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# Biodegradable poly(ε-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen

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**Abstract**

To increase the local concentration of tamoxifen in estrogen receptor (ER) positive breast cancer, we have developed and characterized nanoparticle formulation using poly(ε-caprolactone) (PCL). The nanoparticles were prepared by solvent displacement method using acetone–water system. Particle size analysis, scanning electron microscopy, zeta potential measurements, and differential scanning calorimetry (DSC) were used for nanoparticle characterization. Biodegradation studies were performed in the presence and absence of *Pseudomonas* lipase in phosphate-buffered saline (PBS, pH 7.4) at 37 °C. Tamoxifen loading over different concentrations was analyzed by high-performance liquid chromatography (HPLC) and the optimum loading concentration was determined. In vitro release studies were performed in 0.5% (w/v) sodium lauryl sulfate (SLS) containing PBS at 37 °C. Cellular uptake and distribution of fluorescent-labeled nanoparticles was examined in MCF-7 breast cancer cells. SEM micrographs and Coulter analysis showed nanoparticles with spherical shape and uniform size distribution (250–300 nm), respectively. Zeta potential analysis revealed a positive surface charge of +25 mV on the tamoxifen-loaded formulation. Being hydrophobic crystalline polyester, PCL did not degrade in PBS alone, but the degradation was enhanced by the presence of lipase. The maximum tamoxifen loading efficiency was 64%. Initial burst release of tamoxifen was observed, probably due to significant surface presence of the drug on the nanoparticles. A large fraction of the administered nanoparticle dose was taken up by MCF-7 cells through non-specific endocytosis. The nanoparticles were found in the perinuclear region after 1 h. Results of the study suggest that nanoparticle formulations of selective ER modulators, like tamoxifen, would provide increased therapeutic benefit by delivering the drug in the vicinity of the ER.


*Keywords:* Targeted delivery system; Poly(ε-caprolactone); Tamoxifen; Nanoparticles; MCF-7 cells

## 1. Introduction

One of the major problems facing cancer chemotherapy is the achievement of the required therapeutic concentration of the drug at the tumor site for a desired period of time without causing

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undesirable effects on other organs while circulating in the body. The vascular system of tumors is highly disorganized and unpredictable both in its structure and function. This disorganization serves as a major barrier in the delivery of drugs to solid tumors (Jain, 1994). High viscosity of blood in the tumor significantly hinders drug delivery to poorly perfused regions of the tumor mass (Folkman, 1996; Jain, 1994). Another factor that poses a problem to drug delivery in solid tumors is the abnormally high pressure in the interstitial matrix of the tumor that retards the passage of molecules across the vessel walls and into the interstitial matrix.

Oral administration of the non-steroidal anti-estrogen like tamoxifen is the treatment of choice for the patients with all stages of estrogen receptor (ER) positive breast cancer (Macgregor and Jordan, 1998). The human ER belongs to a family of nuclear hormone receptors. The ER protein consists of 595 amino acids with a molecular weight of 66 kDa that has been separated into six different functional domains. ERα is the conventional ER, but recently ERβ has also been identified. ER is mainly located on the nucleus of the cell (Macgregor and Jordan, 1998). Tamoxifen is the first selective estrogen receptor modulator (SERM; Jordan, 1998) that exhibits good bioavailability upon oral administration and is mainly employed for the long-term prophylactic therapy in high-risk and post-menopausal women (Jordan, 1992, 1999; Lerner and Jordan, 1990; Macgregor and Jordan, 1998). Following long-term therapy, tamoxifen has some major side effects such as endometrial cancer and development of drug resistance, which may lead to further progression of the tumor (Johnston, 1997; Munster and Hudis, 1999; Osborne and Fuqua, 1994). These unwanted side effects of tamoxifen as well as various barriers to the effective administration of drugs demands for targeted delivery to the site of tumor and enhanced uptake by the tumor cells.

To overcome the undesirable side effects of tamoxifen and to increase the concentration at the tumor site, tamoxifen could be entrapped in colloidal drug carriers, such as polymeric nanoparticles, which may provide a better means of delivery in terms of enhanced uptake by the tumor and increased local concentration of the drug at the receptor site. Tumor vasculature is leaky and possesses an enhanced capability for the uptake of particulate drug carriers as compared with the normal blood vessels that have intact and continuous vasculature. Studies have suggested that tumor vasculature is hyperpermeable and selectively takes up macromolecules and colloidal carriers of diameter up to 600 nm. Thus, if a drug loaded particulate carrier could cross the above mentioned barriers they would remain inside the tumor for a longer time releasing the drug either in the vicinity of the tumor cells (Dellian et al., 2000; Maeda et al., 2000) or internalized by the cell and release in the cytoplasm. Thus, such a delivery method could improve the selectivity of treatment by increasing the ratio of tamoxifen absorbed by the tumor to tamoxifen absorbed by other tissues such as the endometrium.

