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Phase Equilibria of Potassium Benzylpenicillin in Water + Butan-1-ol

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Solubility measurements of potassium benzylpenicillin (KBP) in water + butan-1-ol mixtures and liquid-liquid equilibrium data of the ternary system KBP + water + butan-1-ol are reported at 293 K. Solubility values vary from $x(\mathrm{KBP}) = 8.4 \times 10^{-4}$ in pure butan-1-ol to $x(\mathrm{KBP}) = 0.076$ in pure water. KBP enhances the mutual solubility between water and butan-1-ol and the miscibility gap disappears at KBP concentrations higher than $x(\mathrm{KBP}) = 0.006$. At low KBP mole fraction, the KBP partition coefficient between the organic and the aqueous phases is about 0.25. A correlation of the solid-liquid and liquid-liquid equilibrium data by the UNIQUAC model is also reported.

Introduction

Benzylpenicillin (penicillin G) is one of the most important antibiotics; it is used both directly for therapeutic purposes and as raw material for production of more complex antibiotics (semisynthetic penicillins). The molecular structure of benzylpenicillin consists of a condensed thiazolidine-β-lactam ring with a $C_6H_5CH_2$ side chain; owing to the carboxyl attached to the thiazolidine ring, benzylpenicillin exhibits a weak acidic behavior (*1*) with $pK = 2.76$ at 298 K. Benzylpenicillin is commonly produced by a fermentation process followed by several steps of extraction and reextraction in order to obtain a purified product; in the final step the potassium salt of benzylpenicillin (KBP), obtained by extraction with KOH solution, is crystallized from an aqueous solution of butan-1-ol. The downstream process entails significant losses in benzylpenicillin (about 10–15%), and it is worthwhile to investigate how these extraction and purification stages can be improved. Of course, all separation operations stand to benefit from the knowledge of phase equilibrium data that are basic to the process design. Unfortunately, few equilibrium data are reported in the literature (*2–4*) (summarized in ref 5). Values for the solubility of the sodium salt of benzylpenicillin in water and in some organic solvents are also reported (*1*, *5*).

The solubility of KBP in butan-1-ol at temperatures from 283 to 313 K was measured in a previous work (*6*), together with the solubilities in ethanol and propan-1-ol. For ideal dilute solutions, assuming that the difference between the enthalpy of KBP in the solid state and the molar partial enthalpy of KBP in the liquid phase at infinite dilution is temperature independent (*7*), solubility data can be represented by

$$\ln x_1 = a/T + b \tag{1}$$

Fitting of experimental results reported in our previous work (*6*) yields the values of a and b summarized in Table 1.

In the present work we report the solubility of KBP in water + butan-1-ol and ternary liquid–liquid equilibrium data of KBP + water + butan-1-ol at 293 K.

Experimental Section

Materials. Butan-1-ol was a pure grade product by Carlo Erba with a purity claimed of 99.5%. KBP was supplied by Fluka with a purity claimed greater than 99%. Redistilled water supplied by Carlo Erba was used, and dissolved gases (CO_2, O_2) were removed from water by stripping with nitrogen.

Table 1. Parameters in Eq 1 for Potassium Benzylpenicillin Solubility in Alcohols (Experimental Data in ref 6)

solvent	a/K	b
ethanol	−924.3	−4.50
propan-1-ol	−1374.2	−4.08
butan-1-ol	−1700.5	−3.57

Methods. The concentration of KBP was determined spectrophotometrically by a Perkin-Elmer spectrophotometer (Lambda5). Quantitative analysis was carried out by determining the differences between two characteristic absorbance peaks at 264 and 258 nm. Owing to the low solubility of KBP in alcohols, the calibration curve was obtained using solutions of KBP in ethanol and ethanol + water prepared by mass. This calibration procedure is allowed since no significant influence of the solvent on the spectrophotometric readings was observed (*6*). Concentrations ranged from 0 to 1.2×10^{-2} mol/L. An accuracy of $x_1 = \pm 2 \times 10^{-6}$ and a reproducibility of $x_1 = \pm 3 \times 10^{-6}$ were obtained.

Solubility Measurements. The solubility of KBP in water + butan-1-ol was measured by contacting an amount of solid penicillin exceeding the saturation value with a solution of known composition in a thermostated flask with temperature regulated with an accuracy of ±0.1 K. The suspension was stirred magnetically for at least 60 min; then the solution in the flask was allowed to settle for 30 min. The supernatant liquid was sampled and diluted with a water + butan-1-ol solution having the same composition as the mixture under test (for butan-1-ol-rich mixtures) or with pure water (for water-rich mixtures) in order to perform spectrophotometric analysis.

