

Characterization of cylindrical and strip-shaped tamoxifen citrate-loaded biodegradable implants

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The aim of this study was to prepare tamoxifen citrate (TC)-loaded cylindrical and strip-shaped polymeric subdermal implants. The implant was based on poly(ϵ -caprolactone), a low-melting, biodegradable and biocompatible polymer. Polyethylene glycol (PEG 4000) was used to enhance solubility and release of the drug in the phosphate buffer saline pH 7.4. Implants were prepared by a standardized melt manufacturing method. The prepared implants were evaluated for their physicochemical parameters and drug content in implants by UV spectrophotometric method. PCL-based implants were characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry, X-ray diffraction studies (XRD) and scanning electron microscopy (SEM). DSC studies showed that the TC in the implants was in the amorphous state. *In vitro* drug release studies were performed in methanol:phosphate-buffered saline (pH 7.4) at $37 \pm 2^\circ\text{C}$ by using horizontal water bath shaker. Stability study was carried out for 90 days, there was no significant change in drug content and other parameters of the PCL-based formulations.

Key words: Biodegradable, implants, tamoxifen citrate

INTRODUCTION

Biodegradable systems have gained much popularity when compared to nonbiodegradable implants. Biodegradable polymers have many applications in different areas. Especially, for targeting, through subdermal, intratumoral, intracranial, drug delivery systems, free from the toxicological problems and biodegradable in biological fluids to produce biocompatible or nontoxic products, respectively. In general, biodegradable polymers degradation means hydrolysis, esterification or enzymatic process occurs by either erosion, surface erosion and bulk erosion or combination of either one of the above which includes homogenous or heterogeneous.^[1-3] PCL is biodegradable polyester that is widely used in drug delivery applications. It is highly hydrophobic crystalline polymer that degrades very slowly *in vitro* in the absence of enzymes. PCL degrades *in vivo* by hydrolytic and enzymatic action. Polyethylene glycols extensively used to increase the solubility, dissolution rate and bioavailability of poorly water-soluble

drugs.^[4,5] Tamoxifen citrate is low aqueous solubility and is used clinically as a nonsteroidal antiestrogen for firstline endocrine treatment as well as adjuvant therapy in early and metastatic breast cancers in postmenopausal women. Tamoxifen is also approved as a prophylactic agent for women at high risk of developing breast cancer.^[6-8] Tamoxifen is a low-dose therapeutic agent typically given to patients for long periods of time. Hence, this is an ideal drug for the preparation of biodegradable polymeric implants. The present objectives are to develop and preparation of biodegradable tamoxifen citrate-loaded implants in two different shapes by melt technology and their characterization.

MATERIALS AND METHODS

Materials

TC was obtained as gift sample from Khandelwal Laboratories Pvt Ltd., Mumbai, India. Poly(ϵ -caprolactone) (Mn 90,000) was purchased from Sigma Aldrich, Bangalore, India. PEG-4000, (Mw, 4,000) sodium

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dihydrogen orthophosphate, sodium chloride, potassium dihydrogen orthophosphate were purchased from SD Fine Chemicals, Mumbai, India. All other reagents and solvents were of an analytical grade.

Methods

Preparation of the TC-loaded biodegradable polymeric implants

Implants were prepared by melt method. Polymer is melted below its glass transition temperature ($\sim 60^\circ\text{C}$) on hot plate using porcelain tile over it. As the polymer starts melting at its glassy state, previously triturated drug and PEG-4000 were mixed homogeneously into molten polymer. Next temperature was reduced slowly to 42°C , during this time uniform cylindrical and strip-shaped implants were prepared by using two flattened stainless steel rods. Prepared implants were evaluated for their physicochemical characterization. The photographs and composition of the formulations are shown in Figures 1 and 2 and Table 1, respectively.

Physicochemical evaluation of implants

The prepared implants are evaluated for their physicochemical parameters like weight, color, height and surface area. The implant diameter (d), height (h), length (l) and base (b) were measured by using digital vernier calipers and surface area was calculated using the formulae $2\pi r(r+h)$ and $2(hb+hl+bl)$.

