# Paclitaxel-loaded biodegradable nanoparticles developed by direct dialysis and electrodydrodynamic atomization methods

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## Abstract

This paper presents a new method for fabrication of biodegradable polymeric nanoparticles as carriers for anticancer drug paclitaxel. Emulsion solvent evaporation method was frequently employed to prepare polymeric nanoparticles for drug delivery systems in which the emulsifier such as PVA has to be used. In this study, direct dialysis was employed to fabricate paclitaxel-loaded biodegradable polymeric nanoparticles thus avoiding using emulsifier which potentially could remain on the surface of the nanoparticles and affect the biodegradability, biodistribution and drug-release behavior. The nanoparticles were characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM) for their morphology; laser scattering particle size analyzer for particle size distribution; and, zeta potential analyzer for their surface charge. The physical status of paclitaxel in polymer matrix by different theory drug loadings was studied by differential scanning calorimetry (DSC) and X-ray diffractometry pattern. The encapsulation efficiency and *in vitro* release profile were measured by high performance liquid chromatography (HPLC). C6 glioma cell line was used to evaluate the particle cellular uptake and cytotoxicity.

## Introduction

Paclitaxel is FDA-approved for clinical use in ovarian and breast cancer and has shown cytotoxic activity against common solid tumors and a number of leukemias, as well as Walker 256 carcinosarcomas, lung tumors (Rowinsky et al., 1993) and human hepatocellular carcinoma cell lines (Gagandeep et al., 1999). Paclitaxel is a potent inhibitor of cell replication that blocks cells in the late G2 or M phase of the cell cycle (Jordan and Toso, 1993), which binds to cellular microtubules and makes the microtubules polymerization (Jordan and Wilson, 2004). In addition, it was reported that Taxol, a tubulin drug with higher specificity for $\beta_{II}$-Tubulin than for other β-Tubulin isotypes, irreversibly decreases nuclear $\beta_{II}$ content in a concentration dependent manner in C6 cells (Xu and Luduena, 2002). However, the problem of formulating paclitaxel has being remained a large obstacle in the widespread use of the drug. The current formulation has lots of side effects including hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity (Singla et al., 2002). Also, P-gp is an important obstacle preventing paclitaxel entry into the brain (Fellner et al., 2002). Nanoparticle could break down the barriers (Miller, 2002). Nanoparticulate carriers could be an ideal solution for intravenous injection delivery of paclitaxel and other CNS drugs (Lockman et al., 2002). The use of polymeric particles has been shown to be promising in cancer chemotherapy (Feng and Chien, 2003). Commonly, emulsion solvent evaporation method is widely employed for the preparation of nanoparticles. In this method, serious amounts of

emulsifiers are required to stabilize the dispersed oil droplets. In particular, PVA as a stabilizing emulsifier is most frequently used to fabricate nanoparticles. However, PVA has some problems in that PVA remains at the surface of the nanoparticles and then it becomes difficult to remove it. It was known that PVA existing on the surface of nanoparticles changes biodegradability, biodistribution, particle cellular uptake and drug-release behavior (Landry et al., 1995; Lavelle et al., 1995; Sahoo et al., 2002). Dialysis method was developed for the simple preparation of drug carriers such as lipsomes and polymeric micelles (Jeong et al., 1998). However, little work has been done to fabricate nanoparticles by direct dialysis method (Jeong et al., 2001; Jeong et al., 2003). The objective of this study was to develop biodegradable polymeric nanoparticulate drug delivery system by direct dialysis method. Further more, *in vitro* experiments (paclitaxel *in vitro* release, particle cellular uptake and particle cytotoxicity) were performed to study drug transport, particle uptake efficiency and to evaluate the cytotoxicity of this dosage form using C6 glioma cell line. The formulations generated from this work may be considered intravenously injection for administration of paclitaxel.

