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In vitro digestion testing of lipid-based delivery systems: Calcium ions combine with fatty acids liberated from triglyceride rich lipid solutions to form soaps and reduce the solubilization capacity of colloidal digestion products

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ABSTRACT

In vitro digestion testing is of practical importance to predict the fate of drugs administered in lipid-based delivery systems. Calcium ions are often added to digestion media to increase the extent of digestion of long-chain triglycerides (LCTs), but the effects they have on phase behaviour of the products of digestion, and consequent drug solubilization, are not well understood. This study investigates the effect of calcium and bile salt concentrations on the rate and extent of *in vitro* digestion of soybean oil, as well as the solubilizing capacity of the digestion products for two poorly water-soluble drugs, fenofibrate and danazol. In the presence of higher concentrations of calcium ions, the solubilization capacities of the digests were reduced for both drugs. This effect is attributed to the formation of insoluble calcium soaps, visible as precipitates during the digestions. This reduces the availability of liberated fatty acids to form mixed micelles and vesicles, thereby reducing drug solubilization. The use of high calcium concentrations does indeed force *in vitro* digestion of LCTs but may overestimate the extent of drug precipitation that occurs within the intestinal lumen.

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1. Introduction

While a number of formulation and non-formulation strategies have been developed to address the increasing number of new chemical entities that demonstrate oral absorption limited by low aqueous solubility (Williams et al., 2013), approaches that utilize drug pre-dissolved in a lipid vehicle remain popular. The basis for using lipids stems from a number of studies that noted improved absorption and bioavailability of a poorly water-soluble drug (PWSD) following co-administration with a lipid-rich meal (Charman et al., 1993, 1997; Crounse, 1961; Humberstone et al., 1996; Sunesen et al., 2005; Welling, 1996). In a broad sense, lipid-based drug delivery systems (LBDDS) therefore aim to harness the often positive effect of dietary lipids on oral drug absorption (Hauss, 2007; Larsen et al., 2008; Porter et al., 2007, 2008) by circumventing drug dissolution, which in the case of PWSD is often slow and potentially limits the rate and extent of drug absorption, and by increasing the solubilization reservoir in the GI fluids (Cuine et al., 2007; Kleberg et al., 2010; Porter et al., 2004, 2007).

There are many different types of LBDDS and these may be discriminated on the basis of their composition and properties following interaction with endogenous GI fluids. In an effort to facilitate this discrimination, Pouton proposed the Lipid Formulation Classification System (LFCS) (Pouton, 2000, 2006), which classifies LBDDS into five discrete groups (Type I, II, IIIA, IIIB and IV) according to the proportion of oil, lipophilic surfactant, hydrophilic surfactant and cosolvent in the formulation. Type I and II formulations represent the most lipophilic formulations and form coarse and highly turbid emulsions on dispersion in aqueous fluids. Digestion of the dispersed oil phase promotes *in vivo* performance since this forces drug to partition from the poorly dispersed oil droplet phase into more solubilized colloidal phases. In contrast, it is generally well recognized that digestion is not essential to the performance of Type IIIA/B and Type IV systems as they form finer (*i.e.*, nanosized) emulsions and/or micellar phase systems in the GI tract (Pouton, 2006). However, as LBDDS enter the small intestine, digestion of formulation components is inevitable and may significantly impact the subsequent formulation behaviour. Assessment of all types of

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LBDDS during *in vitro* digestion tests is therefore necessary for a complete understanding of formulation performance.

Pancreatic lipase is the main enzyme involved in the digestion of triglycerides (Armand et al., 1996; Carriere et al., 1993). Pancreatic lipase is an interfacial enzyme that, in the presence of a co-lipase 'anchor', will effectively bind to the hydrophobic surface of an oil droplet (Erlanson-Albertsson, 1992). Other enzymes present in the small intestine and thought to play a role in lipid digestion include carboxyl ester hydrolaze (CEH), phospholipase A_2 and pancreatic lipase-related protein 2 (PLRP 2). However, these enzymes are believed to contribute more to the hydrolysis of phospholipids, cholesterol esters and formulation surfactants rather than triglyceride (Bakala N'Goma et al., 2012; Borgstrom, 1993). Pancreatic lipase hydrolyses molecules of triglyceride (TG) to yield two molecules of fatty acids (FA) and a molecule of 2-monoglyceride (2-MG). Further hydrolysis of 2-MG is limited by the regiospecificity of pancreatic lipase towards positions 1 and 3 of the TG molecule (Carriere et al., 1997), although CEH and PLRP 2 may also hydrolyze 2-MG to yield a third FA and glycerol (Bakala N'Goma et al., 2012). However, the activity of CEH in porcine pancreatin extract – the source of pancreatic lipase commonly used in *in vitro* digestion models – has not been determined, and PLRP 2 has not yet been identified in this extract (de Caro et al., 2008). Alternatively, 2-MG may undergo slow isomerization to the relatively less lipase-stable 1-MG to allow further hydrolysis to yield a third FA and glycerol, although this process is usually limited *in vitro* (Mattson and Volpenhein, 1964). The extent of MG hydrolysis to FA and glycerol *in vitro* is therefore unknown. Natural detergents in the small intestine *i.e.*, bile salts and phospholipids (secreted along with pancreatic lipase from the gall bladder in response to lipids entering the small intestine) form mixed micelles that shuttle the products of lipid digestion from the site of production (*i.e.*, the oil:water interface) to the site of absorption (*i.e.*, the enterocyte membrane) (Hofmann, 1963).