In the present study, we have entrapped tamoxifen in poly(ε-caprolactone; PCL) nanoparticles. PCL is a biodegradable polyester that is widely used in drug delivery applications (Pitt, 1990). PCL is much more resistant to chemical hydrolysis and is achiral, a feature that limits the possibility of property modulation through the configurational structure of polymer chains. High permeability to many drugs and a lack of toxicity has made PCL and its derivatives well suited for colloidal drug delivery. It is a highly hydrophobic crystalline polymer that degrades very slowly in vitro in the absence of enzymes and in vivo as well (Pitt, 1990; Ponsart et al., 2000). The in vitro degradation can be enhanced in the presence of the enzyme lipase.

## 2. Materials and methods

### 2.1. Materials

PCL with a number-average molecular weight of 14 800 Da, as verified by gel-permeation chromatography, was purchased from Polysciences Inc. (Warrington, PA). Pluronic® F-68, a non-ionic surfactant composed of poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) tri-

block copolymer, was kindly supplied by the Performance Chemical Division of BASF Corporation. (Parsipanny, NJ). Tamoxifen (free base, mol. wt. 371.5 Da) and *Pseudomonas* lipase (90 units/g) were obtained from Sigma Chemical Company (St. Louis, MO) and rhodamine123 was obtained from Molecular Probes (Eugene, OR). MCF-7 (or HTB-22) cells were purchased from American Type Culture Collection (Rockville, MD). Deionized distilled water (NanoPure II, Barnsted/Thermolyne, Dubuque, IA) was exclusively used for the preparation of aqueous solutions.

### 2.2. Preparation of nanoparticles

The nanoparticles were prepared by solvent displacement method as described by Espuelas et al. (1997). Briefly, 625 mg of PCL was dissolved in 100 ml of acetone by mild heating and sonication. The polymer solution was gently poured into 200 ml of deionized distilled water containing 625 mg of Pluronic® F-68 under moderate magnetic stirring. The resulting suspension of nanoparticles was centrifuged at 12 600 g for 1 h. The supernatant, consisting of acetone and water, was discarded. The pellet was washed twice with deionized distilled water and freeze-dried. In some instances, nanoparticles were also prepared in the absence of Pluronic® F-68.

### 2.3. Characterization of nanoparticles

#### 2.3.1. Coulter particle size analysis

For each batch of nanoparticle suspension prepared, a sample was analyzed for reproducibility of particle size and distribution. Analysis was performed with a Coulter® N4-Plus Submicron Particle Sizer (Coulter Corporation, Miami, FL) at multiple scattering angle detection. The instrument is equipped with appropriate software for size distribution analysis. The suspension was diluted with de-ionized distilled water until a satisfactory intensity concentration could be obtained, which was confirmed by the equation displayed on the Coulter instrument.

#### 2.3.2. Scanning electron microscopy (SEM)

For SEM analysis of the freeze-dried nanoparticles, a random sample was mounted on an aluminum sample mount and sputter-coated with a gold–palladium alloy to minimize surface charging. SEM analysis was performed using an AMR-1000 scanning electron microscope (Amray Instruments, Bedford, MA) at a working distance of 10 mm and an accelerating voltage of 10 kV. The surface morphology of the particles was observed at 13 500 × magnification.

#### 2.3.3. Measurement of surface charge

Zeta potential measurements of the control, rhodamine-loaded, and tamoxifen-loaded nanoparticles in deionized distilled water were performed using a Zeta Phase Analysis Light Scattering (PALS) Ultra-Sensitive Zeta Potential Analyzer instrument (Brookhaven Instruments, Holtsville, NY).

#### 2.3.4. Differential scanning calorimetry (DSC)

DSC was performed on three freeze-dried preparations that included the drug loaded nanoparticles, prepared in the presence and absence of Pluronic® F-68, and nanoparticles without the drug using a Shimadzu DSC-50 (Columbia, MD) differential scanning calorimeter calibrated with indium standards. DSC scan of tamoxifen crystals was used as a control. The thermal behavior was studied by heating approximately 6.0 mg ($\pm$2.0 mg) of the samples in a covered aluminum sample pan under nitrogen atmosphere. Keeping in mind the melting point of polymer, the temperature range used was between 30 and 120 °C with a heating rate of 10 °C/min.

