European Journal of Pharmaceutical Sciences 125 (2018) 102–109

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Synchronizing the release rates of salicylate and indomethacin from degradable chitosan hydrogel and its optimization by definitive screening design

Racheli Sharon Gabbay[a], Ron S. Kenett[b,c], Roberto Scaffaro[d], Abraham Rubinstein[a,*]

[a] The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy Institute for Drug Research, P.O. Box 12065, Jerusalem 91120, Israel
[b] The KPA Group, Ra'anana, Israel
[c] The Samuel Neaman Institute, Technion, Haifa 3200003, Israel
[d] Department of civil, environmental and aerospace engineering, University of Palermo, Viale delle Scienze Ed. 6, 90128 Palermo, Italy

ARTICLE INFO

Keywords:
Dual drug platform
Synchronized release rates
Crosslinked chitosan
Triggered erosion
Multifactorial definitive screening design

ABSTRACT

Three types of ionically crosslinked (with citric acid) chitosan discs were loaded with the highly water- soluble drug, sodium salicylate (SS) and the poorly water-soluble drug, indomethacin (Ind). In separate experiments the hydrated discs were immersed in a de-crosslinking solution comprising of different concentrations of calcium chloride, which induced a controlled erosion of the discs, a process which was optimized to synchronize the release rates of the two drugs over a predetermined period of time. The optimization was accomplished by manipulating six factors: chitosan MW, its amount in the formulation, the concentration of the crosslinker agent, the concentration of the de-crosslinking agent in the dissolution medium, its pH and its temperature. A computerized multifactorial definitive screening design analysis assisted in minimizing the number of experiments. The quotient of the SS to Ind release rates, the difference factor f1, the similarity factor f2 and the combination of f1 and f2 were determined as the experimental responses. The computerized prediction profilers that were used to simulate the contribution of the experimental factors and their effect on the experimental responses led to a successful erodible formulation with a concomitant release of the two drugs over 150 min.

1. Introduction

A variety of diseases could benefit from a combination therapy. Typical examples include tuberculosis (ethionamide together with its booster BDM41906) (Costa-Gouveia et al. 2017), leprosy (a cocktail of rifampicin, clofazimine and dapsone) (Smith et al. 2017), malaria (fosmidomycin together with clindamycin) (Na-Bangchang et al. 2007), HIV (a cocktail of ritonavir, delavirdine and indinavir) (Grodesky et al. 2001) and numerous anti-cancer modalities (Bayat Mokhtari et al. 2017). For this purpose, an assortment of drug carriers has been developed in which two or more drug entities are packed into a single vehicle, most likely micro- or nano particulate systems or polymer drug conjugates (Jang et al. 2016; Zhang et al. 2011).

Combination therapy may be essential when locoregional cancer therapy is required for cases such as melanoma, peritoneal cancer or breast cancer that spread locally, adjacent to the site of the primary tumors, prior to their circulatory invasion. Although a number of locoregional drug administration strategies have been tested in preclinical and clinical trials, most of the locoregional protocols are associated with systemic delivery (Cai et al. 2011). To achieve a viable local delivery of combination therapy in the vicinity of solid tumors, implantable devices are required. Typical examples for current technologies include, drug loaded stents (Suk et al. 2007), brachytherapy (for local combination therapy with radiotherapy) (Tan et al. 2016) and brain wafers such as Gliadel® (Wolinsky et al. 2012).

Using a breast cancer mouse model, we have already shown that a biodegradable implant made of crosslinked chitosan (x-Ct) can be used in brachytherapy for the prevention of post-surgery metastases recurrence in the tumor bed. That implant was biocompatible and the duration of its in vivo degradation was controllable (Azab et al. 2007a; Azab et al. 2007b; Azab et al. 2006). This type of biodegradable platform could be used for locoregional combination therapy. However, the major problem associated with this approach is the possible difference in the physicochemical properties of the drug cargos, namely,

Abbreviations: CA, citric acid; $CaCl_2$, calcium chloride; Ct, chitosan; DoE, design of experiment; Ind, indomethacin; SEM, scanning electron microscope; SS, sodium salicylate; x-Ct, crosslinked chitosan

