Journal of Pharmaceutical and Biomedical Analysis 131 (2016) 156–159

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

# In vitro and in silico investigation of electrospun terbinafine hydrochloride-loaded buccal nanofibrous sheets

Péter Szabó[a,b], Tünde Beáta Daróczi[a], Gergő Tóth[c], Romána Zelkó[b,*]

[a] Gedeon Richter Plc., Formulation R&D, Gyömrői Str. 19-21, H-1103 Budapest, Hungary
[b] University Pharmacy Department of Pharmacy Administration, Semmelweis University, Hőgyes Endre Str. 7-9, H-1092 Budapest, Hungary
[c] Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre Str. 7, H-1092 Budapest, Hungary

ARTICLE INFO

*Article history:*
Received 2 August 2016
Received in revised form 15 August 2016
Accepted 25 August 2016
Available online 26 August 2016

*Keywords:*
Electrospinning
Terbinafine hydrochloride
Buccal fibrous sheet
Nanofiber
In vitro drug release
In silico absorption modeling

ABSTRACT

Terbinafine hydrochloride-loaded nanofibrous buccal films were formulated with the aim to improve the solubility and dissolution behavior; thus, the local effectiveness of the antifungal agent. Poly(vinyl alcohol) and chitosan polymer composites were selected as delivery base in order to enhance the mucoadhesion of the fibrous films. The dissolution of terbinafine hydrochloride was carried out applying a stainless steel disc assembly and the terbinafine concentration was determined by HPLC–MS in selective ion monitoring mode. The prediction of the absorption behavior of the prepared fibrous samples in the human oral cavity was modeled using GastroPlus™ software. The result indicates that the fibrous films enabled fast and complete dissolution of the active agent. The drug absorption from the oral cavity could be minimized by the employment of the proper oral transit model. Because of the limited absorption of terbinafine hydrochloride from the oral mucosa the formulation can be beneficial in local administration in the case of hold and expectorate administration mode.



## 1. Introduction

Nanofibrous systems are deemed to be popular in pharmaceutical research, because of their numerous benefits, including high surface area, porosity, and ability to incorporate actives in amorphous state [1,2]. The most favored technique for fiber preparation is electrospinning, where fibers are formed in an electric field. Besides their biological applications, e.g. tissue engineering, the optimization of certain physicochemical purposes, namely solubility and dissolution characteristics has emerged as a promising area of interest [3–6]. However, drug-containing films represent popular dosage form for local oral administration, numerous papers available in the scientific literature, which have demonstrated the benefits of fiber-based formulations over films. The most conspicuous advantages of fibrous sheets are as follows: faster and complete drug release, enhanced in vitro permeation and improved activity of biological actives [7–11].

Nowadays, a huge number of pharmaceutically active ingredients possess unfavourable physicochemical characteristics, and a significant proportion of them is affected by poor solubility. The latter can be an obstacle for the administration of the drug. An example of such a drug is terbinafine that is used for the treatment of fungal infections. Terbinafine hydrochloride has a hydrophobic nature, and is slightly soluble in aqueous media [12,13].

Fungal infections of oral cavity are quite common in patients with immunodeficiency and in compromised people. Although, a wide range of antifungal agents are available, the outcome of the treatment does not meet the expectations in every case. This therapeutic failure can be traced back to the exceptional features of the oral cavity, such as saliva flush effect [14]. Oral candidiasis is one of the most important opportunistic infections [15].

Several polymers and combination of polymeric excipients were proved suitable for drug loaded fiber formation. Poly(vinylpyrrolidone), poly(vinyl alcohol), poly(lactic acid) are used as much frequently as polymers of natural sources, such as chitosan or alginic acid [16]. Chitosan is a semisynthetic polymer and a derivative of chitin, a natural polymer occurring in the cell walls of fungi and in the exoskeleton of different animals, such as arthropods. Chitosan can adhere to negatively charged surfaces, since it is a cationic polymer at above pH 6.5 [17]. This adhesion behavior can be exploited in the formulation of mucoadhesive dosage forms based on the prosperous interaction between the negatively charged salivary mucus and chitosan [18,19]. Chitosan per se is not a suitable polymer for electrospinning, but its combination with other polymers can enhance its spinnability [3]. Different additives, such as plasticizers, surfactants can modify the surface properties

* Corresponding author.
*E-mail address:* zelko.romana@pharma.semmelweis-univ.hu (R. Zelkó).

http://dx.doi.org/10.1016/j.jpba.2016.08.021
0731-7085/

of chitosan based films, by the alteration of the molecular arrangement of the polymeric fibers [20].

Terbinafine is a synthetic allylamine antifungal agent, which exerts its antimicrobial activity by inhibiting ergosterol biosynthesis [21]. The drug is active both orally, and topically and effective for the treatment of various fungal infections. Candida species are also among the fungi against which terbinafine is potent [22,23]. Oral candidiasis is a common infection in compromised patients, e.g. in HIV-positive individuals. Antiretroviral therapy compromises a combination of drugs with known hepatotoxicity [24]. Accordingly, avoiding systemic exposure to any drugs with potential hepatotoxicity can be favorable. Azole antifungal drugs and terbinafine were also reported to cause hepatic injury [25–28]. Azole antifungals are considered as the first-line choice for the treatment of mucocutaneous candidiasis. On the other hand repeating relapses pose threat to the successful eradication of the fungi, therefore terbinafine could be a substantial alternative in order to avoid resistance [29]. The primary aim of this study is the development of a bioadhesive mucosal drug delivery system exploiting the benefits of fiber based formulations for local antifungal therapy. On the other hand, the authors intended to predict the absorbed amount of the released drug using GastroPlus™ software.

