Selective Separation of Physically Near-Identical Microparticle Mixtures by Interfacial Partitioning

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Interfacial partitioning is a novel technology for the separation of physically near-identical particles, which are particles with similar densities and similar sizes but different chemical compositions. Different crystals partition differently to the interface of a biphasic system, forming a substantial interfacial layer (the interphase) as well as a sediment. Here, the selective separation of ampicillin and phenylglycine crystal mixtures in liquid biphasic systems is studied as a model system. In a characteristic batchwise experiment in one step under specific but nonoptimized conditions in water/alkanol biphasic systems, most phenylglycine crystals (80−90% of the feed) adsorb at the interface, whereas the majority of ampicillin crystals (80−90% of the feed) sediment. Important process parameters such as the interfacial loading, the feed crystal composition, and the volume ratio of the liquid phases have been identified, and their influence on the partitioning has been studied. We also discuss a possible mechanism of the interfacial partitioning of the crystals on the basis of the formation of an emulsion in the interphase. This technology has tremendous potential for its application in the selective separation of small bioparticle mixtures.

Introduction

Most bioproducts (and substrates) are solids when sufficiently pure. The tendency to intensify and compact industrial processes is leading to the handling of systems in which various particles in a mixture form an essential element (e.g., solid-to-solid biocatalysis, solid-state fermentation). This situation presents a new challenge: the selective separation of one type of particles, which is a desired product, from a multicomponent mixture of particles. This separation problem is largely unsolved, particularly when the particles are physically near-identical, that is, very similar in size, shape, and density. Conventional mechanical separation techniques such as centrifugation do not offer the required selectivity and therefore are inefficient.[1] Also dissolving the target product selectively is clearly not desired as it results in an increased number of unit operations of the process, as well as an increased consumption of auxiliary chemicals. This leads to negative economic and environmental impacts of the process. In particular, for food-grade products, minimal processing contributes greatly to the "green" qualification of the product.

An attractive possibility for the fractionation of a mixture of physically near-identical particles seems to be by exploiting the differences in the chemical compositions of the particles, which are manifested as differences in polarity or charge via their outer surfaces. The separation of small bioparticles (100 nm to 100 μm) from a multicomponent particle mixture has been demonstrated using a liquid biphasic system at the laboratory scale. For example, Albertsson[2] studied the partitioning of different cell particles and demonstrated that cells of different strains could be fractionated by appropriate selection of the aqueous two-phase systems (ATPSs) composed of a polymer-rich aqueous solution and a salt-rich solution. Walker and Lyddiatt[3] studied the application of an ATPS for the fractionation of inclusion bodies from *E. coli*, also at the laboratory scale. They found that, by using a specific ATPS, a higher selective fractionation could be achieved than with conventional solid−liquid technologies. Andrews et al.[4] also fractionated different types of bioparticles including proteins, cell debris, and virus-like particles using an ATPS.

In the above studies, it was observed that, in some cases, particles accumulated at the interface, which resulted in the formation of a third phase or interphase. For example, inclusion bodies were separated at the interface from cell debris.[3] Interfacial partitioning was also exploited for the separation of precipitated protein from a protein solution.[5] Their method has been named three-phase partitioning (TPP) and employs *tert*-butyl alcohol and ammonium sulfate to precipitate proteins from solution, similarly to the salting-out method. The protein precipitate was recovered at the interface.

It is evident from earlier observations that bioparticles with near-identical physical properties but different chemical compositions partition differently to the interface, as well as to the top and bottom bulk phases. In a recent work by the authors,[6] interfacial partitioning in a liquid biphasic system was investigated for the recovery of a single type of crystals. Interfacial forces are considered to be responsible for the interfacial partitioning of the crystals, and hence, surface properties such as polarity as well as the physicochemical properties of the "solvents" have an effect on the

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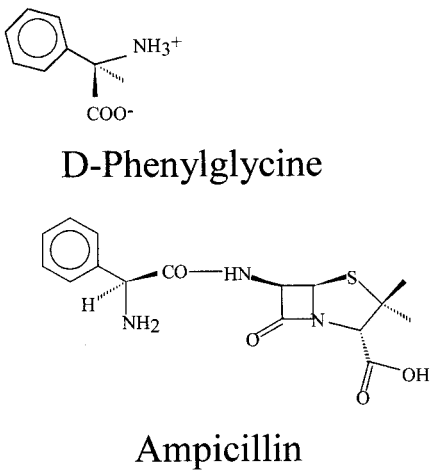

