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Liquid-Liquid Extraction for Recovery of Paclitaxel from Plant Cell Culture: Solvent Evaluation and Use of Extractants for Partitioning and Selectivity

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Abstract

A major challenge in the production of metabolites by plant cells is the separation and purification of a desired product from a number of impurities. An important application of plant cell culture is the biosynthesis of the anti-cancer agent paclitaxel. Liquid-liquid extraction plays a critical role in the recovery of paclitaxel and other valuable plant-derived products from culture broth. In this study, the extraction of paclitaxel and a major unwanted by-product, cephalomannine, from plant cell culture broth into organic solvents is quantified. Potential solvent mixtures show varying affinity and selectivity for paclitaxel over cephalomannine. The partition coefficient of paclitaxel is highest in ethyl acetate and dichloromethane, with measured values of 28 and 25, respectively; however selectivity coefficients are less than 1 for paclitaxel over cephalomannine for both solvents. Selectivity coefficient increases to 1.7 with extraction in n-hexane but the partition coefficient decreases to 1.9. Altering the pH of the aqueous phase results in an increase in both recovery and selectivity using n-hexane, but does not change the results for other solvents significantly. The addition of extractants trioctyl amine (TOA) or tributyl phosphate (TBP) to n-hexane gives significantly higher partition coefficients for paclitaxel (8.6 and 23.7, respectively), but no selectivity. Interestingly, when 20% hexafluorobenzene (HFB) is added to n-hexane, the partition coefficient remains approximately constant but the selectivity coefficient for paclitaxel over cephalomannine improves to 4.5. This significant increase in selectivity early in the purification process has the potential to simplify downstream processing steps and significantly reduce overall purification costs.

Keywords

plant cell culture; paclitaxel; bioseparations; downstream processing; partition coefficient; selectivity

INTRODUCTION

The number of compounds produced by biosynthesis has grown dramatically in recent years, particularly in plant cell culture systems.^{1,2} Cell culture offers a number of challenges in designing economically feasible processes, especially the separation and isolation of a valuable product from an array of impurities. The plant cell culture process for supply of the anti-cancer agent paclitaxel (Taxol®, Bristol-Myers Squibb) has demonstrated commercial success. Paclitaxel is a member of the taxane family of compounds that consist of over 100

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structurally similar chemicals,³ many of which are synthesized in conjunction with paclitaxel in *Taxus* cell culture systems.⁴ Cephalomannine is one of the most difficult taxanes to separate from paclitaxel due to similarities in structure (Figure 1) and physical properties. The conditions for paclitaxel production and recovery from cell culture broth must be chosen not only to efficiently separate paclitaxel from other structurally related taxanes (e.g., cephalomannine), but also to avoid product degradation, which can be significant for secondary metabolites in cell culture.⁵

Separation and purification of paclitaxel from plant cell culture requires several steps including product removal from media and/or culture broth, isolation using an organic solvent, crude purification, and final purification. A general processing sequence for paclitaxel production is summarized in Table 1. The removal of paclitaxel from the feed is accomplished by disrupting the cells and filtering the suspension to remove cellular debris from the aqueous culture medium. As up to 90% of the product can remain bound within the cells, likely in the cell wall,^{6–9} this additional processing step must be added to disrupt the cells and allow for recovery of cell-associated paclitaxel. This can be accomplished through vigorous mixing or sonication, followed by soaking the solid residue in an alcoholic solvent such as methanol or ethanol.¹⁰

Isolation of paclitaxel from plant cell culture broth is commonly accomplished by liquid-liquid extraction (LLE).¹¹ LLE has been shown to recover a high fraction of paclitaxel using organic solvents such as dichloromethane and chloroform, due to the fact that paclitaxel has a much higher affinity for these organic solvents than it does for the aqueous cell culture medium. A number of factors must be considered in evaluating the use of potential solvents for this process, including capacity for partitioning of product, selectivity for desired product over other components, miscibility with the aqueous phase, and property difference from products to allow for adequate separation and recovery.¹² Existing LLE processes for paclitaxel separation show little selectivity for paclitaxel over other taxane molecules, particularly cephalomannine. Achieving a greater selectivity would allow for equipment size reduction and simplification of further downstream processing steps. Chemical stability, availability and cost, and environmental toxicity are also important concerns.

