Oral absorption from surfactant-based drug formulations: the impact of molecularly dissolved drug on bioavailability

Florentin Lukas Holzem , Neil Parrott , Jeannine Petrig Schaffland , Martin Brandl , Annette Bauer-Brandl , Cordula Stillhart

PII: S0022-3549(24)00263-6
DOI: https://doi.org/10.1016/j.xphs.2024.07.017
Reference: XPHS 3483

To appear in: *Journal of Pharmaceutical Sciences*

Received date: 17 May 2024
Revised date: 17 July 2024
Accepted date: 18 July 2024

Please cite this article as: Florentin Lukas Holzem , Neil Parrott , Jeannine Petrig Schaffland , Martin Brandl , Annette Bauer-Brandl , Cordula Stillhart , Oral absorption from surfactant-based drug formulations: the impact of molecularly dissolved drug on bioavailability, *Journal of Pharmaceutical Sciences* (2024), doi: https://doi.org/10.1016/j.xphs.2024.07.017

Oral absorption from surfactant-based drug formulations: the impact of molecularly dissolved drug on bioavailability

Corresponding author:

Martin Brandl

Author names and affiliations:

Florentin Lukas Holzem*[+], Neil Parrott[$], Jeannine Petrig Schaffland[$], Martin Brandl[+], Annette Bauer-Brandl[+], Cordula Stillhart*

[+] *Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark*

[*] *Pharmaceutical R&D, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland*

[$] *Pharmaceutical Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland*

Abstract

Enabling drug formulations are often required to ensure sufficient absorption after oral administration of poorly soluble drugs. While these formulations typically increase the apparent solubility of the drug, it is widely acknowledged that only molecularly dissolved, i.e. free fraction of the drug, is prone for direct absorption, while colloid-associated drug does not permeate to the same extent.

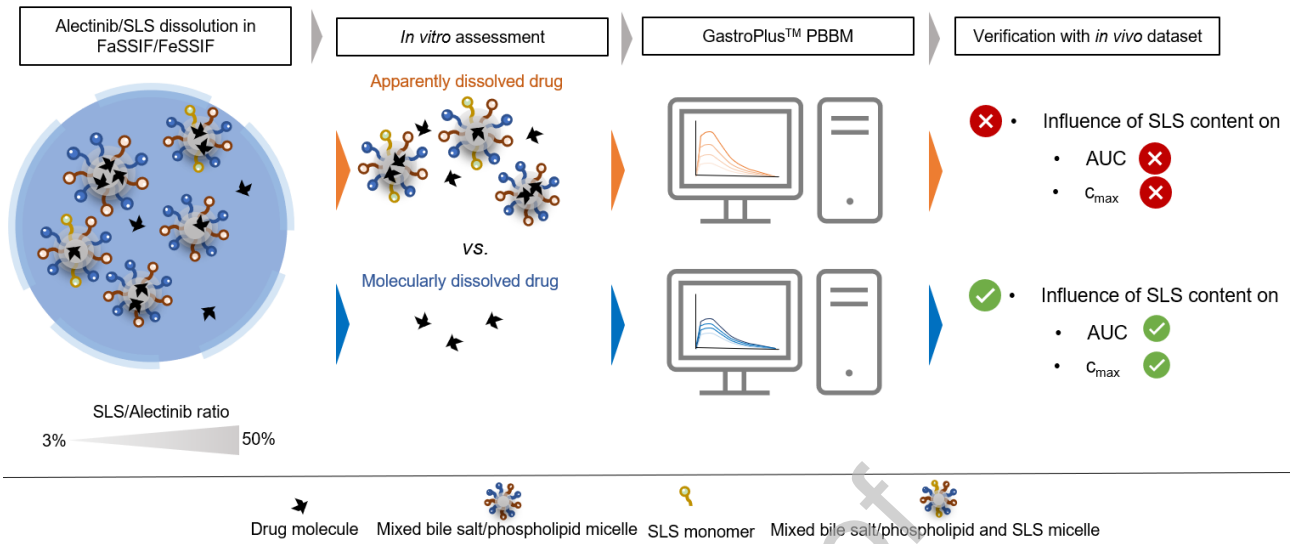
In the present study, we aimed at comparing the effect of molecularly and apparently (i.e., the sum of molecularly and colloid-associated drug) dissolved drug concentrations on the oral absorption of a poorly water-soluble drug compound, Alectinib. Mixtures of Alectinib and respectively 50%, 25%, 12.5%, and 3% sodium lauryl sulfate (SLS) relative to the dose were prepared and small-scale dissolution tests were performed under simulated fed and fasted state conditions. Both the molecularly and apparently dissolved drug concentrations were assessed in parallel using microdialysis and centrifugation/filtration sampling, respectively. The data served as the basis for an in vitro-in vivo correlation (IVIVC) and as input for a GastroPlus™ physiologically based biopharmaceutics model (PBBM).

It was shown that with increasing the content of SLS the apparently dissolved drug in FeSSIF and FaSSIF increased to a linear extent and thus, the predicted in vivo performance of the 50% SLS formulation, based on apparently dissolved drug, would outperform all other formulations. Against common expectation, however, the free (molecularly dissolved) drug concentrations were found to vary with SLS concentrations as well, yet to a minor extent. A systematic comparison of solubilized and free drug dissolution patterns at different SLS contents of the formulations and prandial states allowed for interesting insights into the complex dissolution- / supersaturation-, micellization-, and precipitation-behavior of the formulations. When comparing the in vitro datasets with human pharmacokinetic data from a bioequivalence study, it was shown that the use of molecularly dissolved drug resulted in an improved IVIVC.

By incorporating the in vitro dissolution datasets into the GastroPlus™ PBBM, the apparently dissolved drug concentrations resulted in both, a remarkable overprediction of plasma concentrations as well as a misprediction of the influence of SLS on systemic exposure. In contrast, by using the molecularly dissolved drug (i.e., free fraction) as the model input, the predicted plasma concentration-time profiles were in excellent agreement with observed data for all formulations under both fed and fasted conditions.

By combining an advanced in vitro assessment with PBBM, the present study confirmed that only the molecularly dissolved drug, and not the colloid-associated drug, is available for direct absorption.