Figure 1. Molecular structures of (a) phenylglycine and (b) ampicillin crystals.

partitioning. Differences in the interaction of the crystals with the solvents determine the adsorption of the crystals at the interface to a small or large extent. These results indicate some degree of selectivity. In the present work, the interfacial partitioning phenomenon is studied for its potential application as an industrial solid−solid separation method. In particular, the separation of mixed ampicillin and phenylglycine crystals in several biphasic liquid systems is investigated. This is a very interesting model system that could offer a solution to existing industrial problems.[7,8]

The effect of several parameters, such as the interfacial loading, the feed crystal composition, and the volume ratio of the liquid phases, on the selectivity of the partitioning is studied. The results are discussed on the basis of the formation of an emulsion in the interphase, as in the study of single types of crystals. Finally, some conclusions are drawn in relation to the mechanism of the selective interfacial partitioning. This will allow for the optimization and modeling of interfacial partitioning, which will enable the prediction and design of the separation process.

Material and Methods

Crystals. The particle mixtures studied were composed of crystals of D-phenylglycine (>99% pure) and trihydrated ampicillin (>99% pure) both supplied by DSM (see Figure 1 for the compounds' formulas). Phenylglycine is the byproduct of the enzymatic synthesis of ampicillin, which is a semisynthetic antibiotic. The solubility of both products in water is relatively low (Table 1); therefore, they are often found in solid (crystalline) form. Some other relevant physicochemical data for the crystals are presented in Table 2.

Liquid Biphasic Systems Employed. Saturated water/butanol, water/hexanol, and ATPS systems were chosen to study the partitioning of the above crystals. The bottom phase to top phase volume ratios were 1:2, 2:1, and 4:1, respectively. The ATPS composition was as follows (a) bottom or salt phase, 26.8 wt % phosphate salt and 1.4 wt % PEG; and (b) top or PEG phase, 29.4 wt % PEG and 6.4 wt % phosphate salt. The pH of the top and bottom phases was 7.

Deionized (with a Milli-Q water system, Millipore) water was used in the biphasic systems above. Butanol at 99% purity was supplied by Acros Organics, and hexanol at 98% purity was supplied by Fluka. PEG 600, potassium dihydrogenphosphate, and dipotassium monohydrogen phosphate salts (98%) were purchased from Merck.

The densities and viscosities of the equilibrated top and bottom phases can be found in Jauregi et al.[6]

HPLC Analysis. The HPLC column used was a Hewlett-Packard Zorbax SB-C18 column of 4.6-mm i.d. and 7.5-cm length, packed with C-18 reversed-phase adsorbent particles of 3.5-μm diameter. The flow rate was 1.0 mL/min, and the peaks were detected by a UV detector at $\lambda = 210$ nm. The mobile phase was a mixture of water (80 vol %) and methanol (20 vol %), which was buffered to a pH value of approximately 3 through the addition of 9.6 g/L sodium dihydrogenphosphate monohydrate and 0.5 mL/L of an 85% phosphoric acid solution. Isocratic elution at room temperature was employed. An average relative error of 1% was estimated.

Solubilities. Crystals of ampicillin and phenylglycine were added to the pure solvents (water, butanol, and hexanol) and the top and bottom phases of the biphasic systems, which were then shaken and centrifuged. A sample of the supernatant was analyzed using the HPLC method described above. Experiments were done in duplicate, and the average solubility values are given in Table 1.

Imaging of Partitioning and Interface Formation. Interfacial partitioning of crystals was visualized with the aid of an Olympus camera adaptor, a CCD camera (Olympus MTV-3), and the image-analysis program called Leica Qwin, version pro 2.2.

Size and Shape Distribution Measurements. Optical microscopy as well as image-analysis measurements were performed to characterize the sizes and shapes of the particles. The image-analysis equipment described above was used. The size of the particles is quantified in terms of the length of the longest feret. The shape and average length, as well as the standard deviation of the length as an indication of the width of the size distribution, are reported in Table 2.

