International Journal of Pharmaceutics 504 (2016) 98–109

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Polymeric microcontainers improve oral bioavailability of furosemide

Line Hagner Nielsen[a,*], Ana Melero[b], Stephan Sylvest Keller[a], Jette Jacobsen[c], Teresa Garrigues[b], Thomas Rades[c], Anette Müllertz[c,d], Anja Boisen[a]

[a] Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsteds Plads 345B, 2800 Kongens Lyngby, Denmark
[b] Department of Pharmacy and Pharmaceutical Technology, University of Valencia, Valencia, Spain
[c] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen, Denmark
[d] Bioneer:FARMA, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 25 March 2016
Accepted 26 March 2016
Available online 28 March 2016

Keywords:
Micro devices
Furosemide
Oral delivery
Delivery systems
Oral bioavailability
Intestinal perfusion

ABSTRACT

Microcontainers with an inner diameter of 223 μm are fabricated using the polymer SU-8, and evaluated *in vitro*,*in situ* and *in vivo* for their application as an advanced oral drug delivery system for the poorly water soluble drug furosemide. An amorphous sodium salt of furosemide (ASSF) is filled into the microcontainers followed by applying a lid using Eudragit L100. It is possible to control the drug release *in vitro*, and *in vitro* absorption studies show that the microcontainers are not a hindrance for absorption of ASSF. *In situ* perfusion studies in rats are performed with ASSF-filled microcontainers coated with Eudragit and compared to a furosemide solution. The absorption rate constant of ASSF confined in microcontainers is found to be significantly different from the solution, and by light microscopy, it is observed that the microcontainers are engulfed by the intestinal mucus. An oral bioavailability study in rats is performed with ASSF confined in microcontainers coated with Eudragit and a control group with ASSF in Eudragit-coated capsules. A relative bioavailability of 220% for the ASSF in microcontainers compared to ASSF in capsules is found. These studies indicate that the microcontainers could serve as a promising oral drug delivery system.

1. Introduction

Oral pharmaceutical products represent approximately 70% of the value of the US pharmaceutical market, and oral delivery has for many decades been the preferred administration route for drugs, and continues to be so (Colombo et al., 2009; Perioli et al., 2012). However, there is a tendency for new drug compounds currently in the pipelines of the pharmaceutical industry to have a poor solubility in water and maybe even a low intestinal permeability, meaning that they are classified as class II or IV in the Biopharmaceutics Classification System (BCS) (Agrawal et al., 2014; Amidon et al., 1995; Lipinski, 2001). This trend complicates utilising the oral route for drug delivery purposes. Consequently, many approaches to overcome these obstacles, such as the use of lipid based drug delivery systems, permeation enhancers and nanoparticles, have been suggested (Agrawal et al., 2014; Colombo et al., 2009; Ensign et al., 2012). Sometimes, it can even be necessary to employ more advanced drug delivery systems allowing the potential for targeted and/or sustained delivery in the gastro-intestinal (GI) tract. Advanced drug delivery devices may have the potential to make the treatments more safe and efficient along with more convenient (Ensign et al., 2012). Micro fabricated drug delivery devices have been proposed as advanced drug delivery systems being able to increase the oral bioavailability of drugs (Chirra and Desai, 2012). Of these micro devices, microcontainers have been suggested as promising new advanced oral drug delivery systems (Chirra et al., 2014a, 2014b). Primarily, this is due to the fact that the size and shape of the microcontainers can be controlled very precisely whereby polydispersity, as seen e.g. for many micro- and nanoparticles, is avoided (Randall et al., 2007). Microcontainers are polymeric, cylindrical devices in the micrometre size range (Fig. 1) (Nielsen et al., 2014, 2012). A major advantage is that these devices allow for unidirectional release, as only one side of the microcontainer is open, compared to more conventional microparticles where release can occur from the whole surface area (Eaimtrakarn et al., 2001; van Hoogevest et al., 2011). Moreover, the drug can be protected inside the cavity of the microcontainer from the harsh environment of the stomach until release is desirable e.g. in the small intestine (where most drugs are absorbed) (Ahmed et al., 2002; Ainslie et al., 2009; Eaimtrakarn et al., 2001; Tao et al., 2003). A hydrogel with acyclovir entrapped inside micro reservoirs, thereby facilitating unidirectional release