* Corresponding author.
E-mail address: avrir@ekmd.huji.ac.il (A. Rubinstein).

https://doi.org/10.1016/j.ejps.2018.09.020
Received 17 June 2018; Received in revised form 21 August 2018; Accepted 27 September 2018
Available online 27 September 2018
0928-0987/

differences in their water solubility properties that directly affect the desired concomitant release rate at the site of implantation. This problem could be overcome by designing multi-drug platforms in which the release rate is governed by erosion rather than diffusion over a predetermined period of time. We have used this concept in the past for increasing the bioavailability of poorly absorbed drugs in the gastrointestinal tract by their concomitant release, with absorption enhancer, at similar rates (Baluom et al. 2000; Baluom et al. 2001). At a later stage we upgraded the platform's design by adding a remote component that allowed the degradation of the multi-drug vehicle, to be conducted from a distance, by employing external de-crosslinking buffer solutions of varying concentrations. This, together with appropriate design of crosslinking constituents of the hydrogel vehicle, enabled concomitant release rates of two drug entities with different hydrophilicities, upon exposure to external stimulus. We tested this competence in the context of locoregional cancer therapy, explicitly for the prevention of post-surgery metastasis spread (Nadler-Milbauer et al. 2011; Nadler-Milbauer et al. 2009).

Specifically, the study goals were to (***a***) crosslink chitosan (Ct) with citric acid (CA) to obtain x-Ct erodible hydrogel, (***b***) load the x-Ct with two model drugs, the highly water soluble sodium salicylate (1.25 g/mL in 25 °C, denoted as SS) and the poorly water soluble indomethacin (0.94 mg/L, 25 °C, denoted as Ind,) and assess their erosion-driven release rates upon incubation with calcium chloride ($CaCl_2$) (***c***) manipulate the concentrations of crosslinker, CA, and the de-crosslinking agent, $CaCl_2$, in order to identify the erosion rate which will overlay the release kinetics of SS and Ind over a predetermined period of time and (***d***) assess the effect of Ct type, Ct/crosslinker ratios in the x-Ct hydrogel, pH of the dissolution medium and its temperature on the similarity in the release rate profiles of SS and Ind. The last two parameters were added to the formulation design with the forethought of suggesting the successful hydrogel vehicle to be used in hyperthermic intraoperative peritoneal chemotherapy (HIPEC), commonly practiced in multimodal therapy of peritoneal carcinomatosis. This treatment includes irrigation of the abdominal cavity with a heated cocktail solution of cytotoxic drugs over prolonged periods of time after the surgical removal of the malignant tissues (Nissan et al. 2009; Sugarbaker et al. 2006). An equally important goal was to employ a statistically based factorial design analysis for the rational design and optimization of the x-Ct, in order to identify possible interactions among the significant variables affecting the erosion-driven release rates of the two drugs.

2. Materials and methods

2.1. Materials

The three types of Ct, low molecular weight Ct (MW 50–190 kDa, 20–300 cps of 1% solution in 1% acetic acid), medium molecular weight Ct (MW 190–310 kDa, 200–800 cps of 1% solution in 1% acetic acid) and high molecular weight Ct (MW 310–375 kDa, 890–2000 cps of 1% solution in 1% acetic acid,) were purchased from Sigma, St. Louis, MO, USA. Citric acid (AR), desiccated calcium chloride, sodium hydroxide (CP), hydrochloric acid 37% (AR), acetic acid glacial (AR) and acetonitrile (HPLC grade) were purchased from Bio Lab, Jerusalem, Israel. Water was de-ionized and ultra-filtered by reverse osmosis (Barnstead Nanopure, Waltham, MA, USA).

