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Preparation of polymer nanoparticles loaded with doxorubicin for controlled drug delivery

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ABSTRACT

The preparation of polymer nanoparticles loaded with an active principle, commonly used in cancer treatment, is investigated here from the experimental point of view. The main novelty of this work stands in the use of continuous confined impinging jets mixers in combination with realistic materials, notably the biodegradable and biocompatible copolymer poly(methoxypolyethyleneglycolcyanoacrylate-co-hexadecylcyanoacrylate) together with two forms of the drug doxorubicin. To our knowledge this is the first attempt to use for such a system a device that can be operated continuously and can be easily scaled up. Nanoparticles are produced via solvent-displacement experimenting different solvents; the effect of the other operating parameters is also investigated. Nanoparticles are characterized in terms of their size distribution and surface properties; for a limited number of samples prepared with the optimized preparation protocol further characterization (in terms of drug loading, incorporation and release profiles) is also carried out. Collected results show that the overall approach is capable of producing nanoparticles with controlled particle size distribution, drug loading and good reproducibility and that on the contrary of what reported in the literature the presence of the active principle does play an important role.

Keywords: Controlled drug delivery; Confined impinging jets reactors; Doxorubicin; Mixing; Nanoparticles; Drug incorporation; Biodegradable polymer

1. Introduction

Chemotherapeutic agents commonly lead to the damage of healthy tissues and organs because they act on rapidly proliferating cells by inhibiting DNA synthesis and interfering with the processes of cell division and metabolism. Engineered drug delivery systems for cancer treatment aim to increase the efficacy of chemotherapeutic agents while minimizing the interactions with healthy sites in the body by modifying their bio-distribution and controlling the rate at which the agent is released (Couvreur and Vauthier, 2006).

As it is now well known, biodegradable nanoparticles offer numerous advantages for the treatment of cancer since they are able to circulate through capillaries, passively target tumour tissues and enter cells for intracellular drug delivery. The mechanisms responsible for passive targeting is the so-called enhanced permeability and retention (EPR) effect that allows the passage of drug particulate carriers (between 10 and 500 nm in size) through the highly permeable blood vessels supplying growing tumours and leads to their entrapment as a result of deficient lymphatic drainage (Betancourt et al., 2007). In fact, as it has been reported, the intracellular openings in tumour vascular endothelium can be as large as 2 μm in diameter and the vessel leakiness in tumour vasculature can be up to an order of magnitude higher than that of normal blood vessels. Ideal characteristics for these particles depend on the target organ but generally the particle size distribution (PSD) must be narrow and centred at about 200 nm; the presence of a hydrophilic surface is also highly desirable since it increases the carrier lifetime in the blood stream, therefore allowing its accumulation in the solid tumour. This latter property is generally obtained using copolymers with a hydrophobic part

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linked to a hydrophilic part (typically polyethyleneglycol, PEG) that is eventually displaced on the particle surface protecting the particle itself from negative interactions with the reticulo-endothelial system (Alexis et al., 2008; Joen et al., 1991).

The use of polymeric nanoparticles typically results into improved efficacy, better use of the encapsulated pharmaceutical agents and increased patient compliance and quality of life, especially when the active principle has particularly severe side effects. One such case is doxorubicin, an anthracycline antibiotic that blocks DNA synthesis and transcription by intercalating between DNA nucleotides and inhibiting topoisomerase II, but generates damaging radicals from its metabolism. Doxorubicin has been used clinically for the treatment of lymphoma, acute leukemia, soft-tissue sarcoma and breast, ovarian, testicular, lung, bladder and gastric cancers. Doxorubicin is usually administered intravenously at a maximum dose of 60–90 mg/m^2 at 21-day intervals. A maximum lifetime dose of 550 mg/m^2 is allowed because of known cumulative cardiotoxicity associated with anthracycline treatment (Betancourt et al., 2007). Some doxorubicin formulations based on biodegradable polymers such as poly(lactic acid) (PLA) or poly(lactic-*co*-glycolic) acid (PLGA) have been reported in the literature (see for example Yoo and Park, 2001; Park et al., 2009). These formulations involved microparticles that have a limited clinical potential because they must be administered locally or implanted. In addition, formulations such as these ones, in which doxorubicin is chemically conjugated to the drug carrier are often problematic because modification of the drug may cause changes of its *in vivo* activity and create the risk for non-biocompatible products of drug–polymer metabolism. It is therefore clear that the incorporation of doxorubicin molecules into synthetic polymer nanoparticles represents an interesting possibility that has not been fully investigated in the recent past.

