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Efficient loading and entrapment of tamoxifen in human serum albumin based nanoparticulate delivery system by a modified desolvation technique

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ABSTRACT

Herein, the poorly water-soluble drug, Tamoxifen (Tmx), was loaded in the amphipathic matrix of human serum albumin (HSA) nanoparticles by a modified desolvation method. In order to enhance the drug loading (DL) and drug entrapment efficiency (DEE) (<2% and 10%, respectively), ultrasonication of Tmx-HSA mixture was performed prior to desolvation process. Tmx loading and entrapment efficiency were optimized by employment of the response surface methodology (RSM)-central composite design (CCD) of experiments. Under the optimum conditions of 1.59 mg Tmx/ml concentration, 7.76 pH and 5 h incubation of HSA-Tmx, the DL of 6.7% and DEE of 74% are achievable. Particles with the average size of 195 nm, zeta potential of −21 mV and polydispersity index of 0.09 were produced under these conditions. A more sustained Tmx release behavior was observed from polyethylene glycol (PEG) conjugated nanoparticles in comparison to the non-PEGylated ones. The short-term stability investigation showed no alteration in physicochemical properties of nanoparticles at 4 and 37 °C, but small increase in nanoparticles size was observed after three months of storage at room temperature. This is the first report for efficient production of a Tmx delivery system based on HSA nanoparticles.

Keywords: Human serum albumin nanoparticles; Tamoxifen; Response surface methodology; Ultrasonication; *In vitro* drug release; Storage stability

1. Introduction

The non-steroidal drug tamoxifen (Tmx) is the most commonly used drug for the treatment of estrogen receptor (ER)-positive breast cancers in pre-and post-menopausal women. Breast cancer death rate can be reducing to one third with Tmx treatment (Jordan, 2009; Pears and Bartlett, 2009). It is also the only drug employed for the prevention of breast cancer in healthy women at high risk of developing the disease (Bourassa et al., 2011). In its mechanism of action, the complex of Tmx and ER binds with DNA and alters or blocks the subsequent mRNA transcription leading to cellular apoptosis (Cameron et al., 1997). Commercially, Tmx is only available as tablets of Tmx citrate and consumes orally in a daily dose of 10–20 mg. However, Tmx citrate has shown poor oral bioavailability (<30%) due to its precipitation in the acidic environment

Abbreviations: Tmx, tamoxifen; EPR, enhanced permeability and retention; DDSs, drug delivery systems; HSA, human serum albumin; PEG, polyethylene glycol; DL, drug loading; DEE, drug entrapment efficiency; RSM, response surface methodology; CCD, central composite design; DX-7, Design Expert Software version 7.0.0; Tmx-HSA NPs, tamoxifen loaded HSA nanoparticles; HPLC, high performance liquid chromatography; TNBS, 2,4,6-trinitrobenzene sulfonic acid; ANOVA, analyses of variance; PDI, polydispersity index; DLS, dynamic light scattering; SEM, scanning electron microscopy; SDS, sodium dodecyl sulfate; *F*-value, Fisher variance ratio.

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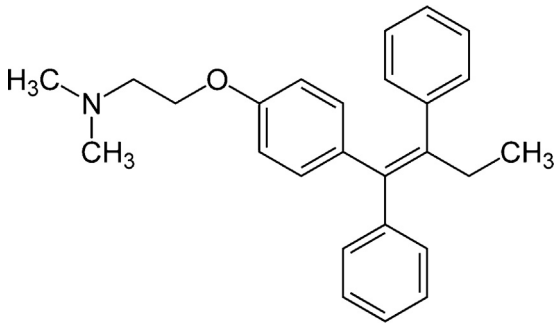

Fig. 1 – Chemical structure representation of the anticancer drug Tmx.

of stomach and extensive hepatic and intestinal first pass metabolism (Shin and Choi, 2009). Therefore, despite the clinical choice of Tmx in advanced stages of breast cancer, it may cause toxic hepatitis, pulmonary emboli and venous thrombosis as common side effects depending on the usage dose and concentration (Delima et al., 2003; Hard et al., 1993; Peyrade et al., 1996). Furthermore, an increased risk of endometrial cancer has been associated with the treatment duration and dose accumulation (Shin et al., 2006). Therefore, developing an injectable delivery system of Tmx is essential for an effective treatment with improved bioavailability and reduced side effects. Fig. 1 schematically represents the chemical structure of Tmx.

