Drug Development and Industrial Pharmacy, 32:1089–1094, 2006

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600683501

Paclitaxel-Loaded Lipid Nanoparticles Prepared by Solvent Injection or Ultrasound Emulsification

Betül Arıca Yegin
Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, 06100 Sıhhiye-Ankara, Turkey

Jean-Pierre Benoît
Inserm U646, 10 rue André Boquel, University of Angers, 49100 Angers, France

Alf Lamprecht
Laboratory of Pharmaceutical Engineering, Faculty of Medicine and Pharmacy, University of Franche-Comté, 25000 Besançon, France

ABSTRACT Lipid nanoparticles were fabricated as an injectable carrier system for paclitaxel. The components for the lipid matrix were based on phospholipids, and sucrose fatty acid ester was used as an emulsifier. Formulation prepared with solvent injection has a slightly larger particle size (187.6 nm) than the formulation (147.7 nm) prepared with ultrasound emulsification. Differential scanning calorimetry results indicated that paclitaxel entrapped in the lipid nanoparticles existed in an amorphous state in the lipid matrix. In vitro drug release was rather slow; only 12.5–16.5% of the drug released from the formulations within 14 days. Lipid nanoparticles demonstrated their potential as a promising pharmaceutical formulation of paclitaxel.

KEYWORDS Paclitaxel, Sucrose fatty acid ester, Lipid nanoparticles, Solvent injection, Ultrasound emulsification

INTRODUCTION

Paclitaxel (PX) is an antineoplastic agent isolated from the bark of the Pacific yew tree, *Taxus brevifolia,* with its novel mechanism of action as a promoter of tubulin assembly. Clinical studies have demonstrated the significant activity of PX against a variety of solid tumors including breast cancer, advanced ovarian carcinoma, lung cancer, head and neck carcinomas, and acute leukemias (Singla et al., 2002). However, the administration of PX usually includes the use of significant amounts of solubilising agents since the solubility of PX in aqueous solution is minimal. One major aspect to address is therefore the solubilisation or dispersion of the highly lipophilic drug in an aqueous formulation. The commercial formulation of paclitaxel consists of a micellar solution of the drug in Cremophor® EL (polyoxyethylated castor oil) containing 50% absolute ethanol. Before administration as a slow infusion, the micellar solution is diluted with saline solution. Since Cremophor® EL has been observed to cause severe hypersensitivity reactions in animals and man, the design of new carriers for PX aims also for the possibility to avoid or reduce adverse effects of the drug and its formulation.

Address correspondence to Alf Lamprecht, Place Saint Jacques, 25000 Besançon, France; Tel: +33 3 81 66 55 48; Fax: +33 3 81 66 52 90; E-mail: alf.lamprecht@univ-fcomte.fr

A variety of approaches has been proposed, such as the use of polymeric micro- and nanoparticles, micro- and nanoemulsions, or liposomes (Singla et al., 2002). Also nanoparticles based on solid lipids demonstrated to be a promising alternative drug delivery system (Miglietti et al., 2000; Cavalli et al., 2001; Chen et al., 2001; Serpe et al., 2004). Several methods were described in the literature for the preparation of lipid nanoparticles, such as high pressure homogenization, emulsification-solvent evaporation method and solvent-injection method (Barichello et al., 1999; Cavalli et al., 2001; Jenning et al., 2002; Schubert & Müller-Goymann, 2003; Garcia-Fuentes et al., 2004; Langguth et al., 2005; Liu et al., 2005). In most cases, a surfactant is added to the formulation of lipid nanoparticles (LNP) to stabilize the emulsion formed during particle preparation. The type of surface active substances or stabilizers involved in the fabrication procedure obviously have an important effect on physical and pharmaceutical properties, such as particle size and zeta potential values being important for physicochemical stability as well as the biopharmaceutical properties of the preparation. However, many of these stabilizers have a low biocompatibility (Youan et al., 2003).

In the present study, a sucrose fatty acid ester was proposed as stabilizer for LNP. Principally, sugar esters are interesting compounds because of their excellent biocompatibility and biodegradability, emulsifying and stabilizing ability against various stresses, and large emulsifying capacity. Sugar esters are widely used not only for pharmaceutical formulations but also in food and the cosmetic industry, where they fulfill various functions (Youan et al., 2003). Surprisingly, little attention was paid to the use of this surfactant type in the design of micro- and nanoparticles. Especially, in the preparation of nanoparticles where usually larger amounts of surfactants are necessary to stabilize the highly increased particle surface, a nontoxic surfactant may be interesting to replace standard surfactants used in this context, for example, polyvinyl alcohol.

