

# The Conflict Between in Vitro Lipolysis and in Vivo Performance of Fenofibrate Self-Emulsifying Drug Delivery System

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## Abstract

The purpose of this work is to examine the correlation between the in vitro solubilization process of lipophilic drug fenofibrate prepared as self-emulsifying drug delivery systems, and the in vivo absorption data. In vitro lipolysis model was performed, and the concentration of drug in blood was measured in rats. The in vitro lipolysis model shows low concentration of drug in the aqueous phase, while the in vivo study demonstrates complete drug absorption orally. Poor in vitro in vivo correlation attributed to significant drug transport through lymphatic rout.

**Keywords:** Vitro Lipolysis, Vivo Performance, Drug Delivery System.

## 1. INTRODUCTION

Fenofibrate is a lipid-regulating drug that has been studied extensively in the treatment of hypercholesterolemia and hypertriglyceridemia (1). Fenofibrate is a highly lipophilic molecule with high permeability throughout the gastrointestinal tract, and it is nearly insoluble in water (1 µg/ml) (2). The limited water solubility of certain active compounds is a severe drawback when administering them orally (3), resulting in poor dissolution and, consequently, limited oral bioavailability (4). To this end, several techniques have been developed to enhance the solubility of active substances in water, including micronization (5), solid dispersion (6), complexation (7), and microencapsulation (8). Further, when the active ingredient is lipophilic, lipid-based formulations, such as self-emulsifying drug delivery systems (SEDDSs), might be more suitable (9). When mixed with water, SEDDSs become an isotropic mixture of oil, surfactant, co-surfactant, and drug that forms a spontaneous oil-in-water (O/W) emulsion (10). This property and the ability of SEDDSs to present an active chemical in its dissolved state constitute a powerful approach (11) that has garnered interest with regard to developing fenofibrate in the form of a SEDDS.

The U.S. Food and Drug Administration (FDA) has described in vitro in vivo correlation (IVIVC) as a predictive mathematical model that describes the relationship between an in vitro dosage form characteristic (such as drug dissolution or release profile) and its in vivo behavior (such as concentration-time profile) (12). The purpose of IVIVC is to reduce the regulatory burden since it can be used as a substitute for additional in vivo experiments. The IVIVC model is used to support the biowaivers and to support the in vitro dissolution profiles as a substitute for in vivo bioequivalence (13).

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A correlation is a measurement of the link between two or more quantitative variables with a correlation coefficient that demonstrates how closely related the variables are (14). There are four levels of correlation regarding IVIV including level A, B, C, and D.

Level A correlation is often calculated using a two-step process that involves deconvolution and a comparison of the amount of drug absorbed (in vivo) to the amount of drug dissolved (in vitro). It is a point-to-point link between in vitro dissolution and the drug input rate that is justified by the linear association between the two (15). Since it permits waivers for changes in manufacturing site, raw material suppliers, and minor changes in formulation, Level-A correlation is the highest level of correlation and the one that is most desired to attain (16).

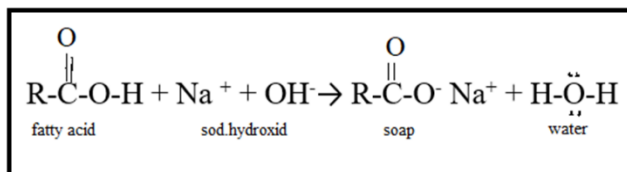
Level B correlation is based on statistical moment analysis. Statistical moments are used to compare the mean in vitro dissolution time (MDT) to the mean residence time or the mean absorption time. Despite of involvement of all of the in vitro and in vivo data level B correlation is not considered to be a point-to-point correlation as a number of different in vivo curves will produce similar mean residence time values. Hence least useful for regulatory purposes and to justify extremes of quality control standards (17).

Level C correlation is a single-point correlation between the in vitro dissolution rate and the AUC, C<sub>max</sub>, T<sub>max</sub>, K<sub>a</sub>, or the time to absorb 10, 25, and 90%. (in vivo) Correlation is not very trustworthy but has a positive effect in the early stages of formulation development because it does not provide a clear picture of the plasma concentration-time profile, which is the crucial aspect that determines the performance of any controlled-release product (14).

Level D It is a rank-order eccentric semi-quantitative or qualitative analysis that is not thought to be useful for regulatory purposes, but it does allow for the differentiation between two main types of correlations that, in essence, serve as a copula for formulation development and any changes made after approval (18).