Graphical abstract



Keywords

- Microdialysis
- Dissolution
- Enabling formulations
- Poorly soluble drugs
- Alectinib
- Physiologically based biopharmaceutics modeling (PBBM)
- Molecularly dissolved drug
- Solubilization

Nonstandard Abbreviations

AF4-MALLS	Asymmetric flow field-flow fractionation combined with multi-angle light scattering
API	Active pharmaceutical ingredient
ASD	Amorphous solid dispersion
CMC	Critical micellar concentration
FaSSIF	Fasted state simulated intestinal fluid
IVIVC	In vitro-in vivo correlation
PBBM	Physiologically based biopharmaceutics model/modeling
PVDF	Polyvinylidene fluoride
SGF	Simulated gastric fluid
SLS	Sodium lauryl sulfate
TPGS	Tocopherol polyethylene glycol succinate

1 Introduction

An increasing number of drug compounds in pharmaceutical pipelines exhibit poor aqueous solubility and so enabling drug formulations are essential to ensure sufficient drug absorption upon oral administration [1-3]. For decades, enabling formulations have been designed and optimized with regard to their ability to improve the (apparent) drug solubility in the widespread belief that this will increase the in vivo oral bioavailability, a belief which, in many cases, does not hold true [4]. Early indications by Levy and coworkers that only free and not colloid-bound drug is readily available for absorption [5] have been widely forgotten. In the past decade, various mechanistic studies have demonstrated that not all "dissolved" drug species are equally able to boost absorption (e.g., [6-10]). In the meantime, it is widely acknowledged that the concentration of free drug, i.e., single drug molecules surrounded by a hydration shell (molecularly dissolved drug) is the driving force for drug absorption. In contrast, colloidal drug associates such as micelle-bound drug (e.g., in bile salt micelles), complexed drug (e.g., with cyclodextrins), or drug present in other colloidal structures (such as drug-rich nanoparticles) may not be readily available for absorption [11]. The starting hypothesis of this work was that discrimination between the molecularly dissolved and the apparently dissolved drug (i.e., the sum of molecularly dissolved and colloid-associated drug) thereby requires particular attention when predicting in vivo drug product performance.

Various approaches have been described to discriminate between molecularly dissolved and colloid-associated drug in a static manner, i.e., upon dispersing drug formulations in buffer or biomimetic media. These approaches include field-flow fractionation, ultracentrifugation, and equilibrium dialysis. Asymmetric flow field-flow fractionation combined with multi-angle light scattering (AF4-MALLS) has been employed for amorphous solid dispersions (ASDs) [7, 12, 13] or drug release-/transfer-studies of liposome associated drug [14], the latter even attempting to capture the kinetic aspect by repeated fractionations over time. Separation may also be achieved by ultracentrifugation as was demonstrated for ASDs [1, 15]. Equilibrium dialysis and ultrafiltration approaches have been described for surfactant-based formulations [9] or for ASDs [16]. However, all these approaches are cumbersome and time-consuming and take too long to resolve the rapid changes which occur during drug dissolution. Until recently, to our knowledge, there were no analytical methods to discriminate molecularly and apparently dissolved drug in vitro in a time-resolved manner. Standard dissolution experiments typically use filtration or centrifugation to separate the dissolved drug from the undissolved fraction, and thus determine the dissolution-time profile. However, these methods typically reveal the concentrations of apparently dissolved drug and, since a significant amount of the total dissolved drug may be present in colloidal states, the resulting data may overestimate in vivo drug absorption. Recent studies have focused on dynamic in vitro methods to discriminate between molecularly and apparently dissolved drug over time by using in vitro microdialysis sampling [8, 17-20]. These studies employed an innovative approach for studying a variety of enabling formulations by providing a close to real-time quantification of molecularly dissolved drug from a variety of enabling drug formulations. Thus, besides giving novel mechanistic insights into the dissolution processes of enabling formulations, this method offers new opportunities to parametrize physiologically based biopharmaceutics models (PBBMs) and thus integrate in vitro data into a mechanistic PBBM, which is key for formulations with more complex absorption behavior.

Besides experimental in vitro biopharmaceutics tools, PBBMs have gained increasing importance in the recent decade as a standard approach to support biopharmaceutics investigations in drug

product development and registration [21-23]. During formulation development, PBBMs are frequently used to guide formulation selection and optimization based on the integration of relevant in vitro data [24, 25]. The models allow simulation of the impact of different factors, such as drug particle size or formulation type, on drug release and absorption. The drug product performance is integrated into a PBBM via a dissolution model, which requires development and verification with an appropriate set of in vitro data [21]. Using this translational workflow, several commercially available PBBMs have demonstrated the ability to predict accurately the absorption from numerous drug compounds, often from standard immediate release formulations [22, 26, 27]. However, commonly used PBBM software is often limited in their ability to describe or parametrize complex biopharmaceutical processes, which are frequently relevant for enabling drug formulations. Hence, the confidence in predicting the in vivo performance of such formulations may be reduced. In this context, one of the factors that may influence the predictive power of PBBMs is the discrimination between different “dissolved” drug species and their relative contribution to drug absorption. Commonly used PBBMs provide limited options to discriminate between different “dissolved” drug species and their ability to permeate across the gut membrane. This limitation is not only due to the software, but also to the quality of input data, which is usually more representative for the apparently dissolved drug, rather than the molecularly dissolved drug. Thus, the model may mispredict absorption leading to inaccurate prediction of in vivo exposures.

The present study aimed at characterizing the contribution of molecularly vs. apparently dissolved drug on oral drug absorption. For this purpose, the very poorly water-soluble drug Alectinib was studied as a model compound under simulated fed and fasted state conditions in vitro and using PBBM. Small-scale in vitro tests were performed using microdialysis and commonly used bench-top centrifugation/filtration sampling to quantify the molecularly and apparently dissolved drug, respectively. The tests were set up to simulate the oral administration of formulations with 3%, 12.5%, 25% and 50% sodium lauryl sulfate (SLS) relative to the Alectinib dose. The dissolution data were used to parametrize a PBBM to predict plasma exposure from the different formulations based on either the apparently or the molecularly dissolved Alectinib. In vivo pharmacokinetic (PK) profiles from Morcos et al. [28] served as a basis for evaluating the experimental and modelling results from this study.

2 Materials and Methods

2.1 Chemicals

Alectinib hydrochloride was obtained from F. Hoffmann-La Roche AG (Basel, Switzerland). Sodium hydroxide was purchased from Sigma-Aldrich GmbH (Buchs, Switzerland).Sodium chloride and glacial acetic acid were purchased from Fisher Scientific AG (Reinach, Switzerland). Sodium phosphate monobasic dihydrate, hydrochloric acid and ethanol (EtOH) were obtained from Merck KGaA (Darmstadt, Germany) and SLS was obtained from BASF SE (Ludwigshafen, Germany). Vitamin E tocopherol polyethylene glycol succinate (Vit E TPGS) was purchased from PMC Isochem (Vert-le-Petit, France), and FaSSIF/FeSSIF/FaSSGF powder was obtained from Biorelevant.com (London, UK). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich GmbH (Buchs, Switzerland). Acetonitrile, dimethyl sulfoxide (DMSO), and purified water were obtained from VWR International GmbH (Dietikon, Switzerland).