Drug content estimation

The drug content in polymeric implants was determined as follows. The implant was dissolved in 20 ml of chloroform by sonicating continuously till clear solution was obtained. To the above solution methanol and PBS pH 7.4 (2:8 ratio) mixture is added, (calibration curve was prepared in the same media). Chloroform was evaporated completely, filtered through a $0.22\text{-}\mu$ nylon membrane (Millipore, Bangalore, India). The obtained solution was suitably diluted and drug content was estimated at 274.0 nm using double beam UV-Visible spectrophotometer against methanol and PBS 7.4 mixture as a blank.

Fourier transform infrared analysis

The Fourier transform infrared analysis (FT-IR) spectral measurements of pure TC, polymer, carrier, physical mixtures (1:1:1 ratio) were taken at ambient temperature using a FT-IR spectrophotometer (Perkin Elmer, Japan, IISc, Bangalore). About 5 mg of samples were mixed with KBr and vacuum-packed to obtain pellets of the material. The implant samples were dissolved in dichloromethane and it is allowed to evaporate to form a film. The obtained film is cast onto

NaCl plates from solution in dichloromethane, which were analyzed. All the spectra acquired scans between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} .

Thermal analysis

The thermal analysis (DSC) thermograms of pure TC, PCL, PEG-4000, physical mixture (1:1:1 ratio) and implant formulations (T1, T3, T5 and T6) were carried out using Mettler Toledo star system, Metallurgy Department, Indian Institute of Science Bangalore, India. Samples were weighed (3.00-5.00 mg) and placed in sealed aluminum pans. The coolant was liquid nitrogen. The samples were scanned at $10^\circ\text{C}/\text{min}$ from 0 to 170°C .

X-ray diffraction analysis

X-ray diffraction analysis (XRD) patterns of the pure TC, polymer, carrier, physical mixtures (1:1:1 ratio) and drug-loaded PCL implants (by using sample holder grid technique) were determined using a diffractometer equipped with a rotating target X-ray tube and wide angle goniometer in Department of Physics, Indian Institute of Sciences (IISc), Bangalore, India. The range (2θ) of scans



Figure 1: TC-loaded cylindrical implant formulations



Figure 2: TC-loaded strip shape implant formulations

Table 1: Composition of the formulations

| Ingredients | T1 (20% w/w) (mg) | T2 (30% w/w) (mg) | T3 (20% w/w) (mg) | T4 (30% w/w) (mg) | T5 (33.3% w/w) (mg) | T6 (25% w/w) (mg) | T7 (33.3% w/w) (mg) | T8 (25% w/w) (mg) |
|-------------|-------------------|-------------------|-------------------|-------------------|---------------------|-------------------|---------------------|-------------------|
| TC | 40 | 60 | 40 | 60 | 100 | 100 | 100 | 100 |
| PEG-4000 | 40 | 40 | - | - | 30 | 60 | - | - |
| PCL | 120 | 100 | 160 | 140 | 170 | 240 | 200 | 300 |

was from 0 to 60° and the scan speed was 2° per minute at increments of 0.02°.

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 and after *in vitro* release studies implants were subjected to surface morphology studies using surface morphology studies (SEM). 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 848, Joel, Japan (Materials department, Indian Institute of Science (IISc) Bangalore, India). The surface morphology of the implants was observed under suitable magnification.

In vitro drug release studies

The *in vitro* release studies of TC implants were carried out in a horizontal water bath shaker at 37±2°C in methanol and pH 7.4 PBS (2:8 ratio) to enhance the complete release of drug, for a period of 120 days for cylindrical-shaped implants and 90 days for strip-shaped implants. The 60 ml screw-capped bottles, containing TC implants in 50 ml of PBS pH 7.4 as release medium, were fixed to stainless steel holders attached to platform that was dipped in water maintained at 37±2°C.^[9] The platform was moved horizontally at an average speed of 100 rpm to induce mixing in the release medium. At periodic intervals of 48 hrs, 50 ml of the release medium was sampled and replace with complete fresh 50 ml release medium to provide the necessary sink condition. The withdrawn samples were suitably diluted and analyzed by UV Spectrophotometer at 274 nm. The filtered solution was used for TC analysis. The cumulative percentage drug release was calculated to establish the drug release profile of the implants.

