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Nanoparticle encapsulation and controlled release of a hydrophobic kinase inhibitor: Three stage mathematical modeling and parametric analysis

Armando Lucero-Acuña[a,b], Roberto Guzmán[a,*]

[a] Department of Chemical and Environmental Engineering, The University of Arizona, Tucson, AZ 85721, USA
[b] Department of Chemical and Metallurgical Engineering, University of Sonora, Hermosillo, SON 83000, Mexico

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ABSTRACT

A mathematical model of drug release that incorporates the simultaneous contributions of initial burst, nanoparticle degradation–relaxation and diffusion was developed and used to effectively describe the release of a kinase inhibitor and anticancer drug, PHT-427. The encapsulation of this drug into PLGA nanoparticles was performed by following the single emulsion–solvent evaporation technique and the release was determined in phosphate buffer pH 7.4 at 37 °C. The size of nanoparticles was obtained in a range of 162–254 nm. The experimental release profiles showed three well defined phases: an initial fast drug release, followed by a nanoparticle degradation–relaxation slower release and then a diffusion release phase. The effects of the controlled release most relevant parameters such as drug diffusivity, initial burst constant, nanoparticle degradation–relaxation constant, and the time to achieve a maximum rate of drug release were evaluated by a parametrical analysis. The theoretical release studies were corroborated experimentally by evaluating the cytotoxicity effectiveness of the inhibitor AKT/PDK1 loaded nanoparticles over BxPC-3 pancreatic cancer cells *in vitro*. These studies show that the encapsulated inhibitor AKT/PDK1 in the nanoparticles is more accessible and thus more effective when compared with the drug alone, indicating their potential use in chemotherapeutic applications.

1. Introduction

The delivery of drugs in a controlled fashion plays a critical role in the effective administration of bioactive molecules for diverse clinical applications. The use of biodegradable polymers as carriers in controlled drug delivery has been widely explored and used in the preparation of micro and nanoparticles with encapsulated drugs. Polymers used to produce nanoparticles have been for the most partpoly(amides), poly(amino acids), poly(alkyl-a-cyano acrylates), poly(esters), poly(orthoesters), poly(urethanes), poly(acrylamides), *etc.* A summary of pharmaceutically used polymers together with their physicochemical characteristics and factors affecting drug delivery abilities, and fundamental drug delivery systems have been reported widely in the literature (Jain, 2000; Khandare and Haag, 2010; Liechty et al., 2010; Moghimi et al., 2001; Parveen and Sahoo, 2008). In this manuscript we report the experimental and theoretical work with poly(esters), more specifically poly-D,L-lactide-co-glycolide (PLGA), one of the few food and drug administration (FDA) approved polymer for drug delivery and other biotechnological applications (Parveen and Sahoo, 2008). PLGA is one of the most successfully used biodegradable and biocompatible polymers for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid, which themselves are biodegraded further to CO_2 and water(Jain, 2000; Kumari et al., 2010). Depending of the ratio of its monomers, PLGA exhibits different biodegradation rates, structural and mechanical properties (Jain, 2000). The encapsulation of hydrophobic and hydrophilic cytotoxic chemotherapeutic agents into biodegradable PLGA nanoparticles is one of the benefits over other delivery systems such as for example liposomes (Park et al., 2009), that for the most part are mainly used to encapsulate hydrophobic drugs. Several anticancer drugs and other different compounds have been encapsulated into PLGA nanoparticles, including paclitaxel, doxorubicin, 5-fluorouracil, 9-nitrocamptothecin, cisplatin, triptorelin, dexamethasone, *etc.* (Kumari et al., 2010). In this work, we report the encapsulation of the hydrophobic kinase inhibitor AKT/PDK1 (PHT-427). The biological action of this inhibitor is on the protein kinase AKT, also known as protein kinase B, a serine/threonine kinase involved in

* Corresponding author.
E-mail address: guzmanr@u.arizona.edu (R. Guzmán).

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the regulation of cell proliferation, survival/apoptosis, angiogenesis, metabolism, and protein synthesis (Mortenson et al., 2004). AKT is known to be a key component of the PtdIns-3-Kinase cell survival signaling pathway which is activated in pancreatic cancers (Mortenson et al., 2004). The drug PHT-427 is a novel AKT inhibitor that binds to the Pleckstrin Homology PH domain of the kinase, thus preventing its binding to phosphatidylinositol (PtIns)-(3,4,5) P3 at the plasma membrane and subsequent activation of AKT(Meuillet et al., 2010; Moses et al., 2009). The emphasis of the work reported here is however, on the development of a three-stage mathematical model that describes the controlled release of this drug under physiological conditions and to assess its application and effectiveness in the cytotoxicity of the PLGA encapsulated drug *in vitro* cancer studies.

