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PII: S0378-5173(23)00714-7
DOI: https://doi.org/10.1016/j.ijpharm.2023.123294
Reference: IJP 123294

To appear in: *International Journal of Pharmaceutics*

Received Date: 28 April 2023
Revised Date: 24 July 2023
Accepted Date: 3 August 2023

Please cite this article as: M. Czajkowski, A-C. Jacobsen, A. Bauer-Brandl, M. Brandl, P. Skupin-Mrugalska, Hydrogenated phospholipid, a promising excipient in amorphous solid dispersions of fenofibrate for oral delivery: Preparation and in-vitro biopharmaceutical characterization, *International Journal of Pharmaceutics* (2023), doi: https://doi.org/10.1016/j.ijpharm.2023.123294

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Hydrogenated phospholipid, a promising excipient in amorphous solid dispersions of fenofibrate for oral delivery: Preparation and in-vitro biopharmaceutical characterization

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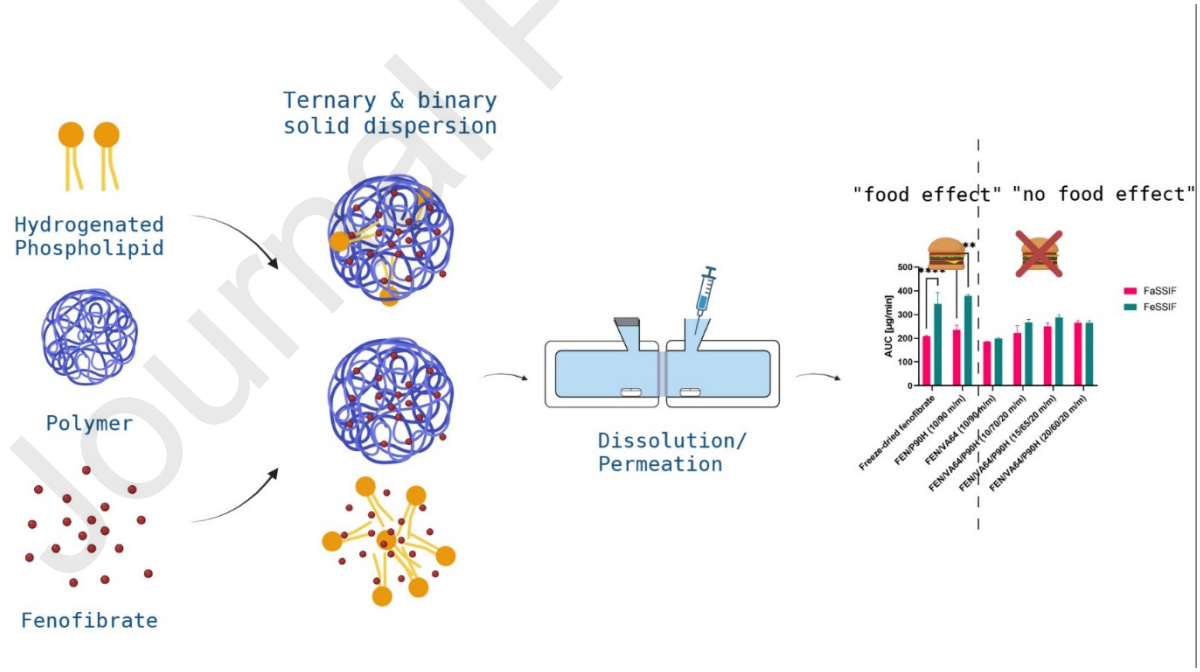
Abstract

Amorphous solid dispersions (ASD) represent a viable formulation strategy to improve dissolution and bioavailability of poorly soluble drugs. Our study aimed to evaluate the feasibility and potential role of hydrogenated phospholipid (HPL) as a matrix material and solubilizing additive for binary (alone) or ternary (in combination with polymers) solid dispersions, using fenofibrate (FEN) as the model drug. FEN, incorporated within ASDs by melting or freeze-drying (up to 20% m/m), stayed amorphous during short-term stability studies. The solubility enhancing potential of HPL depended on the dissolution medium. In terms of enhancing in vitro permeation, solid dispersions with HPL were found equally or slightly more potent as compared to the polymer-based ASD. For studied ASD, in vitro permeation was found substantially enhanced as compared to a suspension of crystalline FEN and at least equal compared to marketed formulations under comparable conditions (literature data). Additionally, while the permeation of neat FEN and FEN in binary solid dispersions was affected by the dissolution medium (i.e., the "prandial state"), for ternary solid dispersions the permeation was independent of the "prandial state" (FaSSIF=FeSSIF). This suggests that ternary solid dispersions containing both polymer and HPL may represent a viable formulation strategy to mitigate fenofibrate's food effect.

Keywords

hydrogenated phospholipids, amorphous solid dispersions, fenofibrate, polymers, supersaturation, enabling formulations,

Graphical abstract



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1 Introduction

Designing oral formulations of poorly water-soluble drugs (i.e., BCS class II and IV), prevalent among recent, non-biologic drug candidates, requires solubility-enhancing formulations. A prominent example of such formulations is amorphous solid dispersions (ASDs). Even though ASDs have been demonstrated to be successful 'candidate enabling formulation', their mechanisms of action have only recently been fully understood (Holzem et al., 2023, 2022a, 2022b)). Thus, formulation development has been done on a trial-and-error basis for decades.

In the past two decades, ASDs have gained increasing attention, as indicated by the number of new drug products approved that use this technology (Zhang et al., 2018). ASDs can enhance oral bioavailability because, due to the amorphous nature of the drug, during dissolution, typically a supersaturation is observed, i.e., a concentration of dissolved drug that is higher than that of its crystalline counterpart, which is closely linked to the formation of sub-micron amorphous particles (Buckley et al., 2013; Frank et al., 2014, 2012; Kanzer et al., 2010; Tho et al., 2010).

Stability maintenance, meaning limiting the tendency of re-crystallization, is challenging for amorphous systems. Molecules in the amorphous state are in a higher free energy state; therefore, the molecules eventually would spontaneously crystallize over some time. In an ASD, the amorphous form of the drug is stabilized by the matrix material (most commonly amorphous polymers such as polyvinyl-pyrrolidone vinyl acetate; PVPVA), in which the drug is molecularly dissolved or dispersed in the solid state. Polymers not only inhibit drug re-crystallization in the solid state by limiting molecular mobility but also, during dissolution, may inhibit re-crystallization (Chauhan et al., 2014).

In addition to polymers, ASDs frequently contain surfactants, thereby forming ternary ASDs. Surfactants serve multiple functions in ASDs: They plasticize polymers aiding processability during hot melt extrusion and improving wettability and drug release (Ghebremeskel et al., 2007). However, low molar mass surfactants like sodium dodecyl sulfate (SDS) and polysorbate 80 can promote molecular mobility and thus promote premature (re-)crystallization within the ASD. To the best of our knowledge, there is just one prior study describing the use of hydrogenated phospholipid as a plasticizer within a melt-extruded PVPVA-ASD of ritonavir (Zhao et al., 2019), yielding a depression of melting temperature by 20 K along with improved in vitro dissolution profile and enhanced oral bioavailability.

A second well-known limitation of polymer-based ASDs is their limited capacity to accommodate drugs within the matrix, such that a continuous, rapid, complete release is achieved.

