Mechanism of Chemical Degradation and Determination of Solubility by Kinetic Modeling of the Highly Unstable Sesquiterpene Lactone Nobilin in Different Media

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ABSTRACT: The objective of this work was first to investigate the chemical degradation of the sesquiterpene lactone nobilin and determine its solubility under conditions of concurrent degradation for partially amorphous starting material; second, to determine the effect of biorelevant media used in the *in vitro* measurement of intestinal absorption on degradation and solubility of nobilin. Purely aqueous medium (aq-TM_{Caco}), fasted and fed state simulated intestinal fluid (FaSSIF-TM_{Caco} and FeSSIF-TM_{Caco}), and two liposomal formulations ($Liposomes_{FaSSIF}$ and $Liposomes_{FeSSIF}$) with the same lipid concentration as FaSSIF-TM_{Caco} and FeSSIF-TM_{Caco} were used. Degradation products were identified by nuclear magnetic resonance and X-ray crystallography and the order of reaction kinetics was determined. Solubility was deduced with a mathematical model encompassing dissolution, degradation, and reprecipitation kinetics that took into account particle size distribution of the solid material. Degradation mechanism of nobilin involved water-catalyzed opening of the lactone ring and transannular cyclization resulting in five degradation products. Degradation followed first-order kinetics in aq-TM_{Caco} and FaSSIF-TM_{Caco}, and higher-order kinetics in FeSSIF-TM_{Caco} and the two liposomal formulations, whereas degradation in the latter media was diminished. Solubility of nobilin increased in the order: aq-TM_{Caco} < FaSSIF-TM_{Caco}, < $Liposomes_{FaSSIF}$ < FeSSIF-TM_{Caco} < $Liposomes_{FeSSIF}$. Improvement of stability and solubility was consistent with the incorporation of the nobilin molecule into colloidal lipid particles. The developed kinetic model is proposed to be a useful tool for deducing solubility under dynamic conditions. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3139–3152, 2014

Keywords: chemical stability; solubility; degradation products; natural products; mathematical model; NMR spectroscopy; kinetics; particle size; food effects

INTRODUCTION

Physicochemical properties such as stability and solubility play an important role in the absorption process of a drug. Therefore, these properties are an integral part of drug profiling programs. Factors that potentially influence stability and solubility in the gastrointestinal tract are the aqueous environment, a pH range from 1 to 8, and substances such as bile salts, phospholipids, digestive enzymes, and lipolysis products.[1,2] The biopharmaceutics classification scheme classifies compounds with respect to intestinal absorption based on their intestinal permeability and their solubility,[3] whereby solubility of the largest dose in 250 mL or less of aqueous media over the pH range of 1–7.5 defines a highly soluble compound.[4] Several drug substances reportedly do not fulfill this requirement including danazol, mefenamic acid, ketoconazole, glyburide, troglitazone, atovaquone, sanfetrinem cilexetil, dantrolene, indinavir, saquinavir, sirolimus, albendazole, 9-nitrocamptothecin, and curcumin.[5–20] Issues of low solubility of a compound are frequently accompanied by poor stability. Hence, chemical instability in the gastrointestinal tract has been reported to compromise absorption of compounds such as curcumin or sirolimus.[13,14] Further examples include penicillin G with a degradation half-life of 7 min that is increased to 15–20 min for amoxicillin. Penicillins show pH-dependent degradation and solubility with a maximum stability and lowest solubility at pH 6–7.[21,22] Other examples of pH-dependent stability and insufficient solubility are prodrugs of 9-β-D-arabinofuranosyladenine against herpes viruses, different HIV protease inhibitors such as 2′,3′-dideoxypurine nucleosides, carbovir, and oxathiin carboxanilide.[23–26]

In recent years, the content of the gastrointestinal tract was considered in connection with its effect on absorption. The bioavailability of the poorly water-soluble drug danazol, for example, was found to be increased by food, whereas its solubility was increased by micelles of bile salts, phospholipids, and lipolysis products.[9,10,15,19] Food effect has become the subject of a guidance for the industry issued by the US FDA.[27] Therefore, several attempts have been made to develop biorelevant media for *in vitro* study to simulate the conditions in the intestine in fasted and fed state.[1,5,6,15,28,29] A quite good correlation between *in vivo* bioavailability and *in vitro* dissolution was

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seen.[6–8,15–17,28,30] Recently, biorelevant transport media for *in vitro* absorption studies with the Caco-2 cell model simulating intestinal fluid in the fasted and the fed state, FaSSIF-TM_{Caco} and FeSSIF-TM_{Caco}, respectively, were developed.[11] The influence of these media on solubility and stability is essential for their role in determining drug absorption.

Solubility is commonly determined experimentally by adding drug in excess to a medium and directly measuring concentration of dissolved drug.[31] This methodology has also been used in the literature for determining solubility of chemically unstable compounds, whereas sampling was performed typically at a predefined time point.[18,21,32–34] The behavior of crystalline and amorphous drugs with respect to reaching equilibrium has been further addressed.[31] No method to determine solubility taking into account chemical degradation and degree of crystallinity, however, has been reported to the best of the authors' knowledge.