This study aimed at the design of LNP by using this surfactant. Furthermore, the influence of the two different preparation methods, the solvent injection technique and ultrasound emulsification, on the encapsulation efficiency, size and size distribution, zeta potential, and the release of the drug were investigated.

MATERIALS AND METHODS

Materials

PX was obtained from Indena SpA (Milan, Italy). Sucrose fatty acid esters (SE; hydrophilic-lipophilic balance: 7 [SE-7], 11 [SE-11], or 15 [SE-15]) were chosen as surface active agent and were supplied by Mitsubishi Chemical Europe GmbH (Düsseldorf, Germany). Solid lipid (Lipoid®S 100; soybean lecithin) was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). All other chemical reagents were obtained from Sigma (Steinheim, Germany) and were of analytical grade.

Preparation of Lipid Nanoparticles

Solvent Injection Technique (LNP1)

PX-loaded LNP were prepared by a modified solvent injection technique (Schubert & Müller-Goymann, 2003). A total of 5 mg of PX and 150 mg of Lipoid®S 100 were dissolved in 5 mL of acetone and then rapidly injected through an injection needle (0.45 mm) into the stirred aqueous phase containing SE. Then the solvent evaporation step was performed in a Büchi Rotavapor RE 121 (Büchi, Flawil, Switzerland) for 30 min, reducing the pressure stepwise down to 10–30 mbar with a diaphragm pump. Blank lipid nanoparticles were prepared without drug.

Ultrasound Emulsification (LNP2)

Second, either blank or PX-loaded LNP were prepared by a simple emulsion (o/w) technique. In brief, PX (5 mg) was dissolved in 5 mL of methylene chloride containing 150 mg of Lipoid®S 100 under magnetic stirring. This organic solution was poured into the aqueous solution (20 mL) containing SE and the emulsion was homogenized with an ultrasonic cell disruptor (Misonix Inc., Farmingdale, NY, USA) at 20 W for 5 min. Thereafter, the organic solvent was removed under reduced pressure as described previoiusly.

By keeping all other preparation parameters constant, the influences by the preparation method and the related solvent on the particle properties should be easily determinable.

Particle Size and of Zeta Potential

The LNP were analyzed for their size distribution by photon correlation spectroscopy (PCS) using a

Malvern Autosizer® 4700 (Malvern Instruments, SA., Worcestershire, UK). To determine the zeta potential values of the LNP, a Coulter Delsa 440 (Coulter Scientific Instruments, Amherst, MA, USA) was used. Blank and PX-loaded LNP were diluted with purified water before analysis and were analysed in triplicate.

Atomic Force Microscopy (AFM)

LNP were diluted with distilled water before analysis. The characterization of PX-loaded particles was performed with a commercial AFM (Autoprobe cp, Park Scientific Instruments, Sunnyvale, CA, USA). The AFM was used in the contact mode. Samples were placed on a mica plate surface by dropping 20 μL of the suspension onto the surface and removing the water under reduced pressure overnight.

Differential Scanning Calorimetry (DSC)

Thermograms of the different samples were obtained from a DSC (DSC 30; Mettler-Toledo, Viroflay, France) equipped with a thermal analysis data system (TC 15; Mettler-Toledo). LNP were lyophilized without cryoprotectant and obtained samples (3–5 mg) were heated in sealed standard aluminum pans from −50°C to 250°C at a scanning rate of 10°C/min under nitrogen purge, with an empty aluminum pan as reference.

Encapsulation Efficiency and In Vitro Release Studies

PX-loaded LNP were dissolved in acetone in appropriate dilutions, and the drug content was analyzed by HPLC according to a method published elsewhere (Shirazi et al., 2001). The nonentrapped amount of PX was determined in the supernatant after centrifugation. All drug release experiments were performed in phosphate buffer (pH 7.4) at 37°C containing 0.5% (w/v) of polysorbate 80. PX-loaded LNP were placed into a dialysis bag (pore size: 50,000 Da) and kept in 200 mL of release medium under constant shaking at 100 rpm. Aliquots of the dissolution medium (500 μL) were withdrawn at predetermined time points and directly analyzed as described above. The dissolution medium was maintained at a constant volume by replacing the samples with fresh dissolution medium. All measurements were performed in triplicate.