* Corresponding author.
E-mail address: lihan@nanotech.dtu.dk (L.H. Nielsen).

http://dx.doi.org/10.1016/j.ijpharm.2016.03.050
0378-5173/

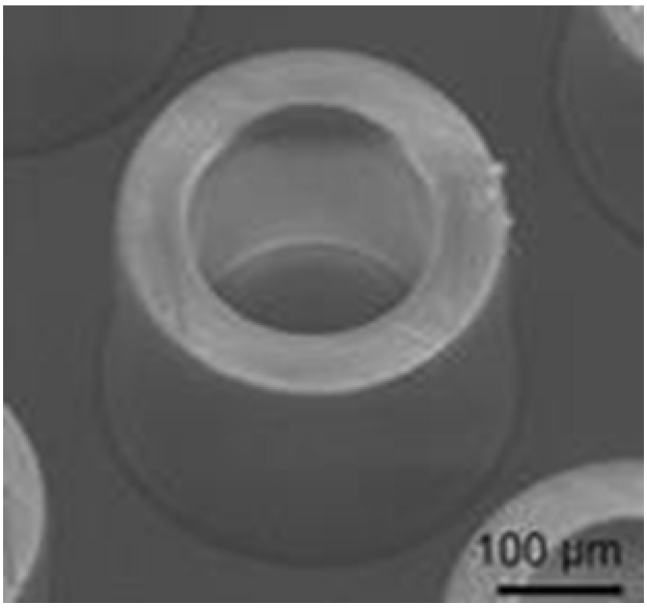

Fig. 1. Scanning electron microscope (SEM) image of a microcontainer made of SU-8.

of the drug, has previously been described. The unidirectional release resulted in a high drug concentration at the micro reservoir-epithelial cell interface and allowed for increased *in vitro* permeation of acyclovir across a Caco-2 cell monolayer. Furthermore, the micro reservoirs led to increased oral bioavailability of acyclovir in mice (Chirra et al., 2014a, 2014b). Other studies have also demonstrated that the unidirectional release of drug from a micro reservoir delivery system resulted in an increased local concentration of the drug in close proximity of the targeted epithelium (Ahmed et al., 2002; Ainslie et al., 2009). The materials for fabrication of drug delivery systems can vary from well-known polymers such as poly-L-lactic acid (PLLA) (Nielsen et al., 2015) and poly(lactic-co-glycolic acid) (PLGA) (García-Díaz et al., 2015; Randall et al., 2007) to naturally occurring protein polymers such as silk fibroin (Wenk et al., 2011). SU-8 is an epoxy based polymer, and is reported suitable as implant material and also as a biocompatible polymer for biomolecular encapsulation (Nemani et al., 2013). In this paper, SU-8 was used as a model polymer for fabrication of the microcontainers, as it has been described in previous research (Ahmed et al., 2002; Nielsen et al., 2014, 2012).