### 2.4. Biodegradation of nanoparticles

There have been a number of reports on biodegradation studies of PCL using high molecular weight polymer (i.e. $>$100 000 Da). However, biodegradation study of low molecular weight PCL nanoparticles has not been reported to our knowledge. We have, therefore, examined the degradation of PCL nanoparticles in the absence and presence of *Pseudomonas* lipase in PBS kept at 37 °C. Briefly, 5.0 mg of the freshly

freeze-dried nanoparticle sample (previously checked for the particle size distribution) was placed in an Eppendorf tube with 1 ml lipase (0.5 mg/ml or 45 units/l) in PBS or PBS alone. Samples were taken out at different time intervals, freeze-dried and the molecular weight differences were analyzed by gel permeation chromatography (GPC). Organic phase GPC was performed using a Hewlett Packard 1100 series isocratic pump, a Rheodyne Model 7125 injector with a 100 μl injection loop, and two PL-Gel mixed-D columns in series (5 μm, 300 × 7.5 mm, Polymer Laboratories, Amherst, MA). Tetrahydrofuran/0.1-M piperidine was used as the eluent at a flow rate of 1.0 ml/min. Data were collected using an Optilab DSP interferometric refractometer (Wyatt Technology, Santa Barbara, CA) and processed using the TRISEC GPC software package (Viscotek Corporation, Houston, TX).

### 2.5. Tamoxifen loading studies

All of the experiments with tamoxifen were performed under subdued light as the drug is highly photosensitive. For the loading studies, a stock solution of tamoxifen (free base) was prepared in acetone. The drug was incorporated into the nanoparticles by adding a predetermined volume of tamoxifen stock solution to the polymer solution and mixing to ensure uniform distribution. Nanoparticles were prepared from this drug–polymer mixture in Pluronic® F-68 as described above. The amount of drug loaded per milligram of polymer and the percent loading was determined by re-dissolving a known amount of the nanoparticles in acetone and then analyzing for the drug content by high-performance liquid chromatography (HPLC) assay.

HPLC analysis of tamoxifen in the nanoparticle formulation was carried out with Water's LC Module 1 (Milford, MA) using the procedure described by MacCallum et al. (1996). A $C_{18}$ reverse-phase column (Zorbax®, Hewlett–Packard) was used as the stationary phase, while the mobile phase consisted of 1.0% (v/v) triethylamine in deionized distilled water added to methanol in the ratio of 11:89 (v/v). The injection volume was 20 μl and the flow rate of the mobile phase was 1.2 ml/min. Tamoxifen was detected at 265 nm using a UV detector. The amount and percent loading of tamoxifen in the nanoparticles was determined from a calibration curve of the drug in acetone.

### 2.6. In vitro tamoxifen release studies

As tamoxifen base is relatively insoluble in the aqueous medium with a solubility of $< 0.1$ mg/ml at 20 °C (Sigma Product Information for product T5648, available at http://www.sigma.com), 0.5% (w/v) sodium lauryl sulfate (SLS) was included in phosphate-buffered saline (PBS, pH 7.4) to enhance the solubility of tamoxifen (Rich et al., 2001). Besides increasing the solubility, SLS inclusion in the release medium also prevented the adsorption of tamoxifen to the container surfaces. Ten milligrams of the freeze-dried, drug-loaded nanoparticles was added to a 10-ml centrifuge tube in the presence of 8.0 ml of SLS–PBS solution and kept in a water-bath at 37 °C. Periodically, the tubes were centrifuged at $12\,600 \times g$ for 10 min, and 4.0 ml samples of the release medium were withdrawn. Following this, 4.0 ml of fresh SLS–PBS was added to the test tubes to maintain sink conditions. The supernatant was filtered through a 0.22-μm membrane filter to ensure that the filtrate was free of most nanoparticles (since the particles are in the size range of 200–300 nm). The concentration of the drug was determined by HPLC assay as described above. The cumulative amount and percent of tamoxifen released from the nanoparticles was determined.

### 2.7. Cell uptake and distribution studies

To prepare the dye-entrapped nanoparticles, rhodamine-123 was added to the polymer–acetone solution before addition of this mixture to the water–pluronic mixture under magnetic stirring. The concentration of the fluorophore was optimized at 1.0% (w/w) of the polymer based on preliminary studies that showed lower concentrations resulted in loss of fluorescence signal due to photobleaching. Being a hydrophobic dye, rhodamine-123 was efficiently loaded in the PCL nanoparticles. We also observed that rhodamine-123 did not leach out of the nanoparticles during

incubation with the cell culture medium. Rhodamine-123-containing nanoparticle suspension was prepared by re-suspending the washed pellet formed after the centrifugation using de-ionized distilled water. The size distribution of the suspension was checked using the Coulter analysis to ensure the desired size range of 200–300 nm. ER positive human breast cancer cells (MCF-7) cells, procured from ATCC, were maintained in Eagle's minimum essential medium supplemented with non-essential amino acids, fetal bovine serum and other essential ingredients in 5% $CO_2$ atmosphere. The MCF-7 cells were sub-cultured twice during the experimental time period. The cell viability was periodically examined using Trypan blue exclusion assay.