## Experimental

In this study, a particular amount of PLGA (50:50) and paclitaxel/coumrain6 with or without surfactants were dissolved in a certain amount of DMF. The resulting solution, with the organic phase, was directly loaded into the dialysis membrane. Following this, the dialysis membrane was put into the external aqueous phase of water. The organic solvent diffused out of the membrane, leading to precipitation and separation of the polymer molecules out of solvent to gather together and form spherical nanoparticles. The external water phase is gently stirred by a magnetic stirrer to aid diffusion. The organic phase was dialyzed against the aqueous phase for 24 hours, with changes of the aqueous phase every 2-3 hours until the organic solvent had been completely removed by diffusing out of the dialysis membrane. Then, the sample solution in the membrane was collected and centrifuged at 11500 rpm for 30 minutes in Eppendorf Centrifuge 5810R. The nanoparticles were obtained as the pellet and further freeze-dried.

## Results and discussion

It is observed that the nanoparticles appear to be round in shape and the surface is smooth from SEM images and AFM images (shown in Figures 1 and 2). The particle size distribution of paclitaxel and coumrain6 loaded PLGA nanoparticles was shown in figure 3 which presents the effective diameters are 161nm and 155.4nm, respectively. In this study the drug loading is 10% and encapsulation efficiency is 58.9%.Zeta potential values of paclitaxel and coumrain6 loaded PLGA nanoparticles measured by zeta potential analyzer are -21.62 ± 0.9mv and -48.65 ± 1.16mv, respectively. The higher zeta potential value indicated more negative charge on the surface and hence more stable particles solution could be obtained. Under such conditions, particles are less likely to aggregate and can be well suspended in water based solution, which is very important for their applications. For the DSC analysis, there was no peak observed at the temperature range of 150-250℃ for the samples (data not shown). The DSC study did not detect any crystalline drug material in the nanoparticles samples. It could thus be concluded that the

paclitaxel formulated in the samples was in an amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution state in the polymer matrix after fabrication. In the fabrication process, the controllable particle size could be achieved by adjusting the polymer concentration. In addition, pure paclitaxel and different drug loading PLGA particles were observed by SEM, which indicated that some paclitaxel crystals could exist in higher drug loading samples (30% and 20%) after fabrication and it was also proved by X-ray diffractometry pattern(data not shown.).