Crude purification of paclitaxel is typically accomplished through evaporation or precipitation to remove the organic solvent for recycle.^{13,14} Final purification or “polishing” of paclitaxel is performed using a series of progressively finer chromatography stages. The crude product is redissolved, typically in methanol, and then resolved into individual taxanes by high-performance liquid chromatography, HPLC. After several chromatography steps, usually three or more, a paclitaxel purity of greater than 99% can be achieved.¹⁵ Because the separation and purification of paclitaxel from cell culture is complex, separation costs constitute a significant percentage of total processing costs; the final two steps of the process, crude purification and polishing of paclitaxel, account for the bulk of these.¹⁶ For this reason, it is desirable to reduce the size and number of chromatography steps necessary to purify paclitaxel. This would be possible if the upstream processing steps (e.g., LLE) were improved to achieve a greater selectivity for paclitaxel over impurities.

In a typical LLE process, the product is to be recovered from a dilute aqueous feed. This feed is contacted with an immiscible solvent phase in a series of stages. The ratio of the concentration of a species in one phase to the other is the *partition coefficient* and can be expressed as:

$$K_p = \frac{C_{\text{SOLVENT}}}{C_{\text{FEED}}} \quad (1)$$

Following the completion of the extraction process, the product-poor aqueous phase (raffinate) is discarded, while the product-rich organic phase (extract) is further treated to purify the product and recover the solvent. The solvent flow rate, feed flow rate, and distribution coefficient are naturally combined into a single parameter, the *extraction factor*:

$$E = \frac{S K_p}{F} \quad (2)$$

The *single-stage fractional recovery* of paclitaxel is defined as the mass of paclitaxel recovered in the extract phase divided by the mass of paclitaxel originally present in the aqueous phase:

$$F_R = \frac{m_{EXT}}{m_{INIT}} \quad (3)$$

The *selectivity* for a single-stage extraction process is the amount of a desired product extracted compared to the amount of an unwanted by-product:

$$S_{ij} = \frac{K_{pi}}{K_{pj}} \quad (4)$$

Often, the presence of an extractant, also called a “reactive” agent or carrier molecule, in the solvent phase can increase the partition coefficient for a valuable component.¹⁷ An extractant in the solvent phase can form a complex with the component in the aqueous phase, which makes the complex soluble in the solvent phase.¹⁸ Addition of extractants has several advantages over more established methods, such as precipitation by calcium salts,¹⁹ including higher recoveries, lower solvent usage, and reduction of waste products. Extractants such as phosphorus based compounds and aliphatic amines have been used for the recovery of variety of carboxylic acids from aqueous streams.^{20–23} In this situation, the partition coefficient depends on the concentration of extractant as well as product concentration.^{20,24} Other factors such as the solvent type and pH of the aqueous stream also influence the selectivity and partition coefficient.¹⁷ Because of the large demand for carboxylic acids, their extraction has been studied for a number of different extractants, organic carriers, and operating conditions.²⁵ To the best of our knowledge, there have been no studies on the use of extractants to recover high-value products from plant cell cultures.

Due to the complex nature of taxane molecules, solution behavior is difficult to predict. This is primarily due to the interaction of the polar and nonpolar regions, which affect solubility, dimerization, and mass transfer rate.²⁶ Because equilibrium behavior is difficult to predict, we have measured the partitioning of taxanes (specifically paclitaxel and cephalomannine) in various solvents under different conditions (e.g., changes in pH). Four different solvent groups were examined: straight-chain alkanes (e.g., n-hexane), halogenated methanes (e.g., dichloromethane), esters (e.g., ethyl acetate), and aromatics (e.g., toluene and hexafluorobenzene (HFB)). The effect of addition of extractants (trioctyl amine (TOA) and tributyl phosphate (TBP)) was also evaluated. We quantify these studies with values for the partition coefficient and selectivity coefficient for paclitaxel over cephalomannine. The partition coefficient can be used to estimate the number of stages and solvent flow required to recover the product. Selectivity coefficient is valuable in determining the size and complexity of downstream processing steps to produce pure paclitaxel. Ultimately, the separation system can be optimized to reduce cost while achieving the required purity for paclitaxel.

