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Research Article

In Situ Lipolysis and Synchrotron Small-Angle X-ray Scattering for the Direct Determination of the Precipitation and Solid-State Form of a Poorly Water-Soluble Drug During Digestion of a Lipid-Based Formulation

Jamal Khan [1], Adrian Hawley [2], Thomas Rades [3], Ben J. Boyd [1, 4, *]

[1] *Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash, Parkville, Victoria 3052, Australia*
[2] *SAXS/WAXS beamline, Australian Synchrotron, Clayton, Victoria 3168, Australia*
[3] *Department of Pharmaceutical Sciences, University of Copenhagen, Copenhagen 2100, Denmark*
[4] *ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia*

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ABSTRACT

In situ lipolysis and synchrotron small-angle X-ray scattering (SAXS) were used to directly detect and elucidate the solid-state form of precipitated fenofibrate from the digestion of a model lipid-based formulation (LBF). This method was developed in light of recent findings that indicate variability in solid-state form upon the precipitation of some drugs during the digestion of LBFs, addressing the need to establish a real-time technique that enables solid-state analysis during *in vitro* digestion. In addition, an *ex situ* method was also used to analyse the pellet phase formed during an *in vitro* lipolysis experiment at various time points for the presence of crystalline drug. Fenofibrate was shown to precipitate in its thermodynamically stable crystalline form upon digestion of the medium-chain LBF, and an increase in scattering intensity over time corresponded well to an increase in concentration of precipitated fenofibrate quantified from the pellet phase using high-performance liquid chromatography. Crossed polarised light microscopy served as a secondary technique confirming the crystallinity of the precipitated fenofibrate. Future application of *in situ* lipolysis and SAXS may focus on drugs, and experimental conditions, which are anticipated to produce altered solid-state forms upon the precipitation of drug (i.e., polymorphs, amorphous forms, and salts).

Introduction

Lipid-based formulations (LBFs) provide an effective means to improve the oral absorption of poorly water-soluble compounds. In particular, lipophilic drugs, or more generally Class 2 drugs as defined by the Biopharmaceutics Classification System (BCS),[1] are well suited to LBF technology, as in these formulations the drug is presented to the gastrointestinal tract in a solubilised form, avoiding the rate-limiting dissolution step during gastrointestinal transit.[2] In addition, LBFs may also drive the lymphatic uptake of some highly lipophilic drugs from the small intestine, which limits the effect of first-pass metabolism on bioavailability.[3,4] The enzymatic degradation of lipidic components during digestion leads to the formation of self-assembled structures in the small intestine,[5,6] such as mixed micelles, which enable the transport of lipophilic drugs to sites of absorption.[7,8] Endogenous bile salts and phospholipids interact with liberated fatty acids from the digestion of lipids to form these self-assembled structures.[9,10]

There is, however, potential for a loss of solubilisation capacity of the formulation during dispersion and digestion.[11] The drug may then become rapidly supersaturated in the formulation, in which case there is an increased risk of drug precipitation. The supersaturation of drug is in itself complex; some drugs persist in a supersaturated state for an extended time interval while others rapidly precipitate.[12] Supersaturating formulations aim to extend this time interval of drug supersaturation for as long as possible,[13-15] as a means to harness the driving force for absorption

* *Correspondence to:* Ben J. Boyd (Telephone: +61-3-99039112; Fax: +61-399039583)
E-mail address: ben.boyd@monash.edu (B.J. Boyd).

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of drug whilst in the supersaturated state. This inhibition of drug precipitation to enhance absorption is commonly achieved with the use of polymeric materials.[16,17] The use of non-ionic surfactants with polyethylene glycol head groups, or "stealth" components, has also recently been investigated as a means to address the rapid loss of solubilisation capacity seen with the digestion of medium-chain (MC) lipids in particular.[18]

In vitro digestion experiments are used to detect drug precipitation during digestion by centrifugation of the digesting medium to sediment precipitated drug. Until recently, drug precipitation during the digestion of LBFs was viewed as inherently detrimental to drug absorption; hence, the strong focus on inhibiting drug precipitation with the use of polymers in supersaturating formulations. This paradigm, however, has come under question with a number of studies showing that the precipitation of some drugs during *in vitro* digestion is not limited to poorly water-soluble crystalline forms. Most notable are the recent findings of Sassene et al.,[19] Stillhart et al.[20] and Thomas et al.,[21] which all point to the presence of non-crystalline cinnarazine,[19] carvedilol[20] and halofantrine[21] in the endpoint pellet phase of digested LBFs containing these weakly basic drugs. The dissolution performance of these noncrystalline drug precipitates, in bio-relevant media, was significantly improved as compared with their thermodynamically stable crystalline forms. Moreover, Williams et al.[22] has shown tolfenamic acid to precipitate as a high-energy polymorph during the *in vitro* digestion of an LBF. The findings above indicate that the solid state of the precipitated drug during digestion may play an integral role in influencing the total amount of absorbed drug.