The desired properties of drug delivery systems (DDSs) include biodegradability, bioavailability, their small size, high content of drug in the final preparation, prolonged circulation in the blood and the ability to passive (*via* the enhanced permeability and retention – (EPR) – effect) or active (*via* specific ligands, such as monoclonal antibodies) targeting of a determined area (Torchilin, 2006). Although DDSs are efficiently employed for water-soluble drugs (Alexis et al., 2010; Gref and Couvreur, 2006; Lince et al., 2011), development of nanoparticulate DDSs for poorly water-soluble pharmaceuticals remains a major challenge and many researchers are working to resolve this problem (Agarwal et al., 2008; Danhier et al., 2009; Lee et al., 2011; Yang et al., 2007).

Human serum albumin (HSA) is a highly water-soluble globular monomeric plasma protein with a relative molecular weight of 67 KDa, consisting of 585 amino acid residues, one sulfhydryl group and 17 disulfide bridges (Elzoghby et al., 2012). Among nanoparticulate carriers, HSA nanoparticles have long been the center of attentions in the pharmaceutical industry due to their ability to bind to various drug molecules, great stability during storage and *in vivo* usage, no toxicity and antigenicity, biodegradability, reproducibility, scale up of the production process and a better control over release properties. In addition, significant amounts of drug can be incorporated into the particle matrix because of the large number of drug binding sites on the albumin molecule (Low et al., 2011; Wagner et al., 2010). For example, 98% of Tmx can bind to HSA at 1.2 M ratio of Tmx per HSA (Bourassa et al., 2011).

Desolvation is the most commonly used technique for the production of albumin-based nanoparticles. The most advantages of desolvation in comparison with other production techniques such as emulsification, thermal gelation, nano spray drying, nanoparticle albumin bound (NAB)-technology and self-assembly are easy and reproducible process and the production of nanoparticles with more controllable drug release properties (Elzoghby et al., 2012). Water-soluble drugs such as doxorubicin and 5-fluorouracil were efficiently loaded in albumin nanoparticles by desolvation technique (Dreis et al., 2007; Maghsoudi et al., 2008). It is not possible to manufacture HSA nanoparticles with efficient loading and entrapment of poorly water-soluble drugs such as paclitaxel and tamoxifen (water solubility less than 1 ppm) by unmodified desolvation technique. Surface modification of HSA nanoparticles is also possible with activated polyethylene glycol (PEG) and drug targeting ligands such as monoclonal antibodies due to the presence of functional amino and carboxylic groups (Kouchakzadeh et al., 2010, 2012, 2013; Elzoghby et al., 2012).

Response surface methodology (RSM) is an assembly of statistical and mathematical techniques that are useful for the modeling and analyses of problems when a response of interest is influenced by several variables and the objective is the optimization of this response. Central composite, Box–Behnken and Doehlert designs are among the principal response surface methodologies used in the experimental design. Central composite design (CCD) is the most popular response surface method due to its capability in building the second order response models. Second-order models have several advantages such as: (1) they are very flexible and can take on a wide variety of functional forms and (2) it is easy to estimate the parameters in a second-order model (Bezerra et al., 2008; Li et al., 2011).

In this study, physical entrapment of the poorly water-soluble drug, Tmx, in amphipathic HSA nanoparticles was investigated. A modified desolvation method was developed to obtain nanoparticles with a higher amount of the drug in comparison with the nanoparticles prepared by the use of the conventional desolvation technique. Drug loading (DL) and drug entrapment efficiency (DEE) were maximized through optimizing the effective parameters of the Tmx concentration, pH and Tmx-HSA incubation time using CCD-RSM. Design Expert Software (DX-7, State-Ease Inc., Version 7.0.0) was used for the generation and evaluation of the statistical experimental design. In order to optimize the Tmx loading in HSA nanoparticles, mathematical model equations were derived by computer simulation programming DX-7. The produced Tmx loaded HSA nanoparticles (Tmx-HSA NPs) under optimum conditions were also PEGylated, characterized and evaluated for the *in vitro* drug release. In addition, the short and long-term physicochemical stabilities of Tmx-HSA NPs were examined.

2. Materials and methods

2.1. *Materials*

Human serum albumin (HSA, fraction V, purity 96–99%), powders of tamoxifen (trans-2-[4(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethylamine, $C_{26}H_{29}NO$, M_w = 371.51 Da), glutaraldehyde 8% aqueous solution, 2,4,6-trinitrobenzene sulfonic acid (TNBS) 5% aqueous solution and protease (500 U/mg) were obtained from Sigma–Aldrich (St. Louis, Mo). Maleimide-poly(ethylene glycol)-succinimidyl carbonate (Mal-PEG-NHS, M_w = 5000 Da) was purchased from JenKem Technology, USA. Sodium dodecyl sulphate (SDS), trehalose and all solvents for the high performance liquid chromatography (HPLC) purchased from Merck, Germany.