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# Synthesis and in vitro studies of biodegradable thiolated chitosan hydrogels for breast cancer therapy

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ABSTRACT

Local drug delivery strategies have gained momentum recently as a promising modality in cancer therapy. In order to deliver Letrozole (LTZ) at the tumor site in therapeutically relevant concentrations, acetyl-polyamidoamine (Ac-PAMAM)–thiolated chitosan (TCS) films were fabricated. LTZ could be loaded at 31% wt/wt in films, which were translucent and flexible. Physicochemical characterization of LTZ via thermal technique revealed information on solid-state properties of LTZ as well as thiolated chitosan in films. While thiolated chitosan was in amorphous form, LTZ seemed to be present in both amorphous and crystalline forms in film. The lack of formulation-induced local inflammatory responses of LTZ-acetyl-polyamidoamine (Ac-PAMAM)–thiolated chitosan (TCS) films a new paradigm for localized chemotherapy based on breast delivery systems.



## 1. Introduction

The biodegradable polymers are suitable biomaterials for the design of polymeric drug delivery devices for many classes of bioactive agents. These polymers have been used in various macromolecular architectures: linear, cross-linked and branched [1]. Many biodegradable polymers have been synthesized in recent times. One of the most important applications of dendrimer is the design of drug delivery systems [2]. Dendrimers offer a number of advantages compared to other architectural forms of polymers that have been used in drug delivery systems. They have narrow polydispersion, they are in the nanometer size ranges, which can allow easier passage across biological barriers, host–guest chemistry can take place either in the interior (binding groups in the interior of dendrimers are called endoreceptors) or on the periphery of the dendrimer (groups involved in completion chemistry on the periphery of the dendrimers are called exoreceptors) [3,4]. Despite the extensive work on dendrimers, which has been grown potentially in recent years, their biopharmaceutical applications, much remains to be done to make their properties suitable for the intended applications [5]. Efforts are now directed at studying the biological and physicochemical properties of the dendrimers with a view to removing the limitations on their use. To change the biodistribution patterns and to prolong circulation in the blood so as to facilitate targeting of specific tissues, hydrophilic polymers have been conjugated with dendrimers to shield positive charges and to create a steric barrier to reduce the potential for non-specific interactions such as opsonization [6,7]. Kopecek has been developed a dendrimer-based system capable of achieving targeting with high specificity and low toxicity. In this research, we have been used Ac-PAMAM dendrimer (G4) and TCS as a base polymer for a drug delivery system [8]. A side from other desirable properties of PAMAM dendrimer, their synthesis can be tailor-made so as to influence the groups at the surface: full generations such as 1, 2 have amine functionalized surfaces, while half generations such as 1.5, 2.5 have carboxylic acid end groups at the surface. Efforts have been made to modify PAMAM dendrimer using TCS. However, either all the primary amines of the PAMAM dendrimer was shielded with TCS or only 10% of the amino groups in PAMAM were covered [9,10]. We have been hypothesized that it is possible to optimize the biological and physicochemical properties of PAMAM dendrimer modified with TCS by graded changes in the TCS block length in a manner analogous to the reports on TCS [11]. Dendritic architecture and uniformly positioned functionality have been recently reported to carry the anti-inflammatory drug indomethacin for it is transdermal delivery as well as anticancer drugs [12]. Recent studies have been reported investigation on the effect of 4.0 generation(G) PAMAM dendrimer present in an anionic phospholipids composition, consisting of hydrogenated

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soyaphosphotidylcholine, cholesterol, diacetyl phosphate and poly(ethylene glycol)-derivatized phosphortidylethanol-amine, on the hydration and liquid crystalline structure formation [13]. To date, three different thiolated CS derivatives have been synthesized: CS-thioglycolic acid conjugates, CS-cysteine conjugates and chitosan-4-thio-butyl-amidine (CS-TBA) conjugates [14]. These TCS have numerous advantageous features in comparison to unmodified TCS, such as significantly improved mucoadhesive and permeation enhancing properties [15]. The strong cohesive properties of TCS make them highly suitable excipients for controlled drug release dosage forms [16]. We have prepared LTZ-loaded Ac-PAMAM–TCS nanoparticles by emulsion solvent evaporation technique to obtain smaller particle size with high entrapment efficiency and sustained release profile. Particle size, morphology, entrapment efficiency, drug–polymer interaction and in vitro release of LTZ-Ac-PAMAM–TCS nanoparticles were evaluated. The influence of % of drug (relation to polymer mass) on formulation performance including particle size, entrapment efficiency, in vitro release was investigated.

## 2. Materials and methods

LTZ and PAMAM generation 4 contains 64 surface primary amino groups were obtained from Dendritech Inc. (Aldrich Co.). Chitosan (≥85% deacetylated), lysozyme, thioglycolic acid and Tween 80 were purchased from Sigma (St. Louis, MO). Soya phosphatidyl choline and phosphatidylglycerol were kindly provided by Merck Co. (Germany). Absolute (ETOH) was procured from Merck KgaA (Darmstadt, Germany). Glycerol and glacial acetic acid were obtained from LOBA chemie.

