European Journal of Pharmaceutics and Biopharmaceutics 117 (2017) 141–150

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Microwave-assisted microemulsion technique for production of miconazole nitrate- and econazole nitrate-loaded solid lipid nanoparticles

Rohan M. Shah *, Daniel S. Eldridge, Enzo A. Palombo, Ian H. Harding *

Department of Chemistry and Biotechnology, Swinburne University of Technology, Melbourne, Australia

ARTICLE INFO

Article history:
Received 23 January 2016
Revised 23 November 2016
Accepted in revised form 10 April 2017
Available online 12 April 2017

Keywords:
Solid lipid nanoparticles
Microwave-assisted microemulsion
Ionic antifungal drugs
Econazole nitrate
Miconazole nitrate
Drug release
Release modelling
Anti-candidal activity
Cytotoxicity

ABSTRACT

The microwave-assisted production of solid lipid nanoparticles (SLNs) is a novel technique reported recently by our group. The small particle size, solid nature and use of physiologically well-tolerated lipid materials make SLNs an interesting and potentially efficacious drug carrier. The main purpose of this research work was to investigate the suitability of microwave-assisted microemulsion technique to encapsulate selected ionic drug substances such as miconazole nitrate and econazole nitrate.

The microwave-produced SLNs had a small size (250–300 nm), low polydispersity (<0.20), high encapsulation efficiency (72–87%) and loading capacity (3.6–4.3%). Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies suggested reduced crystallinity of stearic acid in SLNs. The release studies demonstrated a slow, sustained but incomplete release of drugs (<60% after 24 h) from microwave-produced SLNs. Data fitting of drug release data revealed that the release of both drugs from microwave-produced SLNs was governed by non-Fickian diffusion indicating that drug release was both diffusion- and dissolution- controlled.

Anti-fungal efficacy of drug-loaded SLNs was evaluated on *C. albicans*. The cell viability studies showed that cytotoxicity of SLNs was concentration-dependent. These encouraging results suggest that the microwave-assisted procedure is suitable for encapsulation of ionic drugs and that microwave-produced SLNs can act as potential carriers of antifungal drugs.

1. Introduction

The use of biocompatible (or perhaps more correctly called cell-compatible) [1] lipids has gained huge popularity in the scientific community as carriers for delivery of drugs with high potency, but currently suffer adverse side-effects and poor aqueous solubility [2]. Solid lipid nanoparticles (SLNs) made of biocompatible lipids have garnered an increased attention in the past years as an attractive alternative to those traditional lipid-based drug carriers. SLNs are produced from microemulsion templates by replacing the liquid lipid (oil) with a solid lipid (i.e. lipid that is solid at room and body temperature) or a mixture of solid lipids [3]. The solid core, lipid matrix and colloidal size range have highlighted the capability of SLNs to provide controlled-release of drugs, biocompatibility and improved bioavailability. In addition to these, specific benefits of SLNs has given impetus to significant advancements in their development as drug carriers [4] - (a) avoidance of organic solvents, (b) protection of encapsulated drugs from biological/chemical degradation, (c) improved drug stability, (d) ease of penetration through cell membranes, (e) potential of site-specific delivery and (f) feasibility of production scale-up.

Several approaches for the preparation of lipid nanoparticle dispersions have been reported since these carriers were first described in the early 1990s including high pressure homogenization [5–7], microemulsion [8], solvent evaporation [9] and more. The readers are directed to Shah, Eldridge, Palombo and Harding [3] for a detailed description of these techniques. Recently, our group reported a novel microwave-assisted single-pot microemulsion method for the synthesis of SLNs [10]. This technique has not yet been systematically explored and therefore, the literature available on this technique is limited.

The main objective of this research was to extend our understanding of the microwave-assisted method when incorporating with drugs possessing an ionic structure. Miconazole nitrate and

* Corresponding authors at: Department of Chemistry and Biotechnology, Faculty of Science, Engineering and Technology, Swinburne University of Technology, PO Box 218, Hawthorn, Victoria 3122, Australia (I.H. Harding).

E-mail addresses: rshah@swin.edu.au (R.M. Shah), iharding@swin.edu.au (I.H. Harding).

http://dx.doi.org/10.1016/j.ejpb.2017.04.007
0939-6411/

econazole nitrate (Table 1) were selected for this purpose. These drugs belong to the "azole" group of antifungal drugs and have a common mechanism of action. The fungistatic effect of azoles is due to their ability to interfere with the functioning of ergosterol. Azoles are known to inhibit fungal growth (fungistatic) and are fungicidal only at high concentrations [11].

Due to their lipophilic character, selection of miconazole nitrate and econazole nitrate as model "lipophilic" drugs seems appropriate. According to the Biopharmaceutical Classification System (BCS), miconazole nitrate and econazole nitrate are classified as BCS Class II (high permeability, low solubility) and BCS Class IV (low permeability, low solubility) drugs, respectively [12]. The lipid-based formulations are well suited for BCS Class II and BCS Class IV drugs [2]. In addition to this, they have short half-lives and are known to cause adverse effects such as local irritation, burning sensations, and erythema, rash and skin tenderness. Their incorporation in SLN delivery systems where a lower concentration (and thus less adverse side-affects) can be used without compromising efficiency is thus well worth pursuing.