2.2. Crosslinked Ct discs preparation, characterization and loading with indomethacin and sodium salicylate

In separate studies, three concentrations (2.4, 3.0 and 4.0% w/v) of the three types of Ct (low, medium and high molecular weights) were dissolved in 3% acetic acid and left overnight to remove air bubbles. The Ct solutions were then casted (850 μL) into Teflon molds (15 mm inner diameter). The molds were then dipped slowly in the crosslinker (citric acid, pH 7, denoted as CA) solutions (50, 125 and 200 mM) and left overnight at room temperature. The formed crosslinked Ct (x-Ct) discs were separated gently from the molds with the aid of a spatula and kept sealed in the crosslinking solutions until further use. The water content of the discs was measured gravimetrically by measuring the relative amount of water loss after drying (shelves oven, 80 °C, until a constant weight was achieved). Ten discs participated in each study.

The surface topography and cross-sections of lyophilized discs were monitored by scanning electron microscope (SEM, Quanta 200, Thermo Fischer Scientific). The TIF format of the images was analyzed by software previously described by Scaffaro and coworkers (Lo Re et al. 2015). The software segments each SEM microphotograph by determining pixel intensity and defining threshold values for regions of interest, thus screens pores by their dimensions. In this study the software was tuned to divide the SEM images into three intervals, respectively colored in blue (larger pores), green (intermediate pores) and red (smaller pores).

Mechanical characterization of the wet discs comprised of compression strength test (Material Testing Machine LF Plus, Lloyd, USA) at a cell load of 100 N and a crosshead speed of 1 mm/min, at room temperature. Each measurement was repeated 5 times. Young's modulus of elasticity (storage modulus), yield stress and yield strain were calculated from the stress- strain plots (Kai et al. 2012). Crosslinking density (ν_c) was calculated as follows (Hajighasem and Kabiri 2013):

$$\nu_c = G'/(RT) \quad \text{(I)}$$

where G' is Young's modulus of elasticity, R is the gas constant and T is the temperature.

The poorly water-soluble drug, Ind, and the highly water-soluble drug, SS, were loaded into the various types of the hydrated x-Ct discs by dissolving the SS and dispersing (30 min sonication) the Ind in 3% acetic acid prior to adding the Ct and its crosslinking to form the discs. Possible drug-drug and drug-platform (Ct) interactions were studied and negated. The former was examined by mass spectroscopy (see Fig. S1 in the supplementary section of the manuscript). The latter was examined by isothermal titration calorimetry and verified by differential scanning calorimetry (data not shown).

The equilibrium entrapment efficiency of Ind was determined by extracting the drug with 25 mL of 60% acetonitrile in water followed by a 30 min sonication. After an overnight soak of the ground disc, Ind concentration was analyzed by HPLC (Lichrospher 100 column RP-18, 5 μ, 25 cm × 4 mm, Merck, Germany RP, UV–Vis detector at 318 nm, mobile phase: acetonitrile: water 60:40, flow rate: 1 mL/min) (Haq et al. 2014). The entrapment efficiency of SS was determined by extracting the drug with 25 mL of water followed by a 30 min sonication. After an overnight soak of the ground disc, the SS concentration was analyzed by HPLC (Lichrospher 100 column RP-18, 5 μ, 25 cm × 4 mm, Merck, Darmstadt, Germany; UV–Vis detector at 309 nm; mobile phase: ammonium acetate buffer (pH 4.2): acetonitrile 80:20, flow rate 1 mL/min). Separate, eight point calibration curves were used for calculating the concentration of each drug from the peak areas of the HPLC chromatograms. Entrapment efficiencies (EE, in %) were calculated as follows (Agnihotri and Aminabhavi 2004):

$$EE = \frac{\text{Amount of drug found in the disc}}{\text{Amount of drug loaded into the disc}} \times 100 \quad \text{(II)}$$

2.3. Erosion and drug release kinetics studies

In separate studies, each type of x-Ct discs (made of 24, 30 and 40 mg/mL of each type of Ct: low, medium and high MW) loaded with both SS and Ind, was placed in a USP type I dissolution basket (Vankel VK 7010, equipped with 200 mL small volume round bottom glass beaker) which, in turn, was immersed in 20 mL of a de-crosslinking buffer solution (50, 125 or 200 mM of $CaCl_2$). The studies were conducted at temperatures of 37, 42 and 47 °C and at pH values of 5, 6 and