MATERIALS AND METHODS

To simulate the processing of plant cell culture broth, a conditioned cell culture medium was used as the aqueous phase. *Taxus* cells were cultured in Gamborg's B-5 Basal Medium (Caisson Laboratories, Sugar City, ID) for approximately 14 days as described elsewhere.²⁷ Metabolic products are secreted into the medium, resulting in a mixture of proteins, sugars, lipids, and other plant metabolites. Nontaxane-accumulating cell cultures were utilized so that paclitaxel and cephalomannine concentrations could be strictly controlled. The cell suspension was filtered through Miracloth® (CalBioChem, LaJolla, CA) to remove the cells. The conditioned medium was either used immediately or frozen at -50°C to prevent degradation of proteins and other cellular products.

Stock solutions of paclitaxel (Sigma Chemical, St. Louis, MO) and cephalomannine (Hauser Chemical, Denver, CO) were prepared in methanol at 1 mg/mL and a carefully measured volume was added to the filtered cell culture medium to achieve the desired taxane concentration, typically 7.5 mg/L to 20 mg/L, which is typical of what is observed in our *Taxus* suspension cultures upon elicitation with methyl jasmonate.²⁸ The filtered conditioned medium from unelicited *Taxus* suspension cultures used in this study did not contain appreciable concentrations of taxanes, and therefore paclitaxel and cephalomannine concentrations could be strictly controlled. Within the concentration range evaluated (0.75 – 2.0 % w/v), the partition coefficient did not change significantly for the solvents tested. The medium was vortexed at high speed for one minute to ensure that the taxanes were well-dispersed in solution. 5 mL of media was transferred to a 20 mL scintillation vial and combined with the organic solvent, usually in a 1:1 ratio, to simulate the extraction process. Since solution pH affects partitioning behavior, the pH value was measured with a Beckmann $\phi 45$ pH meter (Fullerton, CA). In some experiments the pH was adjusted with dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH). To prevent interaction with the surrounding environment, all vials were sealed with Parafilm. Mixing was achieved using one of two methods. In the first method, the vials were placed on a shaker (Labline, Boston, MA) at 125 rpm and 24°C . This resulted in only partial mixing of the two liquid phases, requiring long extraction times to reach equilibrium. In the second method, the vials were sonicated for 10–30 minutes using a Ultrasonic Model 75HT sonicating bath (Aquasonic, West Chester, PA), resulting in a more complete dispersion of the two phases and a more rapid approach to equilibrium. After mixing, each sample was transferred to a glass separatory funnel and the mixture was allowed to settle for 10 minutes for complete phase separation. The lower phase was then carefully drained from the bottom of the funnel using a stopcock and the upper phase was poured from the top of the funnel into a clean vial.

The separated phases were next prepared for HPLC analysis to determine the taxane content. For aqueous samples, 1 ml was dried completely using a Savant SC110A Speed Vac Plus® (Brinkmann Instruments, Westbury, NY) equipped with an RVT400 Refrigerated Vapor Trap. The organic phase (1 ml) was evaporated to dryness under a filtered air stream. The necessary time to dry these samples varied from 1–12 hours, depending primarily on the volatility of the organic solvent. After the samples were dried they were resuspended in a known volume of acidified methanol (0.01% acetic acid). The samples were sonicated for 20 minutes to completely disperse the solid residue and then subsequently centrifuged for 10 minutes at 10,000 rpm. The sonication and centrifugation steps were repeated twice to ensure that the residue was completely dissolved.

200 μL of each sample were filtered (0.2 μm PVDF, Pall Gelman, East Hills, NY) prior to HPLC analysis to remove any debris. The filtered samples were analyzed via HPLC, using a Waters 2690 Separation Module. A Metachem Taxil 5 μ reversed-phase C-18 resin column was utilized to separate the components. 30 μL of each sample were injected into the

column, using a mixture of 52-volume % acetonitrile and 48-volume % water as the mobile phase. The output of the column was analyzed using the Waters 996 Photoiode Array Detector. Standard curves for paclitaxel and cephalomannine were developed for each HPLC run and used to quantify the amount of taxane in each sample.