Stability studies

The stability study was carried out for a period of 3 months. The samples were stored at temperature 5±3°C and 30±2°C/65±5% (RH) in a stability chamber. At frequent interval of time on initial, after 15, 30, 60 and 90 days, samples were withdrawn and analyzed for diameter, surface area and drug content.^[10]

RESULTS AND DISCUSSION

Physicochemical evaluation of implants

TC-loaded PCL implants were prepared by melt method. PEG-4000 was added to improve the release characteristics of the drug from polymer matrices. Temperature was kept ~55°C to avoid degradation of drug. Macroscopically, all the developed implants were cylindrical shape and strip shape with smooth surfaces and similar in appearance. All the implants were white in color due to drug and polymer. The implants thickness (diameter) and surface area were measured by using digital vernier calipers. The results of physicochemical characterizations are illustrated in Table 2.

Surface area = $2\pi r(h+r)$ for cylindrical and $2(hb+hl+bl)$ for strip.

where, r= radius, l= length, h= height, b= base.

CL^{**} - cylindrical

F^{***} - Formulations

FTIR analysis

From the FT-IR data, it was observed that pure TC shows bands at 3027 cm⁻¹(C-H sp³), 1731, 1591 cm⁻¹ (C=C Alkanes), 1377 cm⁻¹ (-CH₃ bending), 1237, 1216 cm⁻¹ (C-O ether and C-N amine stretch), 1605 cm⁻¹ (N-H Stretch) and 1474 cm⁻¹ (C=C aromatic ring). Bands of PCL were observed at 2948 cm⁻¹ (C-H sp³) and 1729 cm⁻¹ (C=O stretch). TC-loaded implants (T1, T3, T5 and T6) shows bands at 2948 cm⁻¹ and 1729 cm⁻¹ for C-H sp³ and C=O stretch, respectively, TC peaks were disappeared in formulations due to drug may be completely entrapped in the polymer. In drug polymer interaction studies IR spectral analytical charts revealed that there is no drug polymer interaction in physical mixture and formulations. The results are shown in Figure 3.

Thermal analysis

The DSC thermograms of pure TC, PCL, PEG-4000, physical mixture and implant formulations (T1, T3, T5 and T6) are observed at 147, 61.33, 61.33, 147 and 61.33°C, respectively. The samples were scanned at 10°C/min from 0 to 170°C. The coolant was liquid nitrogen. 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

Table 2: Physicochemical properties of tamoxifen citrate-loaded implants

| F ^{***} | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 |
|--|------------------|------------------|------------------|------------------|-------|-------|-------|-------|
| Percentage TC loaded (w/w) | 20 | 30 | 20 | 30 | 33.3 | 25 | 33.3 | 25 |
| Weight of implants (mg) | 181.8 | 174.6 | 199.4 | 196.1 | 293.5 | 398.8 | 296.4 | 389.5 |
| PEG-4000 | 40 | 40 | - | - | 30 | 60 | - | - |
| Color | White | White | White | White | White | White | White | White |
| Shape | CL ^{**} | CL ^{**} | CL ^{**} | CL ^{**} | Strip | Strip | Strip | Strip |
| Surface area (cm ²) ^a | 1.723 | 1.726 | 1.628 | 1.757 | 4.542 | 4.828 | 4.226 | 4.610 |
| Thickness (D) (cm) | 0.448 | 0.424 | 0.428 | 0.453 | 0.332 | 0.395 | 0.313 | 0.373 |

Surface area = $2\pi r(h+r)$ for cylindrical and $2(hb+hl+bl)$ for strip. where, r= radius, l= length, h= height, b= base. CL^{**}-cylindrical F^{***}-Formulations

peak did not appear which probably may be due to conversion of TC from crystalline state to amorphous or dissolution during the heating involved in the preparation of implant or may be another phenomenon is drug may be present in polymeric amorphous phase. When DSC Thermograms of formulation were compared with the pure TC, no peak appeared which revealed that drug is completely entrapped or in polymeric amorphous phase.^[11] Thermograms are shown in Figure 4.