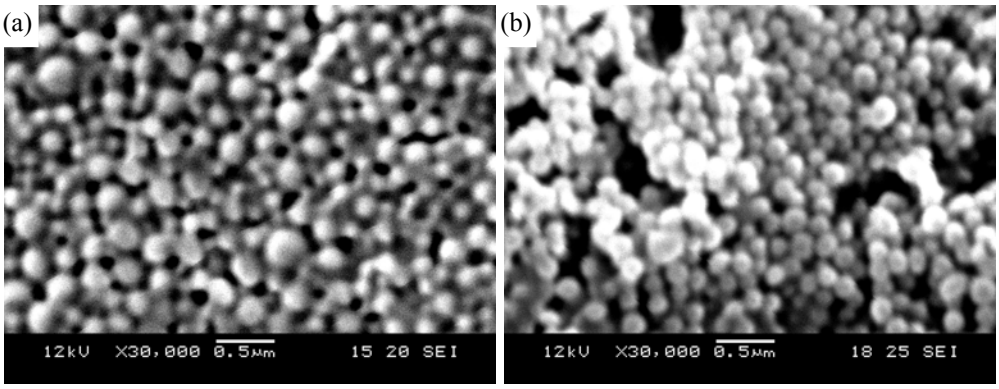


**Figure 1** SEM images (a: paclitaxel-loaded PLGA nanoparticles; b: coumarin6-loaded PLGA nanoparticles).

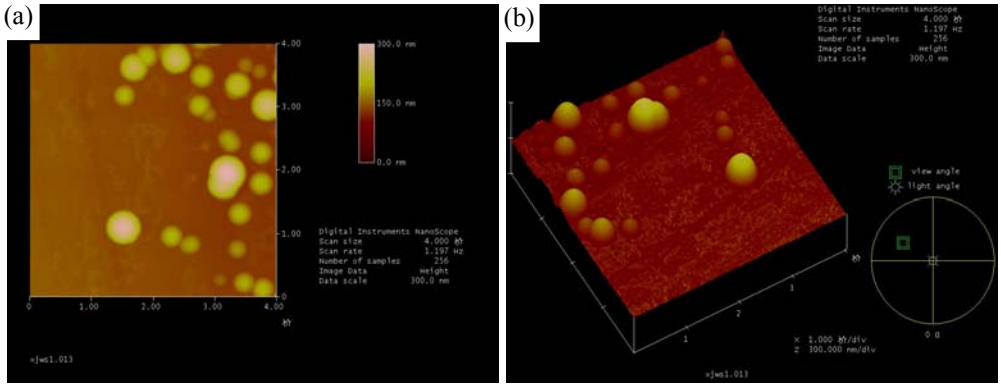


**Figure 2** AFM images of paclitaxel-loaded PLGA nanoparticles. (a: top view; b: surface plot).

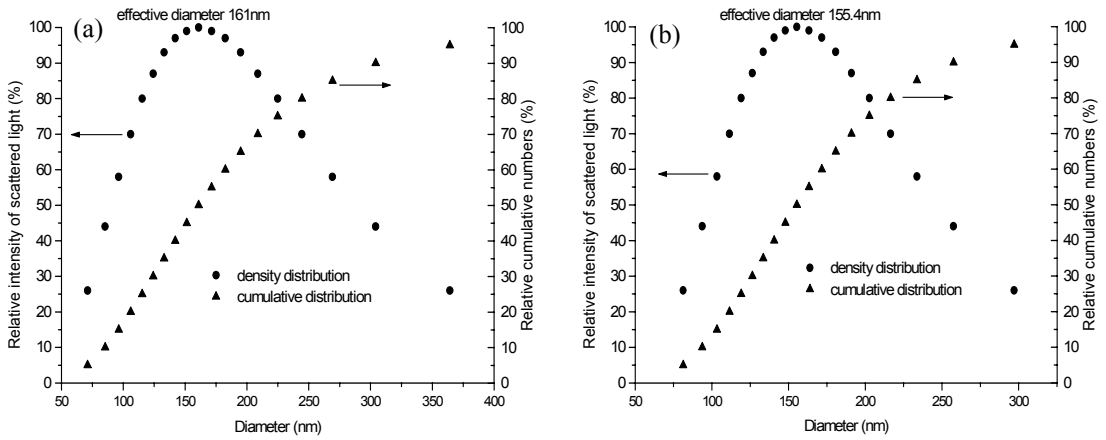


**Figure 3** particle size distribution (a: paclitaxel-loaded PLGA nanoparticles; b: coumrin6-loaded PLGA nanoparticles)

The release curve was shown in Figure 4. High initial burst was attributed to the immediate dissolution and release of paclitaxel adhered on the surface and located near the surface of the nanoparticles. The release profile can be optimized because the surfactant employed plays a significant role in controlling the release profile (Feng and Huang, 2001; Mu and Feng, 2002). Since the degradation time of polymer is about 1-2 months, the release by polymer matrix erosion was not significant in this release period.

