

Preparation and *in vitro* characterization of poly(epsilon-caprolactone)-based tamoxifen citrate-loaded cylindrical subdermal implant for breast cancer

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In the present study cylindrical poly(epsilon-caprolactone) (PCL)-based biodegradable polymeric tamoxifen citrate-loaded subdermal implants were prepared by laboratory-based modified melt extrusion technique. The prepared implants were evaluated for their physicochemical parameters. Drug content in implants by high-performance liquid chromatographic (HPLC) method, differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscope (SEM) studies of tamoxifen citrate-loaded implants. Determination of *in vitro* hydrolytic degradation of polymeric and tamoxifen citrate-loaded implants and *in vitro* drug release was carried out by using indigenously developed dissolution apparatus. DSC and XRD studies proved that the drug is entrapped in the implant. The highest rate of hydrolytic degradation (weight loss) was observed in blank implants when compared to tamoxifen citrate-loaded implants. The studies proved that the developed method have potential in terms of industrial feasibility.

Key words: Breast cancer, cylindrical, implant subdermal, tamoxifen citrate

INTRODUCTION

The production of drug-loaded polymeric implant, wafer, microspheres and hydrogel introduced a new concept in drug administration. The development of implantable drug delivery systems is perhaps the most widely investigated application of biodegradable polymers. Owing to their transient nature, biodegradable polymers do not require surgical removal after their intended application and thus can circumvent some of the problems related to the long-term safety of nondegradable implanted devices. Drug release from a biodegradable matrix type device or implant occurs by one or a combination of three process viz., erosion of the matrix, diffusion through the matrix or combination of both diffusion and erosion mechanism.^[1] Oral administration of the non-steroidal antiestrogen like tamoxifen citrate is the treatment of choice for the patients with all stages of estrogen receptor (ER)-

positive breast cancer.^[2] Antagonizing estrogen is popular treatment strategy because ER overexpression is observed in about 70% of breast cancers, and about two-thirds of breast cancers in postmenopausal women are ER-positive.^[3] Oral tamoxifen citrate undergoes extensive hepatic metabolism and the subsequent biliary excretion of metabolites. Although the plasma antitumor concentration of 4-hydroxytamoxifen citrate are only about 2% of those of the parent compound this metabolite has been reported compound to be about 100 times more than as an estrogen antagonist than tamoxifen citrate.^[4,5] Tamoxifen citrate can have harmful long-term side effects such as the development of endometrial cancer, or an acquired tamoxifen citrate resistance leading to further tumor progression.^[6] 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

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dose-dependent. We have been exploring the development of drug-loaded and drug free, PCL-based subdermal implants and *in vitro* characterization of prepared implants.

MATERIALS AND METHODS

Poly(epsilon-caprolactone) (PCL) (Mn 90,000) was purchased from Sigma Aldrich, Bangalore, India. Tamoxifen citrate was obtained as gift sample from Cipla Pvt. Ltd., Mumbai, India. Sodium chloride, sodium dihydrogen orthophosphate and potassium dihydrogen orthophosphate were purchased from SD Fine Chemicals, Bangalore, India. All high-performance liquid chromatography (HPLC) and analytical grade solvents were purchased from Ranbaxy Chemicals, Bangalore, India.

Preparation of implants

Implants were manufactured by developed melt extrusion method [Figure 1]. Circular cylindrical shaped implants were prepared by incorporating tamoxifen citrate in the PCL. Drug was loaded in a concentration of 10 and 20% w/w in polymer. The uniformly ground and mixed tamoxifen citrate in PCL, were



Figure 1: Fabricated a unit, which consists of mold, rod and plunger (melt extrusion)

introduced into cylindrical mold having a 7.0-mm diameter and 8.0-mm length, which was placed on and attached to a rod having a diameter of 6.9mm (removable). The entire unit was heated by using digital temperature controlled heating mantle upto 60-65°C, once the polymer started plasticizing, another plunger having a diameter of 6.7mm was introduced into the mold. The plunger is adjustable and can move through a distance of 1.0-7.0mm in the mold. The whole unit was cooled to room temperature, after which the plunger and rod were removed and the solidified implant was extruded from the mold. The coded formulations are shown in Table 1.