XRD studies

Figure 5 illustrates the XRD spectra of pure TC, PCL, PEG-400, physical mixture and implant formulations. Sharp peaks were obtained at 9.256, 11.418, 13.571, 15.968, 17.495, 21.071° (2θ) of pure TC. PCL showed peaks at 36.478, 40.301° (2θ). A peak of PEG-4000 was obtained at 24.614, 30.576° (2θ). Physical mixture peaks were found at 9.256, 11.418, 13.571, 17.495, 21.071, 24.614, 27.556° (2θ). In case of TC-loaded implants peaks were obtained at 36.478, 40.301° (2θ). Absent of drug peak in developed implants further justified the presence of drug in the form of amorphous or dissolution state or polymeric amorphous phase.

Surface morphology (SEM)

The surface morphology of blank implant immediately after manufacturing was found to be uniform, homogenous and smooth in surface as shown in Figure 6. Tb Cylindrical drug loaded implants after 120 days *in-vitro* studies of formulation T1, T3. SEM studies revealed that more random fractures like cracks and crevices (clusters and network fibers) on the surface of the sample were observed. In case of formulation T5, T6 (strip shaped) after 90 day studies revealed that various drug-diffusing channels developed on the implant surface.^[3,9,12] The photomicrographs are shown in Figure 6.

In vitro drug release studies

The *in vitro* drug release profile of implant formulations are shown in Figures 7 and 8. Initial fast release or small burst effect phase is considered to result rapid diffusion/dissolution of drug particles at the solid liquid interface. The cumulative percentage drug release of cylindrical-shaped implant formulations (T1, T2, T3 and T4) after 120 days were found to be 66.58±0.75, 75.40±0.58, 53.77±0.43, 56.57±1.39% and in case of strip-shaped implant formulations (T5, T6, T7 and T8) after 30 day were found to be 65.27±0.16, 75.74±0.71, 58.96±0.74, 62.14±0.22%, respectively. The drug released from PCL implants was primarily by diffusion through the various drug-diffusing channels developed on the implant surface as observed in SEM. We have concluded that among all the eight formulations, drug release profiles of TC-loaded PCL implants were related with the shape of the implants and drug loading. Strip-shaped implant (T5, T6, T7 and T8) showed more drug release compared to cylindrical-shaped implants (T1, T2, T3 and T4). Formulations containing PEG-4000 (T1, T2, T5 and T6) also showed highest drug release compared to formulations that did not contain PEG-4000 (T3, T4, T7 and T8), may be due to high wetting system's surface with

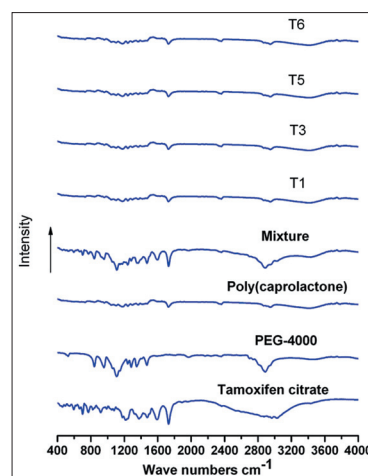


Figure 3: Transmission FTIR spectra of pure TC, PEG-4000, physical mixture, formulation T1, T3, T5 and T6