RESULTS AND DISCUSSION

Paclitaxel Partitioning

Four distinct solvent classes were evaluated for their ability to extract paclitaxel from the aqueous cell culture broth. Each solvent chosen fit at least one of several criteria including ease of use in a bioprocess, previous success in extracting paclitaxel from cell culture, or low environmental toxicity. The data presented are in the form of equilibrium partitioning coefficients, with corresponding single-stage fractional recovery values (Table 2). Lower single-stage fractional recoveries indicate that multiple stages or high solvent flow rates would be necessary to avoid significant product losses. Each solvent was tested in multiple experiments and the equilibrium partition coefficients measured ranged from 1.9 (for n-hexane) to 28 (for ethyl acetate) (Table 2). Because paclitaxel has a phenyl group on the side chain portion of the molecule that many other taxanes lack, an inert aromatic solvent, HFB (C_6F_6), was also evaluated. The HFB-n-hexane mixture did not significantly increase partitioning over n-hexane alone with a measured partition coefficient of only 2.4, which would yield a single-stage fractional recovery of approximately 70%. HFB and n-hexane were tested for a range of compositions (data not shown) and the best results occurred at 10–20% HFB.

Aromatic solvents such as benzene and toluene have shown strong affinity for taxane molecules,²⁹ and these molecules have low miscibility in aqueous solutions and are relatively inexpensive. A partition coefficient of 16.9 was measured for paclitaxel in toluene with a corresponding single-stage fractional recovery of 94%. Both halogenated methanes and esters have also been demonstrated success in the extraction of taxane molecules from cell culture broth.^{16,30} Dichloromethane and ethyl acetate were tested and yielded high partition coefficients of 25 and 28, respectively, with corresponding single-stage fractional recoveries of approximately 97%.

Paclitaxel Selectivity

The capacity of a solvent to extract paclitaxel efficiently is important, but selectivity for paclitaxel over structurally similar compounds also has a significant influence on recovery costs. Identification of a solvent system with demonstrated selectivity for paclitaxel over other taxane molecules (e.g., cephalomannine) has the potential to significantly reduce the costs of separation in the plant cell culture bioprocess. For this reason, the solvents described above were evaluated for selectivity of paclitaxel over cephalomannine (equation 4). Again, multiple experiments were performed with each solvent and the selectivity data are summarized in Table 2. Statistically significant differences from a value of one (i.e., no selectivity) are indicated in the table.

The selectivity coefficients for paclitaxel over cephalomannine ranged from 0.5 (for ethyl acetate) to 4.5 (for the HFB: n-hexane mixture). Ethyl acetate favors extraction of cephalomannine over paclitaxel, which would result in an extract stream with increased concentration of impurities, hence complicating subsequent processing. In addition, ethyl acetate has a solubility of approximately 10% in water at ambient temperature so its recovery is a concern. Dichloromethane and toluene exhibited selectivity coefficients of 0.8 and 1.3 respectively, which were not statistically different from a value of one, and thus had no preference for extracting either paclitaxel or cephalomannine. In addition dichloromethane³¹ and toluene have considerable environmental toxicity. Therefore,

although ethyl acetate, dichloromethane, and toluene exhibited high partition coefficients for paclitaxel, the lack of selectivity and environmental concerns reduces the interest in their use for an improved LLE process.

n-Hexane gives a selectivity coefficient of 1.7, which was statistically greater than a value of one. Therefore, using n-hexane as the organic solvent in a LLE process would increase the ratio of paclitaxel to cephalomannine in the extract stream. A mixture of 80:20 volume percent n-hexane and HFB improved selectivity considerably (selectivity coefficient of 4.5). One possible explanation for higher selectivity of paclitaxel over cephalomannine is because of the difference in side chain structure of paclitaxel and cephalomannine (Figure 1). Paclitaxel has a benzoyl group, whereas cephalomannine has a 2-methyl-2-butenoyl group on the C-13 side chain. HFB molecules can form complexes with benzene.^{32–34} A specific interaction between HFB (in n-hexane) and the benzene moiety in the paclitaxel side chain is potentially the reason for higher selectivity of paclitaxel over cephalomannine in HFB-hexane. n-Hexane and HFB gave significantly lower recoveries of paclitaxel (with partition coefficients in the range of 2.0; Table 2), necessitating a series or cascade of extraction stages. However, the observed selectivity has the potential to significantly reduce the size and cost of subsequent processing steps. It should be noted that although the effects of long-term exposure to HFB have not been as thoroughly studied as for some other traditional solvents, it has demonstrated less toxicity than dichloromethane and toluene.^{35,36} Due to higher selectivity of paclitaxel, the application of n-hexane-HFB (80:20) solvent in a LLE process for paclitaxel recovery would therefore be desirable.