There are two crucial steps for successful delivery of orally administered drugs; drug dissolution and drug absorption (Avdeef and Tsinman, 2008). Early prediction of the intestinal absorption of a compound is essential for further development, making the ability of *in vitro* methods to provide this information extremely important. Cell culture models are one of many methods used to study intestinal absorption (Patel et al., 2006; Pretorius and Bouic, 2009). The Caco-2 cell line is an epithelial cell line originating from a human colonic adenocarcinoma, which represents many of the characteristics and functions found in the epithelium of the small intestine (tight junctions, microvilli, growth factor receptors, and major drug metabolizing enzymes) (Berginc et al., 2012; Hidalgo et al., 1989; Hilgers et al., 1990). The Caco-2 cell model has become a useful *in vitro* tool to predict permeability and absorption of drugs across the small intestinal membrane, and is a valuable set-up to be employed during the development of oral drug delivery systems (Hidalgo et al., 1989). Even though, Caco-2 cells can be a good *in vitro* model for permeation, it lacks the mucus layer, covering the epithelial linings. The mucus layer is two layered; closest to the epithelial cell surface is a firmly adherent mucus layer and on top of that, a loosely transient adherent mucus layer. Both layers vary in thicknesses, ranging from 16 to 29 μm for the firmly adherent layer throughout the small intestine, whereas the loosely adherent layer is reported to have a thickness of varying from 123 to 480 μm in the small intestine (Ensign et al., 2012). The main role of the mucus layer is to protect and lubricate the epithelial lining, and for drugs, the mucus layer may be a physical barrier to absorption. The mucus has variable turnover time in the GI tract, and the turnover time in the intestine is reported not to be longer than 2 h (Crater and Carrier, 2010; Ensign et al., 2012; Lehr et al., 1991a, 1991b; Lehr, 2000). For being able to study the interaction of the drug delivery system with the intestinal membrane and also the mucus layer, *in situ* intestinal perfusion studies are ideal (Lennernas, 1998; Lennernäs, 2014; Lozoya-Agullo et al., 2015; Song et al., 2013). The single-pass intestinal perfusion model and closed-loop rat perfusion are both equally useful for obtaining information about drug absorption and interaction between the intestinal membrane and a drug delivery system. However, the closed-loop model has the advantage of measuring the absorption across a large part of the small intestine (Doluisio et al., 1969; Lozoya-Agullo et al., 2015). Absorption data from intestinal perfusion in rats are found to correlate well with human absorption data (Fagerholm et al., 1996; Lozoya-Agullo et al., 2015).

In the current study, the amorphous sodium salt of furosemide (ASSF) was utilised as a model drug. Furosemide is a class IV compound in BCS. Hence, it has a poor aqueous solubility and a low intestinal permeability. Furosemide is a weak acid with pK_a values of 9.9 and 3.5 (Matsuda et al., 1990), therefore it is possible to utilise the salt form of the drug. ASSF has previously been shown to significantly improve the solubility and dissolution rate of furosemide when compared to the drug in the commonly available crystalline acid form (Nielsen et al., 2013a). The poor intestinal absorption of furosemide is not only influenced by a low solubility, but is further complicated by the occurrence of site-specific absorption, partly in the stomach, but especially in the upper part of the small intestine, leading to a considerable inter- and intra-individual variation in oral drug bioavailability (20–60%) (Granero et al., 2010; Iannuccelli et al., 2000). The intestinal absorption of furosemide is reported to be highly influenced by the dosage form (Granero et al., 2010), and therefore, there is a further need to improve furosemide absorption and specifically reduce the variation in bioavailability. This task could be accomplished through the use of an advanced drug delivery system such as microcontainers. A previous study showed that microwells fabricated with the biopolymer poly-L-lactic acid (PLLA), filled with ASSF, and coated with the pH-sensitive polymer Eudragit L100 made it possible to control the release of ASSF (Nielsen et al., 2015).

The aim of the current study was to evaluate microcontainers *in vitro*, *in situ* and *in vivo* as a potential oral drug delivery system for a poorly water-soluble drug (Fig. 2). The release and absorption of ASSF confined in microcontainers were investigated using Caco-2 cells. Furthermore, intestinal perfusion studies in rats were carried out to explore any interaction between the microcontainers and the small intestinal mucus. Finally, the oral bioavailability of ASSF in rats was determined with and without confinement in microcontainers.

2. Materials and methods

2.1. Materials

Furosemide (>98% purity) was purchased from Fagron Nordic (Copenhagen, Denmark), while taurocholic acid sodium salt hydrate (sodium taurocholate), 2-(*N*-morpholino)ethane sulfonic