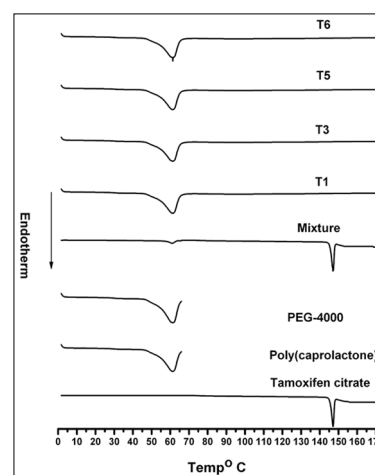


Figure 4: DSC thermograms of pure TC, PCL, PEG-4000, physical mixture, formulation T1, T3, T5 and T6

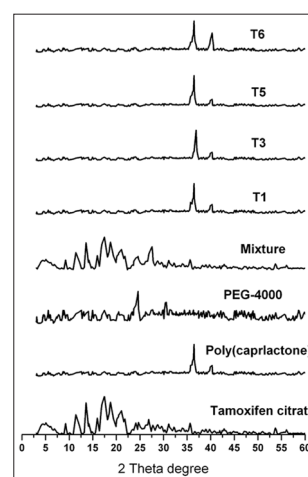


Figure 5: XRD patterns of pure TC, PCL, PEG-4000, physical mixture, formulation T1, T3, T5 and T6

media (dissolution media), formation of continuous polymeric network, creation of more media-filled pores, creation of more cracks and within the release rate-limiting membranes

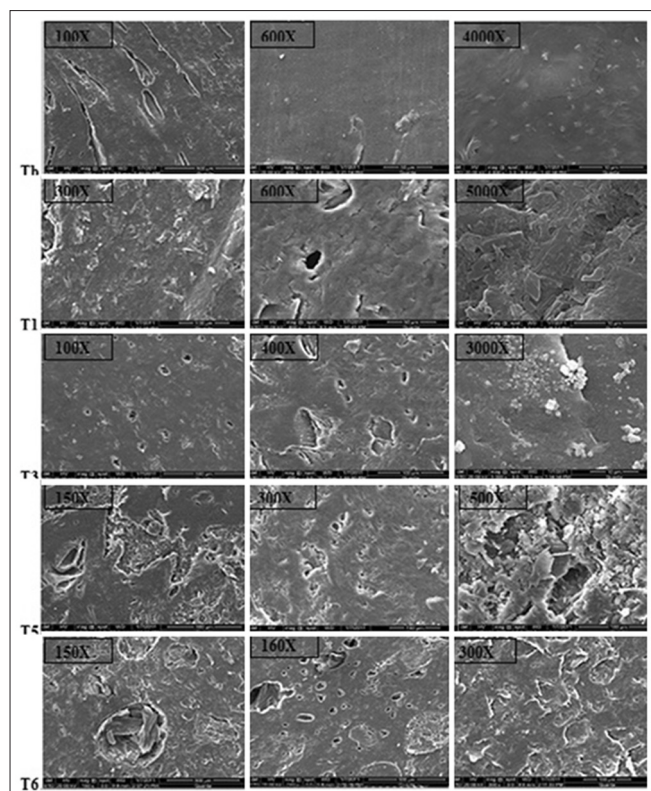


Figure 6: SEM microphotographs, Tb drug-loaded implants immediately after manufacturing (100-4000X), T1 and T3 cylindrical implants after 120 days of *in vitro* release (100-5000x), T5 and T6 strip after 90 days of *in vitro* release (150-500X), in increasing magnifications

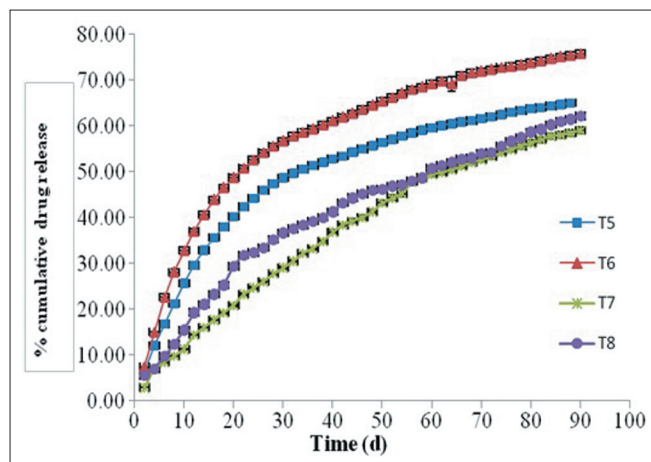


Figure 8: Drug release (TC) from PCL-based strip implants, with 25% w/w drug loading (T6, T8) and 33.3%w/w drug loading (T5, T7)

