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# Injectable in situ forming drug delivery system based on poly(ε-caprolactone fumarate) for tamoxifen citrate delivery: Gelation characteristics, in vitro drug release and anti-cancer evaluation

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**Abstract**

The present study deals with the preparation and characterization of an injectable and in situ forming drug delivery system based on photocrosslinked poly(ε-caprolactone fumarate) (PCLF) networks loaded with tamoxifen citrate (TC). Networks were made of PCLF macromers, a photoinitiation system (comprising initiator and accelerator) and the active ingredient N-vinyl-2-pyrrolidone (NVP) as a crosslinker and reactive diluent. Shrinkage behavior, equilibrium swelling and sol fraction ratios of photocrosslinked PCLF gels were determined as functions of NVP content. It was shown that the crosslinking is facilitated up to a certain concentration of NVP and most of NVP remained unreacted above this value. In vitro drug release, biocompatibility evaluation and activity against MCF-7 breast cancer cell line were also investigated. Accurate but simple bipartite expressions were also derived that enable rapid determination of effective diffusion coefficients of TC in photocrosslinked PCLF/NVP disks. Cytotoxicity assay showed that while the photocrosslinked PCLF network with optimum NVP content exhibits no significant cytotoxicity against MCF-7 and L929 cell lines, 40–60% of the MCF-7 cells were killed after incubation with TC-loaded devices.


*Keywords:* Biocompatibility; Tamoxifen citrate; Photocrosslinking; Poly(caprolactone fumarate); Characterization

## 1. Introduction

There is an increasing interest in injectable, biocompatible and biodegradable polymers for drug delivery and tissue engineering applications. These polymers can be employed as injectable drug delivery systems, especially in cancer chemotherapy [1–8]. Application of injectable devices in local controlled delivery of chemotherapeutics is advantageous over traditional dosage forms, non-biodegradable carriers (e.g. silicone based options) or prefabricated biodegradable ones in many aspects such as ease of administration via a customary syringe, possibility of localized delivery of a precisely predetermined dose, prolonged dosage regimen, and minimal systemic side effects, hence increasing therapeutic efficacy. Various strategies have been reported in preparation of these injectable drug delivery systems including using thermoplastic pastes, in situ precipitation of polymeric solutions, in situ polymerization and ionic gelation [9]. To this end, in situ photopolymeriza-

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tion technique has been widely used in preparation of three-dimensional polymeric networks and seems to be an interesting option to develop novel devices with applications as biomaterials or localized drug delivery due to their ease of fabrication using routine visible light sources found in a dental surgery or operation rooms. This way is also capable of providing rapid conversion of photoreactive macromers into gel-like or completely solid articles under physiological conditions in situ with minimal invasion [10–20].

To develop a liquid injectable polymeric system capable of forming a crosslinked solid implant within the body, the biodegradable polymer should be modified with unsaturated moieties which will be polymerized in situ. Unsaturated polyesters exhibit the properties of good fluidity in dissolved or melted state, ability to solidify at room temperature and good mechanical properties after solidification in some cases such as poly(ε-caprolactone fumarate) (PCLF) [21]. Some fumaric-acid-containing polyesters have been synthesized as injectable and biodegradable materials including poly(propylene fumarate) (PPF), oligo(poly(ethylene glycol fumarate)), poly(propylene glycol-co-fumaric acid) and used as engineered bone and cartilage scaffolds but not for drug delivery to our best knowledge [22–27]. Recently, a new injectable, self-crosslinkable and biocompatible material, PCLF, has been developed by condensation of PCL diol and fumaryl chloride (FuCl) in the presence of triethylamine (TEA) as a catalyst [28]. PCLF, with its characteristic properties of good fluidity and biocompatibility, could be potentially used as an injectable biomaterial using thermal or redox initiator systems; however, PCLF synthesized using TEA has some shortcoming which may limit its application [29]. First of all it could not be photopolymerized efficiently upon its dark brown color that absorbs the light needed for the activation of photoinitiator [29,30]. Secondly, the gel content of the crosslinked PCLF is not high, which causes shrinkage and defects after the extraction of sol part, or renders it unsuitable for controlled release applications. And finally, PCLF network does not exhibit good mechanical properties [29]. To overcome the colorization problem, Wang et al. replaced TEA with potassium carbonate to synthesize a colorless or light-colored PCLF [30]. Sharifi et al. also reported yellowish colored PCLF with medium degree of oligomerization using the same method [31]. Later, to obtain a colorless PCLF macromer with high degree of oligomerization and consequently high degree of unsaturation, Sharifi et al. used propylene oxide (PO) as a new catalyst and proton scavenger. The PCLF synthesized via this method possessed higher degrees of unsaturation and white color which was efficiently photocrosslinked [32].