Table 1. Solubilities of Ampicillin and Phenylglycine in Mutually Saturated Solvents

solvent	crystal	solubility (mM)
water	ampicillin	14.50 ± 0.69
butanol		0.01
hexanol		0.04
top phase[a]		1.40 ± 0.00
bottom phase[a]		15.00 ± 0.57
water	phenylglycine	30.98 ± 1.12
butanol		0.08
hexanol		0.03
top phase[a]		3.50 ± 0.14
bottom phase[a]		25.40 ± 0.00

[a] The biphasic system consisted of water/butanol, 2:1 (v/v), and the feed crystal composition was $X_{pg} = 0.6$. Note that 20% (w/w) water dissolves in butanol and 7% (w/w) butanol dissolves in water.[9]

Table 2. Physicochemical Data for the Crystals Studied

crystal	molecular weight (g mol^{-1})	average size[a] (μm) and shape	density[b] (kg m^{-3})	hydrophobicity[c]
D-phenylglycine	151.17	59 regular cubic	1088	0.341
trihydrated ampicillin	403.46	65 rod	1040	0.678

[a] The size of the crystals is given as the mean of the length.[6] [b] These density values are an average of two measurements with a standard deviation of between 3 and 5%.[6] [c] Reference 10.

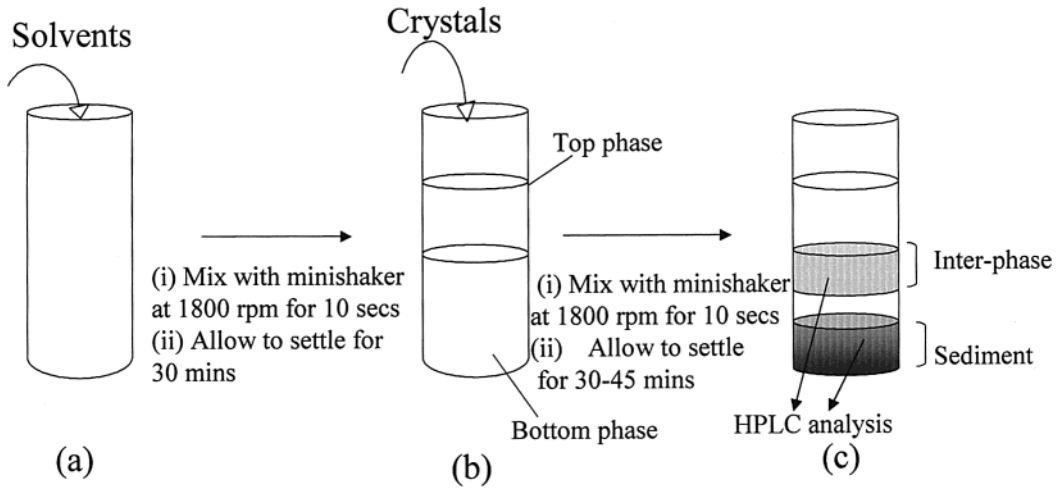

Figure 2. Experimental procedure of partitioning experiments. (a) Solvents were added to a test tube up to a total volume of 3 mL (2.5 mL when the effect of the volume ratio was studied), mixed, and allowed to settle to form the biphasic system. (b) A known amount of each crystal was added and mixed to ensure complete wetting in the biphasic system. (c) Samples (approximately 10 μm, taken with the aid of a Pasteur pipet) from the interphase and sediment were analyzed by HPLC.

Partitioning Experiments. The experimental procedure is depicted in Figure 2. For each experiment carried out in a particular biphasic system two phenylglycine/ampicillin mass ratios were considered (except when interfacial loading effects was studied): (i) 1:1, corresponding to a mass fraction of phenylglycine of X_{pg} = 0.5 (55−60 mg of total crystals) and (ii) 1:1.5, corresponding to X_{pg} = 0.4 (85−90 mg of total crystals).

The compositions of the particle mixture at the interphase and in the sediment are calculated from the concentration data. The mass ratio of phenylglycine and ampicillin crystals in the sample is

$$\frac{M_{pg}}{M_{ampi}} = \frac{[PG]}{[Ampi]} \times \frac{M_w(PG)}{M_w(Ampi)} \tag{1}$$

The weight fraction of phenylglycine crystals, X_{pg}, can then be written as

$$X_{pg} = \frac{M_{pg}}{M_{pg} + M_{ampi}} = \frac{M_{pg}/M_{ampi}}{1 + M_{pg}/M_{ampi}} \tag{2}$$

The mass fraction of ampicillin crystals, X_{ampi}, can be calculated from X_{pg} via

$$X_{ampi} = 1 - X_{pg} \tag{3}$$

Most experiments were duplicated, and the standard deviations of the calculated average mass fractions (X_{pg}) in the interphase and in the sediment ranged between 0.01 and 0.13 (except for the experiment performed at X_{pg} = 0.20, where X_{pg}(i) = 0.61 with a standard deviation = 0.20; see below).