## 2. Materials and methods

### 2.1. Materials

Chitosan (degree of deacetylation: 85%, $M_w$ 50–100 kDa, 85/10 Heppe Medical Chitosan, Germany), poly(vinyl alcohol) (18-88 Ph. Eur., Merck, Darmstadt, Germany) was used in the fiber formation process. Terbinafine hydrochloride (Gedeon Richter Plc, Hungary, Ph. Eur.) was selected as a poorly water soluble drug. In the preparation of drug stock solution, distilled water and polysorbate 60 and glacial acetic acid (Ph. Eur., Molar Chemicals, Hungary) were applied. Potassium dihydrogen phosphate (Ph. Eur., Molar Chemicals, Hungary), sodium hydroxide (Ph. Eur., Molar Chemicals, Hungary) and distilled water were applied in the preparation of the dissolution media. Methanol was obtained from Merck.

### 2.2. Preparation of terbinafine hydrochloride gels

For the preparation of terbinafine hydrochloride stock solution 0.20 g drug was suspended with 1.30 g of polysorbate 60 in a beaker applying a gentle heat. After all, approximately the half of the necessary amount of 2% V/V acetic acid was added to the mixture. The mixture was heated and stirred until a clear solution was formed. Finally, the solution was diluted to 20.00 g with the remaining part of the solvent.

For the gels, 0.40 g of chitosan and 2.00 g of polyvinyl alcohol were homogenized in a 100 ml Schott flask, then 17.60 g of 10 mg/g drug stock solution was added. The mixture was stirred at 80 °C for 2 h until a homogenous system was formed. The gels were cooled to 25 °C and were transferred into a 20 ml plastic syringe for the fiber formation.

### 2.3. Electrospinning

Drug loaded nanofibrous sheets were prepared by electrospinning technique. The applied parameters were as follows: flow rate of 1.0 ml/h (using an Alaris GH infusion pump). The syringe was connected to a 100 Sterican injection needle (metal, 0.90 × 70 mm) through a silicon tube. Fibers were collected after a 15 cm flight on a grounded square static sheet of 625 $cm^2$ in area covered with an aluminum foil. Sheets with uniform diameter and thickness were obtained during 10 min continuous operation of the electrospinning apparatus. The applied voltage was 25 kV.

### 2.4. Morphology study

Morphological evaluation of electrospun fibers was carried out using a scanning electron microscope (JEOL-5610-LV, Tokyo, Japan) after gold coating. The applied accelerating high voltage and working distance were 10 kV and 10–12 mm, respectively. The average diameters of fibers were measured using 239 different randomly selected individual filaments with JSM-5000 (JEOL, Tokyo, Japan) software.

### 2.5. Dissolution test

Dissolution tests of the prepared terbinafine hydrochloride loaded fibrous films were carried out in a Hanson SR8-Plus (Hanson Research, Chatsworth, USA) type dissolution tester using a stainless steel disk assembly according to Ph. Eur. 8. Approximately 18.0 mg of fibrous sample was applied. The temperature of the dissolution fluid was 37 ± 1 °C and the rotation speed was 50 rpm, using rotating paddles and 200 ml of phosphate buffer (pH 6.8, 0.05 M, Ph. Eur. 8). 3.00 ml of samples were taken at predetermined time points using a Biohit Proline 5.00 ml (Biohit OY, Helsinki, Finland) pipette. The terbinafine concentration was determined by HPLC–MS in selective ion monitoring (SIM) mode. HPLC analysis was performed by an Agilent 1260 Infinity LC system in conjunction with an Agilent 6460 triple-quadrupole mass spectrometer (Agilent, Waldbroon, Germany). Chromatography was carried out using a Zorbax Eclipse Plus C18 (4.6 × 100 mm, 3.6 μm) column with a mobile phase of methanol/0.1% acetic acid (50/50 V/V), delivered with 0.7 ml/min flow rate at 30 °C. The mass spectrometer was operated in conjunction with a JetStream electrospray ion source in positive ion mode. MS was set to monitor in SIM mode at $m/z$ 292 $[M+H]^+$. Optimized parameters were the following: fragmentor voltage 125 V, dwell time 200 ms, delta EMV 30 V. Flow and temperature of the drying gas ($N_2$) in the ion source: 10 l/min and 300 °C, pressure of the nebulizer gas ($N_2$): 45 psi, capillary voltage: 3000 V, sheath gas flow and temperature: 11 l/min and 300 °C. Mass spectra were processed using Agilent MassHunter B.04.00 software. The HPLC–MS method was validated according to the ICH guideline Q2 (R1) [30]. Calibration curves were prepared using six concentrations between 2 and 1000 ng/ml terbinafine hydrochloride. Calibration curves were constructed by least-square linear regression analysis with uniform weighting. Intra- and inter-day relative standard deviation (low, mid and high concentrations of the standards in three parallel runs on the same day and on three successive days, respectively) was less than 3.65% and 6.35%, respectively. Three parallel measurements were performed for both the assay and the dissolution study.

### 2.6. In silico absorption assessment

Prediction of probable behavior of the prepared fibrous samples in the human oral cavity was carried out using GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA). The model for the absorption simulation from the oral mucosa was validated on the basis of the results of a previously performed bioequivalence study conducted by Gedeon Richter Plc with the following initial parameters: $AUC_{0\text{-}t}$: 4210.3 ngh/ml, $c_{max}$: 844 ng/ml, $t_{max}$: 1.33 h. Mean plasma concentrations and coefficient of variations were related to the oral administration of a tablet containing 281.25 mg terbinafine hydrochloride. The pharmacokinetic parameters (clearance: 43.78 l/h, biological half-life: 25.8 h, and compartment rate constants: $k_{12}$: 0.12965 1/h, $k_{21}$: 0.03996 1/h) were calculated employing PKPlus module™. The correlation coefficient between the observed and estimated values was