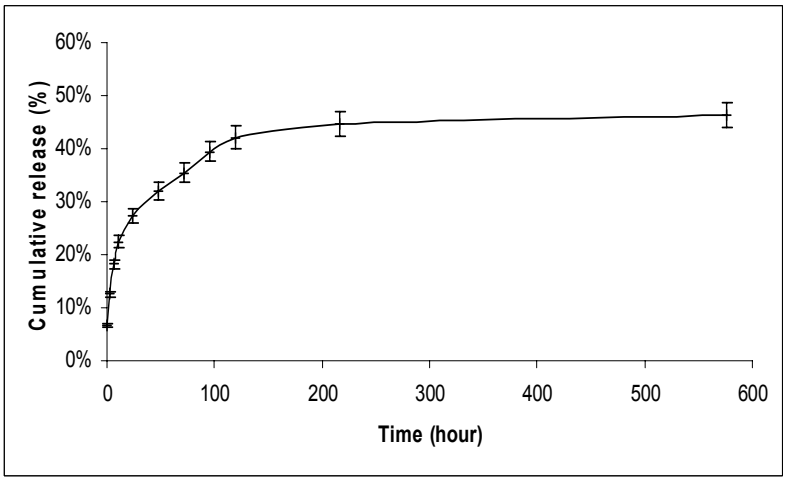


**Figure 4** paclitaxel-loaded PLGA nanoparticles release curve

C6 glioma cell line was used to evaluate the efficiency of PLGA nanoparticle cellular uptake. Nucleus staining was performed using propidium iodide (PI) and observed under confocal microscope using two channel mode. FITC channel was used to observe the coumarin6-labeled PLGA nanoparticles and neutral red channel was used to observe the cells. PLGA nanoparticles uptake progress is shown in Figure 5 at different time intervals, 1 hour and 2 hours, respectively. It is clear that much more particles were engulfed by

cells after 2 hours than after 1hour. Endocytosis is usually considered to occur either constitutively, by the continuous fluid-phase (pinocytic) pathway (less than 150nm), or by phagocytosis, a ligand-induced process responsible for the uptake of large particles (larger than 200nm) (Watts and Marsh, 1992). Also, phagocytosis function was observed in C6 glioma cells in vitro and in vivo (Noske et al., 1982; Zimmer et al., 1995). Phagocytosis, the process by which cells engulf foreign particles (Watts and Marsh, 1992; Desjardins and Griffiths, 2003), could be contributed to the particle cellular uptake. Based on the fluorescence measurements of flow cytometry studies (shown in Figure 6), a significant fraction of the administered nanoparticles could be taken up through nonspecific phagocytosis by the cells. In addition, from particle size distribution the size of approximately 40% of the particles is less than 150nm. Pinocytosis may play partial role in the particle cellular uptake. Therefore, coumarin6-loaded PLGA nanoparticles fabricated in this study could penetrate through c6 glioma cell membrane and be internalized by non-specific endocytosis process.