2.2 Media preparation

Simulated gastric fluid (SGF) was prepared by dissolving 2 g/L sodium chloride in purified water in approx. 90% of the target volume, adjusting the pH to 2.0 with 1 M HCl, and filling up to the final volume.

Blank fasted state simulated intestinal fluid (FaSSIF version 1) was obtained by dissolving 4.47 g/L sodium phosphate monobasic dihydrate, 0.42 g/L sodium hydroxide, and 6.186 g/L sodium chloride in approx. 90% of the target volume of purified water. After adjusting the pH to 6.5 with 1 M sodium hydroxide solution, the final volume was obtained by filling with purified water.

Blank fed state simulated intestinal fluid (FeSSIF) was obtained by dissolving 4.04 g/L sodium hydroxide, 8.65 g/L glacial acetic acid, and 11.874 g/L sodium chloride in approx. 90% of the target volume of purified water. After adjusting the pH to 5.0, the final volume was obtained by filling with purified water.

Ten-fold concentrated blank FaSSIF was prepared by dissolving 44.7 g/L sodium phosphate monobasic dihydrate, 4.2 g/L sodium hydroxide, and 43.86 g/L sodium chloride in approx. 90 % of the final volume of purified water. The pH was adjusted to pH 6.5 with 1 M sodium hydroxide. The final volume was obtained by filling with purified water.

FaSSIF and 10-fold concentrated FaSSIF were prepared by dissolving 2.24 g/L and 22.4 g/L FaSSIF/FeSSIF/FaSSGF powder in blank FaSSIF and 10-fold concentrated blank FaSSIF, respectively. FeSSIF was obtained by dissolving 11.2 g/L FaSSIF/FeSSIF/FaSSGF powder in blank FeSSIF.

2.3 Ultra-high performance liquid chromatography

For the quantification of Alectinib, ultra-high performance liquid chromatography (UHPLC) was applied using an Acquity H-class UHPLC-UV system (Waters, Milford, MA). Samples were separated on an Atlantis Premier BEH C18 AX column (1.7 µm, 2.1 mm x 50 mm) (Waters, Milford, MA) at a column temperature of 40°C and a flow rate of 0.75 mL/min. A gradient method was applied as shown in Table 1 using 0.1% (v/v) TFA in highly purified water and 0.1% (v/v) TFA in acetonitrile. The injection volume was 3 µL and the retention time was 1.9 min. Samples were detected at a wavelength of 265 nm.

Table 1: UHPLC-UV gradient profile used for Alectinib quantification.

Elution time (min)	0.1% TFA in water (%)	0.1% TFA in acetonitrile (%)
Initial	95	5
0.20	95	5
3.00	5	95
5.00	5	95
7.00	95	5
8.00	95	5

2.4 Microdialysis setup

A microdialysis setup was used to quantify the molecularly dissolved drug concentrations during small-scale dissolution tests. The setup consisted of a CMA/4004 syringe pump, 2.5 mL glass

syringes, and microdialysis probes (20 kDa cutoff, polyarylethersulfone membrane) with a 10 mm effective membrane length. Before each experiment, the syringes were filled with perfusion medium, placed into the syringe pump, and connected to the inlet tubing of the microdialysis probes. All parts of the setup were purchased from CMA Microdialysis AB (Kista, Sweden).

A solution of 2% Vit E TPGS in blank FaSSIF and blank FeSSIF was used for microdialysis sampling in FaSSIF and FeSSIF, respectively. The recovery of each microdialysis probe was determined by placing the probes in 20 mL dissolution vessels containing 20 mL of SGF pH 2 at 37°C. The stirring speed for the recovery determination was adjusted to the stirring rates used for the dissolution tests. Thus, the medium was stirred with a magnetic stirrer at 250 rpm and 75 rpm to determine the recovery representative for dissolution tests in FeSSIF and FaSSIF, respectively. SGF pH 2 was spiked with a 5 mg/mL stock solution of Alectinib HCl in DMSO, resulting in Alectinib concentrations of approx. 2.5, 5, 10, 15, 20, 25, and 30 µg/mL. Microdialysis samples were taken 5 min after spiking the medium by placing the outlet tubes in a 200 µL HPLC vial and subsequently collecting the perfusate for 10 min at a flow rate of 1.5 µL/min. All microdialysis samples were diluted 1:1 v/v with EtOH prior to UHPLC quantification. The concentration of Alectinib in the vessel was determined by withdrawing approx. 100 µL of the media and diluting with EtOH prior to UHPLC analysis. All Alectinib concentrations in SGF were above the solubility of Alectinib hydrochloride, and thus, precipitation may occur after a certain time. To exclude subvisible particles that may have formed during the 15 min sampling interval, an additional filtration sample was taken. Approx. 200 µL of the medium was withdrawn by a syringe and filtered through a polyvinylidene fluoride (PVDF) filter with a nominal pore size of 0.2 µm (Merck KGaA, Darmstadt, Germany). After discarding the first three droplets, the filtrate was collected in Eppendorf tubes and diluted with EtOH prior to UHPLC analysis. The recovery values for each probe were calculated according to the procedure described by Fong et al. [19].

2.5 Small-scale dissolution tests with combined microdialysis sampling

Small-scale dissolution tests were conducted to quantify the apparently (assessed after bench-top centrifugation/filtration sampling) and molecularly dissolved (assessed after microdialysis sampling) drug concentration of Alectinib HCl/SLS mixtures at four different ratios, i.e., 3, 12.5, 25, and 50% w/w SLS relative to Alectinib, in FaSSIF and FeSSIF.

Dissolution tests under simulated fed conditions were conducted by weighing Alectinib HCl (48 mg free base equivalent) and 24, 12, 6, and 1.44 mg SLS, respectively, into 20 mL glass vials. The vials were placed in a heating jacket (µDISS profiler) at 37°C and the microdialysis probes were placed in the vessels. At t=0, a volume of 20 mL of pre-heated FeSSIF (resulting in a simulated human dose of 600 mg Alectinib free base equivalent in 250 mL medium) was poured into the glass vials under magnetic stirring at 250 rpm. To determine the concentration of apparently dissolved Alectinib, approx. 200 µL of FeSSIF were withdrawn after 5, 10, 15, 30, 60, 90, 120, 150, 180, 210, and 240 min and filtered through a 0.2 µm PVDF filter. After discarding the first three droplets, the filtrate was diluted with EtOH and quantified via UHPLC. To determine the concentration of molecularly dissolved Alectinib, microdialysis samples were taken at 5-15, 15-25, 25-35, 55-65, 85-95, 115-125, 145-155, 175-185, 205-215, and 235-245 min after the start of the experiment by the procedure described in 2.4.