After the washing steps, rhodamine-123 containing PCL nanoparticle suspension was diluted with sterile PBS and a 100-μl aliquot was added to the MCF-7 cells that were cultured on a glass cover-slip in six-well culture plate. After incubation for specific time intervals, the media was removed and plates were washed with sterile PBS. After the final wash, the cells were fixed with 4% paraformaldehyde and individual cover slips were mounted on clean glass slides with Fluoromount-G® mounting medium (Southern Biotechnology Associates, Birmingham, AL). The slides were viewed with a Zeiss Axioplan-2® (Thornwood, NY) universal microscope with Improvision OPENLAB® 3.0 software for the uptake and distribution of the nanoparticles. Differential interference contrast (DIC) and fluorescence images were obtained at 100 × magnifications and processed using ADOBE PHOTOSHOP® software.

## 3. Results and discussion

### 3.1. Characterization of nanoparticles

The prepared PCL nanoparticles were characterized in terms of mean size and size distribution, morphology, and surface charge. The mean size and size distribution of each batch of the nanoparticle suspension was analyzed using the Coulter particle size analysis. The size distribution profile, as shown in Fig. 1A, represents a typical batch with a mean diameter of 100–300 nm and a narrow size distribution. At the centrifugation

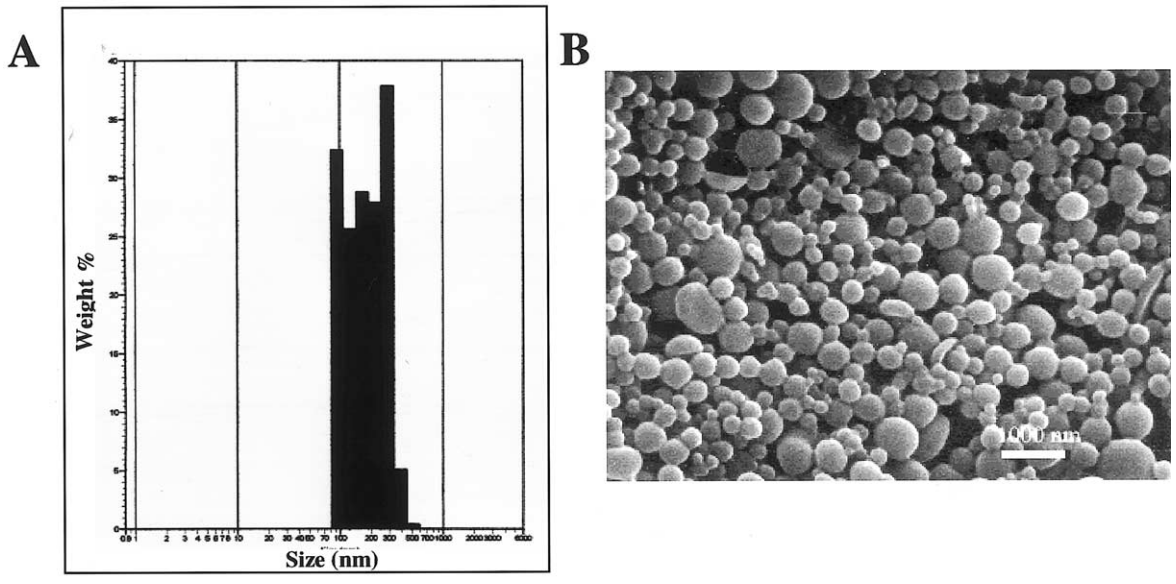


Fig. 1. Results of particle size analysis by Coulter counter (A) and scanning electron micrograph (B) of PCL nanoparticles. The nanoparticles were prepared by solvent displacement method. Original magnification of the electron micrograph was 13 500 × and the scale bar represents a distance of 1.0 μm.

Table 1
Zeta potential values of control and tamoxifen loaded nanoparticles[a]

| Nanoparticle formulations | Zeta potential (mV) |
|---|---|
| Control nanoparticles | 6.7 ± 1.2[b] |
| Tamoxifen-loaded nanoparticles | 25.4 ± 1.4 |

[a] Zeta potentials of the nanoparticle suspension in deionized distilled water were measured using the Brookhaven's Zeta PALS instrument.
[b] Mean ± S.D ($n = 8$).

speed of 12 600 × $g$, we only separated particles in the size range of 200–300 nm. SEM image of the nanoparticles (Fig. 1B) shows distinct spherical particles with smooth surface and confirms the size range of 200–300 nm. Higher magnification SEM showed that the nanoparticles prepared in the presence of Pluronic F-68® were non-porous (data not shown). Nanoparticles prepared in the absence of Pluronic® F-68 were significantly larger with a mean size of 800–900 nm.

