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Formulation, characterisation and *in vitro* studies of doxorubicin-loaded silica–polydimethylsiloxane granules

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ABSTRACT

The goal was to develop a granule-type formulation, characterised as a long-term, zero-order release delivery system for doxorubicin hydrochloride (DOX) and composed of a sol–gel derived silica–polydimethylsiloxane solid matrix with well-defined microstructures. The preparation of the DOX-loaded granule-type formulation was performed using the sol–gel moulding method. A liquid-form of DOX was added to the sol before moulding. Optical microscopy, X-ray diffraction (XRD), Differential Scanning Calorimetry (DSC), Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and N_2 adsorption/desorption studies were employed to characterise the obtained formulation. The influence of different drug loads of DOX per granule (136, 336 and 555 μg) on the release profiles was assessed on a USP Apparatus 4 dissolution and via UV/Vis end analysis. The *in vitro* mineralisation of these formulations associated with the nucleation of the apatite layer on their surface was also examined. The semi-ellipse shape and micrometer-size of the DOX-loaded granule-type formulation was successfully obtained. These formulations exhibited a mesoporous structure, uniform pore size distribution and good monodispersity. Following an initial burst, the slow drug release from all formulations followed zero order kinetics under infinite sink conditions for over 70 days. Besides the formulation's potential properties as a carrier, the material was also surface-reactive during *in vitro* mineralisation.

1. Introduction

Local skeleton drug delivery systems, SDDS, are one the newest pharmaceutical products, whereby a drug is incorporated into a biomaterial designed to function as an implant in bone tissue. The main idea of SDDS is to obtain the continuous release of a drug incorporated at the implant site (Jain and Panchagnula, 2000). The sol–gel process is a very common method for preparing porous bi-functional silica xerogels, which are used in pharmaceutical applications as drug carriers (Lee and Shin, 2007; Margiotta et al., 2007; Quintanar-Guerrero et al., 2009) and as a material with a high bioactivity due to their high porosity and expanded surface area (Kortesuo et al., 2000). When silica xerogels are in contact with a physiological fluid, a surface layer is formed consisting of a semi-crystalline hydroxycarbonate apatite (HCA), which has a structure and composition similar to a biological apatite. This apatite layer may become permanently connected to the bone matrix (Li et al., 1993; Ramila and Vallet-Regi, 2001). The sustained release, for example, of anticancer drugs from these formulations is considered ideal, because their therapeutic use is limited by cumulative dose-dependent irreversible cardiotoxicity. It would be ideal if a single administration could lead to effective chemotherapy that could last for days, weeks, or even months (Feng and Chien, 2003). Recently, methotrexate (Carino et al., 2007), platinum–bisphosphonate complexes (Margiotta et al., 2007) and doxorubicin hydrochloride (Prokopowicz, 2009) have been loaded into silica xerogels, with the aim of using the material as a bone filler acting as a bone substitute while slowly releasing the drug at the site of the tumour. This dual combination of an anticancer drug and a strategic formulation of bi-functional inorganic materials provides novel solutions for the local treatment of bone tumours.

This study was a continuation of the author's research into the design of a bi-functional implantable drug delivery system, releasing doxorubicin hydrochloride at continuous and low concentrations, and exhibiting bioactive characteristics. In this case, bioactivity results in the self-formation of carbonate hydroxyapatite on the delivery surface in the simulated body fluid (Kokubo and Takadama, 2006). Doxorubicin hydrochloride (DOX) was chosen, because it is one of the most potent broad-spectrum antitumor anthracycline antibiotics widely used to treat a variety of primary or secondary bone neoplasms (Fan and Dash, 2001). The synthetic strategy of DOX-loaded silica materials was based on previous studies (Prokopowicz, 2009) using pre-optimised sol–gel reaction

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mechanism conditions (Prokopowicz, 2007). Tetraethoxysilane (TEOS) is used as a precursor in the sol–gel process, because of its non-toxicity (Brinker and Scherer, 1989). At room temperature, the hydrolysis and condensation of TEOS produces both a colloidal suspension (sol) and a gelation of the sol to form an inorganic glass (gel) network. As a result, the labile molecules of the drug are immobilized *in situ* in the oxide matrix formed in this process. The drug molecules remain occluded in the gradually formed gel. Unfortunately, when using TEOS, cracking of the monolith occurs, which results in the irregular shape of silica xerogels. The cracking is a result of the stresses caused by the existence at the liquid–vapour interface of a meniscus, which generates a differential capillary pressure within the network of the xerogel (Brinker and Scherer, 1989; Mosquera et al., 2003). Therefore, other technologies such as spheronisation using wet or dry granulation processes are used; however, these technologies usually require many manufacturing processes and higher production costs. To overcome these pharmaceutical difficulties, increasing interest has been observed in the use of organic–inorganic sol–gel derived composites based on silica. For example, the addition into the silica sol of organic polymer substrates, such as hydroxyl-terminated poly(dimethylsiloxane) (PDMS) or poly(tetramethyleneoxide) terminated with 3-isocyanatopropyltriethoxysilyl (Si-PTMO), leads to the formation of an elastic silica network, which is expected to prevent cracking (Brinker and Scherer, 1989). For biomedical applications, spherical geometry is more preferable than irregular ones when seeking to eliminate non-desirable inflammation reactions from body soft tissues (Langer and Peppas, 1981). Continuous efforts have been aimed at developing a regular-type shaped formulation based on a bioactive silica/PDMS xerogel that could deliver anticancer drugs to the bone with zero-order kinetics. The preparation of a DOX-loaded granule as a three-dimensional solid formulation was performed on the basis of the sol–gel micromoulding in capillaries technique (Xia and Whitesides, 1998). This is a simple, flexible and relatively inexpensive method for producing silica microcomponents, particularly for mass production, providing that precision micro-moulds can be readily made. This technology has typically been used for non-pharmaceutical purposes and, as far as the author is aware, this is the first report where sol–gel drug-loaded granule-type formulations have been obtained in this innovative way.