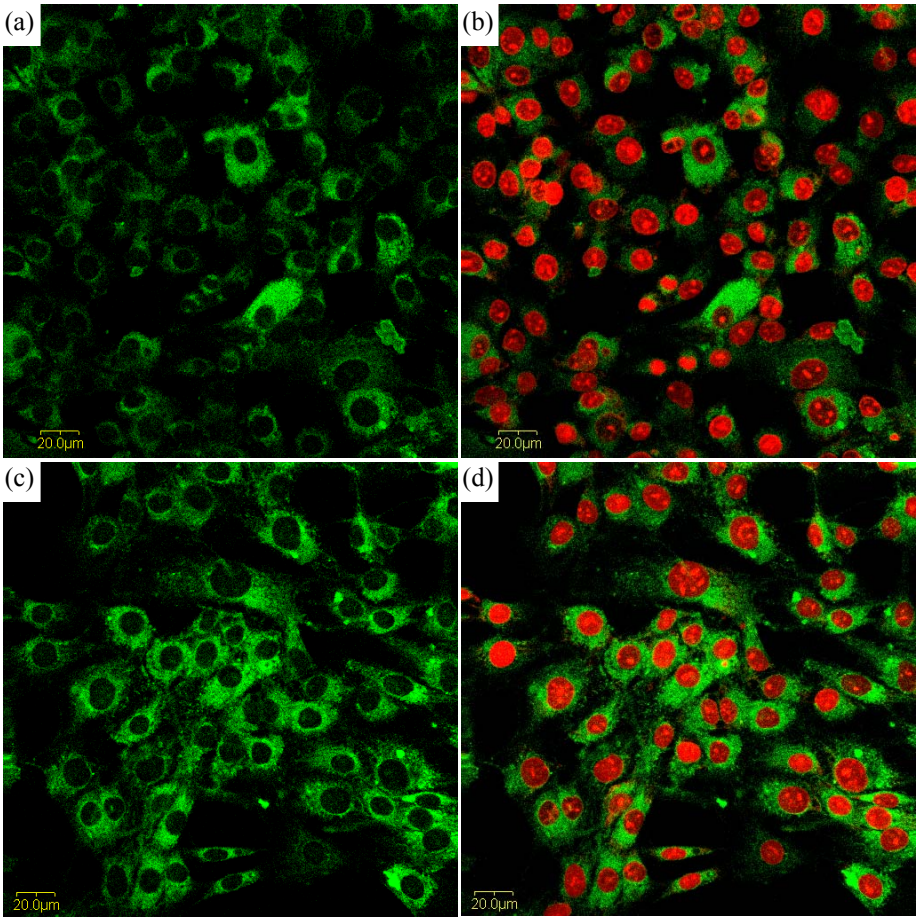


**Figure 5** Confocal fluorescence images of C6 glioma cells with coumarin6-loaded PLGA nanoparticles (a, b: incubated 1 hour; c, d: incubated 2 hours).

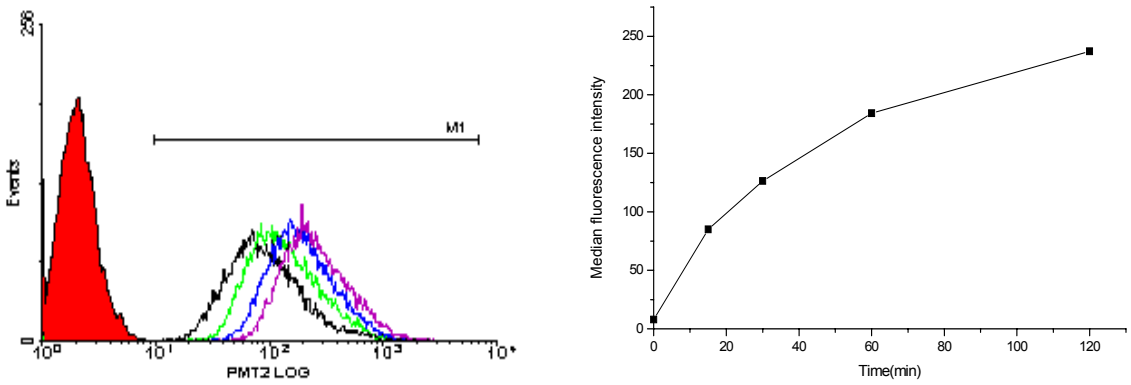


**Figure 6** Particle cellular uptake studied by flow cytometry (Black: 15min; Green 30min; Blue: 60min; Purple: 120min).

The cytotoxic activity of paclitaxel-loaded PLGA nanoparticles fabricated in this study (shown in Figure 7) was evaluated by assessing cell viability by the MTT assay using C6 glioma cell line. Cells were incubated with concentrations of nanoparticles which contained the same amount of drug as that of Taxol® in which paclitaxel concentrations were 15 μg/ml, 30 μg/ml and 60 μg/ml. The concentrations we selected were in the range which corresponds to plasma levels of the drug achievable in humans (Raymond et al., 1997; Fonseca et al., 2002). It was seen that paclitaxel-loaded PLGA nanoparticles could have lower cell viability than Taxol® after incubation 24 hours. Paclitaxel-loaded PLGA nanoparticles could have lower cell viability than Taxol® when the drug concentration is 30μg/ml and 15μg/ml. The amount of paclitaxel released from PLGA polymer matrix was about 27% in 24 hours, respectively (shown in Figure 4). According to the amount of drug of release from particles, the cell toxicity of paclitaxel-loaded nanoparticles seems to be 3 times higher than that of Taxol®. Cell viability was also evaluated with different concentrations of particles and it was found that higher concentration in the rang from 15 μg/ml to 60μg/ml could reduce much more cell viability. Also, cell viability may attenuate with the increase of Taxol® and nanoparticles exposure time. The antitumor effect of paclitaxel is dependent upon sustained therapeutic concentrations of the drug rather than maximal plasma concentrations because paclitaxel-toxicity requires entry of cells into the M phase (Gagandeep et al., 1999). It was studied that increasing the time of paclitaxel exposure results in increasing paclitaxel cytotoxicity (Liebmann et al., 1993). Therefore, the results obtained in this study are in accordance with the literatures. We could conclude that the new drug delivery system developed in this study may have its advantage on cytotoxicity compared to Taxol® because it has sustained release property (shown in Figure 4).