Nobilin, a sesquiterpene lactone of the germacranolide type and a marker compound in the herbal drug of *Anthemis nobilis* L. flowers, was used in this study.[35] No reports about the chemical stability or the solubility of this compound exist in the literature. Nobilin is used as representative of its class of natural compounds that are known for their chemical instability but their intestinal absorption has been scarcely investigated.[36] The aim of this work was to study the mechanism and the kinetics of degradation of nobilin and identify its degradation products and, further, to determine its solubility under dynamic conditions of concurrent dissolution and rapid chemical degradation taking into consideration crystallinity of the starting material. To accomplish the latter goal, the use of a kinetic model that included dissolution rate as a function of particle size distribution of the starting material is proposed. Moreover, the effect of media simulating the environment of the intestinal tract and of vehicles used in *in vitro* Caco-2 absorption studies on the stability and solubility of nobilin was investigated. The ultimate goal was to derive a basic understanding of the role of the media on stability and solubility and the potential consequence of food and formulation for the bioavailability of nobilin.

MATERIALS AND METHODS

Chemicals

D-Glucose, L-glutamine, 4-(2-hydroxyethyl)piperazine-1-ethanesulonic acid (HEPES), sodium chloride (NaCl), sodium hydroxide (NaOH), maleic acid, dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium base powder (without D-glucose, L-glutamine, phenol red, sodium pyruvate, and sodium bicarbonate),[37] and sodium oleate were purchased from Sigma–Aldrich Chemie GmbH (Buchs, Switzerland). Lipoid E PC S and Lipoid S 100 were kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Glycerol mono-oleate was purchased from Danisco (Copenhagen, Denmark) and sodium taurocholate from Prodotti Chimici e di Alimenttari s. p. a. (Basaluzzo, Italy). Methanol (MeOH) and ethanol (EtOH) from J.T. Baker (Deventer, The Netherlands) and sodium acetate and formic acid from Sigma–Aldrich Chemie GmbH were of HPLC grade. Water was purified by reversed osmosis with the water purification system Arium® 61215 from Sartorius (Goettingen, Germany). CD_3OD was purchased from Armar Chemicals (Döttingen, Switzerland).

Purification of Nobilin

Nobilin was kindly provided by Alpinia Laudanum Institute of Phytopharmaceutical Sciences AG (ALIPS) (Walenstadt, Switzerland). It was purified from an ethanolic extract of *Chamomillae romanae flos* (Hänseler AG, Herisau, Switzerland) based on the description of Fischer in Methods of Plant Biochemistry.[38] The received precipitate was further purified by medium pressure liquid chromatography (MPLC, Büchi equipped with a pump manager C-615, a pump module C-605, a variable wave length detector C-635, and a fraction collector C-660). The precipitate was dissolved in EtOH, centrifuged (5810R; Eppendorf AG, Hamburg, Germany), and the supernatant was injected on a Büchi glass column C690 36 × 460 mm packed with silica Polygoprep 100-50 C18 (Macherey Nagel, Oensingen, Switzerland). The mobile phase was MeOH–water (60:40) at flow rate of 40 mL/min under isocratic conditions and the detection wavelength was 218 nm. The nobilin-containing fractions were collected and combined, MeOH was evaporated, water was removed by lyophilization (Labconco, Freezone 2.5 L; VWR, Dietikon, Switzerland), and nobilin was obtained as a yellowish white powder. EtOH for purification was purchased from Alcosuisse (Bern, Switzerland) and all other substances were obtained from Sigma–Aldrich Chemie GmbH. The purity of nobilin was between 86% and 98% as determined by HPLC (see below), the main impurity being the *cis* isomer in the angeloyl rest.

Isolation of Degradation Products

A 1 mg/mL solution of nobilin in EtOH was added to purified water to final concentration of 0.05 mg/mL and stirred for one day at 37°C. The aqueous solution was lyophilized, the resulting white powder dissolved in MeOH–H_2O (70:30) and the degradation products isolated with preparative HPLC–UV (Varian ProStar equipped with a 215 binary pump, a 410 autosampler, a variable wavelength detector, a 701 fraction collector model) using a C-18 reversed-phase column (Reprosil 100 C18, 7 μm, 250 × 20 mm ID; Dr. Maisch HPLC GmbH, Ammerbuch, Germany). The mobile phase consisted of (A) water with 0.1% (v/v) formic acid and (B) MeOH with 0.1% (v/v) formic acid run in gradient mode: 0 min 50:50 (A:B), 0–25 min linear change to 10:90 (A:B), 25–35 min 10:90 (A:B) with a flow rate of 25 mL/min and a runtime of 35 min. Nobilin and degradation products were detected at 220 nm. Fractions were collected every 10 s and those containing nobilin or degradation products were pooled and lyophilized (Alpha 2-4 LD; Crist, Osterode am Harz, Germany). For the degradation experiment, nobilin was previously subjected to an additional purification step using the above preparative method that provided a purity of more than 98%.

Vehicles

Media

The three different transport media aq-TM_{Caco} (purely aqueous medium), FaSSIF-TM_{Caco}, and FeSSIF-TM_{Caco} that are compatible with the Caco-2 cell line were described by Markopoulos et al.[11]

Dulbecco's modified Eagle's medium base powder was dissolved in autoclaved water and supplemented with D-glucose, L-glutamine, NaCl (aq-TM_{Caco}), and HEPES (aq-TM_{Caco}) or maleic acid (FaSSIF-TM_{Caco} and FeSSIF-TM_{Caco}). After adjusting the pH with NaOH and sterile filtration (Supor-200, 0.2 μm

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