Addition of Extractants

The effectiveness of the extractants TOA and TBP for enhancing paclitaxel recovery was also investigated (Figure 2). The addition of TOA to n-hexane resulted in an increase in the recovery of paclitaxel over that obtained with n-hexane alone for mixtures of greater than 15 volume percent TOA. The partition coefficient for paclitaxel increased as the concentration of TOA increased, with a highest partition coefficient value (8.6) measured at a composition of 25:75 volume percent TOA:n-hexane. Further increases in TOA had no significant effect on the partition coefficient (data not shown). The corresponding single-stage fractional recovery of paclitaxel for the 25:75 mixture is 82%.

For addition of TBP to n-hexane, the paclitaxel partition coefficients for n-hexane/TBP mixtures were more than ten times higher than for n-hexane alone (Table 2). Statistically significant increases over n-hexane alone were measured for almost the entire range of volume percentages tested. The highest partition coefficient, approximately 24, was observed at a solvent concentration of 5:95 volume percent TBP:n-hexane. The partition coefficients decreased with TBP concentration beyond this point. At high concentrations of extractant, nonpolar solvents (e.g., n-hexane) may not be able to solvate the TBP-paclitaxel complex effectively, as was observed with extraction of picolinic acid using TBP-sunflower oil.²⁰ This observed decrease might be due to the high viscosity of TBP (3.5 mPa-s for TBP compared to 0.29 mPa-s for n-hexane at 25°C), which makes thorough mixing of the two phases more difficult as the concentration of TBP is increased.²⁰ The addition of TBP to n-hexane resulted in an improvement of up to 30% in single-stage fractional recovery (from 65% to 95%; Table 2). These results demonstrate that both extractants significantly improve paclitaxel partitioning into n-hexane. However, the addition of the high molecular weight extractants will also complicate solvent recovery, which needs to be evaluated when making design decisions. The addition of TBP or TOA to n-hexane did not result in any measurable selectivity for paclitaxel over cephalomannine, with average selectivity coefficients of 1.0 and 0.6, respectively (Table 2).

In terms of cost, HFB, TOA, and TBP are not common industrial solvents and are therefore more expensive than the traditional solvents evaluated (Table 2). Because of this additional cost, recovery and recycle of these components is important and must be considered in process design. Other classes of solvents used in this study, such as n-hexane, dichloromethane, toluene and ethyl acetate are commonly used as carrier solvents in industrial extraction processes. However, dichloromethane poses environmental toxicity hazards, and ethyl acetate is challenging to recover due to partial solubility in aqueous solutions. All of these factors must be considered for solvent selection, in addition to partitioning and selectivity behavior, to design the most efficient process to purify plant metabolites from cell culture.

Effect of pH

In plant cell culture media, secreted paclitaxel remains dispersed in solution due to nonspecific binding with aqueous proteins.³⁷ The conformation of these proteins is highly dependent on pH. For this reason the organic solvents n-hexane, dichloromethane, and ethyl acetate were tested for their ability to extract paclitaxel over a range of pH values. pKa's for both paclitaxel and cephalomannine were predicted to be greater than 11 (using ChemAxon's chemicalize.org database (<http://www.chemicalize.org/>)), suggesting they are not ionizable under typical pH conditions, as has been reported elsewhere.³⁸ At pH values above 8.0, paclitaxel begins to degrade, which limits the range at which the extraction can be conducted.³⁹ Hence, studies were conducted in the range of pH 4.0–8.0 where paclitaxel has been shown to be the most stable and the major species in the aqueous media phase were unionized taxanes.