Preliminary runs showed that higher contact times do not result in higher KBP concentration in the liquid phase. On the other hand the equilibration time is short enough to prevent the formation of any degradation product. In fact, it is known that, in solution, benzylpenicillin can undergo degradation processes with the production of highly cromophoric compounds (*1*, *6*). Benzylpenicillenic acid is the main degradation product and manifests itself in the appearance of an absorption peak at 320 nm. In our experimental runs, all the samples were checked by UV scanning between 240 and 350 nm without detecting the presence of any absorption bands but the KBP ones.

Liquid–Liquid Equilibrium Data. The miscibility gap of the KBP (1) + water (2) + butan-1-ol (3) system and KBP distribution in aqueous and organic phases were determined separately.

The miscibility gap was measured by a synthetic method using two different experimental procedures. The water-

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Table 2. Solubility of Potassium Benzylpenicillin (1) in Water (2) + Butan-1-ol (3) Solutions at 293 K

$x_3/(x_2 + x_3)$	x_1	$x_3/(x_2 + x_3)$	x_1
0.0042	0.0780	0.4382	0.0470
0.0107	0.0806	0.5208	0.0342
0.0204	0.0808	0.5738	0.0347
0.0339	0.0807	0.6207	0.0211
0.0487	0.0821	0.6386	0.0194
0.0686	0.0888	0.6864	0.0133
0.1030	0.0836	0.7084	0.0099
0.1443	0.0798	0.7104	0.0104
0.2284	0.0720	0.7366	0.00705
0.2796	0.0661	0.8783	0.00065
0.3361	0.0602		

rich region of the curve was determined by adding fixed volumes of butan-1-ol to a known mass of KBP and water. The addition was performed in a thermostated cell at T = 293 ± 0.1 K by means of a buret accurate to ±0.02 cm^3. The end point of the titration was detected by a visual inspection of the transition from a homogeneous to heterogeneous mixture. The butan-1-ol-rich side of the miscibility curve was obtained by adding water to known masses of KBP and butan-1-ol. In this case, owing to the low solubility of KBP in butan-1-ol, the mixture is initially heterogeneous: water addition produces firstly a transition from a heterogeneous suspension to a homogeneous mixture and then a transition from a homogeneous mixture to a heterogeneous one.

In both cases, composition measurements were accurate to $w = \pm 0.001$. All the measurements were repeated twice for each mixture; errors in the experimental readings resulted in differences in w values within the w accuracy.

The tie-line values were determined by equilibrating the mixtures of known composition in thermostated glass flasks magnetically stirred for at least 2 h. After a settling period of 2 h, samples of each phase were withdrawn by a syringe and, after dilution, were analyzed spectrophotometrically to obtain the KBP concentration in each phase. Once the KBP concentrations were known, it was easy to determine the complete compositions of the equilibrium phases on each side of the miscibility gap and, thus, to obtain the tie lines. A mass balance check was made between the loaded mixture composition and the equilibrium phase compositions. The mean error in the mass balance was about 1.5%.

Results and Discussion

Solubility in Water + Butan-1-ol. Solubility results of KBP in aqueous solution of butan-1-ol at 293 K are reported in Table 2. The solubility of KBP in pure solvents has been determined in a previous work (*6*). In pure butan-1-ol a solubility of $x_1 = 8.5 \times 10^{-5}$ was obtained (see eq 1 and Table 1). The solubility in pure water is high. A mean value of x_1 = 0.076 ± 0.002 was obtained at 293 K whereas the effect of the temperature in the range from 278 to 308 K was not detectable because it was within the experimental errors. Mixtures of water + butan-1-ol, saturated with KBP, do not exhibit partial miscibility, and the addition of small amounts of water to butan-1-ol considerably enhances the solubility of KBP. The addition of butan-1-ol to water results in a small increase in the solubility of KBP, and a maximum value of about $x_1 = 0.089$ is observed at about $x_3 = 0.07$. In Figure 1, the results are compared with those determined by Inozemtseva et al. (*3*) at 298 K (the low values refer to iodometric determinations, whereas the higher values refer to gravimetric determinations). Values reported graphically by Popova (*4*) in the range from 293 to 318 K have also been included in the plot; this author claims that KBP solubility in water + butan-1-ol is independent of temperature over the temperature range studied.