Physicochemical evaluation of implants

The prepared implants were evaluated for their physicochemical parameters like weight, color, height and area. The implant diameter (d) and height (thickness) (h) were measured by using digital vernier calipers ((Mitutoyo, Japan) and area calculated using the formula. Results are shown in Table 1.

Estimation of drug content in implants

To determine the drug content drug-loaded implant were dissolved in 15 ml of dichloromethane (DCM). Next, 10 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 filtered through 0.22- μ m nylon membrane filter (Millipore, India), clear solution was suitably diluted with methanol:water:triethyl amine (90:10:0.1% v/v) and tamoxifen citrate 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, 50 μ l were injected into Phenomenex C-8 column, (4.6 \times 250mm, 5 μ m) and Phenomenex C-8 guard column cartridge (KJ0-4282, 4.0 \times 3.0mm, 5 μ m). Flow rate 1 ml min⁻¹. The effluent as detected UV spectrophotometrically ($\lambda = 265$ nm).^[7] In order to account for the drug, which could be lost

Table 1: Composition of biodegradable blank and drug-loaded implants and physicochemical evaluation parameters

Formulations	F1	F2	F3	F4
Polymer	PCL	PCL	PCL	PCL
Drug	TC*	TC*	-	-
% Loading/weight	10	20	90 mg	40 mg
Color	White	White	White	White
Shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Weight of implants	98.70 \pm 0.12	49.59 \pm 0.40	88.90 \pm 0.43	39.14 \pm 0.20
Area (cm ²)	1.26 \pm 0.002	0.991 \pm 0.003	1.23 \pm 0.002	0.943 \pm 0.003
Thickness (h) (mm)	0.253 \pm 0.03	0.121 \pm 0.02	0.243 \pm 0.01	0.117 \pm 0.03
% drug recovery	97.46-98.00	98.40-98.90	-	-
Zero order (R2)	0.9740	0.9732	-	-
First order (R2)	0.8360	0.8371	-	-
Higuchi (R2)	0.8190	0.9012	-	-
Korsmeyer-Peppas (R2) and P-value	0.9885 (n=1.15) <0.003	0.9845 (n=0.99) <0.002	-	-

*Tamoxifen citrate; The results reported were average of three determinations (\pm SD)

throughout the above procedure, the recovery efficiency of the procedure was determined by dissolving a known quantity of tamoxifen citrate in DCM and subjecting it to the same procedure as described above.

Fourier-transformed infrared

Infrared spectroscopy (model A-1700 Fourier-transformed infrared (FTIR), Shimadzu Instruments) was performed for pure PCL, physical mixtures of tamoxifen citrate and PCL, and tamoxifen citrate-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

Differential scanning calorimetry (DSC) was conducted using Mettler Toledo Star system (Mettler Toledo DSC 822e). Samples were weighed ($4.00\text{-}6.00 \pm 0.1$ mg) and placed in sealed aluminum pans. The coolant was liquid nitrogen. The samples were scanned at $10^\circ\text{C}/\text{min}$ from 20° to 160° C.

X-ray diffraction studies

X-ray diffraction (XRD) patterns of pure tamoxifen citrate, pure polymer, mixture and drug-loaded PCL implant were determined using a diffractometer equipped with a rotating target X-ray tube and a wide-angle goniometer. The X-ray source was $K\alpha$ radiation from a copper target with graphite monochromator (Bruker AXS D8). The X-ray tube was operated at a potential of 50 kV and a current of 150 mA. The range (2θ) of scans was from 0 to 50° and the scan speed was 2° per min at increments of 0.02° .