The classic dissolution test using dissolution apparatus cannot apply correctly for lipid-based dosage form because it does not demonstrate the harsh enzymatic condition that the dosage form will face when taken orally. Instead in vitro lipolysis test had been routinely used to estimate the amount of oil digested by the lipase enzyme, and the precipitated drug from that oil.

The gastric lipolysis step contributes 10% to 25% of the overall lipolysis, whereas pancreatic enzymes hydrolyze the remaining lipids (19,20) Pancreatic lipase hydrolyzes triglycerides selectively, yielding 1 mol 2 monoglycerides and 2 mol fatty acids per mole triglyceride, these fatty acids can neutralize by a base and form soap as shown in figure (1) below:(21)



**Figure (1):** Soap production from fatty acid and base

However, the hydrolytic activity of lipase enzyme depends largely on a complicated interaction with colipase, bile acids, and calcium (22).

One of the most notable characteristics of pancreatic lipase is that it is active only at the oil-water interface (23). As a result, any process that alters the substrate's surface has a significant impact on its activity, this is especially essential during lipolysis, when amphiphilic digestion products, such as fatty acids and 2-monoglycerides, collect on the substrate's surface, forming liquid crystalline and 'viscous isotropic' phases (24–26). The buildup of these chemicals prevents pancreatic lipase from reaching the oil-water interface. Bile acids displace these amphiphiles from contact with the substrate but, conversely, can block pancreatic lipase (27).

Colipase forms a 1:1 complex with pancreatic lipase, restoring its activity and anchoring pancreatic lipase to the substrate interphase (28). Further, colipase stabilizes pancreatic lipase in an 'open-lid' conformation (29). The lid has a surface loop that covers the enzyme's catalytic center in its inactive conformation, preventing access to the catalytic center (23). As a result, stabilizing the open lid potentiates the hydrolysis of the substrate (30). The presence of bile salts is required for optimal activity of the pancreatic lipase-colipase complex (31). The most important bile acids in human are deoxycholic acid and chenodeoxycholic acid. These bile help move digestive products and formation-derived surfactants away from the interface.

Calcium ions also regulate lipolysis in vivo (32). Calcium is believed to promote the penetration of pancreatic lipase into the substrate surface by removing negative charges from the surface, lowering the enzyme's electrostatic repulsion (33). Also, calcium generates liquid crystals with bile salts (27).

## 2. MATERIALS:

Fenofibrate, fenofibric acid, soybean oil, porcine pancreatic lipase, porcine bile extract, and Sodium Dioctyl Sulfosuccinate (SDOS) were purchased from Hyper Chem® China. Potassium chloride and anhydrous calcium chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, ethyl acetate, and absolute ethanol were acquired from VWR (Herlev, Denmark). All other chemicals were analytical-grade and used as received unless specified otherwise.

### 3. EXPERIMENT & METHODS:

#### 3.1 Formulation:

Soft gelatin capsules were prepared from SDOS and soybean oil in which 145mg and 114mg of fenofibrate and orlistat were dissolved respectively. The formulation was prepared as SEDDS in order to overcome the problem of the low aqueous solubility of fenofibrate. The characteristic of SEDDS was investigated in other works and the in vitro vs in vivo correlation was inspected here.

#### 3.2 In vitro lipolysis:

A mixed gastric/intestinal in vitro lipolysis approach was used in this work, with slight modifications to the method published by Christophersen et al (34), as shown in table (1). An accurately weighted formula equivalent to 145mg of fenofibrate was added to 200ml of 0.1N HCL beaker for gastric lipolysis and allowed to stirrer for ten minute by magnetic stirrer. the test started when lipase enzyme is added, for stimulating gastric condition 10 IU/ml or 2000IU of lipase in 200ml medium (35). and (55mg) of anhydrous CaCl<sub>2</sub> to mimic fasting condition, while 88mg of it to mimic feeding condition which was done separately (36). The pH was controlled during the procedure by titration of KOH solution from burette which was previously prepared by dissolving 10gm of KOH in 100ml deionized water. The pH meter was vertically placed inside the media (fig. 2) to continuously monitor any pH change during the experiment. Gastric lipolysis will last for 30 minutes and samples will be withdrawn at 5,10,15,30 minutes (37).



