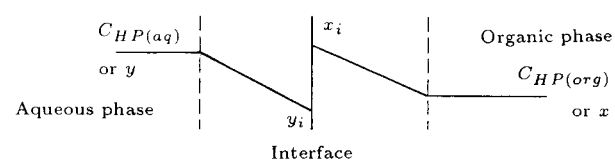

Figure 1. Two-phase extraction system.

The entire two-phase mass transfer effect can be measured in terms of an overall mass transfer coefficient based on the aqueous phase, K_H, using the mass balance relation:

$$-H\frac{dy}{dt} = K_H A(y - y^*), \tag{15}$$

where y^* is the aqueous phase concentration of penicillin G, which is in equilibrium with the organic phase. The quantities t and A are time and mass transfer area, respectively. Using the equilibrium relation between the aqueous and organic phases (Equation 5), the mass balance relation given by Equation 15 is simplified as:

$$-\frac{dy}{dt} = K_H(\frac{A}{H})[y - \frac{H}{KL}(y_0 - y)]. \tag{16}$$

The specific interfacial area, a, is defined by:

$$a = \frac{A}{H}. \tag{17}$$

Using the definitions given by Equations 11, 12 and 17 for the quantities E, E_f and a, respectively, Equation 16 is simplified into:

$$-\frac{dy}{dt} = K_H a(\frac{E_f + 1}{E_f}y - \frac{1}{E_f}y_0), \tag{18}$$

or,

$$-\frac{dy}{dt} = K_H a(\frac{1}{E}y - \frac{1}{E_f}y_0) \tag{19a}$$

$$y = y_0 \quad \text{at} \quad t = 0. \tag{19b}$$

Integrating the ordinary differential equation given by Equation 19a and using the initial condition (Equation 19b) yields:

$$F(y) = \frac{E}{a}\ln[\frac{y_0}{y/E - y_0/E_f}] = K_H t, \tag{20}$$

where $F(y)$ is a function of overall mass transfer coefficient. The overall mass transfer coefficient, K_H, in the two-phase system of extraction is obtained from the slope of $F(y)$ versus time plot. Using Equation 21a, the organic, k_L, and aqueous phase, k_H, mass transfer coefficients can be obtained by drawing $\frac{1}{K_H}$ data points versus $\frac{1}{K}$ values.

$$\frac{1}{K_H} = \frac{1}{k_H} + \frac{1}{K}\cdot\frac{1}{k_L}, \tag{21a}$$

$$K_H = k_H \quad \text{for} \quad \frac{1}{K} = 0. \tag{21b}$$

Equation 21a can be applied in mass transfer resistance analysis of the two-phase extraction system.

MATERIALS AND METHODS

Chemicals

Penicillin G potassium salt was provided by Jaber Ibn-e-Hayan Pharmaceutical Company (Tehran, Iran). All reagents were microbiological or analytical grade and were obtained from Merck AG (Darmstadt, Germany) and Sigma (St. Louis, Missouri, USA).

Microorganism

Lyophilized *Penicillium chrysogenum* 202 ampoule was used for penicillin production, provided by Darou Pakhsh Pharmaceutical Company Research Center (Tehran, Iran). The strain was maintained on slants of malt-agar (45 kg/m^3) at 25°C for 48 h.

Culture Media

1.00×10^{-4} m^3 of the preculture media containing 2.75×10^{-3} kg lactose, 4.00×10^{-3} kg corn-steep liquor, 0.30×10^{-3} kg $NaNO_3$, 0.05×10^{-3} kg KH_2PO_4, 0.25×10^{-4} kg $MgSO_4.7H_2O$, 0.01×10^{-4} kg $ZnSO_4.7H_2O$ and 0.50×10^{-3} kg $CaCO_3$ was prepared. The strains of *Penicillium chrysogenum* were added to the preculture and then incubated in shake flasks at 25°C and 260 rpm for 72 h. The culture media composition was the same as the preculture media. In addition, 0.50 kg/m^3 phenylacetic acid was added to the culture media as the inducer for penicillin G production. The culture condition was maintained at pH of 5.5, 25°C and 260 rpm for 120 h.

Penicillin G Extraction System

n-butyl acetate, *n*-amyl acetate, *n*-heptane, isobutyl-methyl ketone and butanol were used as solvents in penicillin G extraction. The mycelium was separated from the fermentation broth by filtration. Different buffers were used to prepare penicillin G solutions at defined pH values. Buffer solution of KCl-HCl for pH values less than 3.0, citrate buffer for pH values between 4.0 to 6.0 and phosphate buffer for pH values between 6.0 to 8.0 were used. The penicillin G solution was prepared by adding 0.50×10^{-4} m^3 of the fermentation broth and 0.25×10^{-4} m^3 of the organic solvent into a 2.50×10^{-4} m^3 separating funnel, while stirring was performed at 20°C. The pH of the solution was adjusted to the desired value and penicillin G content in the aqueous phase was determined using the hydroxylamine method [10].