In our previous work (Lince et al., 2008) the use of a confined impinging jets mixer (CIJM) for the production of poly-ε-caprolactone (PCL) nanoparticles has been investigated, as well as the development of a smart CIJM able to adjust its geometry depending on the operating conditions for producing particles with desired characteristics (Lince et al., 2009). The role played by mixing in the nanoparticle production process and the importance of an accurate design of the reactor have been extensively investigated both using test reactions (Johnson and Prud'homme, 2003a) and real polymeric nanoparticle precipitation processes based on solvent-displacement (Johnson and Prud'homme, 2003b,c; Lince et al., 2011). The importance of turbulent mixing in CIJM has been extensively investigated both from the experimental and modelling viewpoints (Liu et al., 2009; Gavi et al., 2010) and results have been used to develop novel mixer geometries, such as the multi-inlet vortex reactor (Liu et al., 2008; Cheng et al., 2009). However, most of these works were carried out without considering an active principle that finds realistic applications in the pharmaceutical industry.

In this work, we report on the preparation, characterization and *in vitro* evaluation of nanoparticles constituted by poly(methoxypolyethyleneglycolcyanoacrylate-*co*-hexadecylcyanoacrylate), a grafted co-polymer containing PEG chains, indicated in what follows as P(MePEGCA-*co*-HDCA), loaded with doxorubicin. The drug loaded nanoparticles are prepared through solvent displacement using acetone and tetrahydrofuran (THF) as solvents and water as anti-solvent, carried out in an appositely designed CIJM, followed by solvent evaporation. Nanoparticles were characterized with respect to size, morphology, zeta potential, drug incorporation and loading, release profile and cytotoxicity. Two different types of doxorubicin were considered: the commercially obtained doxorubicin hydrochloride (DOX-HCl), which has minimal solubility in organic solvents and doxorubicin free base (DOX-FB), which presents instead a good solubility in organic solvents but a very low solubility in water. DOX-FB was prepared starting from commercial DOX-HCl and was used in the larger part of the experiments, but for comparison purposes a few tests were also carried out with DOX-HCl. Eventually the therapeutic efficacy and cellular interaction of the nanoparticles was studied *in vitro* in MCF-7 mammarian carcinoma cells.

To our knowledge, this is the first work where the production of nanoparticles loaded with doxorubicin is engineered in a CIJM. Our results show that the use of CIJM can help in properly controlling the operating parameters and the operating conditions, in turn improving the final nanoparticle properties, proving the great potentials of this promising technique that allows for continuous production and is easily scalable to the pilot and industrial scale.

2. Nanoparticle preparation and characterization

2.1. Materials

The copolymer P(MePEGCA-*co*-HDCA) was synthesized as reported in other works (Lince et al., 2011) based on the synthesis of Peracchia et al. (1997, 1998). For each preparation batch a detailed characterization in terms of molecular weight (resulting in about 4.38 kDa), differential scanning calorimetry and nuclear magnetic resonance was carried out. Results show that the copolymer synthesis was successful. A detailed report of these results is included in another work (Lince et al., 2011) where the very same material batch was used for another study. The solvents used for DOX-FB were of analytical grade (Carlo Erba Reagenti, Milan, Italy). Acetone and THF for the nanoprecipitation process as well as Pluronic® and dextrose, were of analytical grade and purchased from Sigma–Aldrich. Doxorubicin hydrochloride (DOX-HCl, 98.5% purity) was purchased from APAC Pharmaceutical, LLC.

The DOX-FB was prepared according to the procedure described by Altreuter et al. (2002), summarised below. DOX-HCl was first dissolved into distilled water at a concentration of approximately 1 mg/mL to form an orange–red solution. The solution was then transferred into a separator funnel and an equal volume of HPLC grade chloroform was then added to the aqueous solution. Drop by drop addition of triethylamine by Pasteur pipette in slight molar excess resulted in a violet aqueous phase in which doxorubicin is unstable and salt-free. Agitation rapidly transferred most of the doxorubicin into the chloroform phase where it appeared deep orange. The chloroform layer was collected and the process of triethylamine addition and extraction were repeated until the aqueous phase was essentially colourless. For the combined chloroform extracts, dehydration was performed by addition of sodium sulphate. The solvent was then removed by rotary evaporation (RV 10 basic, IKA) and by high vacuum pump (Trivac D8B, Leybold). The product was a dry orange–red powder and the yield of reaction was 85%. The resulting solids could be dissolved into many organic solvents, especially aromatic (benzene), halogenated (methylene chloride, chloroform), and polar liquids (dimethylsulfoxide). The solids were stored desiccated under reduced pressure at 4 °C.