In recent years, alternative matrix materials and coformers such as amino acids, polypeptides and sugars were investigated, showing various advantages over the classical polymeric ASD excipients. Selected BCS class II/IV drugs appear to form co-amorphous systems with low molar mass coformers such as sugars (Sekitoh et al., 2021), amino acids (Löbmann et al., 2013) or taurocholate (Aikawa et al., 2023) obviously allowing for higher drug loading capacities as compared to polymer-based ASDs, which above a threshold in drug-load (often from 10% m/m on) show non-congruent and thus substantially deteriorated dissolution behavior. In an attempt to achieve higher glass-transition temperatures and achieve enhanced physical stability as compared to co-amorphous systems, a recent approach is suggesting poly (amino acid)s as excipients for ASDs (Huang et al., 2023)

Natural phospholipids have been previously investigated as an alternative matrix material for solid dispersions (Fong et al., 2015a) and so-called pro-liposomes (Swarnakar et al., 2019). They are amphiphilic lipids consisting of a glycerol backbone esterified to (un)saturated fatty acids and a phosphate group, further connected to a hydrophilic group such as choline. In oral drug formulations, phospholipids are mainly used as solubilizers, wetting agents and emulsifiers. Like other lipid excipients, they cannot be regarded as inert material; in the gastrointestinal tract, they interact with bile

salts forming mixed micelles and are digested by phospholipase A_2 (i.e., enzymatic cleavage of one diacyl phospholipid releases one monoacyl phospholipid and one fatty acid).

It is hypothesized that phospholipid-based solid dispersions may combine the advantages of ASDs and classical lipid-based formulations by stabilizing the amorphous state of the drug and improving the drug-solvent interaction (solubilization), especially in the presence of physiological bile-salts. However, natural phospholipids containing unsaturated lipids are hygroscopic and are susceptible to oxidation (van Hoogevest, 2017). Furthermore, their semi-solid and sticky consistency requires a subsequent optimization of the desired formulation. To achieve this, sugars (Brinkmann-Trettenes et al., 2014; Fong et al., 2015b) and porous materials (Farzan et al., 2020; Jo et al., 2018) have been used.

In this study, we used a semi-synthetic saturated phospholipid (i.e., PHOSPHOLIPON 90H®; P90H), which is produced by hydrogenation of a phosphatidylcholine-rich fraction of soy phospholipid, as a matrix material for solid dispersions intending to replace a synthetic surfactant as a solubilizer. Hydrogenated phospholipids have acceptable powder flow and particle deformation properties (Kolbina et al., 2017; Massing and Bauer-Brandl, 2013) and are less hygroscopic (van Hoogevest, 2017). Kolbina and co-workers prepared extended-release tablets (using water-soluble model drugs) from P90H by direct compression (Kolbina et al., 2017) or hot-melt extrusion (Kolbina et al., 2019), showing that P90H is a versatile oral excipient and can be used in various formulation manufacturing methods.

In contrast to Kolbina and co-workers' study, we prepared solid dispersions in which a poorly soluble model drug was present in the amorphous state. To the best of our knowledge, this study is the first to report using hydrogenated phospholipid as a matrix material for solid dispersions. We combined hydrogenated phospholipid with a polymer conventionally used in ASDs (i.e., vinylpyrrolidone vinyl acetate copolymer; PVP/VA) to form ternary ASDs devoid of synthetic surfactant.

Fenofibrate, a highly lipophilic BCS class II drug (logP 5.6) (Guichard et al., 2000), was used as the poorly soluble model drug. It was previously used as a model drug for ASDs prepared by hot-melt extrusion (He et al., 2010). Most of the fenofibrate marketed formulations exhibit a strong positive food effect. Therefore, fenofibrate medications should be administered together with a meal (Guivarch et al., 2004).

This study shows that hydrogenated phospholipid can be used as a matrix material for solid dispersions – alone or in combination with polymers – stabilizing the amorphous state of a poorly soluble model drug. In the combined dissolution/permeation study, the binary and ternary ASDs comprising hydrogenated phospholipid enhance in-vitro drug permeation compared to raw (crystalline) and are similar to established enabling formulations. Furthermore, ternary ASDs with phospholipid appear unaffected by the prandial state, demonstrating their potential as a formulation strategy to mitigate the food effect of fenofibrate.

2 Materials and methods

2.1 Materials

The vinylpyrrolidone vinyl acetate copolymer (Kollidon® VA64; VA64) was kindly donated by BASF SE (Ludwigshafen, Germany). Hydrogenated soybean phosphatidylcholine (PHOSPHOLIPON P90H; P90H, containing >90% hydrogenated soybean phosphatidylcholine) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Sodium dodecyl sulfate (SDS) was purchased from Caesar & Loretz GmbH (Hilden, Germany). Fenofibrate (FEN), trifluoracetic acid (TFA), dimethyl sulfoxide (DMSO), sodium hydroxide, sodium phosphate monobasic monohydrate, sodium phosphate dibasic dihydrate were purchased from Sigma-Aldrich ApS (Brøndby, Denmark). Purified water was freshly prepared using a Milli-Q® Advantage A10® integral water purification

system (MerckMillipore, Merck A/S, Hellerup, Denmark). Methanol (MeOH) and acetonitrile (ACN) of HPLC-grade, sodium chloride, and *tert*-butanol were purchased from VWR International A/S (Søborg, Denmark). Acetic acid was purchased from Fluka (Honeywell Inc., Charlotte, US). Reagents used in this study were of the analytical grade unless stated otherwise. Simulated intestinal fluid (SIF) powder was purchased from biorelevant.com (London, UK).

FaSSIF containing 3mM sodium taurocholate, 0.75mM lecithin and FeSSIF containing 15mM sodium taurocholate, 3.75mM lecithin were prepared according to the guideline provided by the manufacturer (biorelevant.com). Phosphate-buffered saline (PBS) contained 11.93 g/L of sodium phosphate monobasic monohydrate and 2.41 g/L of sodium phosphate dibasic dihydrate. The pH was adjusted to the value of 6.8 with 0.1M hydrochloric acid or 0.1M sodium hydroxide solution in water; the osmolality was adjusted with sodium chloride to 290 mOsm/kg. 1.5% (m/v) SDS was prepared by dispersing SDS in PBS and leaving it overnight on a magnetic stirrer at 500 rpm.

2.2 Methods

2.2.1 Binary fenofibrate/excipient mixtures – batch melting using Differential Scanning Calorimetry

DSC measurements were carried out using a DSC 8500 system equipped with the Intracooler 2 unit (PerkinElmer Inc., Waltham, MA, USA). The apparatus was calibrated using zinc and indium before use. Before testing, samples were milled for 5 minutes using 2 mm glass beads inside glass vials in an MM200 ball miller (Retsch GmbH, Haan, Germany). Samples (5-10 mg) were weighed into vented aluminum pans and sealed with lids. The composition of the binary mixtures is given in Table 1. DSC analysis cycle consisted of 3 heating and cooling runs from 50 °C to 180 °C with a 100 °C/min constant rate. Samples were held at the endpoints for 2 minutes to ensure equilibration.

Table 1. Composition of binary fenofibrate polymer or fenofibrate phospholipid blends prepared by batch melting using a DSC setup

Sample type	FEN content (% m/m)	Excipient (VA64 or P90H) content (% m/m)
Neat excipient	0	100
Neat FEN	100	0
Binary blends	10	90
	20	80
	30	70
	40	60

[a] The binary blends consisted of fenofibrate and VA64 or P90H

2.2.2 Freeze-dried binary and ternary fenofibrate solid dispersions

Binary and ternary solid dispersions of fenofibrate (FEN SDs) were prepared by freeze-drying from water/alcohol solutions. Stock solutions of fenofibrate (5 mg/mL), polymers (20 mg/mL), and phospholipid (10 mg/mL) were prepared by dissolving the compounds in a 92:8 (v/v) *tert*-butanol water mixture. Aliquots of the stock solutions were transferred to glass vials to obtain mixtures with the desired mass ratios of the components (see Table 2).