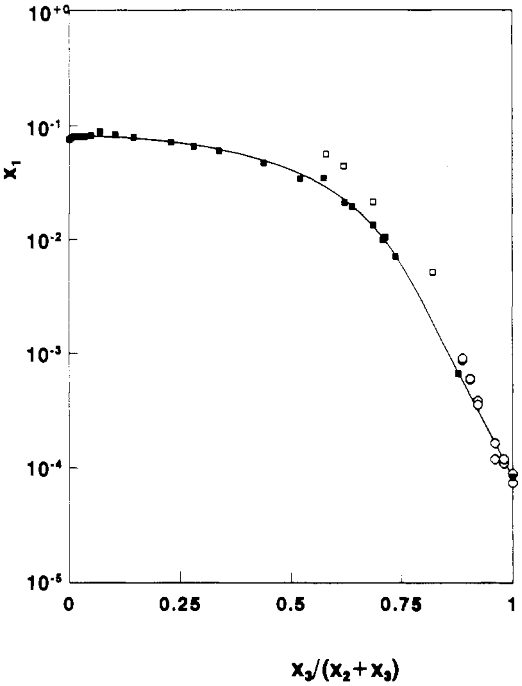

Figure 1. Solubility of potassium benzylpenicillin (1) in water (2) + butan-1-ol (3) at 293 K: ■, this work; ○, ref 3; □, ref 4. The curve is calculated from eq 6 with the UNIQUAC model and fitting parameters reported in Table 5.

Table 3. Miscibility Gap of the Potassium Benzylpenicillin (1) + Water (2) + Butan-1-ol (3) System at 293 K

x_3	10^2x_1	x_3	10^2x_1
0.0198	0.069	0.1410	0.545
0.0202	0.079	0.1738	0.561
0.0208	0.065	0.1974	0.552
0.0224	0.133	0.2518	0.558
0.0263	0.194	0.2785	0.519
0.0328	0.268	0.3080	0.532
0.0456	0.359	0.3594	0.458
0.0678	0.457	0.3735	0.446
0.0879	0.504	0.4073	0.372
0.1080	0.528	0.4716	0.105

Liquid–Liquid Equilibrium. Miscibility gap results, reported in Table 3, show a strong effect of KBP on the mutual solubility of water and butan-1-ol. Complete miscibility is observed at KBP concentrations higher than $x_1 = 0.06$. KBP enhances both butan-1-ol solubility in the aqueous phase and water solubility in the alcohol-rich phase, as shown in Figure 2. The solubility of butan-1-ol in the water-rich phase, $x_{3,\mathrm{w}}$, can be fitted by the equation

$$\ln(x_{3,\mathrm{w}}/x^\circ_{3,\mathrm{w}}) = k_1x_{1,\mathrm{w}} + k_2(x_{1,\mathrm{w}})^2 \qquad (2)$$

where $x^\circ_{3,\mathrm{w}}$ is the solubility of butan-1-ol in pure water and $x_{1,\mathrm{w}}$ is the mole fraction of KBP in the aqueous phase. Similarly, the solubility of water in butan-1-ol, $x_{2,\mathrm{b}}$, is given by

$$\ln(x_{2,\mathrm{b}}/x^\circ_{2,\mathrm{b}}) = k_1x_{1,\mathrm{b}} + k_2(x_{1,\mathrm{b}})^2 \qquad (3)$$

where $x^\circ_{2,\mathrm{b}}$ is the solubility of water in pure butan-1-ol and $x_{1,\mathrm{b}}$ is the mole fraction of KBP in the butan-1-ol-rich phase.

The KBP concentrations in the aqueous and the organic phases at equilibrium were measured spectrophotometrically. These values, together with the data on the miscibility gap, allow us to obtain the tie lines of the system, as reported in Table 4.

The behavior of the ternary system KBP (1) + water (2) + butan-1-ol (3) over the whole composition range (i.e., both