In order to facilitate crosslinking reaction and increasing the degree of conversion and consequently the gel content of the PCLF network, sharifi et al. used N-vinyl pyrrolidone as a reactive diluent [31,32]. Adding about 20% NVP caused a dramatic increase in its degree of conversion (63.33%); however, increasing NVP up to 50% resulted in a decrease in DC% because of lower reactivity of NVP and leaving more unreacted NVP monomer. NVP's homopolymers and copolymers have excellent biocompatibility and the use of NVP as a reactive diluent has been reported in other similar unsaturated polyesters like PPF [33,34].

In order to modulate the physical properties of PCLF such as mechanical and rheological properties, Wang et al. recently reported the synthesis of photo-crosslinkable hybrid network based on PPF and PCLF. Blending of PPF with PCLF not only improved the mechanical properties but also increased its gel fraction content [29].

The objective of this study was to develop an in situ photocrosslinkable drug delivery system using PCLF macromer, a photoinitiator/accelerator system (camphorquinone/dimethyl-*p*-toluidine) and N-vinyl pyrrolidone (i.e. reactive diluent) whereby a gel could be potentially formed in the living tissues after a few minutes of visible light irradiation. By loading of the anticancer drug in uncured composition and subsequent photocuring the device will release the bioactive component sustainedly in the close proximity of body tissues in the tumor site where delivery will be terminated upon device depletion/degradation. Tamoxifen citrate (TC) was employed in this research because of its position in the first line of antiestrogen drugs used in treatment of patients in all stages of estrogen-receptor positive breast cancer [35]. The long term (up to five years) need for oral administration of TC and its undesirable side effects encourage the trials to develop sustained release implantable/injectable devices, also possibly to increase its concentration at tumor site. Five years of adjuvant TC therapy is the recommended treatment regimen which results in a reduction in the relative breast cancer recurrence risk of 46% and the relative risk of death of 26% [36]. In this paper the focus will firstly be on fundamental aspects of gelation or crosslinking characteristics using bonded disk technique [37,38]; elemental analysis and swelling study then evaluation of in vitro release behavior of TC from in situ forming devices and modeling of the drug release profile; and finally in vitro antitumor efficacy against human breast cancer cell line (MCF-7).

## 2. Materials and methods

Poly(ε-caprolactone diol) (PCL diol) of nominal molecular weight of 530 g $mol^{-1}$, fumaryl chloride (FuCl), propylene oxide (PO), N-vinyl pyrrolidone (NVP), camphorquinone (CQ) and calcium hydride were purchased from Aldrich Chemical Co. (Milwaukee, MN, USA). FuCl was purified by distillation at 161 °C and 760 mm Hg. NVP was distilled at ambient temperature under reduced pressure of 30 mm Hg. Dimethyl-*p*-toluidine (DMPT) and solvents were obtained from Merck (Darmstadt, Germany). Anhydrous dichloromethane (DCM) was obtained by distillation after reflux for 1 h in the presence of calcium hydride. Tamoxifen citrate USP (TC) was from Chemische Fabrik Berg GmbH (Ludwigshafen, Germany) from Aldrich. Other solvents and reagents were all of reagent grade and used without further purification.

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