Statistical Analysis

The results were expressed as mean values ± SD. The Student's *t*-test was applied to examine significance of differences. In all cases, $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

LNP Preparation

The solvent-injection method is based on lipid precipitation in which the lipid is dissolved in a water-miscible solvent and rapidly injected into an aqueous phase containing the surfactant. This solvent injection technique relies on the rapid diffusion of the solvent across the solvent-lipid interface with the aqueous phase (Quintanar-Guerrero et al., 1997; Schubert & Müller-Goymann, 2003). On the other hand, the ultrasound emulsification method involves the dissolution of the lipid in a water-immiscible organic solvent, emulsifying the lipid solution in a nonsolvent (mostly water in combination with a surfactant) by using homogenization and precipitating the lipid as particles by evaporating the organic solvent (Hou et al., 2003).

LNP were fabricated by using three different types of SE as emulsifier, but only SE-15 led to the formation of LNP, which allowed the design of stable nanoparticles with both preparation methods. The optimal concentration of SE-15 was found at 1.25% (w/v) in the formulations due to the fact that lower SE-15 concentration led to a high polydispersity of the formulation and significant suspension instability with time (data not shown). Apparently, SE-15 can, by its presence on the nanoparticle surface, protect the particles from aggregation, which is attributed to the formation of a steric barrier between the particles during preparation as observed recently (Youan et al., 2003). The influence of SE concentration and its hydrophilic-lipophilic balance on particle characteristics was described similarly (Youan et al., 2003). Our results are in line with such observations suggesting SE-15 being an interesting alternative surfactant in the development of micro- and nanoparticulate drug delivery systems.

Particle Size, Zeta Potential, and Structural Analyses

LNP produced by both preparation methods appeared spherical under the AFM; however, a distinct flattening was observed, which may be favored by the overnight drying step (Fig. 1). The appearance of LNP was similar for both preparation methods, which suggests a minor influence of the formulation method on the general structure of the LNP. The mean particle size results of either blank or drug-loaded LNP formulations are shown in Table 1. LNP1 and PX-LNP1 formulations prepared with solvent injection technique have a slightly higher mean particle diameter than the LNP2 and PX-LNP2 formulations prepared with the ultrasound emulsification method. This could be explained by the fact that the ultrasound emulsification resulted generally in the formation of smaller droplets during emulsion preparation and subsequently smaller precipitated particles. However, at polydispersity indices of 0.1 to 0.4 it remained doubtful whether this represented a significant difference in the particle size distribution. The incorporation of drug into particles causes no significant effect on the mean particle size for both preparation techniques. Blank and PX-loaded LNP exhibited monomodal distribution for both preparation methods whereas, however, polydispersity was distinctly increased. A reason could be the changed lipid arrangement in the presence of PX. All formulations possessed negative zeta potential; however, variations between –18.9 ± 0.8 mV (blank) and –21.1 ± 1.8 mV (PX-loaded) were minor and without significant influences by the preparation method. Also the strong negative charge may provide a basis for the overall high stability of the suspension. The obtained LNP suspensions were observed to be stable for at least 2 months. This suspension stability was essentially linked to the optimal concentration of SE-15 at 1.25%, as at lower concentrations distinct aggregation tendency was observed after 3 to 7 days. Lyophilized LNP were easily redispersible when sucrose was added (at least 2%), and no significant influence on their particle size was observed by this process.