(thickness and permeability of drug) and also geometry of the devices.^[3,9,12] Release rate kinetics data of implant formulations given in Table 3 illustrates Zero order and Korsmeier-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 Korsmeier-Peppas model demonstrated that drug release occurs mainly through diffusion and erosion process. Since the value of n was in between 0.49-0.81, it indicates anomalous transport.^[3,13]

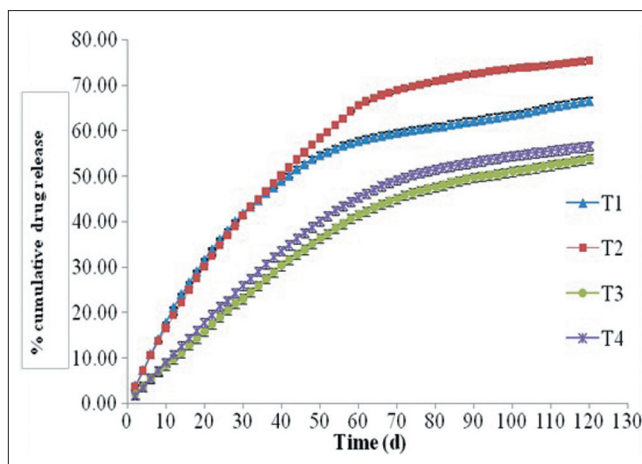


Figure 7: Drug release (TC) from PCL-based cylindrical implants, with 20% w/w drug loading (T1, T3) and 30%w/w drug loading (T2, T4)

Table 3: Release rate kinetics data of implant formulations

| Formulation Code | Regression (R ²) | | | |
|------------------|------------------------------|-------------|------------------|--------------------|
| | Zero order | First order | Higuchi equation | Korsmeier-peppas |
| T1 | 0.803 | 0.890 | 0.925 | 0.926 (n=0.604) |
| T2 | 0.853 | 0.935 | 0.952 | 0.957 (n=0.684) |
| T3 | 0.920 | 0.958 | 0.980 | 0.981 (n=0.792) |
| T4 | 0.899 | 0.943 | 0.972 | 0.970 (n=0.809) |
| T5 | 0.821 | 0.911 | 0.936 | 0.926 (n=0.527) |
| T6 | 0.817 | 0.937 | 0.933 | 0.911 (n=0.494) |
| T7 | 0.968 | 0.993 | 0.994 | 0.992 (n=0.760) |
| T8 | 0.936 | 0.981 | 0.991 | 0.979 (n=0.647) |

Stability studies

Stability studies of the formulations containing TC were carried out to determine the effect of formulation additives on the stability of the drug. Stability studies were performed for cylindrical-shaped and strip-shaped formulations for 90 days. Results showed that there was a slight change in the diameter, surface area due to the softening of the polymer on the surface of the implant and negligible change in the drug content ($\pm 2\%$) was observed.^[10] Thus, it can be concluded that the excipients and method of preparation does not alter the chemical stability of drug entrapped in the PCL implants.

CONCLUSION

We have successfully prepared cylindrical and strip shaped implants with a uniform size and surface. To check the drug excipient compatibility, the FTIR, DSC and XRD studies were carried out and proved that there was no interaction

between drug and excipients in physical mixture as well as in formulations. PCL implants with different drug loadings, exhibited sustained drug release for several weeks, have also been characterized *in vitro*. The stability studies were carried out for period of 3 months as per ICH guidelines. There was no significant change in drug content and other parameters of the PCL-based formulations. Formulation T2 and T6 were found to be most promising formulation because approximately 75% of the TC was released from the implant.

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