Table 2. Composition of binary and ternary fenofibrate solid dispersions prepared by freeze-drying

	Content [mg]			Weight ratio
Sample name	**FEN**	**VA64**	**P90H**	**FEN:VA64:P90H**
Freeze-dried FEN	2	-	-	100:0:0
FEN:VA64 10:90	2	18	-	10:90:0
FEN:P90H	2	-	18	10:0:90
FEN:VA64:P90H 10:70:20	2	14	4	10:70:20
FEN:VA64:P90H 15:65:20	3	13	4	15:65:20
FEN:VA64:P90H 20:60:20	4	12	4	20:60:20

The glass vials were frozen overnight at -82°C and placed in a pre-cooled (-60°C) Christ Gamma 2-16 LSC freeze dryer (Martin Christ GmbH, Germany). The freeze-drying cycle comprised the main drying phase with a shelf temperature of 25 °C and a pressure of 0.1 mbar for 24 h and the final drying phase with a temperature of 50 °C and pressure of 0.01 mbar for 3 h. After preparation, fenofibrate solid dispersions were stored in a desiccator over $CaCO_3$ beads at room temperature until used. As a control, fenofibrate was freeze-dried without polymers and phospholipids. The freeze-dried fenofibrate was used the same day after preparation.

Additionally, freeze-dried dispersions were evaluated using DSC with one heating run from 50 °C to 180 °C with a 100 °C/min constant rate. The selected freeze-dried blends were left at room temperature inside the vented pans for seven days to check their short-term stability.

The solid-state of the freeze-dried solid dispersions was investigated by X-ray powder diffraction (XRPD) analysis using a Rigaku MiniFlex 600 (Rigaku Corporation, Tokyo, Japan) system. The instrument operated at 45 kV voltage and 40 mA current. Samples were exposed to Cu Kα radiation and measured within the angular range of 5°-45° with 0.02 step size mode and scanning speed of 10°/min.

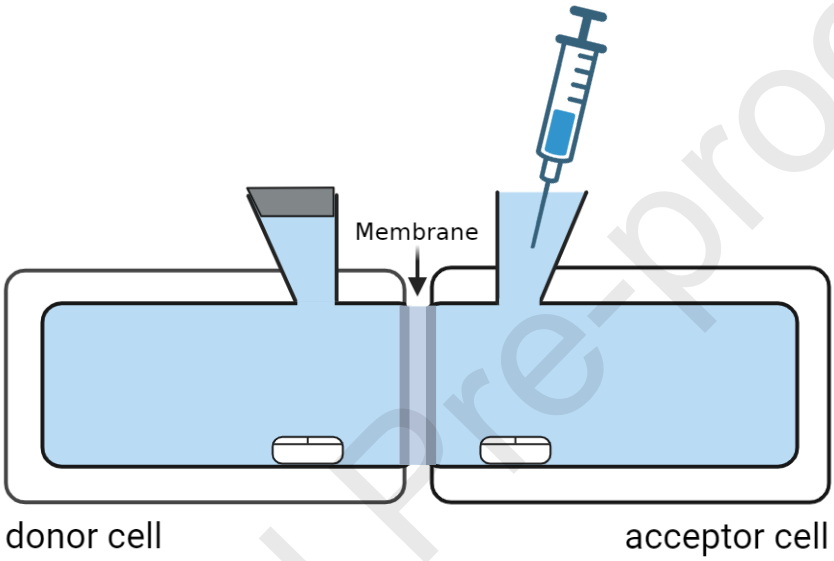

Fig. 1. Side-by-side diffusion cells with a cellulose barrier placed between the donor and acceptor cell used for the in vitro characterization.

2.2.3 Dissolution-/Permeation study

Dissolution/permeation (D/P) testing was performed for freeze-dried binary and ternary mixtures containing FEN using the setup consisting of side-by-side cells (Fig. 1) (PermeGear Inc., Hellertown, PA, USA) with a water jacket, which was separated by a cellulose hydrate barrier (PermeaPlain barrier, Phabioc, InnoMe GmbH, Espelkamp, Germany). Cells were covered with aluminum tinfoil to avoid photodegradation of FEN. The effective permeation area was 1.77 cm^2. The donor and acceptor compartments contained 5 mL and 7 mL, respectively. Both compartments were stirred under a fixed speed of 500 rpm (H-3 stirrer, PermeGear Inc., Hellertown, PA, USA), and the temperature was set to 37 °C.

To assemble the D/P setup, the cellulose barrier was placed between the donor and acceptor chambers. The acceptor compartment was filled with 1.5% SDS in PBS.

20 mg of FEN SDs were dispersed and vortexed for 30 s in 20 mL of biorelevant medium (FaSSIF or FeSSIF), and 5 mL of the dispersions was transferred to the donor compartment using a glass pipette.

During the experiment, samples of 1 mL were withdrawn from both the donor and acceptor compartments after 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min. Samples withdrawn from the donor compartment were centrifuged for 2 minutes at 37°C and 20817 x *g* in a benchtop centrifuge (Eppendorf Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) and filtered through a 0.2 µm Anotop filter composed of alumina-based membrane and polypropylene housing (GE Healthcare, Little Chalfont, UK), discarding the first 0.75 mL of the filtrate. Sampling from the acceptor compartment was delayed by 2 minutes to ensure proper handling. The withdrawn volumes from both compartments were replenished with the appropriate dissolution medium. All samples were immediately diluted threefold with MeOH.

To minimize the effect of non-specific adsorption from aqueous FEN solution, 1 mL glass syringes (FORTUNA® Optima, Poulten & Graf GmbH, Wertheim, Germany) were used for sampling.

2.2.4 Quantification of FEN by HPLC-UV

Fenofibrate was quantified using a Waters 2695 D separation module coupled with a 2487 dual absorbance detector (Waters Corporation, Milford, MA, USA) and a reversed-phase Acclaim® 120 C18 LC-column (150x4.6 mm, particle size 3 µm, pore size 120 Å, Thermo Scientific®, Waltham MA, USA). The column oven was set to 40 °C. The mobile phase comprised 0.1 % TFA and acetonitrile at a 30:70 *v/v* ratio. Chromatographic separation was achieved using isocratic elution with a 1.5 mL/min flow. The injection volume was 50 µL. Fenofibrate was detected at 286 nm after 2.8 min, and the run time was 5.5 min. Two calibration curves were used for quantification in the 0.01–10 µg/mL and 10–100 µg/mL ranges.

2.2.5 Statistical evaluation of data

Statistical analysis was carried out using GraphPad Prism ver. 9.0.0. The area under the concentration-time curve (AUC) was calculated from zero to the last measurable concentration. Two-way ANOVA with Šídák post hoc analysis was carried out. The test compared calculated AUC values data between each media used and between each formulation. The statistical data presented in Fig. 7 show only analysis results for ternary dispersions. The complete data set of statistical analysis can be seen in the supporting information. For p-values ≤0.01, the data were considered significantly different.