The results for the compositions of the interphase layer and sediment are denoted by X_{pg}(i) and X_{pg}(s), respecitively, and are given as functions of the feed composition (X_{pg}). Also, distribution coefficients are defined for phenylglycine, K_{pg}, and for ampicillin, K_{ampi}, as follows

$$K_{pg} = \frac{X_{pg}(i)}{X_{pg}(s)} \tag{4}$$

$$K_{ampi} = \frac{X_{ampi}(i)}{X_{ampi}(s)} \tag{5}$$

The expression for K_{ampi} can be rewritten as a function of X_{pg} and K_{pg} by combining eqs 3 and 4 to give

$$K_{ampi} = \frac{1 - X_{pg}(s)K_{pg}}{1 - X_{pg}(s)} \tag{6}$$

The selectivity parameter for interfacial partitioning, α, of phenylglycine relative to ampicillin is defined as

$$\alpha = \frac{K_{pg}}{K_{ampi}} \tag{7}$$

A high value for the selectivity indicates a good potential for separation of the crystals in the interphase and sediment.

Calculation of Masses of Crystals in the Interphase and Sediment. The masses of each type of crystals in the interphase and sediment are determined from the HPLC analysis data and mass balances. The mass balance equation for phenylglycine crystals reads

$$X_{pg}(i)\, M(i) + X_{pg}(s)\, M(s) = M_{pg} \tag{8}$$

and the mass balance equation for ampicillin crystals is

$$X_{ampi}(i)\, M(i) + X_{ampi}(s)\, M(s) = M_{ampi} \tag{9}$$

where M(i) is the total mass of crystals at the interphase; M(s) is the total mass of crystals in the sediment, and M_{pg} and M_{ampi} are the initial masses of phenylglycine and ampicillin crystals, respectively. Equations 8 and 9 are simultaneously solved, and the total masses of crystals in the interphase and sediment are determined. With a knowledge of the compositions, the masses of each of the crystals in the interphase and sediment can be determined.

In the above method, the mass of crystals dissolved has not been taken into account. This can be estimated from the solubility values in Table 2. The dissolved amount is approximately the same for experiments performed in the same biphasic system under different

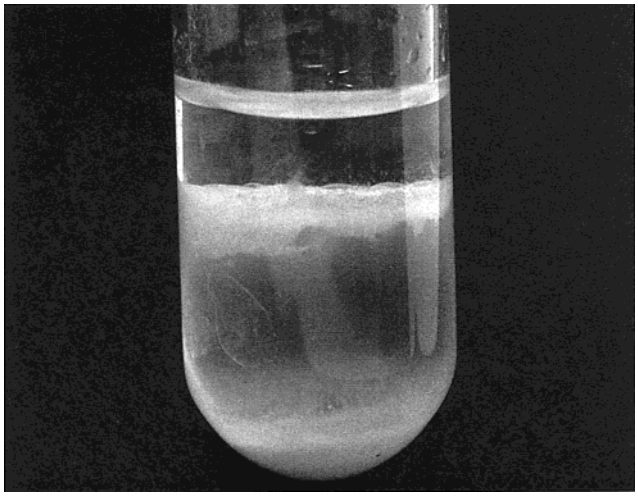

Figure 3. Interfacial partitioning of phenylglycine (33 mg) and ampicillin (57 mg) crystals in water/butanol (2:1, v/v). The interphase mainly contains phenylglycine crystals ($X_{pg} = 0.79$), and the sediment is mostly ampicillin crystals ($X_{pg} = 0.12$).

conditions of feed composition and total mass of crystals. For example, in water/butanol, 2:1 (v/v), 7 and 10 mg of phenylglycine and ampicillin, respectively, dissolve in the bottom phase.

Measurement of the Liquid Composition at the Interphase. Our current understanding is that the interphase layer is a complex dispersion of the bottom and top phases stabilized by ampicillin and phenylglycine crystals. To estimate the liquid composition of the interphase layer, the volume fractions of the top and bottom phases in the interphase layer were measured. For these measurements, small amounts (1.5 mL) of water and *n*-butanol were added to a test tube, mixed, and allowed to form two clear layers. The position of the interface between the two liquids and the surface of the top phase were marked. Crystals were added (44 mg in total) to the biphasic system, and experiments were performed as described above. Again, the upper and lower interfaces of the interphase and the air−liquid surface of the top phase were marked. The changes in volume of the top and bottom phases and the interphase were determined by adding water up to the marks on the tube and weighing.