**Figure (2):** In-vitro lipolysis

After gastric lipolysis is finished, 150ml of 6.8pH phosphate buffer was added to the media, and the pH of the media is corrected with KOH solution until it reach 6.8, media was allowed to homogenized for 10 minutes then intestinal lipolysis is initiated (table2) by the addition of 37,680IU of lipase enzyme with the activity of 20500 U/gm and 1.225gm (3mM) of bile extract with molecular weight 408.5714 gm/mole (both lipase & bile extract are of porcine origin). Also, CaCl<sub>2</sub> of concentration mimicking fasting and feeding condition of intestine was used, 4 mM (444mg for) fast condition & 15 mM (1.665gm) of feed condition (38), fast and feed condition experiment were performed separately (39). Samples were withdrawn at 5,15,30, and 60 minutes after the start of the experiment. All samples were centrifuged at 5000 rpm for 10 minutes to separate undissolved bile salts, filtered through a Millipore syringe 0.45µm, to measure the amount of drug in the aqueous phase, one ml is measured directly by UV-spectrophotometer at 294nm for gastric lipolysis and 303nm for intestinal lipolysis. Another one ml was further diluted with 0.5% SLS buffer solution and detected by UV- spectroscopy at 289nm to measure the amount of drug in both aqueous and lipid phase. The remaining of lipolysis media was filtered by Buchner device were the precipitate was collected and dried then dissolved in 25ml ethyl acetate and scanned at 285nm for any precipitated drug (40).

**Table (1):** Gastric in-vitro Lipolysis

Volume	200ml
pH	1.2
Lipase enzyme	10 IU/ml
anhydrous CaCl <sub>2</sub>	55mg (fasting) & 88mg (feed)
Time	30 minute
Temperature	37°C

**Table (2):** Intestinal in-vitro Lipolysis

Volume	350ml
pH	6.8
Lipase enzyme	37680 IU
Bile extract	3mM (1.225gm)
anhydrous CaCl <sub>2</sub>	444mg (fast)& 1.665gm (feed)
Time	60 minutes
Temperature	37°C

### 3.3 In vivo study:

All in vivo experiments were carried out in accordance with the EC directive 86/609/EEC for animal experiments (license number LA1210261), and by the local research ethics committee, College of Pharmacy, University of Baghdad.

Animals: twelve albino male rats weighing 140-160 gm, eight weeks' old were obtained from the animal house of the College of Pharmacy- University of Baghdad. The animals were maintained on normal conditions of temperature, humidity and light/dark cycles. They were fed standard rodent pellet and they had free access to water.

Dosing: rats were dosed via oral gavage with liquid dosage form; each equivalent to 20mg/kg of fenofibrate (41).

Blood sampling: the rat Retro-Orbital Plexus blood was obtained at the 0.5,1,2,3,4 and 24 h after the administration, respectively, with two rats for each time interval. Blood were placed in heparinized tube and centrifuged at 6000 rpm for 15minutes. The supernatant collected in eppendorf tube and stored in -80 °C refrigerator for future use (42).

Bioanalysis: All plasma samples were quantified for fenofibric acid, the major active metabolite of fenofibrate. Presystemic metabolism of fenofibrate into fenofibric acid is quantitative in rats (43).

100 µL of plasma sample was quantitatively obtained and placed into 2 ml plastic centrifuge tube, added and mixed by 1000 µL acetonitrile for the deproteinization. The tube then vortexed for 30 second and centrifuged at 4000 rpm for 10 minutes. The supernatant transferred into glass tube and re-dissolved with 300 µL methanol, vortexed for 3 minutes and filtered by 0.45 µm Millipore syringe. 20 µL filtrate was injected into HPLC (44,45).

The following were the chromatographic conditions: column: Kromasil C18 (4.6 mm 250 mm, 5 m), mobile phase: 75:24:1, pH = 4.0, methanol-water-10% phosphoric acid 286 nm detector wavelength; 30 °C column temperature; flow 1 ml/min rate (46).

### 3.4 IVIVC model Development:

The relation between the percentage of the drug dissolved and the percentage of the drug absorbed was investigated using linear regression analysis using excel software 2016. The deconvolution procedure was used to obtain in vivo input profiles of the fenofibrate SEDDS dosage form. To determine the validity of the generated IVIVC model, the percent of dissolved is plotted against the dissolution sample points of the target formulation (47). A point-to-point correlation (Level A IVIVC) between the in vitro permeation and in vivo absorption was developed, and validated (48).