Determination of Mass Transfer Coefficients

The Lewis cell was used to determine the mass transfer coefficient in each phase. The cell consists of a cylindri-

cal vessel with the diameter of 8.00×10^{-2} m and height of 1.10×10^{-1} m. The cell was equipped with two flat-blade paddle type impellers, 10 mm from the bottom of the cell and 3.00×10^{-2} m apart from each other, one in aqueous and the other in the organic phase. Each impeller had two rectangular blades of 2.50×10^{-2} m length and 1.00×10^{-2} m height. Buffer solutions of penicillin G potassium salt (5 kg/m^3) were prepared and 1.35×10^{-4} m^3 of each of the aqueous and organic phases were added to the cell ($L/H = 1$). The height of each phase in the cell was equal to 3.30×10^{-2} m. The stirring of the two phases was performed at 5 rpm to prevent the disturbance of the interface plane. The interface area was equal to 50.24×10^{-4} m^2. Penicillin G solution in n-butyl acetate was used in all measurements ($y_0 = 0$). Penicillin G concentration data in aqueous phase was utilized for determining mass transfer coefficients.

RESULTS AND DISCUSSION

Penicillin G recovery from the fermentation broth was carried out by solvent extraction using n-butyl acetate, n-amyl acetate, iso-butyl-methyl ketone, butanol and n-heptane as solvents. Penicillin G is a weak acid ($pK_a = 2.75$) and highly sensitive to heat, acids and penicillinases. Penicillin G is more unstable at higher temperatures and pH values less than 4.0 and more than 8.0 [5-8]. The degree of extraction of penicillin G as a function of pH is shown in Figure 2 for each solvent. The obtained results illustrate that at pH of 1.0, the maximum recovery of penicillin G is achieved (Figure 2). However, in this pH value, penicillin G is highly unstable and decomposes rapidly. Consequently, the subsequent processing of the penicillin G solution is quite impossible. Due to high instability and decomposition of penicillin G, as well as the formation of a stable emulsion by solvent droplets at pH values less than 3.0, attempts have been made to recover penicillin G at pH 3.0. Therefore, a trade-off between the maximum stability and degree of extraction of penicillin G as a function of pH leads to pH = 3.0 as the optimum pH value for penicillin G recovery by extraction process. Furthermore, demulsifiers such as esters of phosphoric acid or quaternary ammonium salts were used in solvent extraction at low pH values to prevent the formation of a stable emulsion by surface active components of the fermentation broth such as solid particles, biopolymers and products of enzymatic decomposition of lipids which are widely used as water soluble salts in antifoam agents [11-14]. Although, the partition coefficient for butanol is lower than n-butyl acetate, the former has a higher value of extraction degree which is due to the antifoam property of butanol that prevents bubble formation in the solvent phase [14-18].

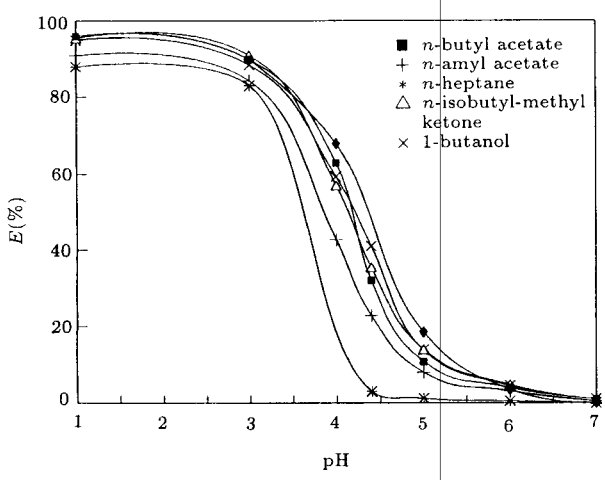

Figure 2. Degree of extraction versus pH. Measurements were carried out at $L/H = 1/2$, 20°C and buffer to broth volume ratio = 1/4. Data points (♦) for n-butyl acetate were taken from the results of Reschke and Schğerl [7].

Adjustments of pH values during extraction were carried out using KCl-HCl, citrate and phosphate buffer, as mentioned above. The experimental results show that when the volume ratio of aqueous to organic phase is equal to 4:1, the minimum variation in pH value occurs [6,19-23]. The maximum variation in pH values was observed when butanol was used as the solvent for penicillin G recovery by extraction process.

The minimum decomposition of penicillin G occurs at pH 3.0 as reported by Likidis et al. [8]. At pH of 3.0, the maximum extraction as well as the minimum decomposition of penicillin G was observed for all of the solvents. Therefore, pH of 3.0 was considered as the optimum pH value for penicillin G extraction and the pH values less than 3.0, due to the high instability of penicillin G at the mentioned pH range, are not illustrated in Figures 3 and 4. There is a wide choice of solvents used in extraction process. In the present study, n-butyl acetate was employed (widely used in industrial extraction of penicillin G due to good solvent properties and low cost [24-26]) as the solvent in mass-transfer analysis.

