TAMOXIFEN LOADED POLY (ε-CAPROLACTONE) BASED INJECTABLE MICROSPHERES FOR BREAST CANCER

HIREMATH G JAGADEESH*, KUSM DEVI V

[1]Department of Pharmaceutics and Pharmacology, East West College of Pharmacy, B.E.L. Layout, Bangalore-91, Karnataka, [2]Department of Pharmaceutics, Al-Ameen College of Pharmacy, Hosur road, Karnataka, Bangalore, 560027, India. Email: jagadeeshgcp@gmail.com, ewcp56@gmail.com

ABSTRACT

The main objective of this study was to develop a polymeric drug delivery system for tamoxifen, intended to be injectable microspheres for breast cancer. To achieve this goal tamoxifen loaded poly (ε-caprolactone) (PCL) microspheres were prepared by solvent evaporation method. Mircoparticles were characterized in terms of particle size, surface morphology, drug physical state by using Master size analyzer (MSA), scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and Differential scanning calirometry (DSC). Tamoxifen loading over different concentration was analyzed by high performance liquid chromatography. *In vitro* drug release studies were performed in phosphate buffer saline (PBS, pH 7.4 at 37º C). Cytotoxicity study by MCF-7 breast cancer cells. The mean particle size of microspheres was 27-47μm and were spherical shape, with smooth surface. The DSC studies revealed that the entrapment tamoxifen in microspheres is in the form amorphous state. The encapsulation efficiency was found to 61-67%. The in vitro release of tamoxifen was relatively fast initially followed by a slower and sustained release. The cytotoxicity against the MCF-7 cells was affected significantly by the released amount of tamoxifen. FTIR and DSC studies demonstrated that did not indicate changes in either chemical stability of the polymer or thermal properties such as glass transition or melting temperature, indicating the absence of chemical degradation or polymer-drug interaction during long term stability studies.

Keywords: Tamoxifen, Poly (ε-caprolactone), Microspheres, MCF-7 cells, Breast cancer

INTRODUCTION

Chemotherapy is complicated procedure in which many factors are involved in determining its success or failure. It carries a high risk due to drug toxicity, and more effective drugs tend to be more toxic. Problems still exist even for successful chemotherapy, and patients have to tolerate severe side effects and sacrifice their quality of life.[1] 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 undesirable effects on the 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.[2] One of the major problems facing cancer chemotherapy is administering the required therapeutic concentration of the drug at the tumor site for the desired period of time without causing undesirable effects on other organs after systemic administration.[3,4] The tumor vasculature, formed by hypersecretion of angiogenic factors, is highly disorganized and tortuous and serves as a major barrier to drug transport. Tumors also have a very high interstitial hydrostatic pressure, which prevents drug entry into the core. Furthermore, the lack of a functional lymphatic system in tumors allows the drug oozing out of the mass to be diluted in the surrounding tissues and fluids.[5,6] Oral administration of the non-steroidal antiestrogen like tamoxifen is the treatment of choice for the patients with all stages of estrogen receptor (ER) positive breast cancer.[7] Antagonizing estrogen is popular treatment strategy because estrogen receptor (ER) overexpression is observed in about 70% of breast cancers, and about two thirds of breast cancers in postmenopausal women are ER-positive.[8] Oral tamoxifen undergoes extensive hepatic metabolism and the subsequent biliary excretion of metabolites.[9] Although the plasma antitumor concentration of 4-hydroxytamoxifen are only about 2% of those of the parent compound,[10] this metabolite has been reported compound to be about 100 times more than as an estrogen antagonist than tamoxifen.[11] Tamoxifen can have harmful long term side effects such as the development of endometrial cancer, or an acquired tamoxifen resistance leading to further tumor progression.[7] Other side effects include liver cancer, increased blood clotting and ocular side effects such as retinopathy and corneal opacities. These effects were reported to be dose dependent. To over come these undesirable side effects, injectable drug delivery is necessary in order to achieve optimum therapeutic outcomes for breast cancer. We have been exploring the development of drug loaded and drug free, PCL based injectable microspheres. *In vitro* characterization of prepared injectable microspheres. To understand growth inhibitory effect we evaluated the responsiveness of cancer cell lines to tamoxifen utilizing human breast cancer cells (MCF-7).