### 2.1. Instruments

Analytical TLCs were run on commercial Merck Co. (Germany) plates coated with silica gel GF250 (0.25 mm thick). Fourier transfer infrared (FT-IR, Bruker, Germany) spectroscopy was used to identified the polymer surface. Spectra was obtained in the wave number range of 400–4000 $cm^{-1}$. Spectra of samples were recorded from KBr in 1:10 (wt/wt) ratio. The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer.

### 2.2. Acetylation of PAMAM dendrimer

The ratio between the acetic anhydride and the dendrimer was adjusted to achieve suitable degree of acetylation, with 100% primary amine groups converted. The amount of acetic anhydride was calculated based on the number of primary amines determined by potentiometric titration of ensure a 1:1 stoichiometric relationship between the acetic anhydride and the primary amino groups of G4-PAMAM dendrimer (when complete (100%) acetylation of dendrimer was planned, 20 mol % excess of acetic anhydride was used). Trimethylamine (10% excess based on the amount of acetic anhydride) was added to quench acetic acid formed as a side product during the reaction. The reactions were carried out in a glass flask and anhydrous methanol solution at room temperature for 24 h. The reaction mixture was dialyzed first in phosphate buffer at pH = 8.0 and then in deionized water. The purified samples were lyophilized and stored at −20 °C.

### 2.3. Synthesis of thiolated chitosan

The chemical modification of CS was performed as previously described. CS (500 mg) was dissolved in 50 mL of 1% acetic acid. In order to facilitate reaction with thioglycolic acid (TGA), 100 mg of ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) was added to the chitosan solution. After EDAC was dissolved, 30 mL of TGA was added and the pH was adjusted to 5.0 with 3 N NaOH. The reaction mixture was stirred and left for 3 h at room temperature. To eliminate the unbonded TGA and to isolate the polymer conjugates, the reaction mixture was dialyzed against 5 mM HCl five times (molecular weight cut-off 10 kDa) over a period of 3 days in the dark, then two times against 5 mM HCl containing 1.0% NaCl to reduce ionic uninteractions between the cationic polymer and the anionic sulfhydryl compound.

### 2.4. Preparation of Ac-PAMAM–(TCS) hydrogel

TCS and Ac-PAMAM hydrogels were dissolved in different concentration (1% to 4%) of acetic acid (1% wt/vol). LTZ (5–20 mg) was dissolved separately in dimethyl formamide (DMF). Then, the TCS–Ac-PAMAM solution (2.5 mL) and the drug solution (7.5 mL) were mixed together to obtain 10 ml of TCS–Ac-PAMAM–LTZ solution. The TCS–Ac-PAMAM–drug solution was added dropwise (using a disposable syringe with a 22-gauge needle) into 40 mL of sodium chloride-saturated Tris–HCl buffer solution containing glutaraldehyde-saturated toluene (GST) in different concentration (1–3 mL). The samples were separated after 1 h of curing time and subsequently decanted, washed twice with 3 mL of 0.05 M Tris–HCl buffer, and samples were dried in vacuum oven at 40 °C. Then, LTZ was dissolved in methanol following which the Ac-PAMAM–TCS conjugate was added. The reaction mixture was stirred for 24 h in the dark, then evaporated using rotaevaporator to remove methanol. The traces were dried under vacuum in order to remove methanol completely. To these traces, deionized water was added. This solution was stirred in the dark for 24 h. Then, the drug–dendrimer complex was extracted, as dendrimer is soluble in water while LTZ is not. The solution was then filtered through PTFE membrane (Millix Millipore) of pore size 200 nm, and then lypophilized to remove water. After approximately 180 min, the sample was sprayed into a liquid nitrogen bath cooled down to 77 K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution.

### 2.5. Preparation of LTZ loaded hydrogel films

The LTZ was used from below approach for its incorporation into Ac-PAMAM–TCS films: Initially, a 10 mg/mL Ac-PAMAM–TCS solution was prepared in 1% (vol/vol) acetic acid was included as a plasticizer at a Ac-PAMAM–TCS: glycerol weight ratio of 2:1. Then, liposomes containing LTZ were prepared by film hydration method using phosphatidyl-choline and phosphatidyl glycerol (9:1, soya origin) at 6–12 mol% drug loading and were dispersed in Ac-PAMAM–TCS solution. Then film was cast by pouring the mixture on a glass plate (area 45.5 $cm^2$) followed by drying under vacuum for 48 h at 37 °C.

### 2.6. Stability of LTZ during film preparation

The following procedure was used to assess the stability of LTZ during the film preparation process. The prepared films were extracted twice with a solvent mixture of 1:1 acetonitrile and ETOH (vol/vol), the extract was evaporated, the residue obtained was reconstituted in mobile phase. Stability-indicating chromatographic method was adopted for this purpose. The method consisted of a Synnetry C18 column (250 mm × 4.6 mm; 5 μm) run using a mobile phase of composition methanol:water (70:30 vol/vol) at a flow rate of 0.5 mL/min, a waters pump (600E), and eluants monitored with waters photodiode array detector(996 PDA) at 227 nm.