The effect of pH on partitioning depends strongly on the solvent tested (Figure 3). For n-hexane, the highest partition coefficients were measured at the lowest and highest pH values, with lower partition coefficients at pH values nearest that of the plant cell culture medium (5.0–7.0). This result may be explained by considering paclitaxel binding to proteins in plant cell culture medium. When the solution pH is similar to the cell culture level, many proteins exist in their natural, folded conformation, which allows them to bind paclitaxel, preventing extraction into the organic phase. A shift in pH outside of this range may cause a fraction of the proteins to denature, releasing paclitaxel to be more efficiently extracted by the solvent. If the solvent has a strong affinity for paclitaxel, changes in the solution pH would have a smaller effect. For extractions in dichloromethane, changing the pH had very little effect on extraction. The partition coefficient for paclitaxel was high (approximately 20) throughout the entire pH range tested. For ethyl acetate, the partition coefficients were lower at the more acidic pH levels tested, with a measured value of 16 at pH 4.0. At a pH of 6.0 or higher, the partition coefficient increased to 30 or greater. These results show that the relationship between partitioning behavior and pH is complex and dependent on solution chemistry. These interactions are not well understood, but must be considered and quantified in process design. Making a minor adjustment to the pH of the aqueous cell culture medium phase can produce substantial improvements in the recovery of paclitaxel using LLE for particular solvent systems.

The aqueous phase pH also affects selectivity for paclitaxel. For n-hexane, pH had a large effect, with the highest selectivity observed at the most acidic pH tested (4.0) (Figure 3). Dichloromethane gave a slightly higher selectivity at pH values in the middle range of approximately 6.0. For ethyl acetate, the aqueous phase pH had essentially no effect on selectivity. Dichloromethane and ethyl acetate had selectivity coefficient values less than one for the entire pH range tested.

The effect of pH on the partitioning behavior of paclitaxel in 90:10 (volume %) mixtures of n-hexane with HFB, TOA, and TBP was also examined (Figure 4). The n-hexane:TBP

mixture showed similar behavior to n-hexane alone (Figure 3), giving the highest partition coefficients at pHs 4.0 and 8.0 with lower numbers at intermediate values. The highest partition coefficient was 14 at pH 4.0, again demonstrating the potential of TBP addition for increasing partitioning of paclitaxel. The partition coefficient of the n-hexane:TOA mixture was highest at the acidic end of the pH range with a measured value of 2.8 at pH 4.0; the partition coefficient decreased to 1.1 at pH 8.0. The mixture of n-hexane:HFB did not demonstrate a pH effect, with partition coefficients approximately 2 for the entire pH range tested.

CONCLUSIONS

Plant cells present unique challenges in the design of processes to synthesize and separate high-value products. An understanding of the behavior of these products in terms of phase partitioning is important in designing the more expensive and difficult downstream purification steps (e.g., chromatography). Specifically, the design of a LLE system that promotes high recovery of paclitaxel and shows selectivity for paclitaxel over structurally related compounds such as cephalomannine is necessary to reduce the overall process cost. Several classes of solvents were evaluated for both recovery of paclitaxel and selectivity over cephalomannine. High partition coefficients were obtained for dichloromethane (25) and ethyl acetate (28), while selectivity was observed for n-hexane and HFB:n-hexane (20:80) mixtures (selectivity coefficients of 1.7 and 4.5, respectively). This is the first report of significant selectivity for paclitaxel over the by-product cephalomannine in LLE. Extractants were tested to improve selectivity and/or partitioning of paclitaxel. The addition of TOA and TBP to n-hexane resulted in significant increases in paclitaxel recovery but did not improve selectivity. In particular, 5:95 volume percent TBP:n-hexane mixture provided high recovery of paclitaxel at a cost relatively similar to traditional solvents employed. However, in order to estimate the potential for economic improvements in LLE for paclitaxel recovery and purification, the overall separation process must be considered, including the cost of recycle and recovery of the solvents used. Changing LLE parameters (e.g., solvent flow rate, extractor size, pH) can have a large effect on the size and cost of subsequent operations in the process. However, economic estimates for equipment size and energy usage for all pieces of equipment from the bioreactor to the final chromatography column are ultimately needed to effectively compare design alternatives.