MATERIAL AND METHODS

Materials

Tamoxifen was obtained as gift samples from Cipla Ltd. Mumbai Central, India. Poly (ε-caprolactone) (PCL) (Mn 90,000) was purchased from Sigma Aldrich, Bangalore India. Dichloromethane (DCM), polyvinyl alcohol (PVA, Mw =30,000-70,000), from SD Fine Chemicals, Bangalore, India). Methanol, triethyl amine (TEA) (HPLC grade) was obtained from Merck, Bangalore, India. 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide dye (MTT), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, (FBS), Dulbecco's phosphate buffered, saline (PBS), trypsin-EDTA (0.5% trypsin, 5.3 mM EDTA tetra-sodium), and the antibiotic agents penicillin, streptomycin (100 U/ml) purchased from Sigma Aldrich, Bangalore, India. Distilled and deionized water was prepared using a Milli-Q Plus System (Millipore) All other materials and reagents were of analytical grade of purity.

Methods

Preparation of poly (ε-caprolactone) microspheres by solvent evaporation method

Drug free and tamoxifen loaded poly (ε-caprolactone) (PCL) microspheres were prepared with oil in water (o/w) solvent extraction/evaporation method. Two different types of tamoxifen loaded systems were prepared, differing in the theoretical loading (20 and 40% w/w). The exact amount of polymer and drug used for the preparation of each type of system are indicated in Table 1. Either 80 or 120 mg of PCL was dissolved in 15 ml dichloromethane (DCM) and sonicated for 20 min separately, after which tamoxifen (20 or 80 mg) was dissolved in this organic phase further sonicated for 10 min. The organic phase was added drop wise (0.8 ml/min) into the external aqueous phase containing polyvinyl alcohol (80 ml) and stirred at 1200±5 rpm (mechanical stirrer) using a stainless steel propeller having 16 mm diameter. The resulting emulsion was

stirred till DCM evaporated. The microspheres were collected by filtration using 0.22µm nylon filter (Millipore, India), washed five times with deionised water and resuspended into 5 ml of deionised

water, frozen in liquid nitrogen and lyophilized. Drug free microparticles were prepared in the manner without adding tamoxifen.

Table 1: Formulation parameters and characteristics of the investigated tamoxifen microspheres

PVA (%)	Theoretical loading %	PCL (mg)	Tamoxifen (mg)	Practical loading % (±S.D)	EE%[a] (±S.D)	Particle size µm (±S.D)	PD[b] (±S.D)
0.4%	20%	160	40	14.2±0.6	67.00±0.4	40.2±08.45	0.69±0.04
	40%	120	80	56.2±0.2	70.25±0.5	41.1±10.25	0.68±0.07
0.8%	20%	160	40	11.2±0.6	60.00±0.2	26.2±08.21	0.80±0.03
	40%	120	80	50.4±0.5	65.00±0.4	28.6±11.33	0.77±0.04

[a] Entrapment efficiency, [b] polydispersity

Drug entrapment in microspheres

To determine the tamoxifen content in the microspheres 5 mg of samples were dissolved in 5 ml of DCM. Next, 5 ml of mixture containing methanol: water: triethyl amine (90:10:0.1% v/v) was then added. A nitrogen gas stream was introduced to evaporate DCM until a clear solution was obtained. Further the solution was filtred through 0.22 µm nylon membrane filter (Millipore, India), clear solution was suitably diluted with methanol: water: triethyl amine (90:10:0.1% v/v) and tamoxifen was determined by HPLC analysis: HPLC system consists of a Shimadzu SPD-10ATVP, binary pump equipped with a normal sample injector SPD-10AVP variable wavelength UV detector and Spincotech station for data analysis: 50µl were injected into Phenomenex C-8 column, (4.6 x 250 mm, 5µm) and Phenomenex C-8 guard column cartridge (KJ0-4282, 4.0 x 3.0 mm, 5µm). Flow rate 1ml min^{-1}. The effluent as detected UV spectrophometrically (λ =265 nm). In order to account for the drug, which could be lost throughout the above procedure, the recovery efficiency of the procedure was determined by dissolving a known quantity of tamoxifen in DCM and subjecting it to the same procedure as described above.

