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Sterile, injectable cyclodextrin nanoparticles: Effects of gamma irradiation and autoclaving

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Abstract

Sterility is required as stated by compendial requirements and registration authorities worldwide for an injectable drug carrier system. In this study, injectable nanospheres and nanocapsules prepared from amphiphilic β-cyclodextrin, β-CDC6, were assessed for their in vitro properties such as particle size distribution, zeta potential, nanoparticle yield (%), drug entrapment efficiency and in vitro drug release profiles. Different sterilization techniques such as gamma irradiation and autoclaving were evaluated for their feasibility regarding the maintenance of the above mentioned nanoparticle properties after sterilization. It was found that amount these techniques, sterilization with gamma irradiation seemed to be the most appropriate technique with no effect on particle size, drug loading and drug release properties. Gamma irradiation causes some chemical changes on β-CDC6 observed as changes in zeta potential but this does not lead to any significant changes for nanoparticle properties. Autoclaving caused massive aggregation for the nanoparticles followed by precipitation, which led to the conclusion that excessive heat disrupted nanoparticle integrity. Sterile filtration was not feasible since nanoparticle sizes were larger than the filter pore size and the yield after sterilization was very low. Thus, it can be concluded that blank and drug loaded β-CDC6 nanospheres and nanocapsules are capable of being sterilized by gamma irradiation.

Keywords: Amphiphilic cyclodextrin; Autoclaving; Gamma irradiation; Nanoparticle; Tamoxifen

1. Introduction

Sterility is a crucial factor for drug delivery systems that are to be directly injected into the organism. Injectable nano- or microparticles can be sterilized by a number of techniques all with considerable advantages and drawbacks. Regarding injectable nanoparticles, alternative sterilization techniques include membrane filtration, gamma irradiation, autoclaving, ethylene oxide sterilization and high hydrostatic pressure sterilization.

Membrane filtration is a safe technique based on physical removal of present microorganisms that does not require excessive heat or radiation causing irreversible effects on the nanoparticles or the encapsulated drug. However, it is very much limited to the size of the particles. Nanoparticles with size exceeding 200 nm are not appropriate for this kind of sterilization. Several authors have stated that filtration is not an effective sterilization method since nanoparticles are similar in size to contaminants and also the filter pore size. Moreover, elasticity and size of the nanoparticles could lead to clogging of the filtration membranes (Allemann et al., 1993; Magenheim and Benita, 1991). Adsorption of the nanoparticle material to the filter is another drawback of this technique reducing the yield of the finished product.

Heat sterilization by autoclaving is a highly effective technique involving high temperatures (120 °C), which may influence decomposition or degradation of active ingredient as well as the nanoparticle material, i.e., polymer. An increase in size of nanocapsules from 200 to 500 nm was reported after moist heat sterilization where Miglyol is used as oil phase surrounded by poly(isobutylcyanoacrylate) (Rollot et al., 1986). This increase in size was attributed to either the swelling of polymeric membrane or expansion of oily phase. No change in size was observed for nanospheres.

Sterilization by gamma irradiation is also an effective method accepted by European Pharmacopeia. The main advantage is its high penetration power and the isothermal character of gamma rays that allows suitable treatment for heat-sensitive materials.

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Moreover, gamma irradiation assures homogeneous sterilization and is useful for packaged products, thus avoiding further risk of microbial contamination. However, gamma irradiation also may exert serious effects on the drug delivery system (Sintzel et al., 1997). Energy transfer may induce fragmentation of covalent bonds and production of free radicals that, in turn, are responsible for the majority of the damage that occurs to irradiated materials as a consequence of chemical attack, e.g., radiation could cause alteration of physicochemical properties, decrease of the amount of active ingredient by partial decomposition or create molecular fragments that may result in a toxicological hazard (Boess and Bögl, 1996; Sintzel et al., 1997; Masson et al., 1997).

Amphiphilic cyclodextrins have been widely investigated as excipients for drug delivery systems in the form of nanoparticles since the last decade (Duchene et al., 1999). They have been reported to give stable nanospheres and nanocapsules without the presence of surfactants achieving high encapsulation efficiency and reduction of burst effect in drug release (Memisoglu et al., 2002, 2003). These nanoparticles are designed for injectable carriers mostly for anticancer agents so sterility is an important factor in the manufacturing of cyclodextrin-based nanoparticles.

The objective of this study is to assess the feasibility of sterilization with different techniques employing heat or gamma-irradiation for the first time on amphiphilic β-CD nanoparticles. Effect of sterilization technique on nanoparticle properties such as size, zeta potential, drug loading, in vitro drug release, thermal behavior and nanoparticle yield was assessed for the first time for amphiphilic β-cyclodextrin nanoparticles in a comprehensive approach.