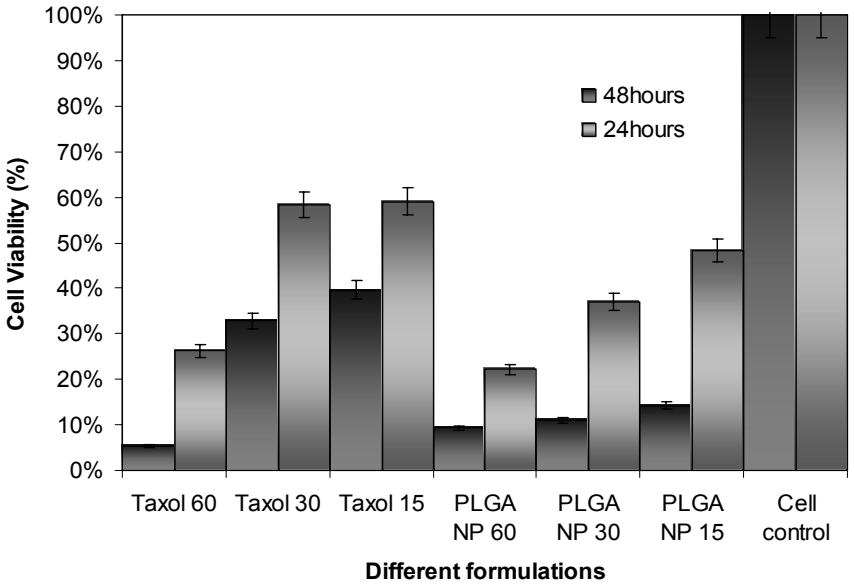


**Figure 7** *In vitro* cell viability of different formulations.

## Conclusions

The PLGA nanoparticles loaded with paclitaxel were obtained successfully by direct dialysis method, which avoided using emulsifier such as PVA. The coumarin6-loaded PLGA nanoparticles could penetrate through C6 glioma cell membrane and be internalized. The results of cytotoxicity test shows that the cytotoxicity of paclitaxel-loaded nanoparticles seems to be 3 times higher than that of Taxol® according to the amount of released drug. These results could be useful for predicting possible dose response of paclitaxel-loaded PLGA nanoparticles *in vivo* tests and clinical trial administration. Based on these studies, the formulations fabricated in this work could be promising for in vivo paclitaxel drug delivery systems. The surface of nanoparticles in this study could be modified and the *in vivo* test of the nanoparticles will be investigated by intravenous injection in the future work.

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### Nomenclature

PLGA Poly (lactide-*co*-glycolide) acid

PVA Poly (vinyl alcohol)
SEM Scanning Electron Microscopy
AFM Atomic Force Microscopy
DSC Differential Scanning Calorimetry
p-gp p-glycoprotein

**References**


DESJARDINS, M. and GRIFFITHS, G. 2003. Phagocytosis: latex leads the way. Current *opinion in cell biology*. 15, 498-503.

FELLNER, S., BAUER, B., MILLER, D. S. et al., 2002. Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. *Journal of Clinical Investigation*. 110(9), 1309-1318.

FENG, S. S., CHIEN, S. 2003. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. *Chemical Engineering Science.* 58, 4087-4114.

FENG, S. S., HUANG, G. F. 2001. Effect of emulsifiers on the controlled release of paclitaxel from nanospheres of biodegradable polymers. *J. Contr. Rel.* 71, 53-69.

FONSECA, C., SIMOES, S., GASPAR, R. 2002. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. *J. Contr. Rel.* 83, 273-286.

GAGANDEEP, S., NOVIKOFF, P.M., OTT, M., GUPTA, S. 1999. Paclitaxel shows cytotoxic activity in human hepatocellular carcinoma cell lines. *Cancer Research*.136, 109-118.

JEONG, Y. I., CHO, C. S., KIM, S. H., KO, K. S., KIM, S. I., SHIM, Y. H., NAH, J. W. 2001. Preparation of poly (DL-lactide –co-glycolide) nanoparticles without surfactant. *J. Appl. Poly. Sci.* 80, 2228-2236.