3 Results and discussion

3.1 Binary fenofibrate/excipient mixtures – batch melting using Differential Scanning Calorimetry

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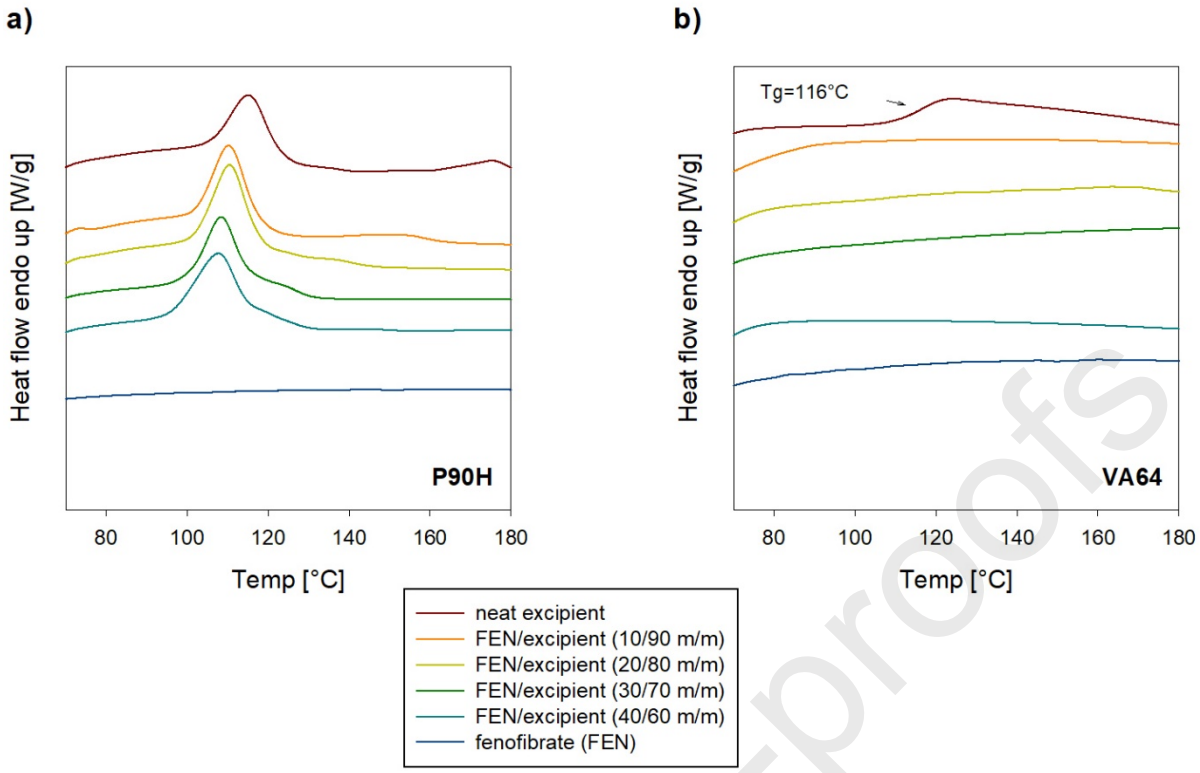

Fig. 2. DSC thermograms of batch-melted FEN/excipient mixtures, a) PHOSPHOLIPON® P90H, and b) Kollidon® VA64. The thermograms were recorded after two previous heating cycles to ensure complete melting and mixing of fenofibrate and the excipient. The curves are plotted with an offset to improve visibility.

One of the most challenging aspects of amorphous solid dispersions is their inherent instability due to the high internal energy, which makes the system prone to re-crystallization during storage. Batch melting in DSC pans is a convenient and fast screening method to evaluate the miscibility of raw materials, drug loading range, and the ability to transition from the crystalline to the amorphous state (Browne et al., 2020; Mura, 1995). Additionally, it is a convenient method to obtain the preliminary stability data of solid dispersions.

Thermal properties of melted fenofibrate, P90H, VA64 and binary mixtures with different fenofibrate-to-excipient ratios are presented in Fig. 2. The thermograms correspond to the third heating run. Two previous heating runs were performed to ensure proper melting and diffusion of fenofibrate throughout the excipient and to dispose of absorbed water. The lack of an endothermic peak corresponding to the melting point of fenofibrate (80-81°C) (Ming-Thau et al., 1994) and, in the case of polymer melts, a single glass transition temperature (T_g) suggested that all of the melts, prepared in situ in the DSC, are fully amorphous.

Fig. 2a shows thermograms of melted binary mixtures of fenofibrate and hydrogenated phospholipid (fenofibrate content 10-40 % m/m). A single endothermic event corresponding to side chain partial melting (Chapman et al., 1967; Kolbina et al., 2019) can be observed in all thermograms independently of the fenofibrate content. On the FEN/P90H (10/90 m/m) thermal curve, an additional endothermic event is visible at 175°C. These events can be attributed to the phase transition of phospholipids from anisotropic solids to fluid anisotropic liquid-crystalline states (Chapman et al., 1967). In the absence of fenofibrate, the onset temperature was 102°C. In the presence of fenofibrate, the onset temperature decreased with increasing fenofibrate content.

Fig. 2b shows the thermograms of binary fenofibrate, and VA64 melts. In Fig. 2b, T_g is seen for the neat polymer. No T_g in the scanning range can be observed with higher FEN loadings. The increasing content of fenofibrate lowered the T_g of the blends. FEN has *a* T_g of -19°C, exhibits a strong plasticizing effect, and reduces the overall T_g of the system (Sailaja et al., 2016). Based on DSC

results, freeze-dried solid dispersions were prepared with a maximal drug loading of 20%. Low storage stability is expected above 20% of drug loading due to the high molecular mobility caused by the plasticizing effect of drug substance (Lehmkemper et al., 2017).