Dissolution tests under simulated fasted conditions were conducted in a two-staged dissolution setup as follows: 48 mg Alectinib free base equivalent (in the form of Alectinib HCl-salt) were

weighed together with the required amount of SLS to obtain 3, 12.5, 25, and 50% w/w SLS/Alectinib mixtures. The powder was filled into size 2 HPMC capsules (Capsugel, Bornem, Belgium) and placed into 20 mL screw-cap vials. At t=0, 18 mL SGF pH 2.0 were poured into the glass vials. The vials were placed in a thermostated oven at 37°C and 5 rpm end-over-end rotation for 30 min. Samples were taken at 5, 15, and 25 min after the start of the experiment by withdrawing 200 µL SGF, subsequent centrifugation at 13,000 rpm (9447 rcf) for 2 min, and dilution of 50 µL of the clear supernatant with EtOH. The centrifugation procedure was preferred over filtration due to extensive clogging of the filter membranes. After 30 min, the medium was transferred into 20 mL glass vials, the microdialysis probes were added and the media were stirred at 75 rpm. A reduced stirring rate of 75 rpm was applied in FaSSIF, compared to dissolution tests in FeSSIF, as Alectinib was shown to be prone to precipitation under simulated fasted conditions. The relatively high stirring rate of 250 rpm applied in FeSSIF was expected to overpredict precipitation and was thus reduced in all experiments using FaSSIF. A volume of 2 mL 10-fold concentrated FaSSIF and 70 µL of 1 M NaOH was added to SGF to obtain FaSSIF pH 6.5. To quantify the apparently dissolved drug concentrations, 200 µL samples were withdrawn after 35, 40, 45, 60, 90, 120, 150, 180, 210, 240, and 270 min, centrifuged (13,000 rpm, 9447 rcf, 2 min), and 50 µL of the clear supernatant were diluted with EtOH prior to UHPLC analysis. To quantify the molecularly dissolved drug concentrations, microdialysis samples were taken at 30-40, 40-50, 50-60, 70-80, 85-95, 115-125, 145-155, 175-185, 205-215, 235-245, and 265-275 min after the start of the experiments as outlined in 2.4. All tests were performed in triplicate.

The influence of the encapsulation, stirring rate, and the sampling method (centrifugation vs. filtration) on apparently and molecularly dissolved Alectinib, were evaluated and are presented in the Supplementary Materials, Figure S1. In brief, it was shown that the stirring rate had a minor influence on the dissolution rate, but no influence on the highest measured concentration, the encapsulation did not influence the highest measured concentration and, the sampling methods for assessing the apparently dissolved drug (centrifugation vs. filtration) were comparable. No influence on the average measured concentration of molecularly dissolved Alectinib at 30-180 min was found.

2.6 PBBM development

GastroPlus™ 9.8 (Simulations Plus, Inc., Lancaster, USA) was used to build a PBBM for Alectinib. Key input data were obtained from a published GastroPlus™ physiologically based pharmacokinetic (PBPK) three-compartment model for Alectinib and are listed in Table 2[29].

Table 2: Key input data used in the Alectinib PBBM. All presented data were obtained from Parrott et al. [29].

Parameter	**Input**	
LogD	1.96 at pH 3.575	
pKa	7.05 (basic)	
Effective permeability	2.5×10^{-4} cm/s	
Thermodynamic solubility of Alectinib-free base	pH	Solubility in µg/mL
	0.97	7.45
	2.33	4.49
	3.13	14.01
	4.10	0.869

	5.15	0.059
	5.95	0.013
	7.30	0.019
	7.90	0.015
	8.94	0.016
	9.63	0.015
	10.99	0.035
	11.65	0.168
Particle diameter	4 µm	
Blood/plasma ratio	2.6	
Free fraction in plasma	0.003	
Clearance from the central compartment (CL)	0.45 L/h/kg	
Clearance from the second compartment (CL2)	4.41 L/h/kg	
Clearance from the third compartment (CL3)	0.426 L/h/kg	
Compartment volume (V_C)	0.43 L/kg	
Compartment volume (V2)	3.32 L/kg	
Compartment volume (V3)	3.02 L/kg	

The modeling work focused on the contribution of apparently and molecularly dissolved Alectinib to the overall Alectinib absorption. For this purpose, the concentrations of apparently and molecularly dissolved Alectinib determined with the four Alectinib/SLS ratios in the dissolution tests by filtration/centrifugation and microdialysis, respectively, were implemented in the PBBM. For simulations in the fed state, concentrations of apparently and molecularly dissolved Alectinib obtained from the small-scale dissolution tests in FeSSIF were used as input for biorelevant solubility. The input concentrations were based on the average concentration measured in the interval of 60 to 240 min of dissolution time after filtration (apparently dissolved drug) and microdialysis (molecularly dissolved drug), respectively. The two-stage dissolution tests of the Alectinib/SLS mixtures under simulated fasted state conditions revealed that precipitation occurs upon media transition from SGF to FaSSIF. We assume that the in vitro setup likely overpredicts precipitation due to the lack of an absorption sink and the experience that the addition of a microdialysis probe in a dissolution vessel may promote precipitation of supersaturated drug compounds [30]. Thus, to predict the oral exposure under simulated fasted conditions, the bile salt solubilization ratio was fitted to the highest observed Alectinib concentration in FaSSIF (measured by either centrifugation or microdialysis), rather than the mean dissolved drug concentrations over time.

The bile salt solubilization ratio was fitted to obtain a simulated duodenal solubility in agreement with the observed in vitro data obtained under simulated fasted and fed state conditions (Table 3). The default dissolution model (Johnson) was applied for all simulations. When entering the biorelevant solubility, by default GastroPlus™ adjusts the diffusion coefficient for drug present in bile salt micelles to account for lower diffusion coefficients of drug in micelles compared to unbound drug. To avoid that GastroPlus™ accounts for a lower diffusion coefficient when using molecularly dissolved drug as input, the adjustment of diffusion coefficients for free and micelle bound drug was disabled.

