Journal of Pharmaceutical Sciences xxx (2017) 1-10

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Using Potentiometric Free Drug Sensors to Determine the Free Concentration of Ionizable Drugs in Colloidal Systems

Thuy Tran [1], Anjan Chakraborty [1], Xi Xi [1], Hugo Bohets [2], Claus Cornett [1], Konstantin Tsinman [3], Thomas Rades [1], Anette Müllertz [1, 4, *]

[1] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark
[2] Octens, Drie Eikenstraat 661, 2650 Edegem, Belgium
[3] Pion Inc., Cook Street 10, Billerica, Massachusetts 01821
[4] Bioneer:FARMA, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 25 February 2017
Revised 10 May 2017
Accepted 11 May 2017

Keywords:
analytical chemistry
colloid
micelles
surfactants
liposomes
emulsions
lipids
in vitro models
NMR spectroscopy
dissolution

ABSTRACT

The present study investigates the use of free drug sensors (FDS) to measure free ionized drug concentrations in colloidal systems, including micellar solutions, emulsions, and lipid formulations during *in vitro* lipolysis. Diphenhydramine hydrochloride (DPH) and loperamide hydrochloride (LOP) were selected as model drugs. Self-diffusion nuclear magnetic resonance studies were performed and confirmed the entrapment of drugs in micelles in Brij 35 and sodium taurodeoxycholate (TDC)/phosphatidylcholine (PC) micellar solutions. The FDS measurements indicated that with a constant level of drug, the percentage of free DPH and LOP decreased from 84% to 57% and from 51% to 18%, respectively, as the concentration of Brij 35 was increased from 4.7 to 22 mM; and from 99% to 46% and from 100% to 21%, respectively, as the concentration of TDC/PC was increased from 0.49/0.04 to 8.85/0.78 mM. During the *in vitro* lipolysis of a lipid formulation, free drug concentration decreased with lipolysis time. The percentage of free DPH was higher than for LOP in the same colloidal system because DPH is less lipophilic than LOP. The study showed that FDS can be used to monitor the free drug concentration in colloidal systems with fast response, no sample treatment and simple data analysis.

Introduction

Colloidal structures, in particular micelles and vesicles, are often formed in the intestinal medium because of the presence of bile salts and phospholipids or the use of surfactants in the formulation. During micellar solubilization, drug partitions between colloidal structures and free form to form a dynamic equilibrium.[1] Only dissolved drug in the free form is directly available for absorption via the enterocytes. When the drug concentration is lower than saturation concentration, the reduced free drug concentration caused by the presence of micelles reduced the flux of estradiol across Caco-2 cell monolayer and the kinetics of dantrolene and griseofulvin transport across rat small intestine membrane.[2-4]

Forming colloidal structures during dispersion and digestion is one of the mechanisms by which lipid-based formulations improve the solubilization and absorption of lipophilic drugs, which are poorly solubilized in intestinal fluids.[5] These colloidal structures comprise of mixed micelles, liposomes, and possibly liquid crystalline structures, self-assembled from mixtures of endogenous amphiphilic substances (e.g., bile salts and phospholipids), lipid excipients, and digestion products (e.g., fatty acids and monoglycerides).[5,6] The colloidal structures provide higher solubilization capacity of the digestion medium with a “reservoir” for free drug to be absorbed. It is also believed that mixed micelles disintegrate at the lower pH conditions prevailing near the epithelial membrane and thus drugs close to the absorption site become free and are readily absorbed.[5]

In vitro lipolysis models have been established based on key characteristics of the human intestinal tract and are used to evaluate the performance of lipid-based formulations in terms of drug solubilization and colloidal structure formation.[7-9] In order to quantify the amount of solubilized drug generated during *in vitro* lipolysis, samples are taken and lipase activity is inhibited, thereafter the sample is separated into 2 different phases (i.e., a supernatant phase and a pellet phase) and the drug is quantified using HPLC.[10] This method allows measuring the total drug concentration

The authors Anjan Chakraborty and Xi Xi contributed equally to the work.
This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2017.05.016.

* *Correspondence to:* Anette Müllertz (Telephone: 00 45 35 33 64 40).
E-mail address: anette.mullertz@sund.ku.dk (A. Müllertz).

http://dx.doi.org/10.1016/j.xphs.2017.05.016
0022-3549/

in supernatant phase, including both free drug and drug entrapped in colloidal structures.

Determining the free drug concentration in a colloidal system can be important when studying the kinetics of absorption, but a straightforward analytical method providing this information does not exist. An analytical device for *in situ* monitoring of free drug concentration during dynamic processes such as dissolution or digestion assays can be a useful tool to effectively evaluate formulations. Potentiometric sensors, also known as ion-selective electrodes, have been of interest in many research fields including chemistry, medicine, environmental and food sciences.[11] In principle, a potentiometric sensor contains an indicator electrode equipped with an ion-selective membrane. After being conditioned, a potential over the membrane will be created depending on the activity of that specific ion dissolved in the contacting solution.[11] Using a voltmeter to measure this membrane potential allows investigating the concentration of the specific dissolved ion in the contacting solution.[11] Indeed, the ion-selective sensors only measure the ionized form which diffuses as a free entity and creates a build-up of charge over the interface between the ion-selective membrane and the contacting solution. The total amount of both ionized and unionized forms of the molecule of interest can be determined by correcting for the various equilibria, either by mathematical or experimental calibration. Potentiometric sensors selectively detecting a specific drug are designated free drug sensors (FDS). FDS selectively detecting loperamide, diphenhydramine, domperidone, or ketoconazole have been used for *in situ* monitoring of solubilized drugs during dissolution tests, with remarkable agreement with HPLC methods with respect to the accuracy of the results.[12,13] However, the utility of FDS to determine free drug concentrations in more complex colloidal systems has not yet been explored.