The results of zeta potential analysis, as shown in Table 1, confirm that the tamoxifen-loaded nanoparticles had a significant positive charge of +25 mV on the surface as compared with the control nanoparticles that had an approximate charge of only +6 mV. The strong positive charge on tamoxifen-loaded nanoparticles was probably due to the preferential surface localization of the drug. Since tamoxifen is a weak base with a p$K_a$ of 8.85, we expect the drug to be positively charged in the nanoparticle suspension. Surface localization of tamoxifen was later confirmed by the burst release observed in the in vitro release profile. The rhodamine-123-containing nanoparticles used for cell uptake and distribution studies had a surface charge of +24 mV, which is similar to that of the tamoxifen-loaded nanoparticles.

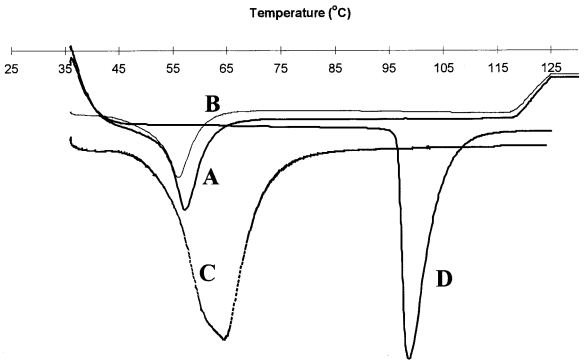


Fig. 2. DSC thermograms of control PCL (A) nanoparticles and those containing tamoxifen prepared in the presence (B) and absence (C) of Pluronic® F-68 and pure tamoxifen crystals (D).

Fig. 2 shows the DSC thermograms of PCL nanoparticles with and without the drug. DSC experiments were carried out to define the physical state of the polymer and the drug in the formulation and to check for the possibility of any interactions between the drug and the polymer within the matrix (Espuelas et al., 1997). The DSC thermograms of the control nanoparticles and the drug loaded nanoparticles, prepared in the presence of Pluronic® F-68, were identical with an endothermic peak at 58 °C corresponding the melting point of the PCL (Fig. 2A and B). The results show that when nanoparticles were prepared in the presence of Pluronic® F-68, the encapsulated drug did not interact significantly with PCL. In contrast, when the tamoxifen-loaded sample that was prepared in the absence of Pluronic® F-68, an increase in the melting point of the polymer was observed with a much wider peak due to slower transition before reaching the melting point of the polymer. In addition, the melting point peak shifted by 7–65 °C (Fig. 2C). The result suggests that when the nanoparticles were prepared in the absence of Pluronic® F-68, the drug may have interacted with the polymer system. The DSC thermogram of tamoxifen alone showed a sharp endothermic melting transition at around 95 °C (Fig. 2D).

### 3.2. Biodegradation studies

As shown in Fig. 3, PCL nanoparticles degrade very slowly in aqueous media due to its hydrophobicity and high crystallinity within the matrix. The crystallinity of PCL varies with its molecular weight and in general, the crystallinity increases as the molecular weight decreases. Even after 140 days in PBS at 37 °C, no significant reduction in molecular weight was observed in PCL nanoparticles (data not shown). Degradation studies of PCL nanoparticles have been done in a medium

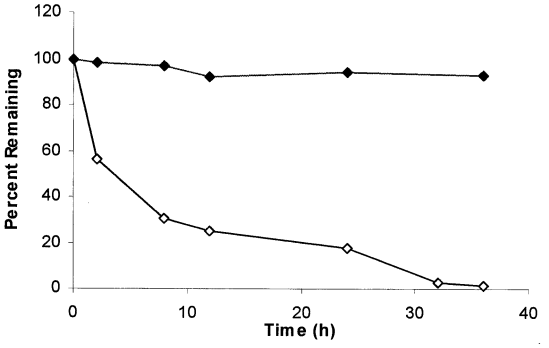


Fig. 3. Degradation kinetics of PCL nanoparticles in PBS (pH 7.4) at 37 °C in the absence (◆) and presence (◇) of *Pseudomonas* lipase. The nanoparticles were incubated with 0.5-mg/ml lipase in PBS and the molecular weight changes were determined by gel-permeation chromatography.

containing the enzyme *Pseudomonas* lipase (Chen et al., 2000; Gan et al., 1999; Wu et al., 2000; Zhihua et al., 1999). Wu et al. (2000) have claimed that the presence of lipase enhances the degradation rate of the polymer by a 1000-fold more as compared with degradation in water alone. In the presence of 0.5-mg/ml (i.e. 45 units/l) lipase, PCL nanoparticles degraded much faster. After 36 h, almost 100% of the polymer was degraded in the presence of lipase, whereas only around 8% of the polymer degraded in the absence of lipase. The serum lipase concentration in healthy adults is in the range of 30–190 units/l (Burtis and Ashwood, 1996).