An in vitro disintegration test of the marketed Alectinib formulation in SGF showed extensive agglutination (internal, unpublished data). This effect is expected to prolong the gastric residence time due to the more difficult pylorus passage of an incompletely disintegrated dosage unit (thus larger particles are assumed to be present in the stomach) or incomplete mixing of a poorly soluble drug with the chyme in the fed state. Thus, the stomach transit time in our PBBM was extended to 0.5 h under simulated fasted state conditions and 2.5 h under simulated fed state conditions.

All simulations were validated against in vivo plasma concentration-time profiles reported by Morcos et al. [28].

Table 3: Fitted bile salt solubilization ratios (BSSR) for simulated fasted and fed state based on input data for apparently and molecularly dissolved drug concentrations.

	Apparently dissolved drug				**Molecularly dissolved drug**			
% SLS (relative to Alectinib)	**BSSR (fed state)**	**Experimental value (fed state) in µg/mL**	**BSSR (fasted state)**	**Experimental value (fasted state) in µg/mL**	**BSSR (fed state)**	**Experimental value (fed state) in µg/mL**	**BSSR (fasted state)**	**Experimental value (fasted state) in µg/mL**
50% SLS	2.3E+7	331	1.56E+8	192	3.32E+6	48	1.57E+7	18
25% SLS	1.75E+7	251	8.7E+7	107	2.67E+6	38	1.28E+7	16
12.5% SLS	1.47E+7	212	7.4E+7	92	2.4E+6	33	9.4E+6	12
3% SLS	1.27E+7	183	3.3E+7	42	2.5E+6	34	6.5E+6	8

2.7 Summary of in vivo reference data

A bioequivalence study has been conducted in healthy adults to assess the effect of increasing SLS content in Alectinib capsule formulations on the oral exposure under fed (n=48) and fasted state (n=49) conditions [28]. Formulations with 25%, 12.5%, and 3% SLS relative to the Alectinib dose of 600 mg were compared against a 50% SLS reference formulation. Table 4 summarizes relevant in vivo PK data reported by Morcos et al.[28].

Table 4: Summary of PK parameters reported by Morcos et al. [28].

		Formulation			
		50% SLS	**25% SLS**	**12.5% SLS**	**3% SLS**
Fed state	**c_{max} (ng/mL)**	271 (82)	232 (59)	206 (50)	204 (57)
	$AUC_{0\text{-}inf}$ (ng·h/mL)	5720 (1530)	5050 (1200)	4600 (1260)	4580 (1240)
	t_{max} (h)	8.00 (4.1-18.0)	8.00 (5.0-18.0)	6.00 (4.0-12.0)	6.00 (4.0-10.0)
Fasted state	**c_{max} (ng/mL)**	106 (53)	92 (35)	67 (24)	42 (19)
	$AUC_{0\text{-}inf}$ (ng·h/mL)	1920 (1000)	1840 (754)	1850 (841)	1630 (792)
	t_{max} (h)	3.25 (1.5-12.0)	3.00 (1.0-5.0)	3.00 (2.0-6.0)	4.00 (2.5-10.0)

c_{max} and $AUC_{0\text{-}inf}$ are reported as arithmetic mean (SD); tmax is reported as median value (range)

Based on the geometric mean ratios and their 90% confidence intervals for c_{max}, $AUC_{0\text{-inf}}$, and $AUC_{0\text{-last}}$ falling in the range of 80 to 125%, the study demonstrated bioequivalence of the 25% SLS formulation compared to the 50% SLS formulation in both fed and fasted state. Under fed state conditions, only minor differences in total exposure (<25% difference in mean $AUC_{0\text{-inf}}$ and c_{max}) were observed between all formulations. In contrast, under fasted conditions, the SLS content substantially influenced c_{max}. While the 25% SLS formulation met the bioequivalence criteria, c_{max} of the 12.5% and 3% SLS formulations decreased to approx. 65% and 40% of the value of the reference formulation, respectively. The overall exposure of all formulations in terms of $AUC_{0\text{-inf}}$, however, fell inside the 80-125% bioequivalence range of the 50% SLS formulation.

The in vivo dataset served as a basis for verification of the predictive power of both, in vitro and PBBM results from the present study.

3 Results, Part 1: In vitro dissolution

Small-scale in vitro dissolution tests under simulated fed and fasted state conditions were performed to assess both, the apparently and molecularly dissolved drug concentrations from Alectinib HCL/SLS mixtures with increasing relative levels of SLS. This test was set up to understand the impact of increasing levels of SLS on Alectinib dissolution and solubilization in biorelevant media and served as a basis for PBBM development.

The dissolution profiles of the different Alectinib HCl/SLS mixtures are presented in Figure 1. The dissolution profiles of apparently dissolved drug (quantified after filtration) in FeSSIF showed a fast, albeit incomplete dissolution (>85% of the dose undissolved), which reached a plateau within 30 min after the start of the experiment (Figure 1A). This plateau was assumed to correspond to the apparent solubility of Alectinib in the presence of the corresponding amount of SLS in FeSSIF. The 50% SLS mixture showed highest apparently dissolved drug concentrations of approx. 320 µg/mL and, overall, a linear increase in apparently dissolved drug with increasing SLS levels (Supplementary materials, Figure S2).

The molecularly dissolved drug concentrations (assessed after microdialysis sampling, Figure 1B) showed a different pattern. The 50% SLS mixture resulted in the highest concentrations of molecularly dissolved Alectinib. However, while the 25% SLS mixture showed slightly higher molecularly dissolved Alectinib concentrations than the 12.5% and 3% SLS mixtures, no substantial difference was observed between the 12.5% and 3% SLS mixtures.

To assess the dissolution behavior of the Alectinib HCl/SLS mixtures under simulated fasted state conditions, a small-scale two-stage dissolution test was performed which consisted of a 30 min phase in SGF pH 2 followed by a 4 h phase in FaSSIF pH 6.5 (Figure 1C and D). In SGF pH 2, major differences in the apparently dissolved drug concentrations were observed for the different SLS levels (Figure 1C). While the 3% and 12.5% SLS mixtures showed only minor dissolution (<2% of the dose), an increase in SLS level to 25% and 50% resulted in high apparently dissolved drug concentrations of approx. 170 and 280 µg/mL, respectively. The initial fast dissolution was followed by a decrease in apparently dissolved Alectinib at 25 min after start of the experiment, which was most prominent for the 50% SLS mixture. Upon media transition to FaSSIF, the 25% and 50% SLS mixtures appeared to supersaturate and subsequently precipitate in FaSSIF, whereas the 12.5% and 3% SLS mixtures showed further dissolution and no apparent supersaturation (Figure 1C). As observed in FeSSIF, the concentration of apparently dissolved

Alectinib in FaSSIF was proportional to the SLS level (Supplementary materials, Figure S2). However, the apparently dissolved drug concentrations did not reach a stable plateau but decreased over the entire duration of the experiment (Figure 1C). A major difference in the dissolution pattern of molecularly and apparently dissolved Alectinib was observed for the 25% and 50% SLS mixtures. The molecularly dissolved concentrations of these two mixtures in FaSSIF were very similar, and, analogous to the concentrations of apparently dissolved Alectinib, the concentrations of molecularly dissolved Alectinib decreased over time (Figure 1D). Although the measured molecularly dissolved concentrations of the 12.5% and 3% SLS mixtures were lower compared to the 25% and 50% SLS mixtures, the precipitation was most prominent for the 12.5% and 3% SLS mixtures.