For these reasons, the aim of the study was to evaluate the applicability of FDS to investigate the concentrations of free drug in different colloidal systems, including micellar solutions, oil-in-water emulsions and during the *in vitro* lipolysis of a lipid-based formulation. Diphenhydramine hydrochloride (DPH) and loperamide hydrochloride (LOP) (Fig. 1) were selected as ionizable model drugs.

Materials and Methods

Materials

Phosphatidylcholine (PC from lipoid S PC) was provided by Lipoid GmbH (Ludwigshafen, Germany). Glyceryl monolinoleate (Maisine 35-1) was kindly donated by Gattefossé (Saint-Priest, France). Polyoxyl castor oil (Kolliphor EL) was provided by BASF (Ludwigshafen, Germany). DPH, LOP, corn oil, polyoxyethylene (23) lauryl ether (Brij 35), pancreatin from porcine pancreas, sodium taurodeoxycholate (TDC) hydrate (>95% pure), tris(hydroxymethyl) aminomethane (Tris), maleic acid, and 4-bromophenylboronic acid (4-BPB; ≥95.0% pure) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride was purchased from VWR (Søborg, Denmark). Sodium hydroxide pellets and dimethyl sulfoxide were obtained from Merck (Darmstadt, Germany). Deuterium oxide (99.9%) was purchased from Deutero GmbH (Kastellaun, Germany). Water was purified by a SG Ultraclear water system (SG Water GmbH, Barsbüttel, Germany).

Methods

Condition and Calibration Process for a Dedicated FDS

Universal potentiometric sensors were provided by Octens BVBA (Edegem, Belgium). The composition of the potentiometric sensors was described in detail by Bohets et al.[19,20] Briefly, the indicator electrode of the potentiometric sensor is made of a hollow polyvinyl chloride (PVC) cylinder with a copper rod glued on the inside wall and filled with a mixture of 60% graphite and 40% high-molecular-weight PVC. The indicator electrode membrane was formed by evaporating the solution containing 100 mg of membrane mixture (65% Mesamoll, 33% PVC, and 2% tetrakis(4-chlorophenyl)borate dissolved in 1 mL of tetrahydrofuran) at room temperature.[20]

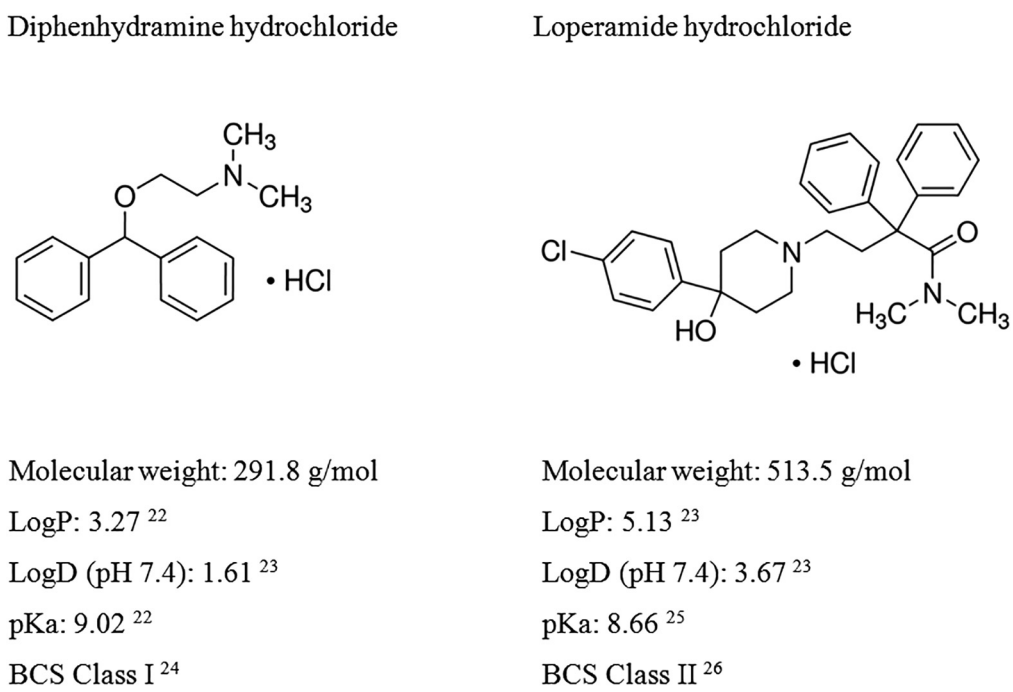

Figure 1. Chemical structures and physicochemical properties of DPH (left) and LOP (right).[14-18]