The GPC data showed a diminishing area of the polymer peak as a function of time rather than shift towards lower molecular weights. This type of degradation profile was supported by the results obtained by other investigators using the laser light scattering method for molecular weight analysis (Wu et al., 2000; Zhihua et al., 1999). Gan et al. (1999) have claimed that the lipase 'eats' the nanoparticles one by one, so that the rate of degradation is dependent on the concentration of the enzyme. Thus, at a fixed enzyme concentration, there is a reduction in the concentration of the nanoparticle that is independent of the change in the molecular weight of the parent polymer. The reduction in concentration is characterized by the reduction in the peak area observed in the GPC. Subsequent analysis of the peak area over the period of time in the presence and in the absence of lipase demonstrated that the PCL nanoparticles were completely degraded after 36 h in the presence of lipase and only about 8% in the absence of lipase.

Enzymatic biodegradation happens mainly on the surface because it is usually difficult for the hydrophilic enzyme to diffuse into a hydrophobic polymer. For hydrophobic polymers, the enzymatic biodegradation follows heterogeneous kinetics. Degradation occurs as a result of adsorption of the enzyme followed by enzymatic hydrolysis. The surface area of the polymer material, such as in nanoparticle formulation, greatly enhances the rate of degradation (Gan et al., 1999). It would not be feasible to directly correlate the in vitro degradation results with in vivo behavior in a complex living system. When administered in vivo, however, we expect that the PCL nanoparticles would degrade faster than in vitro because of the presence of optimum concentrations of lipase and other PCL degrading enzymes in the body. In a study done using rabbits as experimental animals, Chen et al. (1997) found that the in vivo degradation of PCL was significantly quicker than the in vitro degradation.

### 3.3. Tamoxifen loading studies

From the results obtained as shown in Fig. 4, the loading efficiency of tamoxifen in PCL nanoparticles increased linearly with increasing drug concentration to a maximum of 64%. The maximum loading efficiency obtained was a result of the addition of 50 mg of tamoxifen to the PCL solution in acetone containing 625 mg of the polymer, prior to the addition of this mixture to the water–pluronic solution. A plateau was observed at this point and increasing the amount of tamoxifen did not result in enhanced loading. The optimum loading capacity calculated using the above proportion of tamoxifen was 5.1 mg of the drug for every 100 mg of the polymer, which corresponds to 5.1% (w/w) concentration. Thus, 50 mg was considered to be the optimum loading amount per 625 mg of the polymer and all further loading studies were performed based on these loading levels.

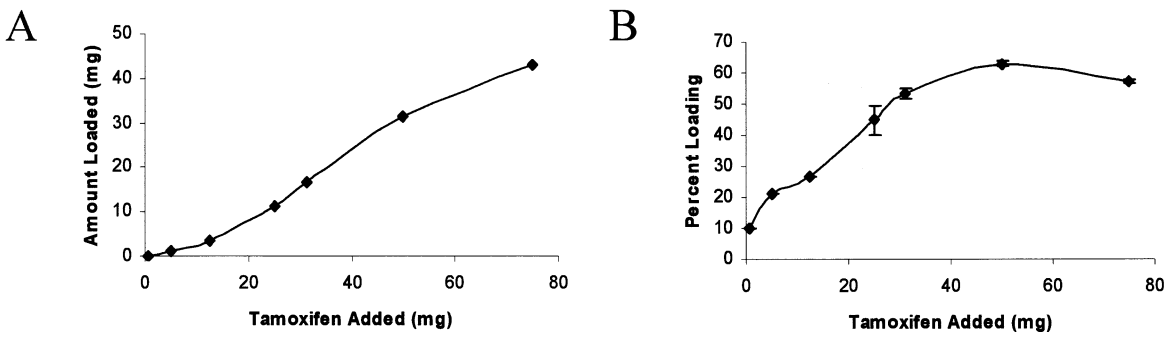


Fig. 4. The amount (A) and percent (B) tamoxifen loaded into PCL nanoparticles as a function of the amount of tamoxifen added to the polymer solution in acetone.

### 3.4. Tamoxifen release studies

From the release profile of tamoxifen, shown in Fig. 5, there was an initial burst effect as about 68% of the entrapped drug was released in the first hour in SLS-containing PBS at 37 °C. The maximum amount of tamoxifen in the nanoparticles was released within a 24-h time interval. The burst release of hydrophobic drugs, like tamoxifen, is attributed to the predominant surface presence of the drug in the nanoparticle formulation. Similar release profile was observed for tamoxifen in the study done by Brigger et al. (2001) when the drug was incorporated in poly(ethylene glycol)-modified cyanoacrylate nanoparticles. They observed 62% of the entrapped drug released within a few minutes when the experiment was performed in cell culture medium and concluded that most of the drug was localized on the surface of the nanoparticles.

To examine the effect of electrolytes in PBS on burst release, we performed the release studies in deionized distilled water instead of PBS keeping all of the other conditions exactly the same. In this case as well, almost 40% of the drug were released within the first hour and 100% was released in 24 h. The release studies were not performed in the presence of the enzyme lipase because in this system, its been shown that the predominant mechanism of release is by diffusion rather than polymer degradation, especially with low molecular weight drugs like tamoxifen. It has been established that PCL and its copolymers have a high permeability to low molecular weight drugs (Pitt, 1990).