The anti-fungal efficacy of microwave-produced SLNs loaded with selected azole drugs is evaluated, in this paper, on *Candida albicans* cells. *Candida* species, including *C. albicans*, are a part of commensal flora that colonize various sites in and on the body, including the skin, the gastrointestinal tract and reproductive tract. *C. albicans* can act as an opportunistic fungal pathogen during host immunosuppression and any alterations in the bacterial microbiota [13]. The progression of fungal infections is often rapid and considered to be serious due to compromisation of patient immunity [14]. The dissemination of *C. albicans* deteriorates the mucosal surfaces and serves as a source of future infections ranging from relatively trivial conditions such as oral and genital thrush to serious super infections, infertility and sterility [15,16]. *Candida* usually invade deeper tissues and blood in immunocompromised people and may lead to severe forms of candidiasis [17].

The encapsulated drug in SLNs can facilitate localised delivery and improve local availability by means of a controlled release pattern. Hence, we further investigated release kinetics of drugs from microwave-produced SLNs. The release profiles were fitted into various drug release models by means of mathematical modelling. Controlled release pattern of drugs from SLNs can advance the anti-fungal activity and skin tolerability of drugs. The research also demonstrates the suitability of microwave-produced SLNs when applied to epithelial cell lines. The development of microwave-produced SLNs in encapsulation of antifungal agents will further assist its development in topical and/or oral formulations and may have a significant advantage for its clinical application.

2. Experimental Section

2.1. Materials

Stearic acid was obtained from ICN Biomedicals Inc. (USA). Miconazole nitrate and econazole nitrate were purchased from Sigma-Aldrich (Australia). Tween® 20 was purchased from Merck (Australia). Ultra-purified water was obtained from a MilliQ® Plus purification system (Millipore, Germany) and all other chemicals and reagents were commercially available and of analytical grade.

Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Invitrogen Corp., Australia), fetal bovine serum (FBS), penicillin G sodium (10,000 units/mL) and streptomycin (10,000 μg/mL) were obtained from Invitrogen Technologies (Australia). Rhodamine 123, Dulbecco's phosphate buffered solution (PBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Australia). Dimethyl sulfoxide (DMSO) was purchased from Merck (Australia). CellMask™ Deep Red Plasma membrane stain and 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) were obtained from Molecular probes (USA).

2.2. Microwave-assisted preparation of solid lipid nanoparticles

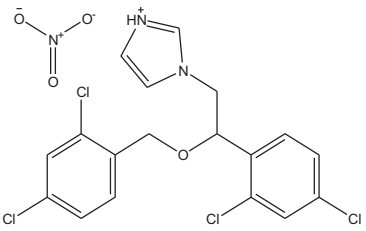
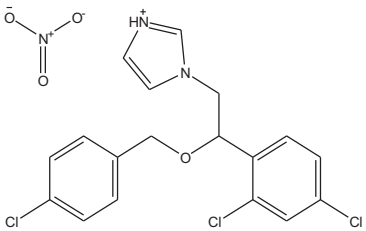
The SLNs were prepared by the microwave-assisted microemulsion technique described in our previous study [10]. Briefly, stearic acid (100 mg), Tween® 20 (150 μl) and water (1.35 mL) were heated at 80 °C with variable microwave power (not exceeding 18 W) for 10 min in a microwave reactor tube with constant stirring using a 2.45 GHz Discover LabMate microwave synthesizer (CEM Corp., USA). This process constitutes a single-pot synthesis of the o/w microemulsion. Miconazole nitrate or econazole nitrate (5 mg) was added to other formulation ingredients in the reactor tube before subjecting them to microwave heating to produce drug-loaded SLNs. On completion of the microemulsion synthesis, the hot o/w microemulsion was dispersed immediately into cold water (50 mL, 2–4 °C) under constant magnetic stirring to generate SLN dispersions.

2.3. Particle characterisation

2.3.1. Measurement of particle size, polydispersity index (PI) and zeta potential

The mean hydrodynamic diameter and polydispersity index (PI) were determined by dynamic light scattering (DLS) using a 90Plus Particle Size Analyzer (Brookhaven Inst., USA). The instrument uses a 35 mW red diode laser (λ = 659 nm) with a photodetector at 90°

Table 1
Physicochemical properties of antifungal drugs [46–48] used as model drugs in this study.

Physicochemical properties	Miconazole nitrate	Econazole nitrate
Structure		
Chemical name	1-[2-(2, 4-dichlorophenyl)-2-[(2, 4-dichlorophenyl) methoxy] ethyl]-1H-imidazole, mononitrate	1-[2-(4-chlorophenyl) methoxy]-2-[(2, 4-dichlorophenyl) ethyl]-1H-imidazole, mononitrate
Molecular weight	479.1 g mol^{-1}	444.7 g mol^{-1}
pK*a*	6.70	6.65
log P	6.25	5.20
Aqueous solubility	100 μg/mL	800 μg/mL