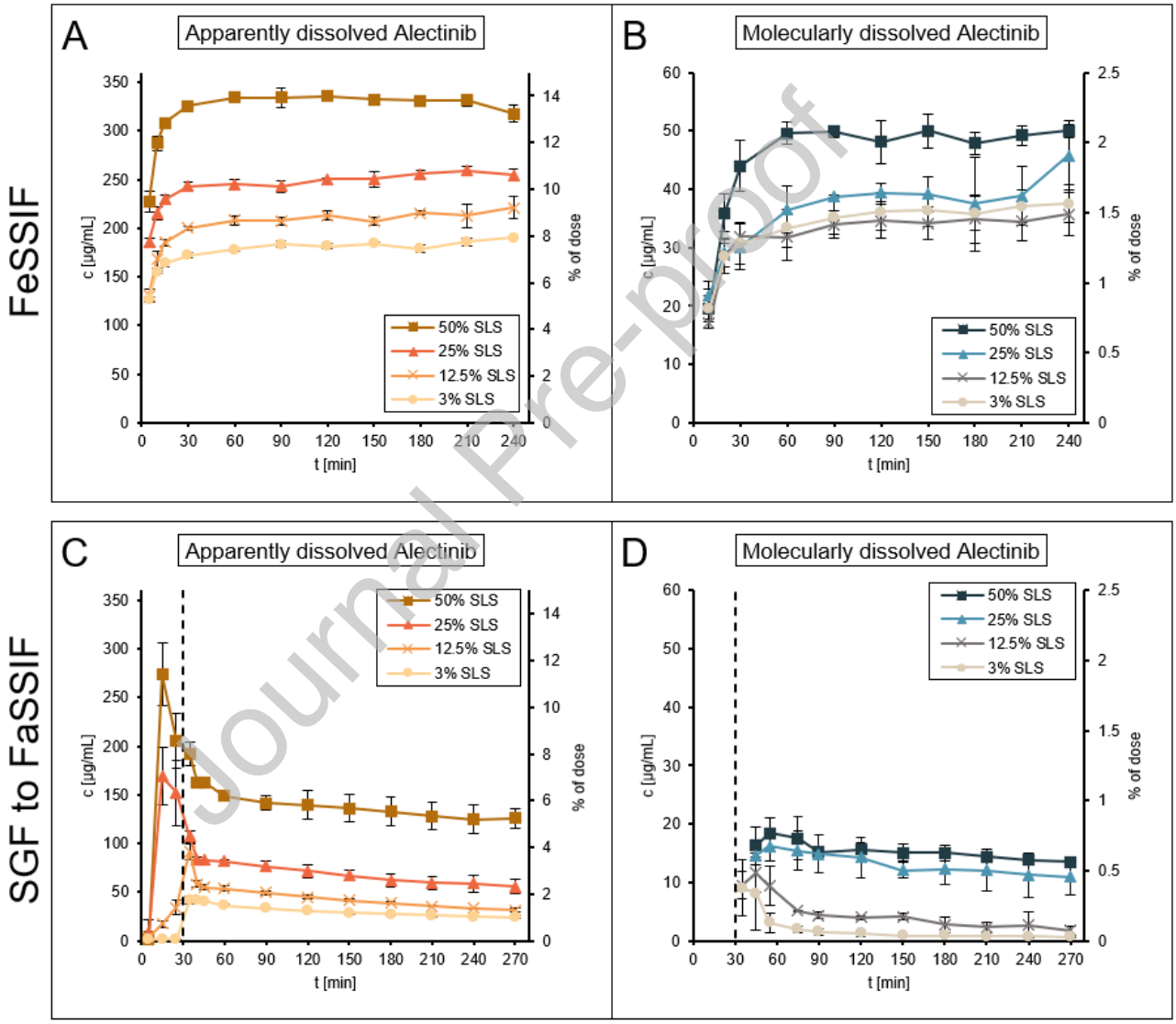


Figure 1: Alectinib concentrations (mean±SD) in small-scale dissolution tests of Alectinib/SLS mixtures with 50%, 25%, 12.5%, and 3% SLS (relative to Alectinib dose) under simulated fed state (FeSSIF, A and B) and fasted state (SGF to FaSSIF, C and D) conditions. The dashed lines indicate a media transition from SGF to FaSSIF. Apparently dissolved drug (A and C) was assessed after filtration and bench-top centrifugation in FeSSIF and SGF/FaSSIF, respectively. Molecularly dissolved Alectinib (B and D) was assessed after microdialysis sampling. All tests were performed in triplicate (n=3). Error bars are partly covered by the symbols.

The apparently dissolved and molecularly dissolved drug concentrations measured in vitro were furthermore plotted against the in vivo reference data and are presented in Figure 2.

Figure 2A and B present the correlation of the in vitro c_{max} (apparently and molecularly dissolved drug concentrations, respectively) and in vivo c_{max}. Both correlations exhibited a reasonable linear fit and thus, both in vitro methods (filtration/centrifugation and microdialysis) resulted in an adequate prediction of in vivo performance in terms of c_{max}. Nevertheless, two datapoints of the correlation based on apparently dissolved drug fell outside the linear fit (Figure 2A, datapoints indicated by arrows). Both points are allocated to the 50% SLS mixture under simulated fed and fasted state conditions, which suggest that the effect of SLS on the in vivo c_{max} was overpredicted for this formulation. A better correlation was observed when plotting the in vivo c_{max} with the in vitro c_{max} of the molecularly dissolved drug concentrations (Figure 2B). The improved linear fit (R^2 of 0.98) was mainly attributed to the 50% SLS mixture (dark blue datapoints), which falls closer to the linear correlation for simulated fed and fasted state conditions.