In vitro hydrolytic degradation of blank and tamoxifen citrate implants

Cylindrical, blank and drug-loaded implants prepared by melt extrusion method, were placed in 25 ml screw capped bottles containing pH, 7.4 phosphate buffer saline (PBS) containing 0.4% (w/v) sodium lauryl sulfate (SLS) to increase solubility of the drug at $37 \pm 2^\circ\text{C}$ for a period of 30 days using an apparatus was indigenously designed and fabricated to conduct *in vitro* release studies. Screw capped bottles, containing implants in 25 ml of pH 7.4 PBS as release medium, were fixed to stainless steel holders attached to a mechanical stirrer and the entire platform immersed in water maintained at $37 \pm 2^\circ\text{C}$. The platform was rotated at an average speed of 100 rpm to induce mixing in the release medium. At specific time intervals, the blank and drug-loaded implants were taken out of the buffer, weighed wet and dry under vacuum. The hydrolysis of the polymer was determined by decrease in weight of implant and tamoxifen citrate content in the remaining implants.^[7,8] At each time point, the formulation was examined for tamoxifen citrate content in the degraded sample by developed HPLC method.

In vitro drug release studies

In vitro release studies of tamoxifen citrate-loaded implants were carried out at $37 \pm 2^\circ\text{C}$ in pH, 7.4 PBS containing 0.4%

(w/v) SLS^[8] for a period of 30 days using specially designed and fabricated dissolution apparatus.^[7,9] SLS was used to increase the solubility of tamoxifen citrate in the buffer solution and prevent adsorption of the tamoxifen citrate on the surface of the screw-capped bottle. In briefly, the screw capped bottles containing 10 mg tamoxifen citrate-loaded implants 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 $37 \pm 2^\circ\text{C}$. 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 HPLC for tamoxifen citrate content by solvent extraction method, i.e., 5 ml of 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 section 2.4. All the release experiments were conducted in triplicate.

Surface morphology studies

The examination of surface of polymeric drug delivery systems can provide important information about the porosity, crystallinity and microstructure of the system. Immediately after manufacturing, blank implants were subjected to surface morphology studies using scanning electron microscope (SEM) (JEOL Model JSM - 6390LV). Drug-loaded implants were also subjected to SEM before and after *in vitro* drug release studies. The polymeric implants were first dried under vacuum. Samples were glued to aluminum sample holders and gold-coated under argon atmosphere. The coated samples were finally analyzed using JSM 840. The surface morphology of the implants was observed under suitable magnification.

In vitro drug-release kinetics of tamoxifen citrate

In order to determine the order of drug release, drug release profile of all the formulations evaluated were fitted into zero order, first order, Higuchi and Krosmeier-Peppas models.^[10] The results are shown in Table 1.

RESULTS AND DISCUSSION

Characterization of implants

Implants were prepared by indigenously developed melt extrusion method using specially designed stainless steel molds. The average thickness (mm) and weight (mg) of the blank implants were found to be 0.243 ± 0.01 , 0.117 ± 0.03 and 88.90 ± 0.43 , 39.14 ± 0.20 , respectively. Similarly, in case of 10 and 20% w/w tamoxifen citrate-loaded implants of thickness and total weight of implants was 0.253 ± 0.03 , 0.121 ± 0.02 and 98.70 ± 0.12 , 49.59 ± 0.40 , respectively. The total area of the blank implants was 1.23 ± 0.002 to 0.943 ± 0.003 cm^2 . The drug-loaded implants area was found

to be 1.26 ± 0.002 and 0.991 ± 0.003 . The obtained results showed that percentage recovery of tamoxifen citrate from 10 and 20% w/w implants was above 98%. The obtained results are shown in Table 1. Macroscopically all the implants were found to be cylindrical in shape, smooth in surface, similar in appearance and similar in color. The PCL-based tamoxifen citrate implants were white in color due to drug and polymer being white in color. The implant diameters showed very little difference even though the mold of the unit had the same diameter. The slight difference is attributed to solidification from the melt and contraction properties of polymer. Area, weight and thickness of the implants depended on the different concentration of polymer loading. All average weights of the implants reflected the amount of polymer actually loaded. The recovery efficiency of pure tamoxifen citrate as compared to drug recovery from a mixture of drug and polymer was found have a deviation of less than $\pm 5\%$.