Morphology (SEM)

The shape and surface characteristics of microspheres were visualized using scanning electron microscopy (SEM). The microspheres were first dried under vacuum. Samples were glued to aluminum sample holders (Materials department, Indian Institute of Science (IISC) Bangalore, India) and gold coated under argon atmosphere. The coated samples were finally analyzed using JSM 840. The surface morphology of microspheres was observed under suitable magnification.

Particle size analysis

In order to analyze particle size drug loaded lyophilized microspheres were dispersed in deionzed water, vortexed for 10 min and sonicated for 5 min before sampling. Particle size was determined by laser scattering light (Malvern Laser Analyzer Instruments, Strides Arco Lab, Bangalore, India). Polydispersity was determined according to the equation below.[12]

Polydispersity=D (0.9) x D (0.1)/D (0.5) Where D (0.9) corresponds to particle size immediately above 90% of the sample. D (0.5) corresponds to particle size immediately above 50% of the sample. D (0.1) corresponds to particle size immediately above 10% of the sample. Microsphere size and polydispersity were determined.

Fourier transform infrared (FTIR)

Infrared spectroscopy (model A-1700 FTIR, Shimadzu Instruments) was performed for pure PCL, physical mixtures of tamoxifen and PCL, and tamoxifen-loaded microspheres. Samples were mixed with KBr and vacuum packed to obtain pellets of the material, which were analyzed. All the spectra acquired scans between 500 and 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Thermal studies (DSC)

Differential scanning calorimetry (DSC) was conducted using Mettler Toledo Star system, (Indian Institute of science, Bangalore, India). Samples were weighed (4.00-6.00 ± 0.1 mg) and placed in sealed

aluminum pans. The coolant was liquid nitrogen. The samples were scanned at 10°C/ min from 20° C to 160° C.

***In vitro* drug release studies**

In vitro release studies of tamoxifen loaded microspheres were carried out at 37±2° C in pH. 7.4 phosphate buffer saline (PBS) containing 0.2% (w/v) sodium lauryl sulfate (SLS)[13] for a period of 30 days using specially designed and fabricated dissolution apparatus.[14] SLS was used to increase the solubility of tamoxifen in the buffer solution and prevent adsorption of the tamoxifen on the surface of the screw-capped bottle. In briefly, the screw capped bottles containing 10 mg tamoxifen loaded microspheres in 25 ml of PBS pH. 7.4 as release medium were fixed to stainless steel holders attached to a mechanical stirrer and platform was immersed in water maintained at 37±2° C. The platform was rotated at an average speed of 75 rpm to induce mixing in the release medium. At periodic intervals, initially at 24 h and then followed by every 2 days upto 30 days, 10 ml of the release medium was sampled and 10 ml of fresh release medium was replaced to provide the necessary sink condition. Samples were analyzed by high performance liquid chromatography (HPLC) for tamoxifen content by solvent extraction method, ie, 5 ml of dichloromethane (DCM) was added to withdrawn samples to which 5 ml containing methanol: water: triethyl amine (90:10:0.1% v/v) was added and the mixture was vortexed vigorously. Further same procedure was followed as described in entrapment efficiency section. All the release experiments were conducted in triplicate.

***In vitro* cytotoxicity studies by MCF-07 breast cancer cell-lines**

Human breast carcinoma MCF-7 (procured from National Cell Science Centre Pune, India) cells was grown in monolayer in Dulbecco's modified Eagle's medium (DMEM). The cells were cultured in media supplemented with 10% heat-inactivated foetal bovine serum and pencilillin (200 U/ml), streptomycin (200 µg/ml) and neomycin (10 µg/ml) at 37° C in a humidified atmosphere containing 5% carbon dioxide (CO2). MCF-7 cells (100 µL) at density of 4x10^{4} cells/ml growing cells were seeded in 96-well plates in the complete growth culture medium.[15] Tamoxifen stock solution was made in cell culture grade ethyl alcohol and was diluted in DMEM media to get the desirable concentration[16]. After culturing for 12 h, and treated for 48 h with various concentrations of tamoxifen 5 to 25 µg/ml.[17] The 40% theoretical drug loading correspondence practical loaded 65.00±0.4, 0.8% PVA based, developed PCL microspheres was selected for the *in vitro* cytotoxicity studies due to promising results. The microspheres containing 25, 50, 100, 250, and 500 µg/ml of tamoxifen loaded and blank microspheres (control) 500 µg/ml were transferred with 100 µL of medium. After predetermined incubation periods, 20 µL MTT solution was added to each well. After 48 h of incubation at 37° C, the medium was removed and any formazan crystals formed were solubilized with 100-µL dimethylsulfoxide (DMSO)[18]. After slow shaking for 10 min, the amount of formazan was then determined from optical density at 570 nm by an ELISA microplate reader (EL310, Bio-Tek Instruments Inc., Winooski, VT, USA). All the experiments were performed in triplicate. This assay depends on the cellular reductive capacity to metabolise the yellow tetra zolium salt, (3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide dye (MTT), a highly coloured formazan product. Cell morphology and location of