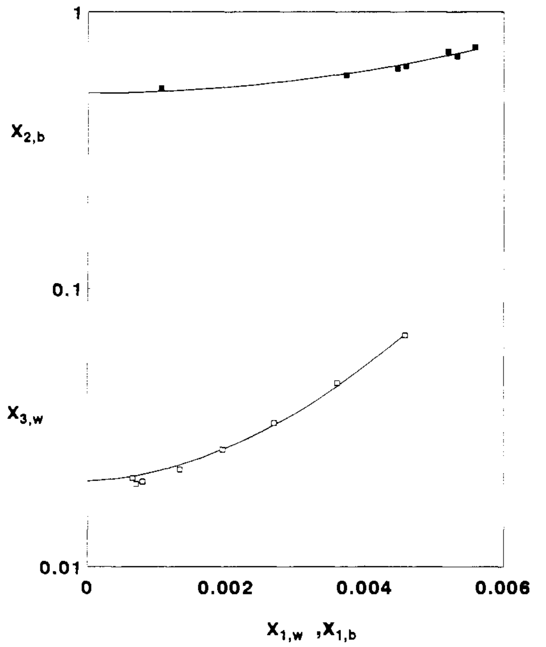

Figure 2. Effect of potassium benzylpenicillin (1) on water (2) + butan-1-ol (3) mutual solubility at 293 K: ■, organic phase; □, aqueous phase. Curves are calculated according to eqs 2 and 3 with $k_1 = 45.2$ and $k_2 = 4.92 \times 10^4$ for the aqueous phase and $k_1 = 1.83 \times 10^{-5}$ and $k_2 = 1.16 \times 10^4$ for the organic phase.

Table 4. Tie-Line Values for Potassium Benzylpenicillin (1) + Water (2) + Butan-1-ol (3) at 293 K

water-rich phase		butan-1-ol-rich phase	
$100x_{1,w}$	$100x_{3,w}$	$100x_{1,b}$	$100x_{3,b}$
0.0703	2.1041	0.0428	48.9572
0.0959	2.0297	0.0811	47.7189
0.1280	2.1720	0.0811	47.7189
0.2020	2.5980	0.1490	46.4510
0.2390	2.8610	0.1810	44.3190
0.2530	3.0470	0.1920	44.2080
0.2620	3.2380	0.2030	43.8970
0.3290	4.1710	0.2390	42.1610
0.3920	5.5080	0.2800	40.5200
0.4050	5.7950	0.3050	39.4950
0.4190	6.1810	0.3140	38.9860
0.4300	6.5700	0.3340	36.2660
0.4700	8.6300	0.4220	31.1780

solid–liquid and liquid–liquid equilibria) can be described if a suitable expression for the Gibbs free energy is available. In particular, for liquid–solid equilibrium, the thermodynamic condition of equality of the chemical potential of KBP in the solid phase, μ_1^{sol}, and in the liquid phase can be written as

$$\mu_1^{\mathrm{sol}} = \mu_1^{*\mathrm{b}} + RT \ln x_1 \gamma_1^{*\mathrm{b}} = \mu_1^{*\mathrm{b}} + RT[\ln x_1 + \ln \gamma_1 - \ln \gamma_{1(x_2=0,x_3=1)}] \quad (4)$$

where the superscript *b refers to a reference state at infinite dilution in pure butan-1-ol, whereas γ (without a superscript) refers to activity coefficients according to the pure component reference state (Raoult's activity coefficients). Accounting for the KBP solubility in pure butanol, $x^{\circ}_{1,\mathrm{b}}$, we obtain

$$\ln x_1 = \ln x^{\circ}_{1,\mathrm{b}} - \ln \gamma_1 + \ln \gamma_{1(x_2=0,x_3=1)} \quad (5)$$

or

$$x_1\gamma_1 = x^{\circ}_{1,\mathrm{b}}\gamma_{1(x_2=0,x_3=1)} \quad (6)$$

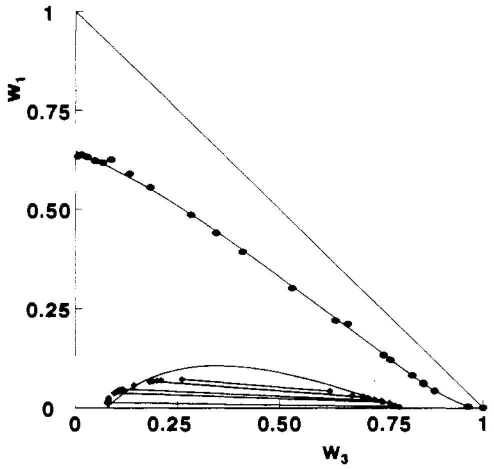

Figure 3. Ternary diagram for liquid–liquid and solid–liquid equilibria of the potassium benzylpenicillin (1) + water (2) + butan-1-ol (3) system at 293 K: ●, solid–liquid equilibrium data; ◆, liquid–liquid equilibrium data. The curves are calculated from eqs 6 and 7 with the UNIQUAC model and fitting parameters reported in Table 5.

On the other hand, the liquid–liquid equilibrium condition is given by

$$\gamma_{i,\mathrm{w}}x_{i,\mathrm{w}} = \gamma_{i,\mathrm{b}}x_{i,\mathrm{b}} \quad (7)$$

where the subscript i (=1–3) refers to the components, whereas the subscripts w and b refer to the water- and butan-1-ol-rich phases, respectively.