FTIR

Figure 2 shows the typical spectra's of pure tamoxifen citrate, PCL, a physical mixture of tamoxifen citrate and PCL and drug-loaded implants. The spectrum of tamoxifen citrate shows characteristic absorption bands at 3027 cm^{-1} (=C-H stretching), 1507 and 1477 cm^{-1} (C=C ring stretching) and 3180 cm^{-1} (-NH₂).^[7] 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} (CH₂) symmetric stretching 2865 cm^{-1} (CH₂). 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.

DSC and XRD

Samples were subjected for DSC studies. A sharp and large melting onset/peak/endset peak of pure tamoxifen citrate, pure PCL and physical mixture of drug and polymer at $146.22/148.6/151$, $54.60/61.44/62.56$ and $55.70/64.31/65.60/145/96/148.26/150.61^\circ\text{C}$ [Figure 3]. Figure 4 shows the pure tamoxifen citrate and polymers were characterized by prominent diffraction peaks in the range of $10\text{-}50^\circ$ (2θ) during XRD studies. The characteristic peaks of tamoxifen citrate were observed at, 6.52 , 9.84 , 18.18° (2θ), respectively. In case of pure PCL characteristic peaks were observed at, 19.73 , 22.12 and 24.97° (2θ). Physical mixture of drug and polymer when subjected for XRD, same prominent characteristic peaks of drug and polymer were observed in mixture at 6.43 , 9.72 , 18.21 and 19.55 , 21.98 and 24.78° (2θ). The drug peaks did not appear in the formulation while only PCL characteristic peaks were appeared at 19.01 , 21.27 and 23.48° (2θ), respectively. The generated DSC thermograms, their respective endothermic values and XRD data indicated that the drug and the method of preparation of implant had little effect on the thermal properties of the polymer. However, drug peak did not appear which probably may be due to conversion of tamoxifen citrate from crystalline state to amorphous or dissolution during the heating involved in

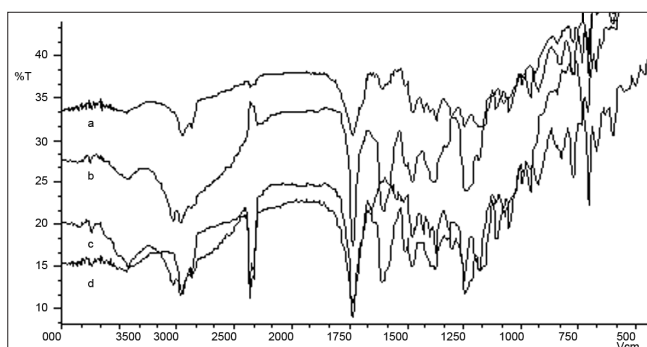


Figure 2: Transmission FTIR spectra of (a) tamoxifen-loaded implant, (b) physical mixture of drug+PCL, (c) pure PCL, (d) pure tamoxifen citrate

