Prepurification of paclitaxel by micelle and precipitation

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ABSTRACT

A novel prepurification method was developed aiming at increasing yield and purity, also reducing solvent usage for purification of paclitaxel. This method was a simple and efficient procedure, for the isolation and prepurification of paclitaxel from the biomass of *Taxus chinensis*, consisting of micelle formation, followed by two steps of precipitation. The use of a micelle and precipitation in the prepurification process allows for rapid separation of paclitaxel from interfering compounds and dramatically reduces solvent usage compared to alternative methodologies. This prepurification process serves to minimize the size and complexity of the HPLC operations for paclitaxel purification. This process is readily scalable to a pilot plant and eventually to a production environment where multikilogram quantities of material are expected to be produced. As much as possible, the process has been optimized to minimize solvent usage, complexity, and operating costs.

INTRODUCTION

Paclitaxel (Taxol®) is an FDA approved drug for the treatment of ovarian cancer, breast cancer, Kaposis sarcoma. It has also shown broad spectrum activity against human solid tumors such as melanoma, breast and lung. Paclitaxel is obtained from the bark of the Pacific yew(*Taxus brevifolia*), a limited resource found in the Pacific Northwest. This supply source has been sufficient to meet paclitaxel needs for clinical trials and early marketing efforts but is not expected to be adequate to meet long-term market demand. In 1995, Bristol-Myers Squibb(BMS) announced that their future supplies of paclitaxel would be obtained by semisynthesis from 10-deacetylbaccatin III(10-DAB). The precursor compound is obtained from needles and twigs of *Taxus baccata*. Recently, paclitaxel production via plant cell culture systems can compete economically with either of these supply sources and represents an environmentally sound and reliable supply alternative to

existing sources.[1,2] One of the goals of this present work was to minimize solvent usage by avoiding chromatographic separation techniques in the early stages of the purification process. A second goal established for the prepurification scheme was to achieve a crude paclitaxel product containing a minimum of 60% paclitaxel on a weight basis so as to minimize process scale HPLC costs

MATERIALS AND METHODS

Suspension cells originated from *Taxus chinensis* were maintained under darkness at 24℃ with shaking at 150 rpm. Suspension cells were cultured in modified Gamborgs B5 medium supplemented with 30 g/l sucrose, 10 μM naphthalene acetic acid, 0.2 μM 6-benzylamino purine, 1 g/l casein hydrolysate and 1 g/l MES. Cell cultures were transferred to fresh medium every two weeks. In prolonged culture for production, 1 and 2 % (w/v) maltose were added to culture medium at day 7 and day 21, respectively and 4 μM $AgNO_3$ was added on the initiation of culture as an elicitor. After culture, biomass was recovered using a decanter (Westfalia, CA150 Clarifying Decanter) and a high-speed centrifuge (α-Laval, BTPX 205GD-35CDEFP). A Hewlett-Packard 1100 HPLC was used for all analytical characterizations of intermediate or finished products. HPLC system with Curosil PFP column (Phenomenex, 4.6 x 250 mm, d_p=5 μm) was used for quantitative analysis. Elution was performed by gradient condition with a mixture of acetonitrile and water from 65:35 (v/v) to 35:65 (v/v) (flow rate=1.0 ml/min). Injection volumes were 10 μl and effluent was monitored at 227 nm (paclitaxel) or 255 nm (internal standard) with photo diode-array detector. Purity determinations of intermediate and finished products were made using an internal standard assay to compare the paclitaxel content of the test material to the paclitaxel content of the reference paclitaxel. An internal standard solution containing 1000 μg n-propyl paraben in 1 ml methanol was mixed in equal portions with accurately prepared stock solutions (~1000 μg/ml) of test and reference samples. These solutions were analyzed by HPLC. Response ratios were calculated as the ratio of the peak areas for paclitaxel and internal standard and the ratios were corrected to reflect a sample concentration of 1000 μg/ml. Purity values were calculated by comparing the response ratio determined for the test sample to that obtained for the reference paclitaxel. Results for HPLC was averaged to arrive at a final purity value.[3]