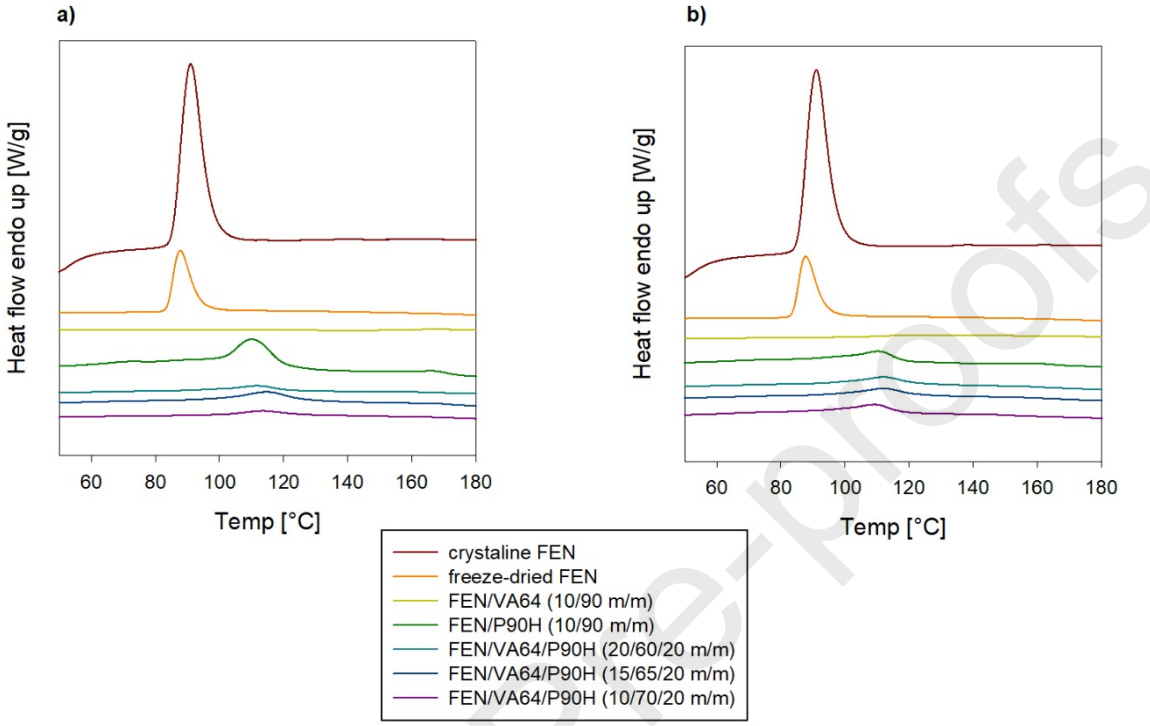

Fig. 3. Short-term stability of freeze-dried FEN and binary and ternary solid dispersions investigated by DSC. DSC thermograms of neat fenofibrate (FEN), freeze-dried FEN, binary blends FEN/excipient (P90H, VA64 10/90 m/m) and ternary FEN/excipient (FEN/P90H/VA64 various mass ratios presented in Table 2, a) before storage (i.e., first heating run performed on day 1), and b) after seven days of storage (i.e., first heating run performed on the freeze-dried formulations). Solid dispersions were stored for seven days under ambient temperature and humidity conditions.

A short-term stability study was performed on the freeze-dried binary and ternary freeze-dried blends to assess the re-crystallization inhibition properties of the excipients. The binary and ternary dispersions prepared by freeze-drying were amorphous at day 1 (Fig. 3a), except neat FEN, which remained crystalline following freeze-drying as indicated by a melting peak at 80°C which corresponds to the melting peak of crystalline FEN (80°C) (Fig. 3a). After 7 days, no re-crystallization of fenofibrate occurred in any of the binary and ternary freeze-dried dispersions. This shows the ample potential of excipients to stabilize the amorphous state. No water sorption was observed for binary and ternary blends. The onset temperature observed of phospholipid was the same at the beginning and after 7 days since the start of the study (Table 3). The decrease in the onset of PL phase transition is similar to that observed in batch-melted binary blends (Fig. 2a), with additional phase transition broad events visible at a low drug-to-excipient ratio.

Table 3. Onset temperatures of endothermic events from the DSC thermograms of neat fenofibrate (FEN), freeze-dried FEN, binary blends FEN/excipient (P90H, VA64 10/90 m/m) and ternary FEN/excipient (FEN/P90H/VA64 various mass ratios presented in Table 2, a) before storage (i.e., first heating run performed on day 1), and b) after seven days of storage (i.e., first heating run performed on the freeze-dried formulations). Solid dispersions were stored for seven days under ambient temperature and humidity conditions; (nd - not determined).

Formulation name	**Onset temperature [°C]**

	Day 0	Day 7
Crystalline FEN	82.1	82.0
Freeze-dried FEN	82.5	81.9
FEN:VA64 10:90	nd	nd
FEN:P90H	100.0	100.2
FEN:VA64:P90H 10:70:20	101.2	101.5
FEN:VA64:P90H 15:65:20	101.9	102.1
FEN:VA64:P90H 20:60:20	101.7	101.9

This study shows that both preparation methods were suitable for obtaining binary and ternary solid dispersions of fenofibrate containing hydrogenated phospholipid at different mass ratios. It is worth mentioning that even though both solvent-based and non-solvent-based methods seemed to be suitable manufacturing methods, the properties of the solid dispersions prepared by either method may differ (e.g., different porosities of the solid material), which could affect their performance (Kolbina et al., 2017).

3.2 XRPD study of freeze-dried binary and ternary solid dispersions

Binary and ternary solid dispersions were prepared by freeze-drying and analyzed by XRPD. The diffractograms shown in Fig. 4b demonstrate that the crystal-specific pattern of fenofibrate, with the most prominent peaks corresponding to FEN crystalline lattice marked in red as a reference, is absent in all the freeze-dried binary and ternary solid dispersions. A broad halo, typical for amorphous materials, can be observed in diffractograms of binary and ternary solid dispersion of fenofibrate. That means freeze-drying is a potential method to obtain amorphous dispersions of binary (FEN/P90H, FEN/VA64) and ternary (FEN/VA64/P90H) blends.

In contrast, it was impossible to obtain fully amorphous fenofibrate without excipients (i.e., neat freeze-dried FEN) as indicated by the diffraction curve (Fig 4a and 4b), which shows intensive sharp peaks. The above results are in agreement with the DSC analysis. Additionally, the XPRD pattern of crystalline FEN differs from the freeze-dried. The peak shift towards lower angles can be attributed to the change in the d-spacing of the FEN crystalline lattice, which is disrupted during the freeze-drying process (Lehmkemper et al., 2017). On the other hand, freeze-drying of crystalline fenofibrate may lead to different polymorphic forms since the substance tends to form polymorphs upon various conditions (Tipduangta et al., 2018).