microspheres were assessed by inverted microscope and photographed. (Bio-Tek Instruments Inc., Winooski, VT, USA). The IC50 value was defined as the drug concentration required inhibiting the growth by 50% relative to controls.

Cell Viability % = NT/Nc

Where NT and Nc are the number of surviving cells in the group treated with tamoxifen

loaded microspheres and in the untreated cell group (control), respectively.

Stability studies

The 40% theoretical drug loading correspondence practical loaded 63.00±0.4, 0.8% PVA based, developed PCL microspheres were lyophilized was selected for the stability as per ICH guidelines at 30°C/65% RH upto 5 months. Periodically (initial, 15 days, 2 month, and 5 months) samples were removed and checked for SEM, FTIR and DSC studies.

RESULTS AND DISCUSSION

Characterization of microspheres

The encapsulation efficiency of tamoxifen in the PCL based microspheres was rather high (60.0-67%), the encapsulation efficacy is decreased even though the water solubility of tamoxifen (0.4 µg/ml), it is expected that almost all the tamoxifen would incorporated in the PCL polymer matrix. The tamoxifen, which remained in solution, may be the result of its increased solubility due to PVA surfactant formation of the micelles in the aqueous phase, irrespective of the theoretical drug loading and PVA

concentration in the outer aqueous phase during microparticle preparation Table 1. This can be explained by the high lipophilicity of the drug, minimizing tamoxifen loss into external water phase. Clearly, the PVA concentration in the latter had significant effect on the resulting microparticle size. At 0.4% (w/v) PVA, much larger microparticles were obtained (mean size= 44-47) than at 0.8 % PVA (mean size = 26-28). This can be attributed to the more pronounced decrease in surface tension between the organic and aqueous phase during microparticle preparation (PVA acting as a surfactant) and to the increase in viscosity of the external water phase (PVA acting as viscosity inducing agent with increasing PVA concentration). The decrease in microparticle size with increasing PVA concentration in the outer aqueous phase has an important consequence for the resulting encapsulation efficiencies of tamoxifen. The encapsulation efficiency of smaller microparticles (prepared with 0.8% PVA in the outer aqueous phase) was lower than that of larger microparticles (prepared with 0.4%). Polydispersity of microsphere found within the range in between 0.68-0.80. The particle size distribution (mean diameter) and polydispersity variation observed in the drug loaded PCL microspheres due to four factors are deemed essential in the ultimate determination of microspheres particle size namely, the concentration of polymer in the organic phase, the polarity of the solvents, the internal/external phase ratio and concentration of surfactant.[19]

Surface morphology

As showen Figure 1, the PCL microparticles containing tamoxifen presented a smooth surface without apparent porosity. Moreover, microspheres were of good morphological characteristics, spherical with smooth surface, no drug crystals were observed on the surface and without any aggregation homogeneously distributed.
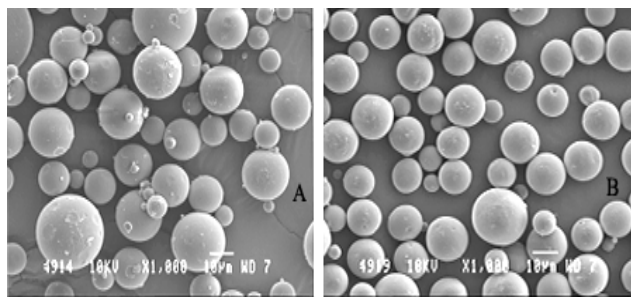

Fig. 1: Scanning electron micrograph (A) tamoxifen loaded PCL microspheres (0.4% PVA, (B) tamoxifen loaded PCL microspheres (0.8% PVA). Original magnification of the electron micrograph was 1,000x and scale bar represents a distance of 10 µm.