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NOMENCLATURE

C_{FEED}	Concentration of product in feed to extraction process
C_{SOLVENT}	Concentration of product in organic phase
E	Extraction factor
F	Feed flow rate
F_R	Single-stage fractional recovery of product from aqueous phase
K_p	Partition coefficient

K_{Pi}	Partition coefficient of species i (desired product)
K_{Pj}	Partition coefficient of species j (unwanted impurity)
m_{INIT}	Mass of product present in cell culture medium
m_{EXT}	Mass of product recovered in organic phase
S	Solvent flow rate
S_{ij}	Selectivity for component i over component j

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Figure 1.
Chemical structure of paclitaxel (A) and cephalomannine (B). Both molecules feature a core of three fused rings attached to a side chain derived from phenylalanine.

Figure 2.

Partition coefficient of paclitaxel in a mixture of n-hexane in either (A) TOA or (B) TBP as a function of volume percentage of extractant. Extraction was conducted at the pH of plant cell culture medium (6–7) at a temperature of 24°C. Each data point represents the average of three samples with error bars showing one standard deviation. * Indicates a statistically significant difference from n-hexane alone at 95% confidence level as measured by the Student's t-test.

Figure 3.

Partition coefficient and selectivity for paclitaxel over cephalomannine in organic solvents n-hexane, dichloromethane, and ethyl acetate as a function of aqueous pH. Extractions were conducted at a temperature of 24°C. The pH was adjusted by addition of small volumes (< 0.1 ml) of HCl and NaOH. Each data point represents the average of three samples with error bars showing one standard deviation.

Figure 4. Partition coefficient for paclitaxel in mixtures of n-hexane with HFB, TOA, and TBP (90:10 volume percentage) as a function of aqueous pH. Extractions were conducted at a temperature of 24°C. The pH was adjusted by addition of small volumes (< 0.1 ml) of HCl and NaOH. Each point represents the average of three samples with error bars showing one standard deviation.

Table 1

Sequence of process steps for separation of paclitaxel from large-scale plant cell culture.

	Cell Culture	Disruption/Filtration	LLE	Evaporation-Crystallization	Chromatography
Equipment/Supplies	Bioreactor, cells, nutrients, oxygen	Physical agitator or sonicator	Extraction unit, solvent	Vacuum crystallizer	Normal phase or reversed-phase column, solvent
Major costs	Sterilization, equipment	Filtration equipment, energy	Solvent recovery and make-up	Equipment, energy	Equipment, labor, solvent
Fractional recovery % [†]	N/A	85–95	80–90	75–90	75–85
Purity weight %	0.01–0.04	0.1–0.5	1–4	60–75	98.5–99.5

[†] Estimated fractional recovery from each stage based on U.S. Patent 5,407,816⁴⁰ and U.S. Patent 6,136,989⁴¹.

Table 2

Comparison of solvent performance for extraction of paclitaxel from plant cell culture.

Solvent	Partition coefficient (KP) [†]	Single- stage fractional recovery	Selectivity coefficient (Sij) [†]	Relative cost [‡]
n-hexane	1.9 ± 1.6	65%	1.7 ± 0.2 [*]	1.64
dichloromethane	25.1 ± 12.9	96%	0.8 ± 0.2	1.01
ethyl acetate	27.5 ± 12.6	97%	0.5 ± 0.2 [*]	1.38
toluene	16.9 ± 2.7	94%	1.3 ± 0.4	1.00
HFB:n-hexane (20:80)	2.4 ± 1.2	70%	4.5 ± 2.5 [*]	18.65
TBP:n-hexane (5:95)	23.7 ± 2.7 ^{**}	95%	1.0 ± 0.6	1.97
TOA:n-hexane (25:75)	8.6 ± 2.3 ^{**}	82%	0.6 ± 0.3	3.87

[†]Extractions were conducted at the pH of the plant cell culture medium (6–7) and room temperature (approximately 24°C). Each data point represents the average value of 12–36 samples measured in 2–4 separate experiments showing one standard deviation.

[‡]solvent price indexed in relation to the price of toluene (assigned an index cost of 1.00) which is the least expensive of the tested organic solvents estimated from Sigma-Aldrich, 2012.

^{*} Statistically different at 95% confidence level from a value of unity according to Student's t-test for mean vs. constant.

^{**} Statistically significant at 95% confidence level over n-hexane alone as measured by the Student's t-test.