## 4. RESULT AND DISCUSSION:

### 4.1 In vitro lipolysis:

As shown in figure (7) During the 30-min gastric step of the SEDDS, nearly 20% of the fenofibrate was dispersed in the aqueous phase. However, after starting the intestinal step, the amount of fenofibrate that was dispersed in the aqueous phase declined steadily, reaching 13% at 60 min (49). The dispersion of fenofibrate remained virtually unaltered during the gastric step for the formulation, indicating that any lipolysis that occurred did not have a significant impact on the drug's dispersion.

The dispersion of fenofibrate in a biorelevant medium depends on the amount and type of surfactant that is used (50). The high solubilization of fenofibrate in dispersed lipid formulations in gastric media can be attributed primarily to the excipients in the formulations. Despite the elevated bile salt levels in the intestinal media, the solubilization capacity of fenofibrate was lower in the intestinal versus gastric medium, due to the increased volumes of media in the intestinal stage and the resulting dilution of the SEDDS excipients, given that fenofibrate solubilization is based predominantly on a formulation's excipients rather than bile salts (51).

The dilution of the final withdrawal at 60 min of intestinal lipolysis with 0.5% SLS phosphate buffer allowed us to determine the percentage of drug in the oil phase, obviating the need for ultracentrifugation, as in other work (52). Because the amount of FENO that is measured by SLS solution represents the total amount in the lipid and aqueous phases.

The outcome of in vitro lipolysis shows that 13% of fenofibrate dispersed in aqueous phase and 87% in oil phase with no precipitation of drug at the end of intestinal lipolysis. There was no difference between fast and fed condition in regards to amount of drug in aqueous or oil phase due to anti-lipase Orlistat was incorporated into the formula.

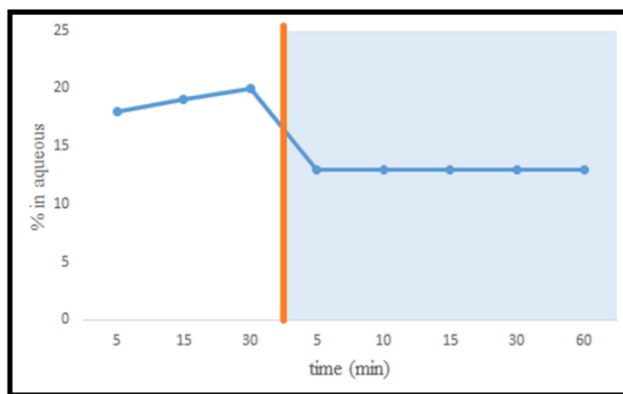


Figure (3): In vitro lipolysis of formula

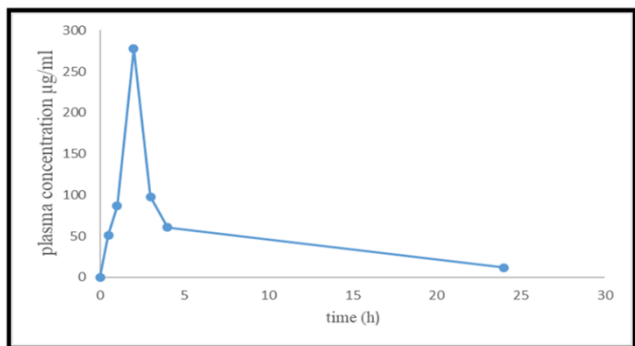
### 4.2 In vivo study:

A calibration curve of fenofibric acid in the mobile phase was conducted using HPLC since fenofibric acid is the main



active constituent of fenofibrate in the body (53).

The plasma concentration-time profiles of fenofibric acid after oral administration are shown in figure (8), the corresponding  $T_{max}$ ,  $C_{max}$ ,  $AUC_{0\rightarrow24}$ , and relative bioavailability are listed in Table 3. The pharmacokinetic profiles were similar to those of fenofibric acid, which were recently published (49,54,55). The fed condition was not performed, and only the fasting state experiment was simulated, due to the physiology of the rat's gastrointestinal tract. Because the rat lacks a gallbladder, bile is continuously secreted into the duodenum, regardless of dietary status (56).