Results and Discussion

Selective Interfacial Partitioning: Formation of an Interphase and Sediment. Approximately 30 min after mixing, the top and bottom phases were transparent, with no particles in suspension, and a stable and thick interphase layer and sediment were formed (Figure 3). This interphase can be up to 0.5 cm thick, the thickness being proportional to the amount of phenylglycine in the feed. The fraction of sediment, however, increases with the ampicillin in the feed. In the water/butanol system (Table 3), most of crystals in the interphase (70%) were phenylglycine, whereas the sediment consisted mostly of ampicillin crystals (86−93%). Rather high selectivities were achieved, specifically $\alpha = 13.1$ and 31 for two different initial masses of feed crystals (Table 3). In the water/hexanol system, efficient separation of phenylglycine from ampicillin crystals was achieved as well (Table 3). When the water/hexanol ratio was 1:2 (v/v), the interphase layer was almost in contact with the sediment in the bottom phase. Therefore, an increase in the volume ratio of water (2 mL of water/1 mL of butanol) resulted in improved separation of the layers and improved separation of the crystals. Maximum selectivity ($\alpha = 90.3$) was observed in the water/hexanol system.

Table 3. Crystal Composition of the Interphase and Sediment

biphasic system	feed crystals (mg)	X_{pg}	X_{pg}(i)	X_{pg}(s)	α
water/butanol 2:1 (v/v)	56	0.48	0.68	0.14	13.1
	89	0.36	0.70	0.07	31.0
water/hexanol 1:2 (v/v)	64	0.50	0.66	0.16	10.2
water/hexanol 2:1 (v/v)	53[a]	0.52	0.82	0.11	36.9
	90	0.33	0.79	0.04	90.3

[a] The duplicate experiment was performed with 72 mg of total solids.

In the ATPS, most crystals partitioned to the interface, and a small fraction to the air−liquid surface of the top phase. The thickness of the interphase increased with increasing ampicillin content in the feed mixture. Because no selectivity was achieved with this particular ATPS, subsequent experiments were performed with aqueous/organic systems.

In a previous study by the authors with a single type of crystal,[6] the formation of an interphase layer with an emulsion-like structure was observed. In fact, some droplets were visualized at the surface of the interphase layer and near the glass walls. In the experiments listed in Table 3, this emulsion-like structure is not clearly shown. However, it was observed that the interphase developed mostly from the liquid−liquid interface down to the bottom phase. This suggests that a water-in-organic emulsion is formed of the dispersed bottom phase in the continuous top liquid phase. This type of emulsion is stabilized by phenylglycine crystals,[6] and therefore, the adsorption of this type of crystals within the interphase is favored. Because the adsorption of ampicillin crystals to the interface is less favored, they form a sediment in the bottom phase. As a net result, ampicillin crystals are displaced by phenylglycine crystals.

Jauregi et al.[6] observed that single-component crystals of both types partitioned to the interface of water/butanol and water/hexanol systems, which clearly demonstrates the displacement effect. These results indicate some direct or indirect interaction between the two types of crystals that alters their partitioning behavior. It should also be noted that there is a limit to the amount of particles that can be loaded to the interface, as shown by Jauregi et al.[6] Above this saturation capacity, additional particles sediment. In the case of mixtures of particles, the resulting sediment composition is different from the feed composition.

Effect of Feed Composition. According to the above observations, the composition of the crystal feed influences the selectivity of the partitioning. In Table 3, the results of two different feed compositions are shown for $X_{pg} = 0.4$ (0.3 in the water/hexanol system) and $X_{pg} = 0.5$. The results differ mainly in the sediment composition. An increased amount of ampicillin in the feed mixture, and thus an increase in the total solids in the feed, results in an increased amount of ampicillin in the sediment and, hence, an increased selectivity. In this set of experiments, the total amount of crystals in the feed was also varied, but the total amount of crystals was kept constant to study the effect of feed crystal composition.

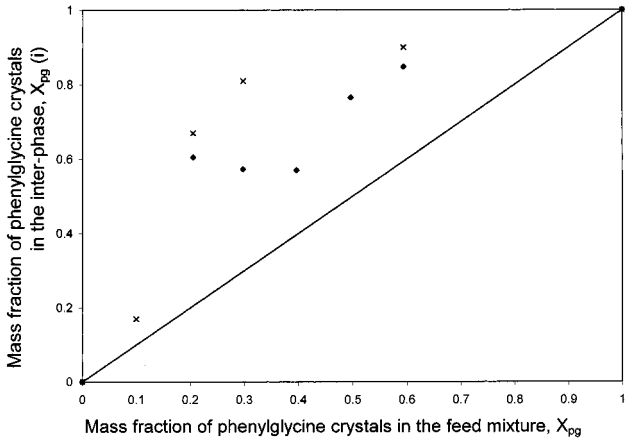

Figure 4. Effect of the feed crystal composition, X_{pg}, on the crystal composition at the interphase, X_{pg}(i), for series A (◆) and series B (×).