RESULTS AND DISCUSSION

Several solvents or combinations of solvents have been tested for the extraction of biomass. Methanol gave the highest paclitaxel recovery with the least amount of solvent usage and was therefore chosen for all subsequent process development work. Also the number of extraction was at least 4 times to obtain high yield (>99%) from biomass. Several different modes of operation are possible in the extraction of biomass and solvent usage is greatly impacted. In this work, we have used a batch extraction process in which biomass is sequentially extracted with fresh solvent at each four extraction stages. In the batch mode of operation, equilibrium (i.e., the ratio of paclitaxel in biomass to paclitaxel in the extraction solvent) was reached within 5 min and neither the equilibrium nor the extraction time have been greatly affected by temperature (5℃ ~ 50℃). After four times extraction of biomass with methanol, the concentration of methanol extract was accomplished in rotary evaporators at a vacuum of 635 mmHg and 40℃. This crude extract was used for the following processes to optimize the prepurification process of paclitaxel.

The approach taken in this work was to transfer paclitaxel in the crude extract to an aqueous surfactant (N-cetylpyridinium chloride, CPC) solution(5%,w/v), allowing organic solvents to be used for lipid removal. An equal mixture of hexane and MtBE (methyl t-butyl ether) was used to extract lipids from the surfactant solution leaving paclitaxel solubilized within the micelles. Although paclitaxel exhibited appreciable solubility in the MtBE/hexane mixture, it partitions favorably into the surfactant solution and losses of paclitaxel into the lipid phase were minimal. Once lipids had been removed, paclitaxel was extracted into MtBE, thereby separating it from the surfactant. Two precipitation steps were incorporated into the prepurification process. The first of these precipitates paclitaxel from MtBE using hexane, resulting in a powdery precipitate which is recovered by filtration. This precipitate contains, in addition to paclitaxel, phenolic compounds, the main component of which is catechin.[4] The precipitate was suspended in methylene chloride(CH_2Cl_2), solubilizing paclitaxel but leaving catechin and the majority of the phenolics undissolved. Thus, by subsequent filtration, paclitaxel was separated from phenolics. Paclitaxel was precipitated from the CH_2Cl_2, by addition of hexane, as a light brown to yellow precipitate recoverable by filtration. This precipitate was the crude paclitaxel which was subjected to process scale HPLC separation. The results obtained for

the recovery/prepurification of paclitaxel from biomass are summarized in Table 1. The precipitation was performed to obtain paclitaxel of over 65% purity which also guarantees high yield and purity while minimizing the load borne on the columns employed in a subsequent HPLC purification step.

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REFERENCES

1. Stull, D. P., Scales, T. A., Daughenbaugh, R., Jans, N. A. and Bailey, D. T., Taxol (Paclitaxel), strategies to increase the supply of a new anticancer drug. *Applied Biochemistry and Biotechnology,* 1995, 54, 133-140.
2. Kwon, I. C., Yoo, Y. J., Lee, J. H. and Hyun, J. O., Enhancement of taxol production by in situ recovery of product. *Process Biochemistry,* 1998, 33, 701-707.
3. Kim, J.H., Kang, I.S., Choi, H.K., Hong, S.S., Lee, H.S., A novel prepurification for paclitaxel from plant cell cultures. *Process Biochemistry*, 2002, 37, 679-682.
4. Kim, J.H., Choi, H.K., Hong, S.S. and Lee, H.S., Development of high performance liquid chromatography for paclitaxel purification from plant cell cultures. *Journal of Microbiology and Biotechnology*, 2001, 11, 204-210.

Table 1. Results obtained for the recovery/prepurification of paclitaxel from 200g of biomass

Process step	Paclitaxel(mg)	Yield(%)	Purity(%)
Biomass	39	-	-
MeOH extract*	38	97	<0.5
MtBE extract	32	82	29.4
Precipitation** (Crude paclitaxel)	31	80	65.8

*Four times extraction

**Two steps of precipitation