2. Material and methods

2.1. Materials

Amphiphilic β-cyclodextrin modified on the secondary face with 6C aliphatic esters, β-CDC6, was synthesized and purified as described previously. The chemical structure, purity and selective substitution of β-CDC6 were previously described by different techniques such as H NMR spectrometry, Fourier transform infrared spectroscopy, elemental analysis and fast atom bombardment mass spectrometry (Memisoglu et al., 2002) (Fig. 1). Miglyol 812® (Condea Chimie, Germany), trigyceride of capryc/caprylic acid was used as oil in the preparation of nanocapsules, Tamoxifen citrate, model drug, was a kind gift of Teva Pharmaceuticals Inc. (Israel). Acetone was extra pure (Carlo Erba, Italy) for the preparation of nanoparticles. All other reagents were of analytical grade and were used as received.

2.2. Methods

2.2.1. Nanoparticle preparation

2.2.1.1. Preparation of blank nanoparticles. Nanoprecipitation technique was used (Fessi et al., 1998) to prepare the nanoparticles. Briefly, β-CDC6 (1 mg) was dissolved in acetone (1 mL) to give an organic phase, which was added with an

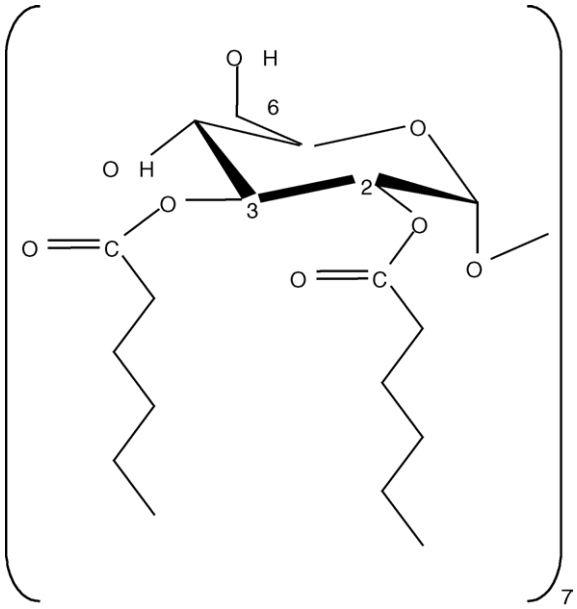

Fig. 1. β-CDC6, selectively substituted and pure amphiphilic β-cyclodextrin per-modified on the secondary face with 6C aliphatic esters.

Eppendorf injector under room temperature at constant stirring to an aqueous phase consisting only of deionized water. Organic solvent was evaporated under vacuum to give nanosphere dispersion of desired volume, which was 2 mL in this study. To obtain nanocapsules, the only difference is the addition of 50 μL of Miglyol 812®, to the organic phase. Organic/aqueous phase ratio was kept at 1:2 (v/v) for nanospheres and nanocapsules.

2.2.1.2. Preparation of drug loaded nanoparticles. Tamoxifen citrate loaded nanoparticles were prepared with a novel technique reported previously (Memisoglu et al., 2003). In this technique, nanoparticles are prepared directly from pre-formed drug:amphiphilic β-CD inclusion complexes. Tamoxifen:β-CDC6 inclusion complexes of 1:1 molar ratio were prepared by co-lyophilization technique and characterized by DSC, FT IR spectroscopy, SEM and MALDI TOF as 1:1 inclusion complexes (unpublished results). Fixed amounts of tamoxifen citrate and β-CDC6 are readily soluble in ethanol were dissolved in 20 mL ethanol. Then 40 mL water is added to obtain a suspension of drug and cyclodextrin. Suspension is left to equilibrate under stirring for 7 days at room temperature and ethanol was evaporated under vacuum to give an aqueous suspension which was then lyophilized (HETO Lyolab Freeze Dryer, UK) yielding the complex in powder form.

Nanoparticles were prepared by weighing and dissolving the TMX:β-CDC6 complex (1 mg) in acetone and further addition of drug solution containing 200 μg TMX in the organic phase during preparation. Organic phase was added to aqueous phase as described in Section 2.2.1.1 and nanoparticles were obtained after evaporation of organic solvent.

2.2.2. Sterilization of nanoparticles

2.2.2.1. Autoclaving (heat sterilization). Samples were divided into two groups of equal volume after preparation. They were put