In vitro digestion tests are designed to simulate the above described digestion processes so that the fate of drug may be monitored as the physical and chemical nature of LBDDS change. It is also customary to relate this outcome to the extent of formulation digestion. Detailed descriptions of *in vitro* digestion models have been already provided elsewhere (Sek et al., 2002; Williams et al., 2012a). Following digestion, drug that is solubilized within a colloidal aqueous phase digest (containing micelles and vesicles) is expected to be in rapid equilibrium with drug in free solution providing a reservoir of drug that is highly available for absorption (Boyd et al., 2003; Porter et al., 2007). In contrast, drug dissolved into the LBDDS that subsequently precipitates during digestion (collecting within the pellet phase) is thought to represent drug that is poorly available for absorption since re-dissolution is required, and the dissolution of solid PWSD is usually poor unless the drug forms a more rapidly dissolving amorphous precipitate (Sassene et al., 2010). One of the experimental complexities of *in vitro* digestion models is the difficulty in achieving complete digestion of the lipid substrate. This issue is particularly the case for highly lipophilic, long-chain lipid formulations (Williams et al., 2012b), and stems from the fact that most *in vitro* digestion models are 'closed' systems. As such, the absence of a sink for removal of digestion products (such as that provided *in vivo* by FA and MG absorption) causes a progressive increase in the concentration of lipid digestion products which, depending on the solubilization capacity of the digestion medium for the digestion products, ultimately results in accumulation of the lipid digestion products at the oil droplet surface, suppressing further digestion of the remaining oil phase (Brockerhoff, 1968; Brockerhoff and Jensen, 1974; Fave et al., 2004; Scow et al., 1979). Since this phenomenon is attenuated *in vivo* by absorption, strategies to 'force' lipid digestion to completion *in vitro* have been sought, and the most common approaches include an increase in the bile salt reservoir (Li et al., 2011) and the addition of a FA complexant, *e.g.* calcium ions either within the digestion media or *via* continuous addition (Alvarez and Stella, 1989; MacGregor et al., 1997; Patton and Carey, 1979; Patton et al., 1984; Zangenberg et al., 2001a,b). The effect of increasing calcium concentration on the phase behaviour of digested lipids and the resultant impact on drug solubilization, however, is not well understood. The first aim of the current study was therefore to determine whether increasing calcium concentration could be used to push the *in vitro* digestion of a model long-chain triglyceride (LCT) to completion. The second aim was to probe the solubilization capacity of the digests formed by this approach. In the current studies, soybean oil was chosen as a model long-chain lipid substrate, and fenofibrate and danazol were employed as model PWSD (Fig. 1) in order to represent two types of drug candidates for formulation in LBDDS, that is, a highly lipophilic drug (fenofibrate) and a hydrophobic but less lipophilic (lipid soluble) drug (danazol).

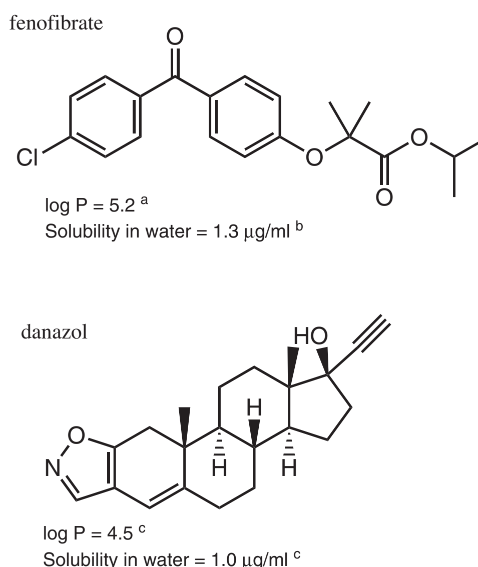

Fig. 1. The chemical structure of model drugs fenofibrate and danazol investigated in this study. [a]Munoz et al. (1994); [b]Sheu et al. (1994) and [c]Bakatselou et al. (1991).

2. Materials and methods

2.1. Materials

Danazol was obtained from Sterling Pharmaceuticals Pty Ltd. (Sydney, Australia). Fenofibrate, soybean oil (the long-chain triglyceride), sodium taurodeoxycholate > 95% (NaTDC), pancreatin extract (from porcine pancreas, P7545, 8× USP specifications activity), calcium chloride dihydrate, Tris-maleate, and the lipid digestion inhibitor 4-bromophenylboronic acid were purchased from Sigma–Aldrich Co. (St. Louis, MO). Lecithin (*ca.* 99.2% egg-phosphatidylcholine (PC), Lipoid E PCS) was purchased from Lipoid (Lipoid GmbH, Ludwigshafen, Germany). 1.0 M sodium hydroxide (Univol) was purchased from Ajax Finechem Pty Ltd. (Sydney, Australia) and was diluted with water (Milli-Q water purification system, Millipore, Bedford, MA) to achieve a 0.2 M titration solution. Methanol and chloroform used in this work were HPLC grade from Merck (Melbourne, Australia).

2.2. In vitro digestion experiments

In vitro digestion experiments were performed as previously described (Sek et al., 2002). Briefly, 250 ± 5 mg of soybean oil was directly weighed into a water jacketed-glass reaction vessel

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