Fourier transform infrared (FTIR) measurements

Figure 2 showed that typical spectra of pure tamoxifen, PCL, a physical mixture of tamoxifen and PCL and a drug-loaded microspheres. The spectrum of tamoxifen shows characteristic absorption bands at 3027 cm^{-1} (=C-H stretching), 1507 and 1477 (C=C ring stretching) and 3180 cm^{-1} (-NH2). PCL displays a

characteristic absorption band at strong bands such as the carbonyl stretching mode around 1727 cm^{-1} (C=O), asymmetric stretching 2949 cm^{-1} (CH$_2$) symmetric stretching 2865 cm^{-1} (CH$_2$)[20]. No changes in the spectrum of the physical mixture and drug-loaded microspheres were evident by FTIR spectroscopy. The strong bands such as the carbonyl peak were clear at all points.
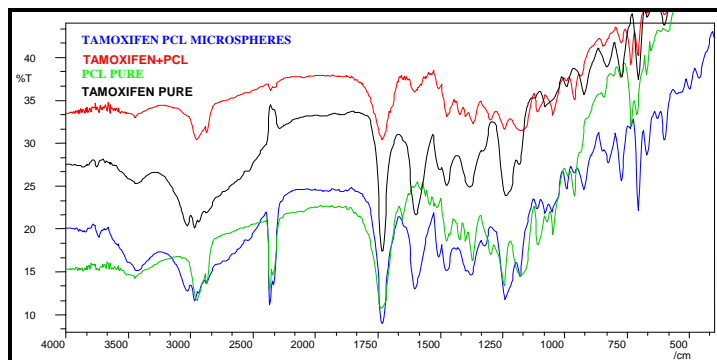

Fig. 2: Transmission FTIR spectra of pure tamoxifen, pure PCL, a physical mixture of drug +PCL and drug loaded microspheres.

Thermal characterization of PCL microspheres

The DSC technique can provide qualitative and quantitative information about the physicochemical status of drug in microspheres, which is reported to be involved in the endothermic or exothermic process. The related thermal transitions include melting, recrystallization, decomposition and out gassing or a change in heat capacity. DSC is useful to monitor different samples of same material to assess their similarities or differences or the effects of additives on the thermal properties of a material. Using the DSC analysis of drug, polymer materials and produced microspheres, the nature of the drug inside the polymer matrix can be assessed, which may emerge in solid solution, metastable molecular dispersion or crystallization.[21] In order to identify the mechanism of sustained

drug release, we first characterized the physical state of the drug within the microparticles.

Samples were subjected for DSC studies. A sharp and large melting onset/peak/endset peak of pure tamoxifen, pure PCL and physical mixture of drug and polymer at 146.22/148.6/151, 54.60/61.44/62.56 and 55.70/64.31/65.60/145/96/148.26/150.61 °C. However, the melting peak was absent on the DSC thermograms of microparticles containing tamoxifen, indicating that the drug was dispersed in the microparticles as an amorphous form.[22] This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to increased solubility and finally to an improved biological activity. The generated thermograms are shown in Figure 3.
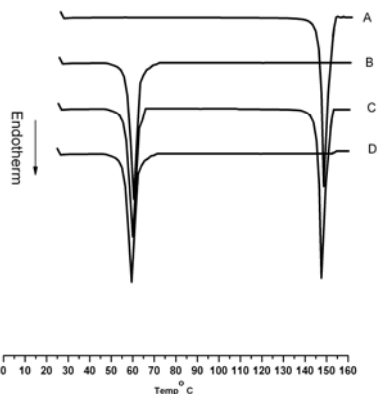

Fig. 3: DSC thermograms of pure tamoxifen (A), pure PCL (B), mixture of tamoxifen+PCL (C) and tamoxifen loaded PCL microsphers (D). The experiment was carried with crimped aluminum pans and a heating rate of 10° C/min.