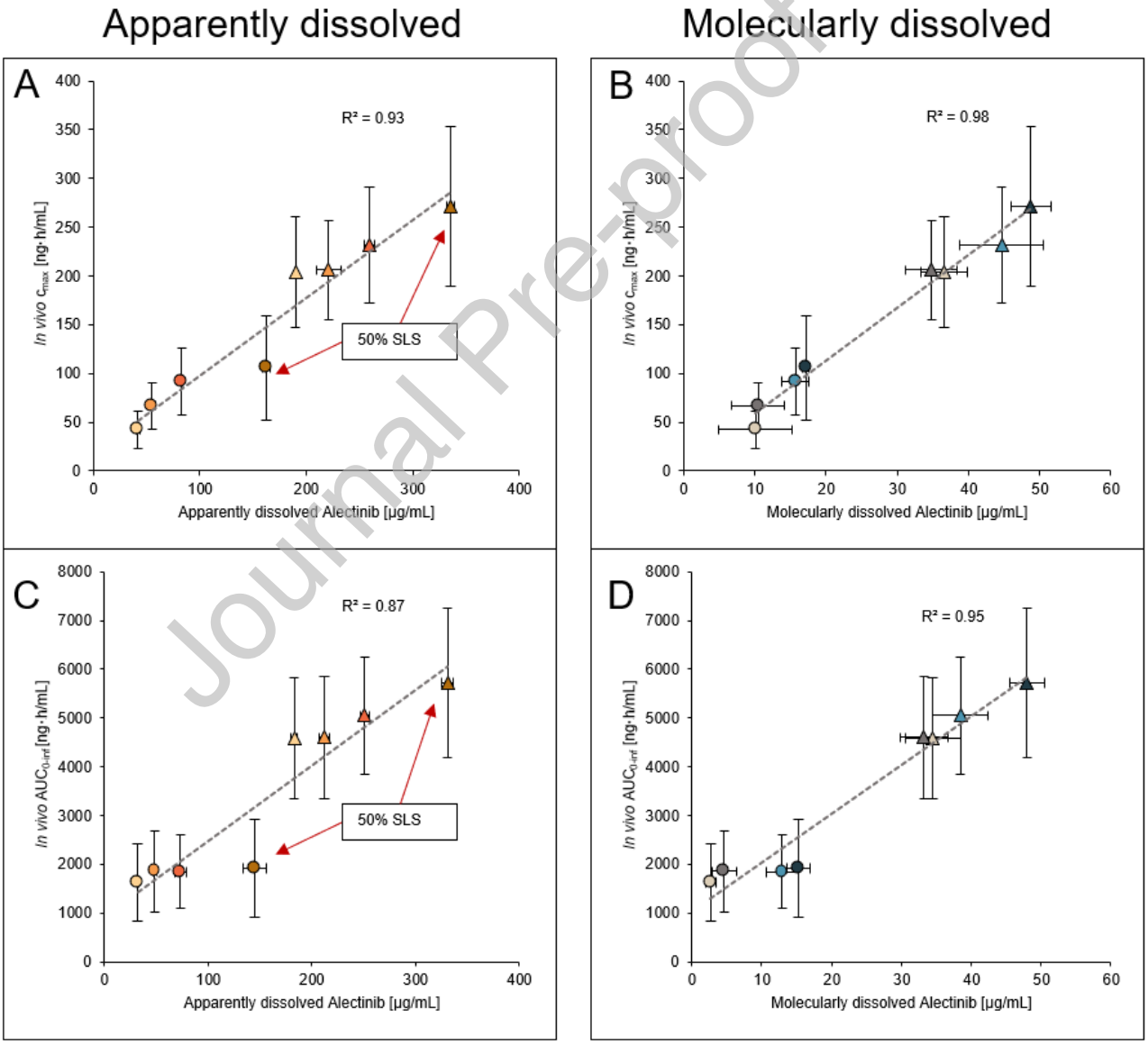


Figure 2: Correlation between observed in vitro and reference in vivo data based on apparently dissolved drug concentrations (A and C) and molecularly dissolved drug concentrations (B and D), respectively. In vivo c_{max} was

correlated with the highest measured in vitro concentration (A and B) in FeSSIF and FaSSIF, respectively. In vivo AUC_{0-inf} was correlated with mean measured apparently dissolved (C) and molecularly dissolved (D) drug concentrations in FeSSIF and FaSSIF, respectively. (Simulated) fasted state and fed state conditions are presented as circles and triangles, respectively. The color coding refers to the Alectinib/SLS mixtures shown in Figure 1. In vivo reference data were obtained from Morcos et al. [28].

4 Discussion, Part 1: In vitro dissolution

The first part of the study aimed to determine the concentrations of apparently vs. molecularly dissolved Alectinib over time in simulated gastrointestinal fluids and to correlate the values with observed exposures in humans. It is acknowledged that the molecularly dissolved drug is the drug fraction directly available for absorption from the gastrointestinal lumen. Even though not being directly available for absorption, colloid-associated drug species may provide a reservoir eventually offering an absorption advantage over the drug in the solid state (e.g., [7-9, 31]). Hence, understanding the relative contribution of molecularly dissolved vs. colloid-associated drug molecules to oral absorption is fundamental for the accurate prediction of in vivo drug product performance, especially for enabling or supersaturating formulations, such as surfactant-based and lipid-based formulations, where the drug is transiently colloid-associated. Recent publications have presented microdialysis as a valuable method to quantify the dynamic concentration profile of molecularly dissolved drug in an in vitro dissolution and combined dissolution/permeation assay [8, 17-20].

Alectinib is a very poorly water-soluble oral compound that is administered as a capsule formulation containing the HCl-salt with SLS as solubilizing agent. The presence of SLS was shown to increase bioavailability after oral administration [28]. As a surfactant, SLS may exert several functions: besides improving wettability, it forms micelles in aqueous media and is thus expected to increase the (apparent) solubility of Alectinib via inclusion in micelles. As such, the SLS-based Alectinib formulation is an ideal model system to evaluate the relative contribution of molecularly dissolved vs. colloid-associated drug to the oral absorption process. In this study, the apparently and the molecularly dissolved Alectinib concentrations as a function of SLS concentration were extensively investigated in biorelevant media.