The system under study is quite complex, because KBP undergoes dissociation and hydrolysis reactions in aqueous solution (*8*). A rigorous model for this equilibrium is not the aim of this work. Therefore, mole fractions and activity coefficients must be considered as referred to the apparent chemical species. We try to represent these activity coefficients by the UNIQUAC model, which is very often able to smooth experimental results with good accuracy; of course, for such a complex system, the interaction parameters can be regarded only as fitting parameters. Interaction parameters $u_{2,3}$ and $u_{3,2}$ and geometric parameters r_2, q_2, r_3, and q_3 for water + butan-1-ol are reported in the literature (*9*). Therefore, two interaction parameters for each binary system, KBP + water and KBP + butan-1-ol, and two geometric parameters for KBP must be obtained from fitting of the results. Parameter estimation has been carried out by the least-squares method by minimizing the following objective function:

liquid–liquid equilibrium

$$F = \sum_k \sum_l \sum_i (\ln w_{i,l,k}(\mathrm{exp}) - \ln w_{i,l,k}(\mathrm{cal}))^2 \quad (8)$$

where the subscript i (= 1–3) refers to the components, l to the liquid phases in equilibrium, and k to the tie lines. $w_{i,l,k}(\mathrm{exp})$ are the experimental mass fractions, while $w_{i,l,k}(\mathrm{cal})$ are the mass fractions of the calculated tie line lying closest to the considered experimental tie line. Mass fractions have been chosen in order to have comparable values for all the components.

solid–liquid equilibrium

$$F = \sum_k (\ln x_{1,k}(\mathrm{exp}) - \ln x_{1,k}(\mathrm{cal}))^2 \quad (9)$$

where the subscript k refers to the different water/butan-1-ol ratios.

Unfortunately, it was not possible to describe both liquid–liquid and solid–liquid equilibrium results with the same set

Table 5. UNIQUAC Parameters of Potassium Benzylpenicillin (1) + Butan-1-ol (2) + Water (3)[a]

	$u_{3,1}/R$ (K)	$u_{1,3}/R$ (K)	$u_{1,2}/R$ (K)	$u_{2,1}/R$ (K)	r_1	q_1
liquid–liquid equilibrium	22.9	−129.8	−371.0	800	11.0	17.1
solid–liquid equilibrium	319.8	−228.7	−1.7	−125.1	11.0	17.1

[a] $u_{2,3}/R = -0.0661$ K, $u_{3,2}/R = 250.05$K, $r_2 = 3.4543$, $q_2 = 3.052$, $r_3 = 0.92$, and $q_3 = 1.4$ (from ref 9).

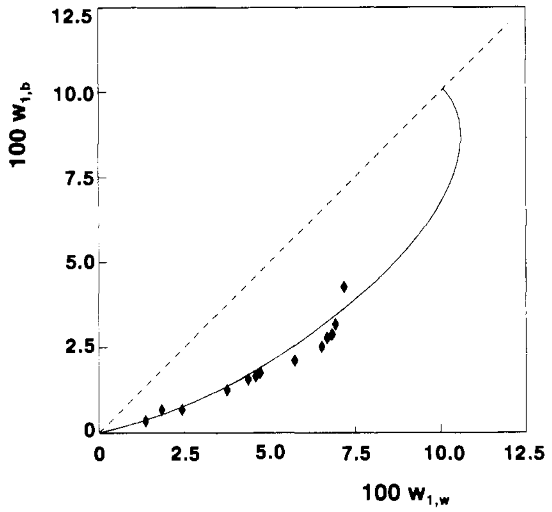

Figure 4. Distribution of potassium benzilpenicillin (1) between the organic (b) and aqueous (w) phases.

of parameters. This behavior is not surprising if one considers the well-known inadequacy of the UNIQUAC model (or other available classical models) to describe different kinds of equilibria with a single set of parameters, even for systems simpler than this one. Therefore, liquid–liquid and solid–liquid results have been fitted separately, and the best fitting parameters are reported in Table 5. Figure 3 shows that the theoretical model is not fully successful for describing liquid–liquid equilibrium: in fact, whereas KBP distribution is represented with sufficient accuracy (see Figure 4), the model predicts a miscibility gap larger than experimentally observed. Of course, solid–liquid equilibrium behavior is easier to represent, and very good fitting results are obtained (see Figures 1 and 3). In this case, good fitting results are also obtained with just four interaction parameters, assuming for *r* and *q* the values obtained from fitting of liquid–liquid equilibrium data.

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