***In vitro* drug release studies**

Figure 4 showed that release behavior of tamoxifen from PCL microparticles, which indicates sustained pattern for upto 30 days. At the initial stage, PCL based microspheres burst effect related to the drug entrapped near the surface of the microspheres[23] was remarkably small, and it was followed by a very slow release stage. Such a small initial burst is an especially interesting phenomenon, which is probably due to the low permeability of water in PCL[24]. To be released, preferentially, the diffusion path must be filled up by water.[25,26] The burst effect must not be considered a negative circumstance in all cases. At the later stage, the drug release was more slowly and sustained, whose rate is determined by the diffusion/erosion of polymer matrix. This fact can be explained as follows. Although PCL is a biodegradable polymer biodegradation of

the PCL is considerably slower than that of other degradable polymers.[27] Therefore, since PCL is hardly degraded during the diffusion process, the diffusion through the polymer is the only possible mechanism of drug release. Irrespective of drug initial drug content, the relative release rate slightly increased with increasing PVA concentration. This can at least partially explained by the decrease in microparticle size, resulting in decreased diffusion pathway lengths and coarse crystalline microstructure. Based on these facts, the amorphous region will be wide open and form a coarse crystalline microstructure through which the drug will diffuse rapidly. Thus it was found that internal crystalline microstructure compared with particle size effect plays an important role in drug release. However, the percentage drug release at the end of 30 days period from 20% and 40% drug loaded was found to be 24-30% and 46-58% respectively.
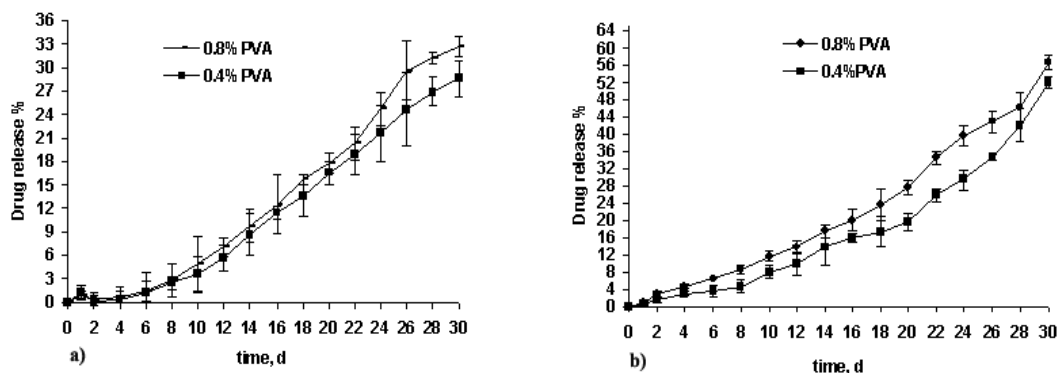

Fig. 4: Tamoxifen release from PCL based microparticles in PBS pH 7.4: (a) 20% theoretical drug loading and (b) 40% theoretical drug loading (the PVA concentration in the outer aqueous phase during microparticle preparation is indicated in the figures). Data represents mean ± SD, *n*=3.

In vitro anti tumor activity studies

Figure 5 showed that the effect of tamoxifen and tamoxifen loaded PCL based microspheres on human breast cancer MCF-7 cells. The action of tamoxifen and formulation on cell growth was observed after 48 h of addition. Tamoxifen dose response against different concentration of drug was observed on MCF-7 cells. The MTT assay shows that concentration at 5µg/ml very little growth inhibition was observed (cell viability was 82.23±1.8 %). There was linear increase in growth inhibition, with concomitant increase in tamoxifen concentration. At concentration of 20 µg/ml 50% of growth inhibition of cells was observed after 48 h of the culture. Complete cessation of cell growth was observed at a concentration 25µg/ml. Morever, different concentration of tamoxifen loaded PCL based microparticles action was observed on MCF-7 cell line culture. The MTT assay shows that the cells treated with at a concentration of 25µg/ml slight inhibition was observed (cell viability was 86.94±2.92). Microspheres added at a concentration 100µg/ml 50% of growth inhibition cells were observed after 48 h of the culture. Complete cessation of cell growth was observed at a concentration of 250 and 500 µg/ml. Viability of the cells incubated with blank PCL microparticles remained at about 95% relative to the non-toxic control during the period of incubation. This indicates that with a concentration of 500 µg/ml PCL polymer imparts no cytotoxic effects to the cells. Obtained results were found to be statistically significant (p<0.05). As expected, incubation with free tamoxifen (20µg/ml) interfered with the cytoskeleton architecture and rapidly altered cell morphology in culture (Figure 6 A&B).