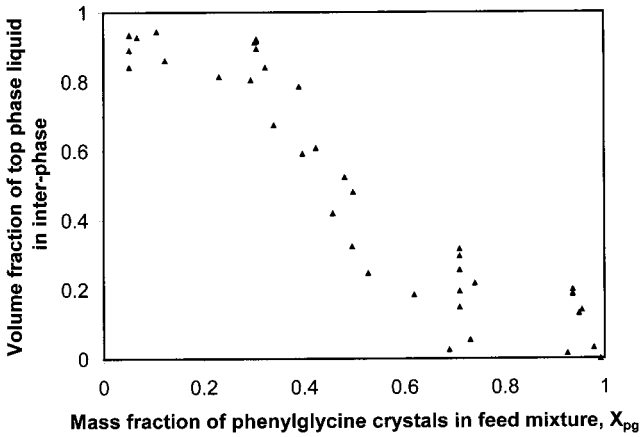

Figure 5. Effect of the feed crystal composition, X_{pg}, on the volume fraction of the top phase in the interphase.

Table 4. Effect of Feed Crystal Composition for Constant Total Solids Amount[a]

feed X_{pg}	X_{pg}(i)	X_{pg}(s)	α	sediment phenylglycine (%)	ampicillin (%)
0.10[b]	0.22 (0.10)	0.02 (0)	13.8	12.0 (0)[c]	65.3 (0)[c]
0.20	0.61	0.05	29.8	17.5	86.1
0.30	0.57	0.14	8.2	29.7	77.8
0.40	0.57	0.05	27.2	3.9	52.4
0.50	0.77	0.11	28.2	8.7	72.5
0.60	0.85	0.07	74.4	3.9	74.8

[a] The crystal compositions in the interphase and sediment and the mass percentages of each of the crystals in the sediment in relation to their initial masses are shown. [b] These data are from the B set of experiments, but the remaining data in the table are from the A set of experiments. [b] After mixing, all of the crystals partitioned to the interphase, and no spontaneous sedimentation (only very few crystals sedimented) was observed. In parentheses are indicated the corresponding values if no sedimentation is considered. The values in the table correspond to the values measured after additional (manual) shaking was applied.

Experiments were carried out in water/butanol, 2:1 (v/v), at different feed compositions. The total amount of crystals was kept constant at approximately 100 mg. Two series of experiments were performed: Series A with $X_{pg} = 0.20$, 0.30, 0.40, 0.50, and 0.60 (experiments were duplicated) and series B with $X_{pg} = 0.10$, 0.20, 0.30, and 0.6 (only the first point, $X_{pg} = 0.10$, was duplicated). In series A, phenylglycine crystals were weighed first and added to the biphasic system, followed by ampicillin, and in series B, ampicillin crystals were weighed first and added to the biphasic system, followed by phenylglycine. The results are shown in Figure 4.

It is noteworthy that, when the feed was composed mainly of ampicillin, $X_{pg} = 0.10$, no spontaneous sedimentation was observed (only very few crystals left the interphase after mixing). Only after additional manual shaking was some sediment formed. The sediment was mainly composed of ampicillin crystals [X_{pg}(s) = 0.02, $\alpha = 13$], so sedimentation is essential to obtain separation. When the phenylglycine content in the feed was increased, a more stable interphase was formed, and the phenylglycine composition at the interphase increased well above the parity line in Figure 4. This again suggests that the presence of phenylglycine crystals play an important role in stabilizing the interphase.