Burst release of the drug has also been observed in solid lipid nanoparticles (SLN) when prepared by the hot homogenization method. In a review article, Muller et al. (2000) have discussed the mechanism of burst release from SLN as being mainly attributed to the temperature of preparation and the presence of surfactant in the aqueous solution. When the hydrophobic drug-containing oil phase is added to the surfactant-containing aqueous phase, the drug preferentially partitions into the hydrophobic domain of the surfactant assemblies. Once the nanoparticles solidifies in the aqueous phase and the surfactants attach to the hydrophobic nanoparticle surface, the drug cannot diffuse back into the solid core. As such, there will be a higher concentration of the drug on the surface of the nanoparticles (Muller et al., 2000). We have examined this theory in the case of tamoxifen-containing PCL nanoparticles by preparing them in the absence of Pluronic® F-68. As shown in the inset of Fig. 5, there was a significant reduction in the burst when the nanoparticles were prepared in the absence of Pluronic® F-68. It is, however, important to note that these nanoparticles were significantly larger (800–900 nm), and as such, would not be applicable for tumor-targeted delivery. The burst release observed may also be attributed to the drying conditions, low molecular weight of the drug, and the smaller size of the

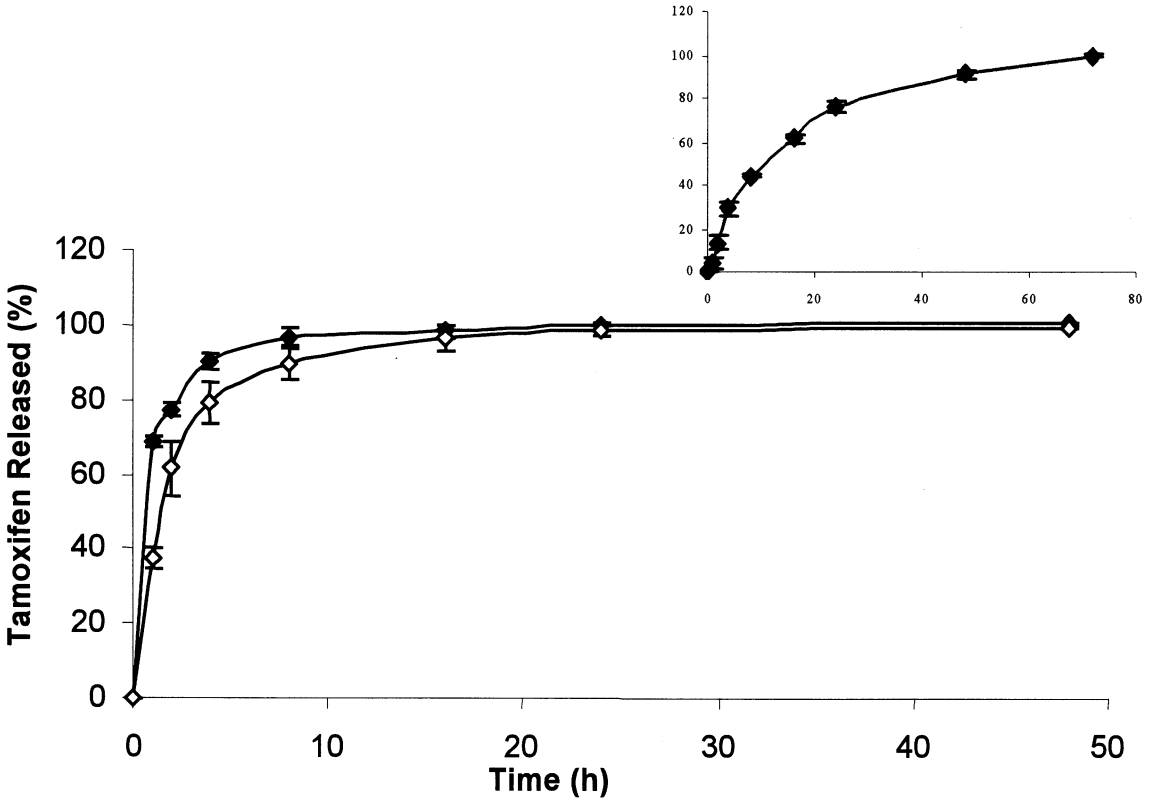


Fig. 5. Release kinetics of tamoxifen from PCL nanoparticles in 0.5% (w/v) SLS-containing phosphate-buffered saline (PBS, pH 7.4) (◆) or deionized distilled water (◇) at 37 °C. The release studies were performed in deionized distilled water to determine the effect of electrolytes in PBS on the burst release behavior. The inset shows the release of tamoxifen from PCL nanoparticles that were prepared in the absence of Pluronic® F-68.

particles (Huang and Brazel, 2001). The lower molecular weight (14 600 Da) of the polymer used in this study might also have contributed to the burst release observed. In a study done by Cohen et al. (1991), burst from PLGA microspheres was reduced as the molecular weight of the polymer was increased. Additionally polymer crystallinity is also known to affect drug release kinetics with more crystalline material exhibiting faster release than the amorphous material. Ge et al. (2000) observed a much faster release of nifedipine from PCL/poly(L-lactide) copolymer nanoparticles and attributed the release kinetics on high crystallinity of the matrix. They suggested that in a highly crystalline matrix, microchannel structures are formed that could lead to a high effective surface area for drug diffusion.