Drug release kinetics from biodegradable devices have been presented in the literature by combining and using different mechanisms of release (Fredenberg et al., 2011; Lao et al., 2011). Mathematical models in this respect, involve processes that consider, in a first stage, first order rates for an initial burst release, combined with a second stage that considers bulk degradation of the polymer in microparticles (Gallagher and Corrigan, 2000) and nanoparticles (Corrigan and Li, 2009). In these particular cases the bulk degradation of the polymer is considered analogous to the thermal decomposition of potassium permanganate crystals (Fitzgerald and Corrigan, 1993; Prout and Tompkins, 1944). In other mathematical models reported in the literature, the mechanisms of release involve a combination of first order rate initial burst with diffusion of the drug through polymeric microspheres (Batycky et al., 1997; He et al., 2005; Raman et al., 2005). Other more complex models of release from biodegradable polymeric microspheres involve three mechanisms: drug diffusion, drug dissolution, and polymer erosion and are described by three equations that consider drug concentration in the liquid phase, a virtual solid phase, and an effective solid phase (Zhang et al., 2003). Similar complex systems model the entire drug release profile as the summation of the three mechanisms: first order burst release, first order bulk degradation of the polymer, and diffusional release, however, in a sequential time intervals for each release stage (Arifin et al., 2006; Lao et al., 2009; Siepmann et al., 2002).

In this manuscript, the release of the encapsulated kinase inhibitor is analyzed by an effective mathematical drug release model that considers for the entire drug release process, a combined contribution of three mechanisms of release in a simultaneous fashion. The release stages involve a first order rate burst release, the bulk degradation of the polymer release or nanoparticle relaxation (Fitzgerald and Corrigan, 1993), and the release by diffusion of the drug through the nanoparticle. A parametric study of the model to assess the main parameters that affect the release of the drug under specified physiological conditions was also performed. *In vitro* studies helped corroborate the effectiveness of the model to predict the release of the drug by measuring and comparing the expected cytotoxicity effects of the drug released concentration with pancreatic cancer cells.

2. Materials and methods

2.1. Materials

PLGA acid terminated (50/50 DL-lactide/glycolide copolymer, IV midpoint 0.2 dl/g) was purchased from Purac Biomaterials, Gorinchem, The Netherlands. Dichloromethane (CH_2Cl_2) was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Poly(vinyl alcohol) (PVA) with an average molecular weight of ~31,000 a.m.u. and 86.7–88.7 mol% hydrolysis were obtained from Sigma–Aldrich, Inc. (St. Louis, MO). The kinase inhibitor and anticancer drug PHT-427 was provided by the laboratory of Emmanuelle J. Meuillet in the Arizona Cancer Center at The University of Arizona (Tucson, AZ) and has a solubility reported by suppliers as ≥4.6 mg/mL (Chemscene.com, n.d.; Selleckchem.com, n.d.).

2.2. Preparation of PLGA nanoparticles

The chemotherapeutic drug PHT-427 was dispersed into PLGA nanoparticles (PHT-427-PNP) by following the single emulsion–solvent evaporation technique (Cascone et al., 2002; Danhier et al., 2012; Jain, 2000; Yang et al., 2007). Briefly, 50 mg of PLGA and 2.5 mg of PHT-427 were dissolved in 5 mL of CH_2Cl_2. Dichloromethane was used because is not miscible with water and also has a lower boiling point, which facilitates the evaporation process in further steps. In addition, the drug used in this study is relatively soluble in this solvent. Next, 10 ml of 5% PVA aqueous solution was added to the organic phase. The mixture was emulsified in an ice bath with a XL2020 ultrasonicator (Misonix Inc., Farmingdale, NY, USA) operating at 55 W power output for one minute to obtain a single emulsion system. The emulsion was later broken by the evaporation of the organic solvent using a rotary evaporator at 38 °C and low agitation velocity during 1 h. Next, a 10 min centrifugation cycle at 850 rcf was employed to remove agglomerations, using an IEC Centra-4B centrifuge (International Equipment, Inc., Nashville, TN, USA). The solution was washed with three centrifugation cycles at 4900 rcf for 50 min. During the first two centrifugation cycles, the supernatant was removed and the complex PHT-427-PNP was resuspended in 10 mL of distilled water. On the final centrifugation cycle, the nanoparticles were resuspended in 7.5 mL of 10 mM sodium phosphate buffer with pH 7.4. All experiments were performed by triplicate. Free drug polymeric nanoparticles (PNP) were prepared in the same way but without the drug.

2.3. Nanoparticle size and zeta potential analysis

Nanoparticle sizes and zeta potentials were measured with a Zetasizer Nano ZEN3600 particle size analyzer (Malvern Instruments, Westborough, MA, USA). The refraction index used in the experiments was 1.33 with water as the dispersant. The size analysis of each sample consisted of an average of 10 measurements.

2.4. Scanning electron micrographs (SEM)

The morphological characteristics of PHT-427-PNP were observed using a S-4800 field emission scanning electron microscope (Hitachi Corporation, Tokyo, Japan). The samples were prepared by their immobilization onto carbon-coated 400-mesh copper grids (Ted Pella Inc., Redding, CA, USA).

2.5. Drug loading and encapsulation efficiency

The drug loading and encapsulation efficiency were determined by first dissolving a known mass of PNP with 1 M sodium hydroxide, followed by the addition of hydrochloric acid to equilibrate the pH of the solution at 7.4. The PHT-427 content was analyzed by spectrophotometry at 270 nm using a spectrophotometer UV-1800 Shimadzu Co., Ltd., Japan. The concentration of PHT-427 was determined by standard calibration curves in 10 mM PBS buffer pH 7.4. The drug loading (*DL*) was determined as the mass ratio of PHT-427 entrapped in the nanoparticles to the mass of PHT-427-PNP recovered (Lei et al., 2011), using the following formula:

$$DL\% = \frac{\text{amount of PHT-427 in nanoparticles} \times 100}{\text{amount of PHT-427-PNP obtained in the process}} \quad (1)$$