Liquid Composition of the Interphase. "Pure" ampicillin crystals stabilize butanol-in-water emulsion, whereas "pure" phenylglycine crystals stablize water-in-butanol emulsions.[6] For increasing phenylglycine contents in mixtures of the two crystals, a transition from a butanol-in-water emulsion to a water-in-butanol emulsion is expected. In Figure 5, the liquid composition of the interphase is plotted versus the feed crystal composition. For an ampicillin-rich crystal feed (X_{pg} = 0.10−0.30), the interphase is mainly composed of the butanol-rich top phase. This seems to be consistent with an interphase layer developing toward the top phase and suggests that a butanol-in-water emulsion is formed. This emulsion seems to lead to selective phenylglycine adsorption as well. When the phenylglycine content is increased from $X_{pg} = 0.1$ to $X_{pg} = 0.3$, the overall liquid composition remains the same. The volume fraction of the top phase reduces gradually between $X_{pg} = 0.3$ and 0.6, to remain virtually constant at $X_{pg} > 0.6$. This seems to parallel the formation of mixed emulsions for X_{pg} = 0.3−0.6 and of a water-in-butanol emulsion at higher phenylglycin contents. The uptake of phenylglycine and increasing displacement of ampicillin at increasing overall phenyglycine content supports this mechanism. For $X_{pg} < 0.3$, an increasing phenylglycine content promotes sedimentation of ampicillin. In the intermediate region, $0.3 < X_{pg} < 0.6$, the capacity of the interphase to adsorb solids doubles from 30−40 mg to 60−70 mg (Table 4), mostly through an increased phenylglycine uptake. At high phenylglycine content, $0.5 < X_{pg} < 0.6$, the capacity remains constant, as does the overall crystal composition.

The two sets of experiments in Figure 4 differ quite significantly, particularly in the intermediate region where the formation of mixed emulsions can occur. Differences between the two sets could also be due to the preparation method. In series B, ampicillin crystals were first weighed and added to the biphasic system. Ampicillin crystals remain mostly in the bottom phase while phenylglycine crystals are being weighed. This could have had the same effect as prewetting ampicillin in the bottom phase, which might enhance its affinity for this phase and hence increase the selectivity.

Effect of Interface Loading. In these experiments, the initial mass of crystals was varied to determine the capacity of the interphase to adsorb solids. The feed composition was kept constant ($X_{pg} = 0.5$). A limited capacity for the adsorption of solids in the interphase should result in increased particle sedimentation at increased overall particle loading to the system. The results in Table 5 show that an increase in the total amount of crystals from 56 to 167 mg (in the water/

Table 5. Variation of the Crystal Loading for Constant Feed Composition[a]

biphasic system	feed crystals (mg)	X_{pg}(i)	X_{pg}(s)	selectivity α	sediment PG (%)	ampi (%)
water/butanol	56	0.70	0.14	13.1	10.0	61.4
2:1 (v/v)	82	0.93	0.05	252.4	4.4	93.1
	100	0.77	0.11	27.1	8.7	72.5
	167	0.92	0.15	65.2	15.7	92.2
water/hexanol	53	0.84	0.04	126.0	3.2	80.7
2:1 (v/v)	72	0.80	0.18	18.2	14.8	75.6
	170	0.92	0.04	276.0	4.0	91.7

[a] $X_{pg} = 0.5$. [b] The mass percentages of phenylglycine and ampicillin crystals sedimented in relation to their initial masses are also indicated.

Table 6. Effect of the Volume Ratio of the Liquid Phases[a]

	feed					sediment	
biphasic system	X_{pg}	crystals (mg)	X_{pg}(i)	X_{pg}(s)	α	PG (%)	ampi (%)
water/butanol 2:1	0.48	56	0.68	0.14	13.1	10.1	60.4
	0.36	89	0.70	0.07	31	11.7	75.8
water/butanol 4:1	0.49[b]	54	0.59	0.17	7	8.1	39.5
	0.33[c]	83	0.73	0.04	64.9	7.1	82.5

[a] The mass percentages of phenylglycine and ampicillin crystals sedimented in relation to their initial masses are also indicated. [b] Very little sediment was formed, so additional (manual) shaking was applied. [c] All experiments were duplicated except for this one.

butanol system) indeed resulted in increased ampicillin sedimentation and increased selectivity, with α ranging from 13.1 to 252.4. There is, however, a considerable spread in the selectivity, mostly because of the variation of the small amounts of phenylglycine in the sediment.

In the water/hexanol biphasic system, an increase in the total amount of crystals in the feed from 53 to 170 mg resulted in an increased ampicillin content in the sediment and also a decrease of phenylglycine in the sediment and, hence, an increased selectivity (α = 126 and 276 respectively). Thus, maximum selectivity seems to be achieved in the water/hexanol system at the maximum crystal loading, whereas the selectivity in the water/butanol system seems to be a maximum at intermediate crystals loadings.

Effect of the Liquid-Phase Volume Ratio. The type of emulsion formed was governed by the particle feed composition as well as by the volume ratio of the liquid phases. This affects the crystal uptake and interfacial selectivity, as shown by some experiments performed in a 4:1 (v/v) water/butanol biphasic system. These results are compared to those performed in 2:1 (v/v) water/butanol in Table 6.