Experimental data of $F(y)$ as a function of time is plotted in Figure 3. The overall mass transfer coefficient, K_H, increases by increasing the stirring rate of aqueous and organic phases. However, in the present study, the stirring speed of 5 rpm was used to prevent interface disturbances. Figure 3 shows that decrease in pH results in increase of the overall mass transfer coefficient and, hence, the rate of penicillin G recovery for the pH range between 3.0 and 7.0. Mass transfer coefficients for aqueous and organic phases were obtained by plotting $1/K_H$ versus $1/K$ values. The experimental data illustrates that the values of both mass transfer resistances for aqueous ($1/k_H$ =

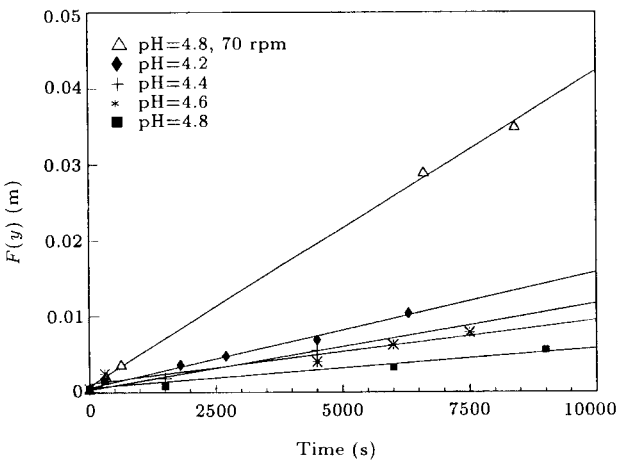

Figure 3. The values of mass transfer function, $F(y)$, at different time intervals of extraction operation. The overall mass transfer coefficients were obtained from the slope of these lines. Measurements were carried out at 30°C and 5 rpm for n-butyl acetate solvent.

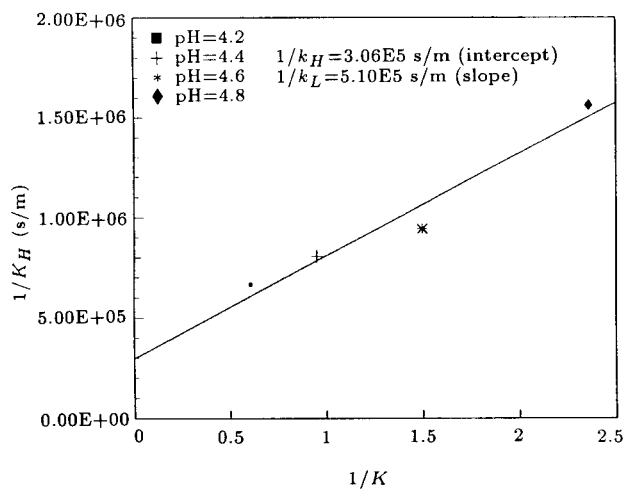

Figure 4. Overall mass transfer resistance, K_H^{-1}, (min/cm), versus partition coefficient values, K^{-1}, in two-phase extraction system. The slope of the line is equal to k_L^{-1} and the intercept is equal to k_H^{-1}.

3.06×10^5 s/m) and organic phases ($1/k_L = 5.10 \times 10^5$ s/m) are considerable, i.e., the overall mass transfer resistance depends on the properties of both phases (Figure 4).

CONCLUSIONS

Penicillin G can be isolated with highest degree of extraction at low pH values, since decreasing the pH value increases the overall mass transfer coefficient in extraction operation at the pH range between 3.0 to 7.0. Mass transfer resistances for aqueous and organic phases were obtained experimentally. The results for aqueous ($1/k_H = 3.06 \times 10^5$ s/m) and organic phases ($1/k_L = 5.10 \times 10^5$ s/m) reveal that both resistances are in the same order of magnitude and must be considered in extraction operation and equipment design.

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NOMENCLATURE

a	specific interfacial area (m^{-1})
A	mass transfer area (m^2)
$C_{HP(aq)}$	penicillin acid concentration in aqueous phase (kmol/m^3)
$C_{HP(org)}$	penicillin acid concentration in organic phase (kmol/m^3)
$C_{P^-(aq)}$	penicillin acid anion concentration in aqueous phase (kmol/m^3)
E	degree of extraction (%)
E_f	extraction factor
$F(y)$	mass transfer function defined by Equation 20 (m)
H	aqueous phase volume (m^3)
H^+	proton
HP	penicillin acid
K	partition coefficient of physical extraction
K_a	association constant of penicillin acid
k_H	mass transfer coefficient in aqueous phase (m/s)
K_H	overall mass transfer coefficient based on aqueous phase (m/s)
K_i	intrinsic partition coefficient of physical extraction
k_L	mass transfer coefficient in organic phase (m/s)
L	organic phase volume (m^3)
P^-	penicillin acid anion
t	time (s)
x	solute concentration in the organic phase (bulk)
x_i	solute concentration in the interface for organic phase
y	solute concentration in the aqueous phase (bulk)
y_0	initial concentration of the solute in the aqueous phase
y_i	solute concentration in the interface for aqueous phase

y^* solute concentration in the aqueous phase which is in equilibrium with the organic phase

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