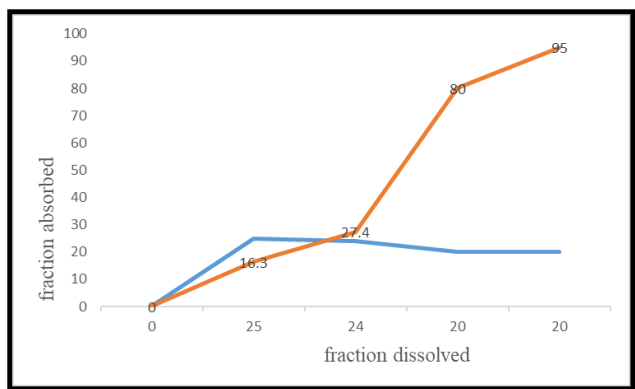
**Figure (4):** Plasma concentration of fenofibric acid ( $\mu\text{g/ml}$ ) vs time (h)

**Table 3:** Pharmacokinetic Parameters of Fenofibrate for SEDDS

$T_{max}$ (h)	2
$C_{max}$ ( $\mu\text{g/ml}$ )	277.66
$AUC_{0\rightarrow24}$ ( $\mu\text{g}\cdot\text{hr/ml}$ )	15,490.5
F%	95

#### 4.3 IVIVC Development:

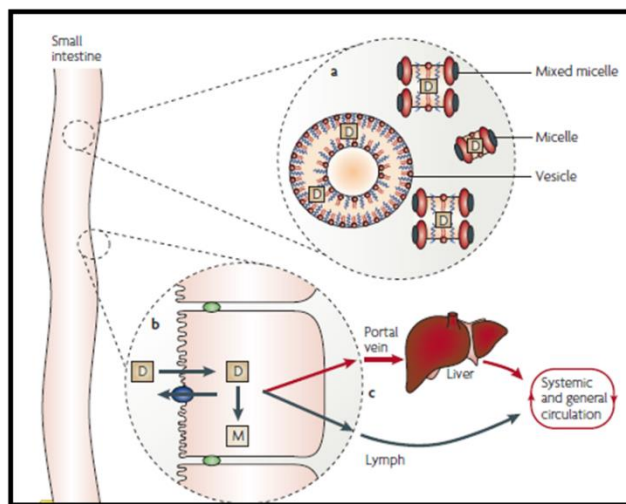
As illustrated in figure (9) the in vitro in vivo correlation was bad since the regression value ( $r^2$ ) was 0.157 and the  $p$ -value  $> 0.05$ , indicating a significant difference and bad correlation between the two experiments. The same outcome was obtained by Tho Thi et al (57) and Yuanlong Xu et al (58).



**Figure (5):** In vitro in vivo correlation of

The blue line in vitro & orange line in vivo

The passage of FENO into general circulation can be mediated by both portal vein and lymphatic channels (a mix of both), as shown in figure (6). The lipid-based dosage form can improve the absorption of the drug in three different ways (a): drug uptake, efflux, disposal, and the production of metabolites (M) within the enterocyte via interacting with enterocyte-based transport and metabolic processes. (b): or by changing the route by which drugs are transported to the systemic circulation (intestinal lymphatic system vs. portal vein), which can lessen first-pass drug metabolism because intestinal lymph is sent straight to the systemic circulation without first passing via the liver. (c): A representative transport protein is shown by a blue oval, while cellular junctions are represented by green ovals (26).



**Figure (6):** Absorption of lipid-based dosage form

For highly lipophilic drug ( $\log P > 5$ , and solubility more than 50mg in 1gm long chain triglyceride) as in fenofibrate, lymphatic absorption is preferred, but because the velocity of fluid flow in the portal vein is higher than that of the mesenteric lymph, drugs are typically delivered to the systemic circulation through the portal vein in most cases (59).

The discrepancies between the in vitro and in vivo results in the current studies imply that fenofibrate administration as SEDDS is more important for the in vivo performance than the drug's initial physical state (dissolved or suspended) and the solubilization profile obtained from in vitro lipolysis. This contrasts with earlier research that linked in vivo performance to the amount of drug solubilized in the aqueous phase following in vitro lipolysis (60,61).

#### 5. CONCLUSION:

The in vitro lipolysis model is useful for the optimization of oral lipid formulations. However, when lymphatic transport is a significant route of absorption, the in vitro lipolysis data may not be predictive for actual in vivo absorption.

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