For $X_{pg} = 0.5$ in the different biphasic systems, at an increased water-phase volume ratio (4:1), a decrease in selectivity is observed as a result of increased adsorption of ampicillin at the interphase. In this case, only 40% of ampicillin crystals sedimented, whereas 60% of ampicillin crystals sedimented in a 2:1 (v/v) water/butanol system. However, when both the ampicillin content in the feed ($X_{pg} = 0.33$) and the total amount of solids were increased, most of the ampicillin sedimented in both biphasic systems (76 and 83% for 2:1 and 4:1 water/butanol, respectively), with the selectivity being higher at increased water-phase volume ratio (α = 31 and 64.9, respectively).

Again, these results can be explained on the basis of the emulsion-like interphase. When the water-phase volume is increased up to 4 times that of butanol, a butanol-in-water emulsion, favoring ampicillin adsorption, is formed.[6] This agrees with the increased adsorption of ampicillin at the interphase. Furthermore, it was observed that ampicillin formed less stable emulsions and that adsorption at interphase is less favored for ampicillin than for phenylglycine. Therefore, an increase in ampicillin crystals in the feed ($X_{pg} = 0.33-0.36$) results in increased sedimentation of ampicillin crystals and higher selectivity.

Experiments were also performed with water/hexanol systems, but the interphase layer and sediment were difficult to distinguish visually.

Table 7. Effect of Centrifugal Force at Constant Feed Composition[a]

centrifugal speed (rpm)	feed crystals (mg)	X_{pg}(i)	X_{pg}(s)	selectivity α	sediment PG (%)	ampi (%)
0	82	0.93	0.05	252.4	4.4	93.1
1800	61	0.41	0.76	0.22	38.5	6.6
3600	50	0.40	0.85	0.12	37.8	12.5

[a] $X_{pg} = 0.5$. [b] The mass percentage of phenylglycine and ampicillin crystals sedimented in relation to their initial mass is also shown.

Effect of Centrifugal Force. The particles employed in this work are still sufficiently large to be affected by forces such as a centrifugal field. The use of a centrifugal field allows for variations in the magnitude of the interfacial forces relative to gravity and buoyancy. Experiments were performed in water/butanol, 2:1 (v/v), with $X_{pg} = 0.5$ under gravity conditions and for two different rotational velocities, 1800 and 3600 rpm, as described in the Materials and Methods section. The results are shown in Table 7.

It is interesting to note that the selectivity of partitioning has been reversed upon application of centrifugal force, i.e., ampicillin is preferentially partitioned at the interphase, and phenylglycine partitions to a large extent (around 40% at both speeds) to the sediment with a subsequent reduction in selectivity.

These results confirm that the selectivity of interfacial partitioning of phenylglycine relative to ampicillin crystals under gravity conditions is driven by a difference in interfacial interaction and not by differences in density or size.

Conclusions

Interfacial partitioning of bioparticles using water/alkanol biphasic systems seems to be a novel and potent process for the separation of particles. It results in high selectivities for mixed ampicillin/phenylglycin crystals with $X_{pg} = 0.5$ in 2:1 (v/v) water/butanol (α = 252.4) and 2:1 (v/v) water/hexanol (α = 276) but is expected to be a more general tool, even when conditions favor the formation of a butanol-in-water emulsion.

The interphase is not a single-crystal layer but a thick emulsion-like layer with a thickness of at least 0.5 cm, and it is stabilized by the crystals. In this work, phenylglycin crystals seem to favor the formation of a water-in-butanol emulsion. Ampicillin crystals favor an interphase, rich in the top phase, which might be a butanol-in-water emulsion.

Phenylglycine crystals displace ampicillin crystals from the interphase, and a sediment is formed that is composed mainly of ampicillin crystals. When a majority of the crystals in the feed mixture are ampicillin, they tend to adsorb at the interface, which results in an unstable interphase that can easily be disrupted by

applying additional (manual) shaking. Adsorption of ampicillin crystals at the interphase, rather than phenylglycine crystals, is not favored, even when conditions favor the formation of butanol-in-water emulsion.

Further research should be carried out to obtain a better understanding of the mechanisms of interfacial partitioning for the prediction and optimization of processes. However, it has been shown here that this is a promising technology of particular relevance for biotechnological applications where the development of innovative technology for the selective separation of solid products of similar sizes and densities is necessary.

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