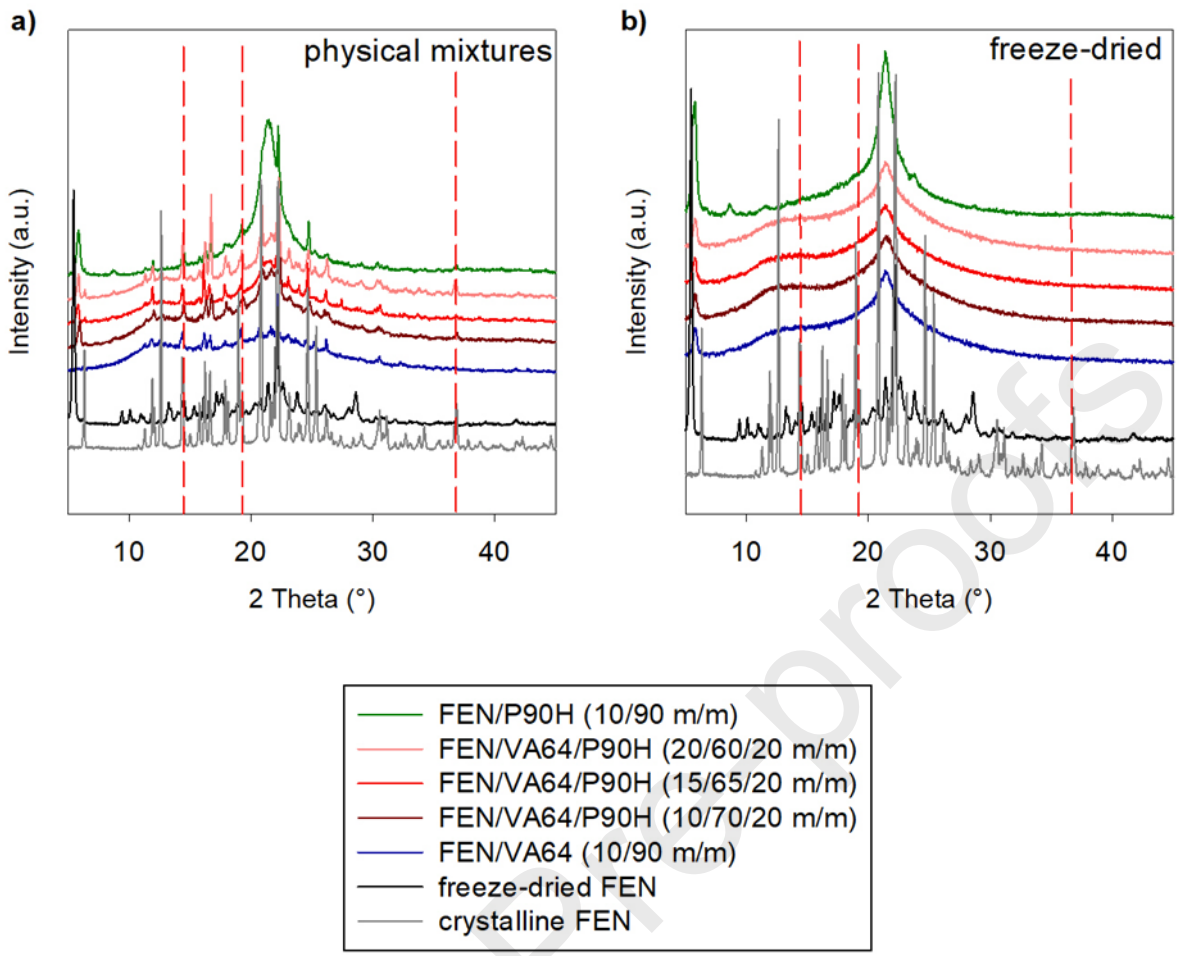

Fig. 4. X-ray diffractograms of binary FEN/excipient and selected ternary FEN/VA64/P90H blends at various drug loadings: a) physical mixtures, b) freeze-dried samples.

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3.3 Dissolution/permeation study of freeze-dried binary and ternary solid dispersions of fenofibrate

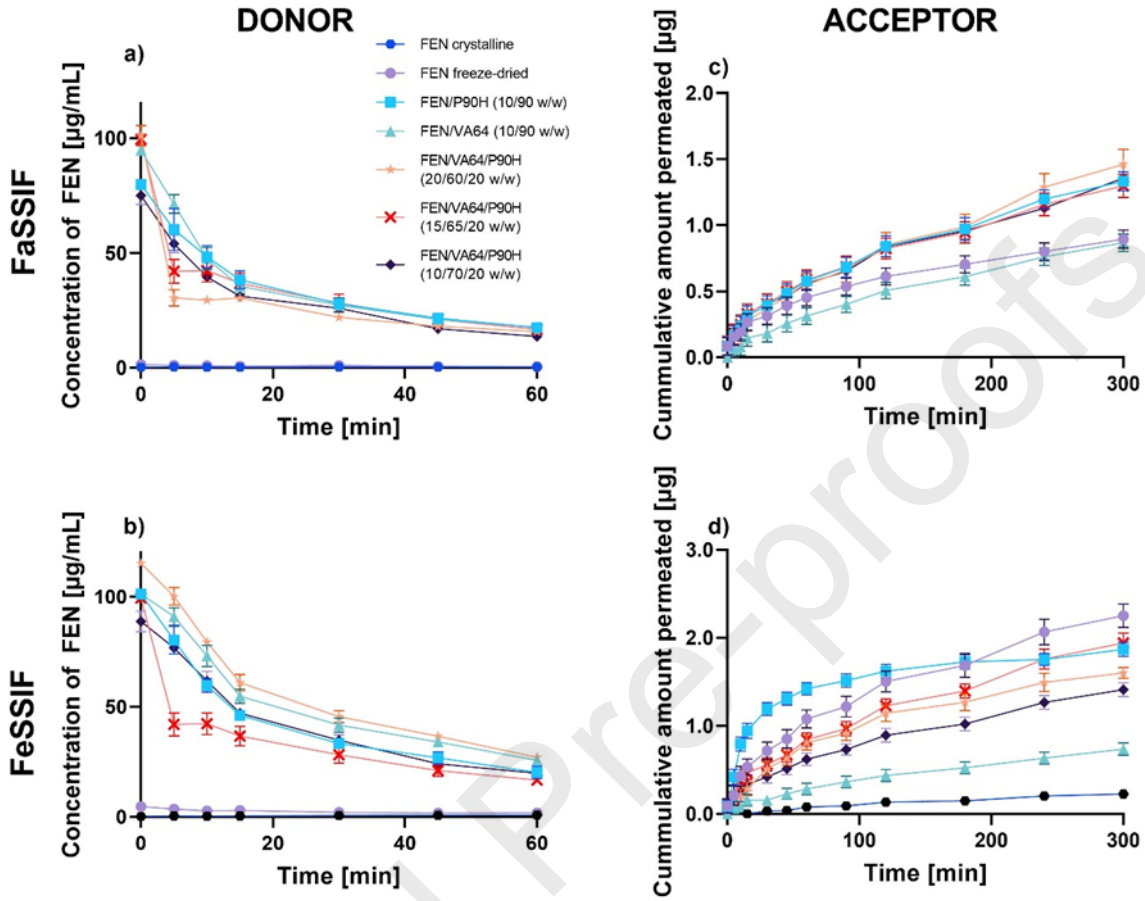

Fig. 5 Results of the dissolution/permeation study performed in the side-by-side setup. The dissolution profiles (a, b) and the permeation profiles (c, d) of neat (crystalline) fenofibrate, freeze-dried fenofibrate and binary and ternary solid dispersions of fenofibrate prepared by freeze-drying. Binary solid dispersions (i.e., FEN/VA64 and FEN/P90H) had a FEN loading of 10%. Ternary solid dispersions (i.e., FEN/VA64/P90H) had different FEN loadings (10-20%). Two dissolution media were used in the donor compartment: FaSSIF (a) and FeSSIF (b). 1.5% SDS in PBS pH 7.4 was used as a medium in the acceptor compartment. Each data point represents the mean ± SD (n = 3).

For the combined dissolution/permeation experiment, amorphous solid dispersions were prepared by freeze-drying, then hydrated and vortexed right before the experiment. The dissolution media were FaSSIF (Fig. 5a) and FeSSIF (Fig. 5b). Fig. 5a-b show FEN concentration changes during dissolution/permeation experiment determined for fenofibrate binary (FEN/VA64 and FEN/P90H, 10/90 m/m) and ternary solid dispersions (FEN/VA64/P90H, various ratios), in the donor compartment. The observed concentration profiles (named dissolution profiles) describe complex processes ongoing in the donor compartments, which involve dissolution, enhanced (and prolonged) supersaturation, followed by precipitation possibly also recrystallization. Dissolution profiles of FEN in all formulations, except that of neat freeze-dried fenofibrate, showed characteristics typical for amorphous solid dispersions with higher initial drug concentrations (supersaturation), followed by gradually decreasing drug concentrations over time, reflecting precipitation or recrystallization. Initially, studied solid dispersions temporarily increase FEN solubility relative to crystalline fenofibrate. The generation of a metastable supersaturated state is known as the "spring effect" (Guzmán et al., 2007). The initial concentration increase is followed by a concentration decrease over time due to precipitation and possibly also crystallization; however, the decreased concentrations are further stabilized and do not reach a concentration as low as observed for crystalline fenofibrate. The

ability to maintain supersaturation for an extended time is described as the "parachute effect" (Guzmán et al., 2007). The observed performance of binary and ternary solid dispersions in the donor compartments of enhanced and prolonged solubilization of FEN may further contribute to increased absorption and bioavailability of FEN in vivo. It is worth mentioning, that the mechanistic understanding of the dissolution / supersaturation / precipitation processes of ASDs has very recently undergone a major revision and extension (Holzem et al., 2023, 2022a, 2022b; Nunes et al., 2023a, 2023b). Obviously, there initially occurs spontaneous formation of submicron drug-rich amorphous precipitates, which subsequently give rise for enhanced concentrations of molecularly dissolved drug above the amorphous solubility (supersaturation). Furthermore, re-crystallization in many of the studied cases occurs much slower (later), if observed at all.