Similar effects were observed with tamoxifen (100 and 250 µg/ml) loaded particles (Figure 6 D&E), but not with blank particles despite their long lasting adhesion to the cell wall (Figure 6 C&D). The decrease in cell viability with increasing tamoxifen concentration in the medium is due to the increasing in tamoxifen concentration in biodegradable microspheres, which results in a proportionate increase in dose, resulted in increased reduction in cell proliferation. The cell line was sensitive to the released drug when it was exposed continuously to tamoxifen for 48 h. The reduction rate of cell viability from drug loaded microspheres could be explained with respect to the dependence on release of drug from the microspheres. The maximum efficacy, with reduction in cell proliferation of > 90%, was seen with 500 µg/ml of tamoxifen loaded microspheres. The cytotoxicity against the MCF-7 cells was affected significantly by the released amount of tamoxifen from microspheres. As a result of these, we supposed that tamoxifen was released from PCL microspheres sustained and continuously.
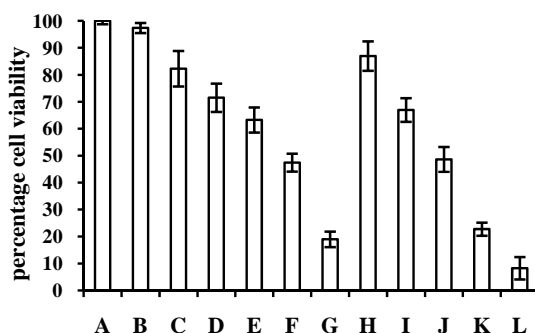

Fig. 5: Results of the MTT assay A: Air, B: blank microspheres, pure tamoxifen dose response against different concentration of drug was observed on MCF-7 cells (C-F: 5, 10, 15, 20 25µg/ml. MCF-7 human breast carcinoma cells were treated with tamoxifen loaded PCL microspheres (H-L, 25, 50, 100, 250, 500µg/ml). Data represents mean ± SD, *n*=3.
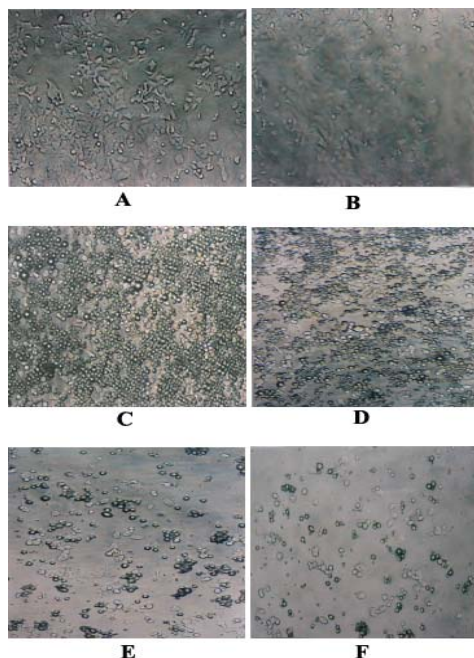

Fig. 6: Cell morphology of MCF-7 breast cancer cell lines treated tamoxifen loaded PCL microspheres during 48 h: A: tamoxifen 20µg/ml, B: tamoxifen 20µg/ml treated after 48 hr, C: blank microspheres, D: blank microspheres treated after 48h, E&F: tamoxifen 100 and 250 µg/ml treated PCL microspheres observed after 48h.

Stability studies

The lyophilized PCL based tamoxifen loaded microparticulates when subjected for stability for the period of 5 months at 30° C with 65% RH as per ICH guidelines. Figure 7 shows (A) after 15 days when sample subjected for SEM revealed that the no aggregation of the microparticulates was observed, however after 2 month slightly aggregation of the microparticles was observed, incase of the sample tested after 5 months aggregated in nature and slightly swollen was observed on the surface the microparticulates, these phenomenon may be attributed due the low glass transition temperature of the polymer and absorbed moisture during storage at higher relatively humidity and may be presence residual solvent. However these microparticulates readily redisperable when stored correctly. Again, FTIR and DSC studies demonstrated that did not indicate changes in either chemical stability of the polymer or thermal properties such as glass transition or melting temperature, indicated the absence of chemical degradation or polymer-drug interaction. Figure 8 illustrates that FTIR of PCL tamoxifen loaded microparticlulates