In vitro lipolysis testing and subsequent characterisation techniques may be used to examine the solid-state aspects of drug precipitation. The solid-state characterisation of precipitated drug from *in vitro* digestion experiments has, thus far, been largely limited to X-ray diffraction (XRD), crossed polarised light microscopy (CPLM) and dissolution testing, performed on the pellet phase of endpoint digested contents. Although XRD and CPLM can reveal the crystallinity and morphology of precipitated drug at the endpoint of *in vitro* digestion, these techniques provide no real-time information with regard to the solid-state aspects of drug precipitation during digestion. Another drawback of performing XRD and CPLM on pellet phases produced during *in vitro* digestion experiments is the extensive sample handling and time required to produce the pellet phase containing the solid drug material in question, which often involves ultracentrifugation of samples for up to 90 min, as well as analysis time with benchtop XRD instruments requiring at least 10 min per sample. This extensive sample treatment and analysis time needed for the conventional XRD and CPLM methods brings into question the relevance of the solid drug material measured with respect to what actually takes place during digestion, and highlights the need for developing suitable real-time methods capable of monitoring the precipitation of drug and its solid state during digestion.

In-line Raman spectroscopy was recently applied to monitor the precipitation kinetics of the neutral and poorly water-soluble drug fenofibrate, in real-time, during the *in vitro* digestion of an LBF.[23] Raman spectroscopy proved effective in this instance; however, extensive data analysis was necessary to distinguish precipitated drug from the hydrolysis products of digestion, and the analysis relied on *a priori* knowledge of the reference crystalline form to correlate with the solid state of precipitated drug. Hence, the need to develop a method that provides the *in situ* lipolysis of LBFs with real-time determination of the solid-state form of the drug becomes apparent. Boetker et al.[24] have recently demonstrated the direct *in situ* elucidation of transitions between different solid-state forms of drugs in a suspension using synchrotron X-ray scattering. Warren et al.[25] have also recently established the use of *in situ* lipolysis and small-angle X-ray scattering (SAXS) for the purpose of following the formation of colloidal structures during the digestion of lipids. In this study, we demonstrate the potential for convergence of these approaches to enable the real-time solid-state structural elucidation of precipitated drug (fenofibrate) during the digestion of a typical LBF (self-nano-emulsifying drug delivery systems, SNEDDS). The precipitation kinetics was also independently measured by high performance liquid chromatography (HPLC), and the presence of crystalline material was verified using CPLM.

Experimental

Materials

Fenofibrate was purchased from AK Scientific (Union City, California). Captex 355® [MCT composed of 59% caprylic acid (C_8), 40% capric acid (C_{10}), <1% lauric acid (C_{12}) as stated in the product information], and Capmul MCM® [mono/diglycerides composed of caprylic acid (C_8) in glycerol], were obtained from Abitec Corporation (Janesville, Wisconsin). Cremophor EL® was purchased from BASF Corporation (Washington, New Jersey). Tris maleate (reagent grade), bile salt (sodium taurodeoxycholate, >95%), and 4-bromophenylboronic acid (4-BPBA, >95%) were purchased from Sigma-Aldrich (St. Louis, Missouri). Calcium chloride dihydrate (>99%) was obtained from Ajax Finechem (Seven Hills, New South Wales, Australia). Sodium chloride (>99%) was purchased from Chem Supply (Gillman, South Australia, Australia). HPLC grade methanol and acetonitrile were purchased from Merck (MA, USA). Phospholipid (1,2-dioleoyl-sn-glycero-3-phosphocholine, DOPC) was obtained from Trapeze Association Pty. Ltd. (Clayton, Victoria, Australia). USP grade pancreatin extract was purchased from Southern Biologicals (Nunawading, Victoria, Australia).

Selection and Preparation of Drug and Formulation

Fenofibrate was chosen as the poorly water-soluble model compound for this study, as it has previously been shown to precipitate extensively at the endpoint of *in vitro* digestion in its thermodynamically stable crystalline form.[22,23,26] Hence, it was deemed suitable for the purpose of validating the proposed *in situ* lipolysis and SAXS method, as characteristic diffraction peaks were anticipated to appear when the drug first precipitates during the experiment and increase in scattering intensity over time, to correspond with the progressive precipitation of drug. Moreover, the use of fenofibrate allowed for a comparison of the *in situ* technique described here, and the *in situ* Raman technique described by Stillhart et al.[23]

The formulation was a MC-SNEDDS, and was prepared in 1 g batches with 0.3 g of Captex® 355, 0.3 g of Capmul® MCM, 0.3 g of Cremophor EL® and 0.1 g of ethanol all weighed into the same 20 mL glass scintillation vial. The resultant mixture was vortexed for 1 min before the drug was incorporated. This produced a formulation that was an optically clear solution, which self-emulsified upon agitation in the lipolysis vessel to form a nano-emulsion. MC lipids were selected because of their higher solubilisation capacity for fenofibrate compared with LC lipids,[27] and because the rapid enzymatic breakdown of the MC lipids was expected to result in the rapid precipitation of drug.

The loading of fenofibrate in the formulation was selected according to previously established equilibrium solubility values for the drug in a similar MC-SNEDDS formulation, where the equilibrium solubility was shown to be 143.1 mg/g.[22] The drug loading (200% of solubility) was chosen in order to provide a large fraction of precipitated drug and increase the likelihood of detection. The term "super-SNEDDS" has been used to describe such formulations containing supersaturated levels of drug.[28] Hence, 286.2 mg of