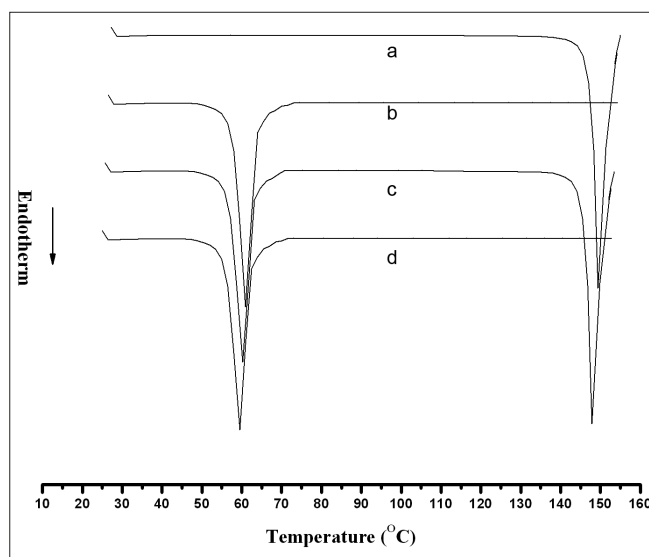


Figure 3: DSC thermograms of pure tamoxifen (a), pure PCL (b), physical mixture of drug+PCL (c) and (d) drug-loaded implant. The experiment was carried with crimped aluminum pans and a heating rate of $10^\circ\text{C}/\text{min}$

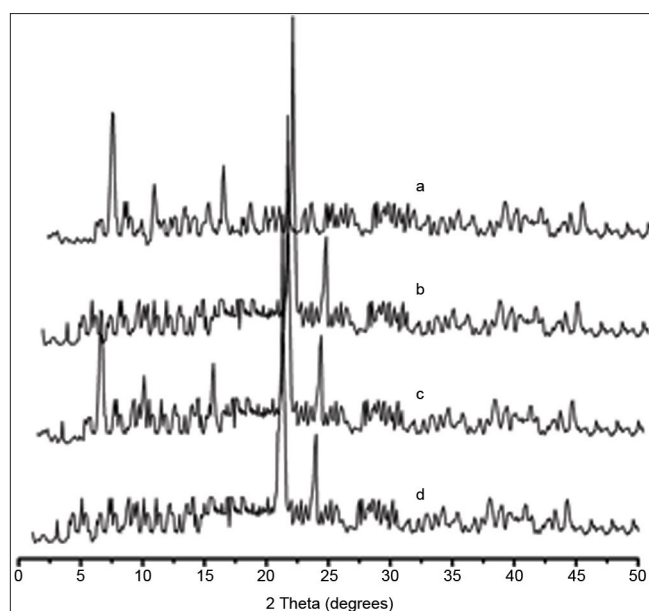


Figure 4: X-ray diffraction studies of pure drug (a), pure PCL (b), physical mixture of drug+PCL (c) and (d) drug-loaded implant

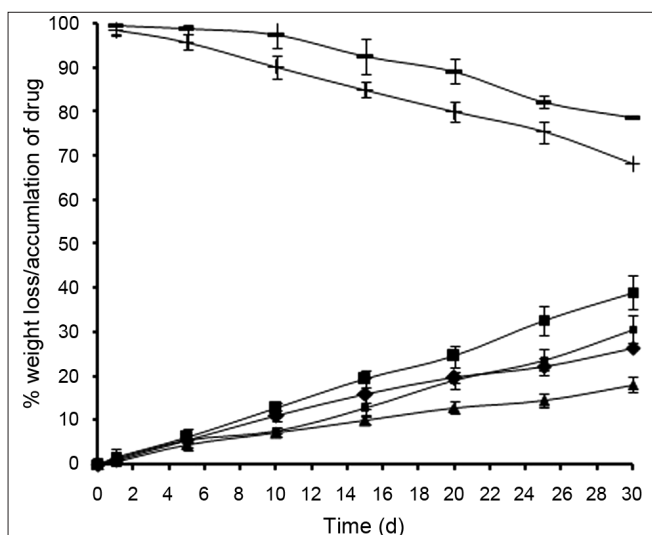


Figure 5: *In vitro* hydrolytic degradation of blank and drug-loaded implants and accumulation of tamoxifen citrate in the implants, F1 (▲), F2 (•) F3 (■), F4 (■) and F1 (-), F2 (+), respectively