### 3.5. Cell uptake and distribution studies

ER positive MCF-7 breast cancer cells were used to examine the efficiency of nanoparticle uptake and distribution. Since there was very small amount of administered nanoparticles in the washing medium (examined by fluorescence measurement), majority the administered dose was probably internalized by the cells through non-specific endocytosis. After 1 h, DIC image (Fig. 6A) show intact cells and the fluorescence image (Fig. 6B) show a high concentration of nanoparticles localized in the cytoplasm and in the perinuclear area. Trypan blue exclusion results confirmed that the cells had remained completely viable assuring that the polymer did not induce any cytotoxicity. Further studies are in progress to

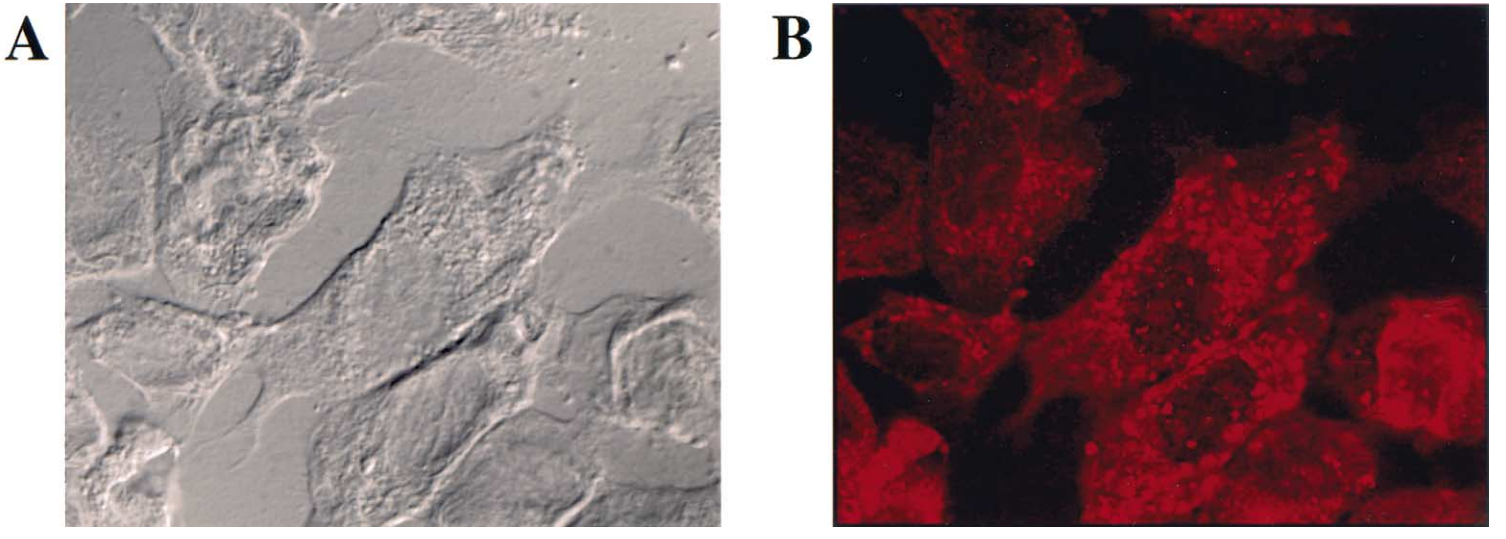


Fig. 6. DIC (A) and fluorescence confocal images (B) of rhodamine-123-labeled PCL nanoparticles in MCF-7 breast cancer cells. The dye-labeled nanoparticles were incubated with the cells for 1 h at 37 °C. Images were acquired with a Zeiss Axioplan-2® microscope at a 100 × magnification.

determine the change in tamoxifen concentrations in MCF-7 cells as a function of time after administration in the nanoparticle formulations.

## 4. Conclusions

Our studies indicate that tamoxifen entrapped in biodegradable poly(e-caprolactone) nanoparticles could serve as a useful form of targeted drug delivery system towards breast cancer. A significant uptake of nanoparticles was observed in the ER positive MCF-7 cell line, leading to an enhanced local concentration of the drug.

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