The binary and ternary solid dispersions containing phospholipid were initially dispersed in the biorelevant media containing bile salt, FaSSIF and FeSSIF (Figure 5 a, b). Almost a complete solubilization of fenofibrate is observed for both binary and ternary dispersions in both dissolution media. In FaSSIF, which contains 3 mM sodium taurocholate, complete initial solubilization of fenofibrate (100 µg/mL) was reached for binary solid dispersion containing neat VA64 and for ternary solid dispersions with a drug loading of 15 and 20%. In FeSSIF, which contains 15 mM sodium taurocholate, all solid dispersions reached complete initial fenofibrate solubilization. These results suggest that the bile salt present in the biorelevant media aids hydration and formation of colloidal species such as micelles/mixed micelles, which increases the apparent solubility of the drug substance. These micelles/mixed micelles are small colloidal structures, and they are not separated from molecularly dissolved fenofibrate upon benchtop centrifugation and filtration through the 0.2 µm filter (Holzem et al., 2022a; Jacobsen et al., 2019). Additionally, the bile salts and phospholipids within the biorelevant medium also directly increase the apparent solubility of fenofibrate as it is a highly lipophilic compound with high affinity for mixed micelles formed by bile salts and phospholipids from the dissolution media (Hens et al., 2015) This was especially visible when FeSSIF was used (i.e., initial freeze-dried fenofibrate concentration was 1.33, and 4.72 µg/mL in FaSSIF and FeSSIF, respectively).

Correspondingly to Fig. 5a-b, Fig. 5c-d show the permeation profiles of fenofibrate, which were obtained during the combined dissolution/permeation study. All permeation profiles show that the fenofibrate concentration in the acceptor compartment increased over time, with faster permeation at the beginning and leveling off toward the end of the experiment. Additionally, the area under the curve (AUC) of the permeation profiles is given in Fig. 6. When comparing the amount of fenofibrate accumulated in the acceptor chamber with published data gained with comparable in-vitro setups and barriers, it is obvious that all of the binary and ternary ASDs studied here cause a substantially enhanced permeation in comparison to raw (crystalline) fenofibrate and superior to that observed with nano-milled fenofibrate (Lynnerup et al., 2023). Furthermore, an earlier dynamic dissolution/permeation study on marketed fenofibrate products (Sironi et al., 2017) reports flux values of slightly below 100 ng cm^{-2} $hour^{-1}$ for raw crystalline fenofibrate, around 150 ng cm^{-2} $hour^{-1}$ for the microcrystalline marketed product and slightly below 200 ng cm^{-2} $hour^{-1}$ for the nanocrystalline marketed product. All flux values observed with the various ASDs here rank in the same order of magnitude, some even slightly higher, indicating a good candidate-enabling potential.

The amount of fenofibrate reaching the acceptor compartment depended both on the formulation and the dissolution medium. Noteworthy, even though high apparent supersaturation of FEN is reached for binary FEN/VA64 dispersion, it does not correspond to the increased permeation. Still, to the contrary, the lowest amount of FEN permeates for polymer-based formulation. In the case of all formulations containing hydrogenated phospholipid, the increased supersaturation leads to higher amounts of permeated FEN compared to crystalline FEN in both FaSSIF and FeSSIF. For the binary FEN/P90H solid dispersion, the permeated amount of fenofibrate was much higher than that observed for crystalline fenofibrate and comparable to that of freeze-dried fenofibrate, yet with significant differences in the permeated amounts from FaSSIF and FeSSIF. In contrast, in the case of ternary solid dispersions, even though the permeated amounts of fenofibrate in FeSSIF are lower than those detected for FEN/P90H, they are comparable in FaSSIF.

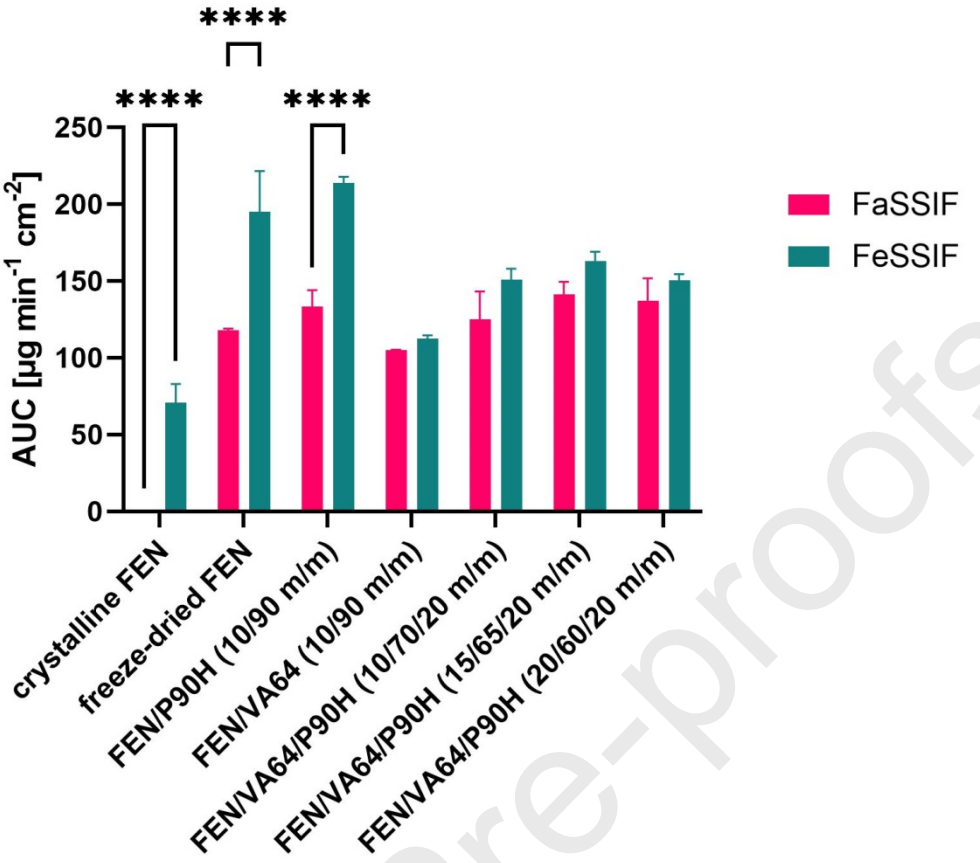

Fig. 6. Area under the curve (AUC) of the amount of normalized flux values. Data reported as a mean ± SD (n=3). The asterisk (****) represents significant differences at p>0.0001. All other differences between the media were not significant.

The presence of bile salts in the dissolution medium (i.e., FaSSIF and FeSSIF) affected the permeation of fenofibrate for some but not all formulations (Fig. 6). The most pronounced changes in fenofibrate permeation were observed for crystalline and freeze-dried fenofibrate and the binary and FEN/P90H. The permeation of crystalline and freeze-dried fenofibrate and FEN in binary FEN/P90H dispersion increased profoundly when FeSSIF was used as a dissolution medium yielding the highest final fenofibrate amount in the acceptor among all samples of 2.25 µg for the freeze-dried fenofibrate.