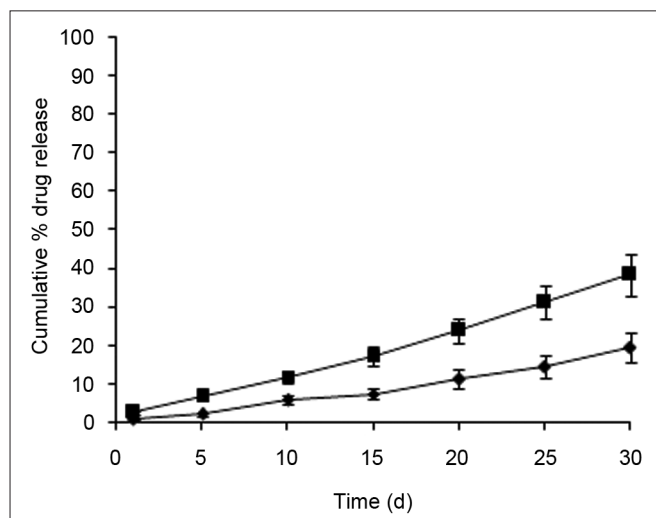


Figure 6: *In vitro* drug release profile of tamoxifen citrate-loaded implants, F1 (•) and F2 (■)

the preparation of implant and another phenomenon can be predicated is elevated temperature and a slow rate of cooling enable the chains to be mobile and to realign themselves may be resulting in a semicrystalline or amorphous nature or drug may be present in the polymeric amorphous surface.^[11]

***In vitro* hydrolytic degradation and drug release studies**

The highest rate of degradation (weight loss) was observed in blank implants when compared to tamoxifen citrate-loaded implants [Figure 5]. The degradation rate of 10% drug-loaded F1 implant was slower when compared to 20% w/w drug-loaded F2, implant. The tamoxifen citrate accumulation in 10% w/w drug-loaded implant was more when compared to 20% w/w drug-loaded implants. Moreover, the *in vitro* drug release aspects also showed the same phenomenon from 10% w/w tamoxifen citrate-loaded implant samples was very