JEONG, Y. I., CHEON, J. B., KIM, S. H., NAH, J. W., LEE, Y. M., SUNG, Y. K., AKAIKE, T., CHO, C. S. 1998. Clonazepam release from core-shell type nanoparticles in vitro. *J. Contr. Rel.* 51, 169-178.

JEONG, Y. I., SHIM, Y. H., CHOI, C. Y., JANG, M. K., SHIN, G. M., NAH, J. W. 2003. Surfactant-free nanoparticles of poly (DL-lactide-co-glycolide) prepared with poly (L-lactide)/poly(ethylene glycol). *J. Appl. Poly. Sci.*89(4), 1116-1123.

JORDAN, M. A., TOSO, R.J., THROWER, D. 1993. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl. Acad. Sci. USA*. 90, 9552-9556.

JORDAN, M. A. and WILSON, L. 2004. Microtubules as a target for anticancer drugs, *Nature Reviews Cancer*. 4, 253-265.

LANDRY, F. B., BAZILE, D. V., SPENLEHAUER, G., VEILLARD, M., KREUTER, J. 1995. Release of the fluorescent marker Prodan® from poly (D,L-lactic acid) nanoparticles coated with albumin or polyvinyl alcohol in model digestive fluids (USP XXII). *J. Contr. Rel.* 44, 227-236.

LAVELLE, E. C., SHARIF, S., THOMAS, N. W., HOLLAND, J., DAVIS, S. S. 1995. The importance of gastrointestinal uptake of particles in the design of oral delivery systems. *Adv. Drug Deliv. Rev.*18, 5-22.

LIEBMANN, J. E., COOK, J. A., LIPSCHULTZ, C., TEAGUE, D., FISHER, J., MITCHELL, J. B. 1993. Cytotoxic studies of paclitaxel (Taxol®) in human tumour cell lines. *Br. J. Cancer.* 68, 1104-1109.

LOCKMAN, P. R., MUMPER, R. J., KHAN, M. A. 2002. Nanoparticle technology for drug delivery across the blood brain barrier. *Drug Development and Industrial Pharmacy*, 28(1), 1-13.

MILLER, G. 2002. Drug targeting: breaking down barrier. *Science.* 297, 1116-1118.

MU, L., FENG, S. S. 2002. Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (Taxol®). *J. Contr. Rel.* 80, 129-144.

NOSKE, W., LENTZEN, H., LANGE, K., KELLER, K. 1982. Phagocytotic activity of glial cells in culture. *Experimental Cell Research.* 142, 437-445.

RAYMOND, E., HANAUSKE, A., FAIVRE, S. et al. 1997. Effects of prolonged versus short-term exposure paclitaxel (Taxol®) on human tumor colony-forming units. *Anti-Cancer Drugs.* 8(4), 379-385.).

ROWINSKY, E.K., WRIGHT, M., MONSARRAT, B., LESSER, G.J., DONEHOWER, R.C. 1993. Taxol: Pharmacology, metabolism and clinical implications. *Cancer Surveys*. 17, 283-301.

SAHOO, S. K., PANYAM, J., PRABHA, S., LABHASETWAR, V. 2002. Residual polyvinyl alcohol associated with poly (D, L- Lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. *J. Contr. Rel.* 82, 105-114

SINGLA, A. K., GARG, A., AGGARWAL, D. 2002. Paclitaxel and its formulations. *International Journal of Pharmaceutics.* 235, 179-192.

WATTS, A. and MARSH, M. 1992. Endocytosis: what goes in and how?. *Journal of Cell Science.* 103, 1-8.

XU, K. L., LUDUENA, R. F. 2002. Characterization of Nuclear β□-Tubulin in Tumor cells: A possible novel target for Taxol. *Cell motility and the Cytoskeleton* 53, 39-52.

ZIMMER, C., WEISSLEDER, R., POSS, K., BOGDANOVA, A., WRIGHT, S.C., ENOCHS, W. S. 1995. MR imaging of phagocytosis in experimental gliomas. *Radiology.* 197, 533-538.