2. Materials and methods

2.1. Materials

Tetraethoxysilane (TEOS), hydroxyl-terminated poly(dimethylsiloxane) (fluid PDMS linear chains 25 cSt, M_W = 1100 g/mol), triethylphosphate (TEP), calcium chloride ($CaCl_2$), sodium lauryl sulphate (SLS), hydroxyapatite crystals (HA, $Ca_{10}(PO_4)_6(OH)_2$) and doxorubicin hydrochloride (DOX, M_W = 580.0 g/mol, crystalline powder-purity 99.0%) were obtained from Aldrich–Sigma. All other reagents were obtained from Prochem-Poland.

2.2. Fabrication of DOX-loaded granule-type formulations

Silica–polydimethylsiloxane–calcium phosphate (SiO_2/PDMS/CaP) formulations were synthesised via the modified sol–gel moulding technique. Briefly, formulations obtained during the synthesis contained 60 wt.% of silica, 20 wt.% of hydroxyl-terminated polydimethylsiloxane (PDMS), 15 wt.% of calcium chloride ($CaCl_2$) and 5 wt.% of triethylphosphate (TEP). Three batches of DOX-loaded SiO_2/PDMS/CaP formulations were prepared as follows: each of the silica sols was prepared by adding PDMS (0.21 mL) to a 1% surfactant solution containing sodium lauryl sulphate (SLS) which was obtained by dissolving 0.004 g of SLS in deionized water (0.368 mL). After 20 min of ultrasonic treatment (20 kHz) in an ice bath (−4 °C), a stable and homogenous PDMS-in-water submicron emulsion was obtained. At the same time, TEOS (2.1 mL) with ethanol (0.671 mL) was stirred for 5 min at room temperature. This solution was slowly added to the mixture of $CaCl_2$ (0.15 g) dissolved in a TEP (0.047 mL) and deionized water (0.368 mL). Next, the PDMS-in-water emulsion was slowly dropped into the former (TEOS–$CaCl_2$–TEP) solution. The mixture was stirred for 10 min and the pH was adjusted to 2 by using 140 μL of 0.01 M HCl. Then, each of the formed sols was stirred in a covered flask for 24 h at 50 °C. Thereafter, 80 μL of 0.2% ammonium hydroxide was added to provide drug stability. The pH of the sols increased to 5.5–6.0 (DOX is stable in the pH range of 4.5–6.5 (Fan and Dash, 2001). After 10 min of stirring, 0.8 mL of the water solutions containing 0, 2, 5, and 8 mg of DOX was added separately to the prepared sols (called the initial amount of DOX in the sol-mixture) and stirred for 10 min. After that, a pre-defined quantity of the sol-mixture was poured into fourteen polypropylene moulds (300 μL per one mould of the dimensions: 8 mm in depth and 5 mm in diameter). The transition of sols into gel forms lasted 10 min, independent of the presence of the drug. The wet DOX-loaded gels in the moulds were slowly dried to a constant weight for 20 days at +4 ± 0.5 °C, 60 ± 10% RH and then freeze-dried for 48 h at −50 °C and a pressure of 2 Pa (Alpha 1–2 LD Freeze-Dryer, Germany). Fig. 1 shows a schematic representation of DOX loading into the SiO_2/PDMS/CaP sols through the formation of solid DOX-loaded SiO_2/PDMS/CaP granules.

2.3. Determination of the drug content

The drug content in the DOX-loaded granules was determined as follows. Briefly, three DOX-loaded granules were randomly selected from each batch of the test formulations, separately weighed, and then pulverized using a small mechanical ball mill. The particles were suspended in a water–ethanol (50:50) mixture giving a 0.1 mg/mL concentration and sonicated for 20 min of ultrasonic treatment (20 kHz) in an ice bath (−4 °C). The DOX concentration in the suspension was measured via UV/Vis spectroscopy at λ_{max} = 480 nm (UV/Vis spectroscope, Jasco model V-500) against the relevant water–ethanol particle suspensions (lacking DOX) as references. Analytical studies were conducted in accordance with the requirements for quantitative analyses, calibrating the detector with a standard solution of the test substance dissolved in 0.1 mg/mL of water–ethanol blank particle suspensions. The recovery of DOX was calculated as the standard deviation of the mean from three independent experiments. The loading efficiency of DOX was calculated as the mass ratio of the amount of DOX incorporated in granules to that used in the granule preparation.

2.4. Uniformity of weight and size analysis

The uniformity of the weight of the DOX-loaded granule was tested in accordance with the British Pharmacopoeia test (2005). The granule size and shape was evaluated via optical microscopy with a calibrated eyepiece micrometer. The DOX-loaded granule-type formulations were accurately weighed both together and separately. The results (means of the fourteen samples for each batch) are expressed in terms of weight, surface area, volume and standard deviation (SD).

2.5. Fourier transform infrared spectroscopy (FT-IR)

The bulk structure of the ground samples was studied via Fourier transform infrared spectroscopy (FT-IR) in the operating range