The presented in vitro data show that the permeation behavior of neat fenofibrate was strongly dependent on the prandial state as mimicked by the different biorelevant dissolution media. The total AUC of permeated neat fenofibrate increased with the increasing amount of bile salts and phospholipids present in the dissolution medium (see Fig. 6). This observation is reasonable considering that the impact of prandial state on fenofibrate absorption is complex (Hens et al., 2015) and depends on the supersaturation induced by the formulation as revealed by in-vitro dynamic dissolution/permeation studies as demonstrated by Sironi et al. (Sironi et al., 2017). This can exacerbate adverse drug reactions and/or abate the therapeutic effect.

While the increase of bile salts in the dissolution medium affected the permeation of both crystalline, freeze-dried fenofibrate and binary dispersion FEN/P90H positively (i.e., a positive "food effect") (Fig. 6), there was no influence of the medium on FEN dissolution profiles obtained for binary and ternary solid dispersion FEN/VA64. Increasing the bile salt concentration in the dissolution medium (i.e., FeSSIF) did not decrease fenofibrate permeation from the FEN/VA64 and FEN/VA64/P90H solid dispersions. This effect is also visible when the AUC of the permeation curves is calculated.

In the presence of FeSSIF, the highest concentration of permeated fenofibrate among all samples was observed for FEN/P90H, which can be seen as a sharp increase in fenofibrate concentration within the first 2 hours of the experiment reaching a plateau at 1.86 µg of fenofibrate. In contrast to the binary solid dispersions, fenofibrate permeation from ternary solid was "immune" to changing dissolution media, and no "food effect" was observed for these formulations (i.e., there was no statistical difference between the ternary dispersions and between different dissolution media used ($p>0.01$) as indicated on Fig. 6.

Evaluating the biopharmaceutical performance of formulations that form solubilizing colloidal species, such as the ternary solid dispersions studied here, is difficult. Traditional biopharmaceutical methods rely on determining how much of the drug is "dissolved". However, in the presence of colloidal species (of different sizes), the dissolved amount depends on the technique used to separate the solid material from the aqueous phase (e.g., filter pore size, centrifugation conditions). To indicate this, the term "apparent solubility" is often used. The presence of colloidal species challenges measuring the molecularly dissolved amount of fenofibrate, which is regarded as important for the permeation process (Sironi et al., 2017). This becomes clear from a previous study on phospholipid-based dispersions of celecoxib comparing mono- and diacyl unsaturated phosphatidylcholine (Jacobsen et al., 2019). There, it was shown that the monoacyl-based phospholipid dispersions yielded much higher apparent solubilities than their diacyl counterparts. However, when the formulations underwent in vitro dissolution/permeation testing, there was no to little difference between them in terms of permeation. A later in vivo study in rats proved that mono- and diacyl phospholipids affect celecoxib absorption similarly (Jacobsen et al., 2021). This suggests that the in vitro performance of phospholipid-based dispersions is mostly dependent on the amorphous-driven increase in the molecularly dissolved drug, which can permeate across the barrier. Therefore, dissolution/permeation testing should be the preferred biopharmaceutical testing method for solid dispersions containing phospholipids, as we also have presented in this study.

4 Conclusion

Hydrogenated phospholipid (HPL) was demonstrated to be suited to serve as a novel excipient for amorphous solid dispersions of the poorly water-soluble model drug fenofibrate, either alone or in combination with a commonly used hydrophilic polymer. A variety of binary and ternary ASDs was successfully prepared via co-melting and/or freeze-drying from organic solutions: within all the blends, the drug was in the amorphous state as determined by DSC-analysis and powder diffraction (XRPD) right after preparation. While freeze-dried neat fenofibrate showed extensive re-crystallization during short-term stability studies (seven days at ambient temperature and humidity), did the drug within all binary and ternary ASDs maintain its amorphous state, even though both pure P90H matrices and ternary matrices exhibited a (liquid-crystalline) phase-transition, which is characteristic for phospholipid monohydrate.

During a combined dissolution/permeation (D/P) study, the potential of hydrogenated phospholipid as ASD excipient became evident:

In the D/P study, a solubility-enhancing potential of binary (FEN/HPL) and ternary (FEN/HPL/polymer) ASDs was observed, dependent on the dissolution medium (FeSSIF>FaSSIF). Regarding enhancing permeation, hydrogenated phospholipid as a matrix-forming agent in binary and ternary systems was as effective or even more effective than polymer-based dispersion. While the permeation of pure fenofibrate (crystalline and freeze-dried) and fenofibrate from binary solid dispersions was affected by the "prandial state" as mimicked by the different dissolution media, the permeation from ternary solid (FEN/HPL/polymer) dispersions was independent of the "prandial state". Interestingly, there was no impact of drug load observed on the dissolution/permeation within the range of drug loads studied here (10 to 20% m/m).

To summarize, using 20% of the HPL as an excipient in the ternary dispersion (FEN/VA64/P90H) seems beneficial with two advantages compared to binary systems. 20% HPL in

FEN/VA64/P90H contributed to the increase in FEN permeability compared to the binary FEN/VA64 system while maintaining evident indifference to the bile salt concentration in contrast to FEN/P90H binary blend.

Further investigations are needed to fully explore whether ternary solid dispersions containing both polymer and hydrogenated phospholipid may represent a viable formulation strategy to enhance fenofibrate absorption. Especially long-term stability against re-crystallization will need to be studied, and last but not least, if the presented potential of hydrogenated phospholipid as ASD excipient holds true in vivo.

CRediT authorship contribution statement

Mikołaj Czajkowski: Investigation, Data curation, Formal analysis, Writing – original draft; Ann Christin-Jacobsen: Data curation, Writing – original draft; Annette Bauer-Brandl: Methodology, Supervision, Writing - review & editing; Martin Brandl: Conceptualization, Methodology, Supervision, Writing - Review & Editing; Paulina Skupin-Mrugalska: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – Review & Editing

Declaration of competing interest

The authors have no conflict of interests to declare.

Acknowledgments

The authors acknowledge the Phospholipid Research Center, Heidelberg, Germany, for financial support, under grant number PSM-2020-085/1-1.

The authors are grateful for fruitful discussions enabled by funding from the Nordforsk program Nordic University Hub ("NordicPop" project #85352).

Graphical abstract created with BioRender.com

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Mikołaj Czajkowski: Investigation, Data curation, Formal analysis, Writing – original draft; Ann Christin-Jacobsen: Data curation, Writing – original draft; Annette Bauer-Brandl: Methodology, Supervision, Writing - review & editing; Martin Brandl: Conceptualization, Methodology, Supervision, Writing - Review & Editing; Paulina Skupin-Mrugalska: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – Review & Editing

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Paulina Skupin-Mrugalska reports financial support was provided by Phospholipid Research Center. Mikolaj Czajkowski reports financial support was provided by Phospholipid Research Center. Martin Brandl reports a relationship with Phospholipid Research Center that includes: funding grants. Ann-Christin Jacobsen reports a relationship with Phospholipid Research Center that includes: funding grants. Paulina Skupin-Mrugalska reports a relationship with APV Mainz that includes: travel reimbursement. Martin Brandl reports a relationship with APV Mainz that includes: travel reimbursement. Ann-Christin Jacobsen reports a relationship with APV Mainz that includes: travel reimbursement. Annette Bauer-Brandl reports a relationship with APV Mainz that includes: travel reimbursement. Martin Brandl reports a relationship with University of Maryland Baltimore that includes: travel reimbursement. Annette Bauer-Brandl reports a relationship with University of Maryland Baltimore that includes: travel reimbursement. Annette Bauer-Brandl has patent PermeaPad patent owned by SDU licensed to out-licensed to InnoMe.

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