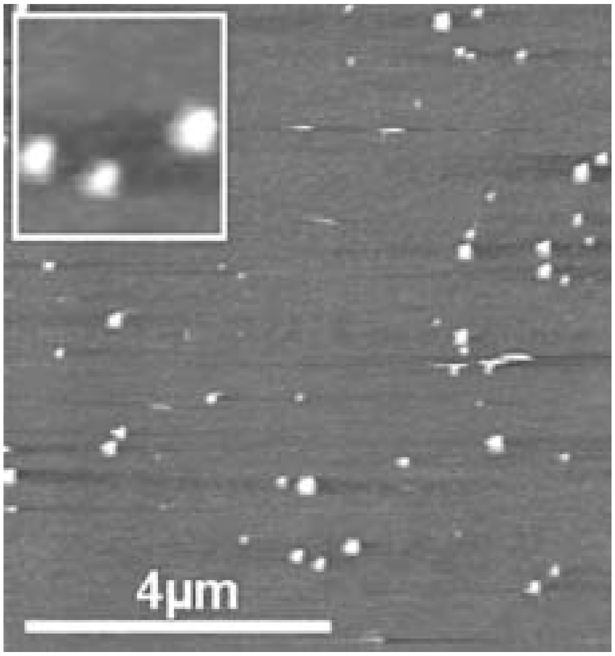

FIGURE 1 Atomic force microscope (AFM) Image of Blank LNP Prepared by the Solvent Injection Method. The Insert Shows an Image Segment of the Lower Middle With a Five-Fold Magnification. The Scale Bar Represents 4 μm.

LNP were analyzed by DSC to investigate the crystal habit of paclitaxel and potential changes or interactions between the drug and excipients (Clas et al., 1999). Fig. 2 shows the DSC curves of freeze-dried blank LNP, PX-loaded LNP, and the respective physical mixtures of the excipients. The LNP did not show the melting peak of PX at 230°C, suggesting that PX is present in LNP in the amorphous form or molecularly dispersed. Similar results were reported by several authors (Cavalli et al., 1995, 2000). Structural studies elucidated the formation of miscible systems of PX with the lipids and proposed an interaction between PX and lipids close to their hydrophilic headgroups explained by intermolecular forces or geometric accommodation (Zhao et al., 2004; Zhao & Feng, 2005). Similar observations were reported for other drugs entrapped into lipid matrices (Yang & Zhu, 2002). The DSC analysis of camptothecin solid lipid nanoparticles

TABLE 1 The Characteristics of the Blank and PX-Loaded Lipid Nanoparticle Formulations

Formulation code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)
LNP1	171.1 ± 2.3	0.124 ± 0.003	–18.9 ± 0.8	—
PX-LNP1	187.6 ± 1.4	0.396 ± 0.008	–21.2 ± 1.2	92.2 ± 6.4
LNP2	142.3 ± 2.3	0.151 ± 0.004	–21.1 ± 1.8	—
PX-LNP2	147.7 ± 0.6	0.238 ± 0.009	–33.7 ± 1.5	89.1 ± 7.2

Mean values ± SD of $n = 3$ batches.

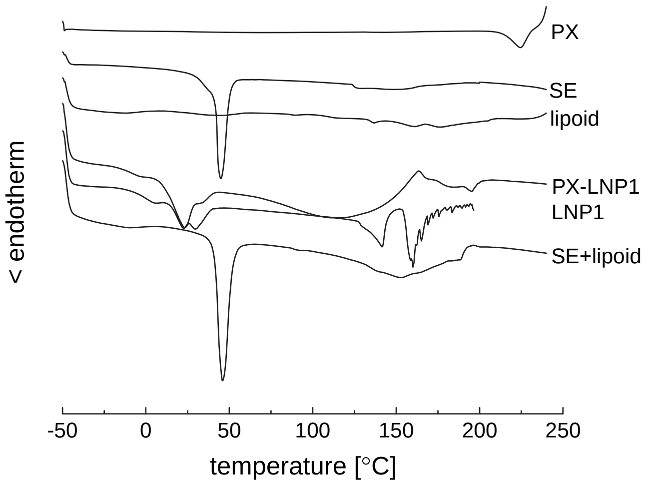

FIGURE 2 Differential scanning calorimetry (DSC) Thermograms of Excipients and Drug, Their Respective Physical Mixture and the Nanoparticle Formulations, Blank and PX-Loaded LNP (SE: Sucrose Ester). For Clarity Reasons, the Figure Shows Only Results of LNP1 (Solvent Injection Method) as Thermograms Obtained for LNP2 Were Similar.

prepared by high-pressure homogenization showed that camptothecin was in its amorphous state. Besides, an interaction between SE-15 and the phospholipids was observed shifting the SE peak to temperatures of 25°C to 30°C. This may raise the question of whether LNP behave in the same way under storage conditions and in the body at 37°C, which remains to be studied.