after storage at 30° C for five months. No physicochemical interaction was observed. Figure 9 illustrates that the DSC thermograms of PCL based tamoxfen loaded microparticulates stored 30° C for five months. PCL was not significantly affected by the temperature. The samples tested after 15 days, 2 and 5 months endothermic peak at 57.80/58.31/62.81, 57.98/60.45/61.77 and 55.98/57.47/59.80°C. The melting temperature remained relatively constant. But in case of after 5 months, DSC tested sample was slightly short and broadened endothermic peak was observed when compared to other two thermograms. This may be due to the stored for longer duration and exposure to higher relative humidity and presence of residual solvent. It would be ideal to quantify the amount of residual dichloromethane in the PCL microspheres using sealed cap head space analysis using gas chromatography-mass spectrometry (GC-MS). However, we believe this would be extremely difficult owing to small sample size, low concentration of dichloromethane and difficulty in extracting residual dichloromethane from within the microspheres.
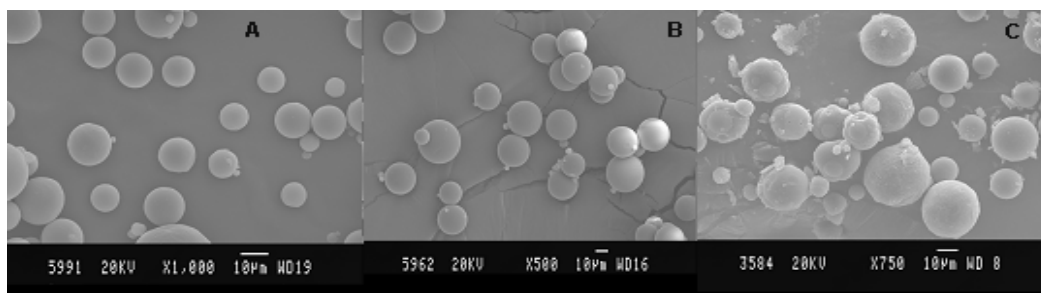

Fig. 7: SEM microphotographs of tamoxifen loaded PCL microspheres during stability at different time intervals. Intial (A) 15 days, (B) 2 months, (C) 5 months.
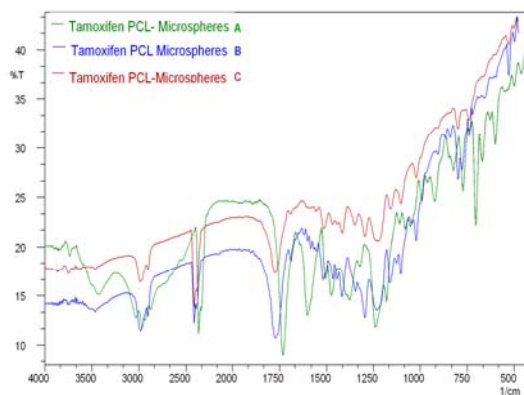

Fig. 8: FTIR Profile of tamoxifen loaded PCL microspheres during stability at different time intervals. Intial (A) 15 days, (B) 2 months, (C) 5 months.
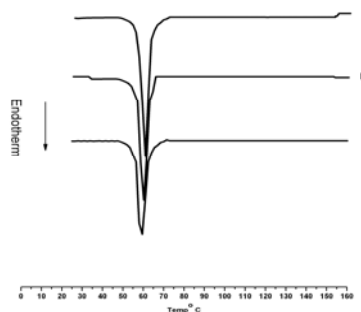

Fig. 9: DSC thermograms of tamoxifen loaded PCL microspheres during stability at different time intervals. Intial (A) 15 days, (B) 2 months, (C) 5 months. The experiment was carried with crimped aluminum pans and a heating rate of 10° C/min.

CONCLUSION

Injectable chemotherapy with tamoxifen loaded microspheres appears to be a promising injectbale (either locally or systemic) treatment modality for breast cancer. The obtained results indicated that the potential use of microspheres using poly (ε-caprolactone) for sustained release of lipophilic drug such as tamoxifen. These results strongly prove that the formulation developed in this study could be used for rodents and clinical trials. However, these data also suggest that injectable microspheres chemotherapy can be a less toxic therapy for breast cancer and an especially valuable modality for neoadjuvant (preoperative) treatment. The data from these studies are being used to identify the most efficacious doses and delivery modalities for use in further studies with larger group sizes and *in vivo* studies are future subjects.

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