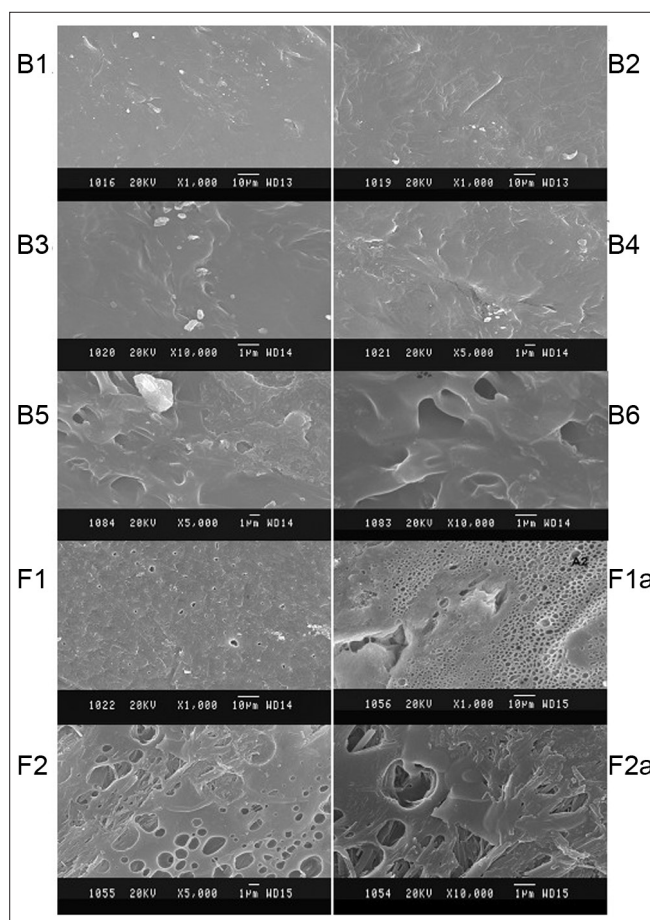


Figure 7: Scanning electron micrographs of the melt extruded drug-loaded PCL samples, freshly prepared blank implants of PCL (B1) and (B2). Blank implants after 5 days *in vitro* studies (B3) and (B4) and after 30 days (B5) and (B6). Similarly, 10% w/w drug-loaded implant after 5 days *in vitro* drug release studies (F1) and after 30 days (F1a). 20% w/w drug-loaded implant after 5 days *in vitro* studies (F2) and after 30 days (F2a), respectively

slow when compared to 20% w/w tamoxifen citrate-loaded implant samples, The percentage cumulative drug release from F1 and F2 after 30 days was found to be 19.577 ± 1.45 and 38.485 ± 2.24 , respectively [Figure 6]. The degradation of implants, drug accumulation and *in vitro* drug release, depends upon the polymer concentration, geometry of implant, density of the matrix, cosolvent, formation of microchannel/pores/water filled channels in the implant and the dissolution media, which causes hydrolysis of polymer.^[7,11-14]

SEM

The surface morphology of blank and drug-loaded implants subjected immediately after manufacturing prepared by developed melt extrusion (B1 and B2 blank implants) drug-loaded implants (not shown) using PCL found to uniform, homogenous and smooth in surface. The blank (B3 and B4) and drug-loaded implants F1 and F2 SEM studies revealed porous homogenous surface at the end of 5th day as compared to implants subjected to *in vitro* release studies at the end of 30 days [Figure 7]. The 30th day implants (B5 and

B6), drug-loaded implants F1a and F2a showed numerous random porous/cracks/crevices was observed. Thus the drug release from PCL implants occurred by diffusion through the various drug-diffusing channels developed on the implant surface [Figure 7]. Comparison of hydrolytic degradation of blank and drug-loaded implants indicated less hydrolytic degradation in blank implants which may be attributed to the crystalline network, low uptake of water into the system, high density and porosity of blank implants as compared to drug-loaded implants. Implants subjected to SEM studies before and after drug release studies clearly indicated that hydration of implants/formation of drug diffusing channels/cracks/crevices on the surface of implants Depending upon the composition, dimension and sometimes even the method of preparation different physical and chemical phenomena may be involved in the rate of drug release kinetics from polymer, which includes: geometry of the of the system, porosity and density of the matrix, water penetration into the system, dissolution/degradation of the matrix, precipitation and re-dissolution of the degradation products, structural changes within the system occurring during release, such as creation/closure of water-filled pores and diffusion of drug through the polymer/osmotic effects, respectively^[7,11-14]

Drug release kinetics

In case of all the formulations developed the percentage cumulative drug release versus time plots showed small burst phase followed by slow and constant drug release mimicking zero-order release. Initial fast release or small burst effect phase is considered to result rapid diffusion/dissolution of drug particles at the solid liquid interface. The magnitude of burst effect was dependent on the proportion of tamoxifen citrate on the outer surface of the implant. Zero order and Korsmeyer Peppas model gave a good fit for the drug release profiles of all implants with greater regression coefficients in comparison to other models. The fitting of these data to the Korsmeyer-Peppas model demonstrated that drug release occurs mainly through diffusion and erosion process. Since the value of n was in between 0.64-1.15, it indicates anomalous transport and super case II transport mechanism^[10] [Table 1].

CONCLUSIONS

The present study clearly indicates tamoxifen citrate-loaded biodegradable PCL polymeric implants have a tremendous potential as novel local/systemic drug delivery system for the treatment of cancer. *In vitro* studies demonstrated that tamoxifen citrate loaded with PCL implants were capable of providing a constant zero-order drug release. Incorporation

of high drug loading, evaluations and optimizing the *in vitro*–*in vivo* correlations are may be future subjects.

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