Encapsulation Efficiency and In Vitro Release Study

The initial PX loading in both formulations (PX-LNP1 and PX-LNP2) was 0.2% (w/v) and encapsulation efficiencies of PX-LNP1 and PX-LNP2 formulations were 92.2 ± 6.4% and 89.1 ± 7.3%, respectively. PX was dissolved in the organic phase before the preparation step for both preparation methods. Acetone and methylene chloride have different miscibility with water; a changed drug encapsulation behavior could be postulated. However, PX has a low solubility in water and the extraction of the drug toward the aqueous phase by the diffusion of the organic solvent is limited. Such an observation is in line with findings from other studies (Lamprecht et al., 2001; Arıca & Lamprecht, 2005).

The in vitro release kinetics of PX from LNP is shown in Fig. 3. The cumulative amounts of PX released in 14 days were about 12.5% and 16.5% for the lipid nanoparticles prepared with solvent injection (PX-LNP1) and ultrasound emulsification (PX-LNP2), respectively. The drug release from the LNP prepared with different preparation methods was nearly linear, indicating almost zero-order release kinetics in the 14 days after an initial release of less than 1% in the first day. This was probably due to the hydrophobic nature of the drug being released (Attawia et al., 2001), but also a lipophilic interaction between drug and LNP matrix lipids may retain the drug release over such a long period. The release from LNP2 was slightly faster, which could be based on smaller particle diameter leading to an increased interface facilitating the drug diffusion toward the surrounding release buffer.

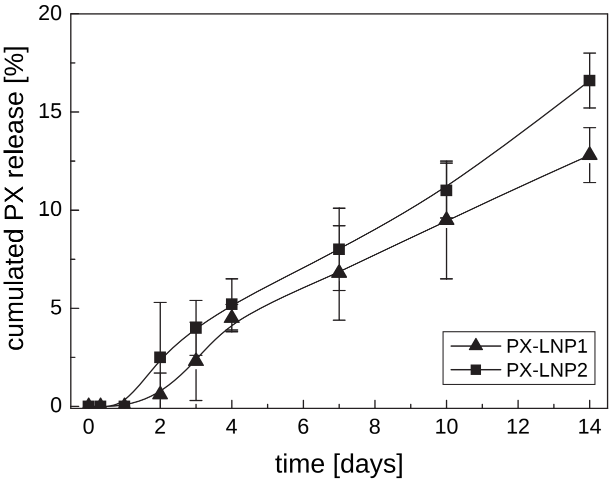

FIGURE 3 Dissolution Profiles of PX From Lipid Nanoparticles Prepared by Solvent Injection (PX-LNP1) and Ultrasound Emulsification (PX-LNP2) Methods. Data Are Shown as Mean Values ± Standard Deviation of Three Experiments.

CONCLUSIONS

PX containing LNP were prepared by a solvent injection and an ultrasound emulsification method. The lipophilic drug PX can be successfully entrapped into the LNP using either ultrasound emulsification or solvent-injection techniques. It has been also shown that SE is an efficient emulsifier for producing LNP and might be an alternative stabilizer in the production of various kinds of nanoparticles. The described lipid nanoparticles appear to be interesting as a controlled release system since they provide a sustained release in vitro.

REFERENCES

Arıca, B., & Lamprecht, A. (2005). In-vitro evaluation of betamethasone loaded nanoparticles. *Drug Development and Industrial Pharmacy, 31,* 19–25.

Attawia, M. A., Borden, M. D., Herbert, K. M., Katti, D. S., Asrari, F., Uhrich, K. E., & Laurencin, C. T. (2001). Regional drug delivery with radiation for the treatment of Ewing's sarcoma. In vitro development of a taxol release system. *Journal of Controlled Release, 71,* 193–202.

Barichello, J. M., Morishita, M., Takayama, K., & Nagai T. (1999). Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. *Drug Development and Industrial Pharmacy, 25*, 471–476.

Cavalli, R., Aquilano, D., Carrlotti, M. E., & Gasco, M. R. (1995). Study by X-ray powder diffraction and differential scanning calorimetry of two model drugs, phenothiazine and nifedipine, incorporated into lipid nanoparticles. *European Journal of Pharmaceutics & Biopharmaceutics, 41*, 329–333.

Cavalli, R., Caputo, O., & Gasco, M. R. (2000). Preparation and characterization of solid lipid nanospheres containing paclitaxel. *European Journal of Pharmaceutical Sciences, 10,* 305–309.

Cavalli, R., Gasco, M. R., Barresi, A. A., & Rovero, G. (2001). Evaporative drying of aqueous dispersions of solid lipid nanoparticles. *Drug Development and Industrial Pharmacy, 27,* 919–924.

Chen, D. B., Yang, T. Z., Lu, W. L., & Zhang, Q. (2001). In vitro and in vivo study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. *Chemical Pharmaceutical Bulletin, 49,* 1444–1447.

Clas, S. D., Dalton, C. R., & Hancock, B. C. (1999). Differential scanning calorimetry: applications in drug development, *Pharmaceutical Science Technology Today, 2,* 311–320.

Garcia-Fuentes, M., Torres, D., Martin-Pastor, M., & Alonso, M. J. (2004). Application of NMR spectroscopy to the characterization of PEG-stabilized lipid nanoparticles. *Langmuir, 20*, 8839–8845.

Hou, D., Xie, C., Huang, K., & Zhu, C. (2003). The production and characteristics of solid lipid nanoparticles (SLNs). *Biomaterials, 24,* 1781–1785.

Jenning, V., Lippacher, A., & Gohla, S. H. (2002). Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization. *Journal of Microencapsulation, 19*, 1–10.

Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Lehr, C. M., Maincent, P., & Kawashima, Y. (2001). Design of rolipram loaded nanoparticles: comparison of two preparation methods. *Journal of Controlled Release, 71*, 297–306.

Langguth, P., Hanafy, A., Frenzel, D., Grenier, P., Nhamias, A., Ohlig, T., Vergnault, G., & Spahn-Langguth, H. (2005). Nanosuspension formulations for low-soluble drugs: pharmacokinetic evaluation using spironolactone as model compound. *Drug Development and Industrial Pharmacy, 31,* 319–329.

Liu, J., Zhu, J., Du, Z., & Qin, B. (2005). Preparation and pharmacokinetic evaluation of Tashinone IIA solid lipid nanoparticles. *Drug Development and Industrial Pharmacy, 31,* 551–556.

Miglietta, A., Cavalli, R., Bocca, C., Gabriel, L., & Gasco, M. R. (2000). Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. *International Journal of Pharmaceutics, 210,* 61–67.

Quintanar-Guerrero, D., Allémann, E., Doelker, E., & Fessi, H. (1997). A mechanistic study of the formation of polymer nanoparticles by the emulsification-diffusion technique. *Colloid Polymer Science, 275,* 640–647.

Schubert, M. A., & Müller-Goymann, C. C. (2003). Solvent injection as a new approach for manufacturing lipid nanoparticles: evaluation of the method and process parameters. *European Journal of Pharmaceutics & Biopharmaceutics, 55,* 125–131.

Serpe, L., Catalano, M. G., Cavalli, R., Ugazio, E., Bosco, O., Canaparo, R., Muntoni, E., Frairia, R., Gasco, M. R., Eandi, M., & Zara, G. P. (2004). Cytotoxicity of anticancer drugs incorporated in solid lipid nanoparticles on HT-29 colorectal cancer cell line. *European Journal of Pharmaceutics & Biopharmaceutics, 58,* 673–680.

Shirazi, F. H., Bahrami, G., Stewart, D. J., Tomiak, E., Delorme, F., Noel, D., & Goel, R. (2001). A rapid reversed phase high performance liquid chromatographic method for determination of PX (VP-16) in human plasma. *Journal of Pharmaceutical and Biomedical Analysis, 25,* 353–356.

Singla, A. K., Garg, A., & Aggarwal, D. (2002). Paclitaxel and its formulations. *International Journal of Pharmaceutics, 235,* 179–192.

Yang, S. C., & Zhu, J. B. (2002). Preparation and characterization of camptothecin solid lipid nanoparticles. *Drug Development and Industrial Pharmacy, 28,* 265–274.

Youan, Bi-Botti C., Hussain, A., & Nguyen, N. T. (2003). Evaluation of sucrose esters as alternative surfactants in microencapsulation of proteins by the solvent evaporation method. *AAPS Pharm Sci. 5,* Article 22.

Zhao, L., & Feng, S. S. (2005). Effects of lipid chain unsaturation and headgroup type on molecular interactions between paclitaxel and phospholipid within model biomembrane. *Journal of Colloid and Interface Science, 285,* 326–335.

Zhao, L., Feng, S. S., & Go, M. L. (2004). Investigation of molecular interactions between paclitaxel and DPPC by Langmuir film balance and differential scanning calorimetry. *Journal of Pharmaceutical Sciences, 93,* 86–98.