When comparing the apparently (Figure 1A) and the molecularly dissolved Alectinib concentrations (Figure 1B) in FeSSIF, a high Alectinib solubilization capacity of SLS was observed. Similar to our observations in small-scale dissolution tests, a linear increase in apparent solubility was reported for other poorly soluble compounds in biorelevant media with increasing SLS concentrations [32]. SLS is known to form mixed SLS/bile salt/phospholipid micelles in biomimetic media [33] and the increase in solubilization capacity has been attributed to the formation of larger and more hydrophobic mixed micelles compared to micelles without SLS [32]. It is commonly accepted that SLS contributes to the micellar drug solubilization, and one would expect that the concentration of molecularly dissolved drug is independent of the SLS concentration. However, while SLS remarkably increased the apparently dissolved Alectinib in FeSSIF, as expected, the molecularly dissolved drug concentration was found (slightly) increased with increasing SLS levels too (Supplementary materials, Figure S3). One may speculate that the substantial range of concentrations of ionic surfactant may play a role in this context: SDS may influence the dissociation of the Alectinib HCl salt, its hydration shell e.g. as a consequence of altered hydrogen-bonding between the water molecules (change in water structure), as well as alter the Alectinib precipitation behavior in terms of surface wetting and surface pH, and/or other factors.

Of note, the observed enhancement of molecularly dissolved Alectinib concentrations (which may be called supersaturation) appears to be rather stable during the entire dissolution experiment in FeSSIF (over a period of 6 hours), whereas during two-stage dissolution testing (SGF/FaSSIF) this only holds true for the higher SLS-concentrations (25 and 50%). In contrast, at the lower SLS-concentrations (3 and 12.5%), (molecular) supersaturation appears to decline after about an hour, a behavior that has repeatedly been described as “spring and parachute”, yet with the important difference that “spring and parachute”-type of dissolution/precipitation curves described in literature typically refer to the apparently dissolved drug concentration. Interestingly, when comparing the supersaturation patterns of the apparently dissolved with those of the molecularly dissolved alectinib, one can hypothesize that at high SLS-concentrations the colloid-associated alectinib concentration declines upon media change, while the free alectinib concentrations are barely affected (molecular supersaturation is maintained), which is not the case for low SLS concentrations (collapse of molecular supersaturation). This may indicate the role of SLS micelles as a reservoir even under non-sink conditions as in our experiments.

The PK profiles in fed state showed a minor, non-linear increase in overall Alectinib exposure with increasing SLS contents in the formulation, while the effect was more pronounced in the fasted state as outlined by Morcos et al. [28] (a summary on the in vivo PK data is given in chapter 2.7). It was therefore interesting to compare the in vitro dissolution profiles of the apparently dissolved and molecularly dissolved drug with the in vivo PK data. When plotting apparently dissolved drug concentrations with in vivo c_{max}, a linear fit was observed for SLS levels between 3 and 25%, but the 50% SLS mixtures were clear outliers (Figure 2A), while the use of molecularly dissolved drug resulted in a remarkable better fit across all SLS levels (Figure 2B). Hence, as expected, the increase in apparently dissolved drug did not quantitatively translate into a higher in vivo exposure and suggested that the apparently solubilized Alectinib was not readily available for absorption. The overprediction of the 50% SLS mixture based on apparently dissolved drug can be explained by the increased apparent solubilization of Alectinib in mixed micelles. The increased solubilization, however, was not accompanied by a proportional increase in molecularly dissolved drug, which is acknowledged to be the main driver of absorption.

By plotting the mean in vitro concentrations (0-240 min) against the observed in vivo $AUC_{0\text{-}inf}$ (Figure 2C and D), the plot resulted in a comparable outcome to that observed for the correlation of c_{max}. Although a generally better linear fit was observed for the molecularly dissolved drug, a minor discrepancy between the linear fit existed for the 3% and 12.5% SLS mixtures under simulated fasted conditions (SGF to FaSSIF, Figure 2D beige and gray circles). As mentioned above, these two SLS levels exhibited the most pronounced precipitation (Figure 1D) and it can be hypothesized that the observed precipitation was caused by vigorous hydrodynamic conditions, as well as by the absence of an absorption sink, which are both influence factors lowering the biomimetic capability of the in vitro setup. Moreover, a recent publication demonstrated that the microdialysis sampling itself may promote drug precipitation [30]. When adjusting the plot of the two concentrations to the in vitro c_{max} (to neglect later precipitation), a better fit (R^2 of 0.98) was obtained (data not shown). We therefore assumed that the in vitro setup overpredicted the precipitation of Alectinib under simulated fasted state conditions.

The present in vitro data once more demonstrated the importance of assessing the molecularly dissolved drug concentrations when predicting oral absorption. This is particularly important for enabling formulations such as supersaturating or micelle-based drug formulations, as a remarkable

amount of the total drug may be present in colloidal structures and thus may not be immediately available for absorption [4]. Moreover, the present data demonstrates the ability to rank formulations by considering molecularly dissolved drug over time, confirming the hypothesis of Buckley et al. [4] that enabling formulations should be ranked by their absorption potential and thus based on molecularly dissolved drug. This may be less relevant for drug compounds and formulations, where most of the drug is present in its molecularly dissolved state.

In addition to the in vitro assessment, our study utilized the presented in vitro dissolution data to develop a PBBM that links the observed in vitro properties to the in vivo PK profiles of Alectinib formulations with increasing levels of SLS, as outlined in the following sections.

5 Results, Part 2: PBBM

A PBBM was built to predict the oral exposure of Alectinib formulations with 50%, 25%, 12.5%, and 3% SLS based on (i) apparently and (ii) molecularly dissolved drug. In vitro data of small-scale dissolution tests (Figure 1) were incorporated in the model as outlined in 2.6. Hence, the aim was to link the understanding of drug product performance gained from in vitro studies to the in vivo absorption behavior and thus further strengthen the mechanistic understanding of drug absorption from this enabling formulation.

Figure 3 presents the simulated plasma concentration-time profiles for the four Alectinib formulations using either apparently or molecularly dissolved drug concentrations under fed and fasted state conditions. When implementing the apparently dissolved drug concentrations (Figure 1A and C) into the PBBM, the model clearly overpredicted the oral drug exposure for all formulations in both fed and fasted state (Figure 3A, C, E, and G). Overall, c_{max} was overpredicted 3- to 6-fold, while the highest mismatch was observed with the 50% SLS formulation (4.4-fold and 6.1-fold overprediction in fed and fasted state, respectively; Figure 3A).

When comparing the simulations based on molecularly dissolved drug (Figure 3B, D, F, and H) with the human PK data, an excellent agreement was obtained with all four formulations in both fed and fasted state conditions. The positive food effect (approx. 3-fold) was captured accurately with this approach. Furthermore, the influence of the SLS content on the oral absorption of Alectinib was very well captured. This includes the observed decrease in c_{max} in the fasted state with increasing SLS contents, while only a minor influence was shown in the fed state.

Additional plotting of observed vs. simulated PK parameters showed a remarkable better correlation for simulations based on molecularly dissolved drug than for simulations based on apparently dissolved Alectinib (Figure 4).

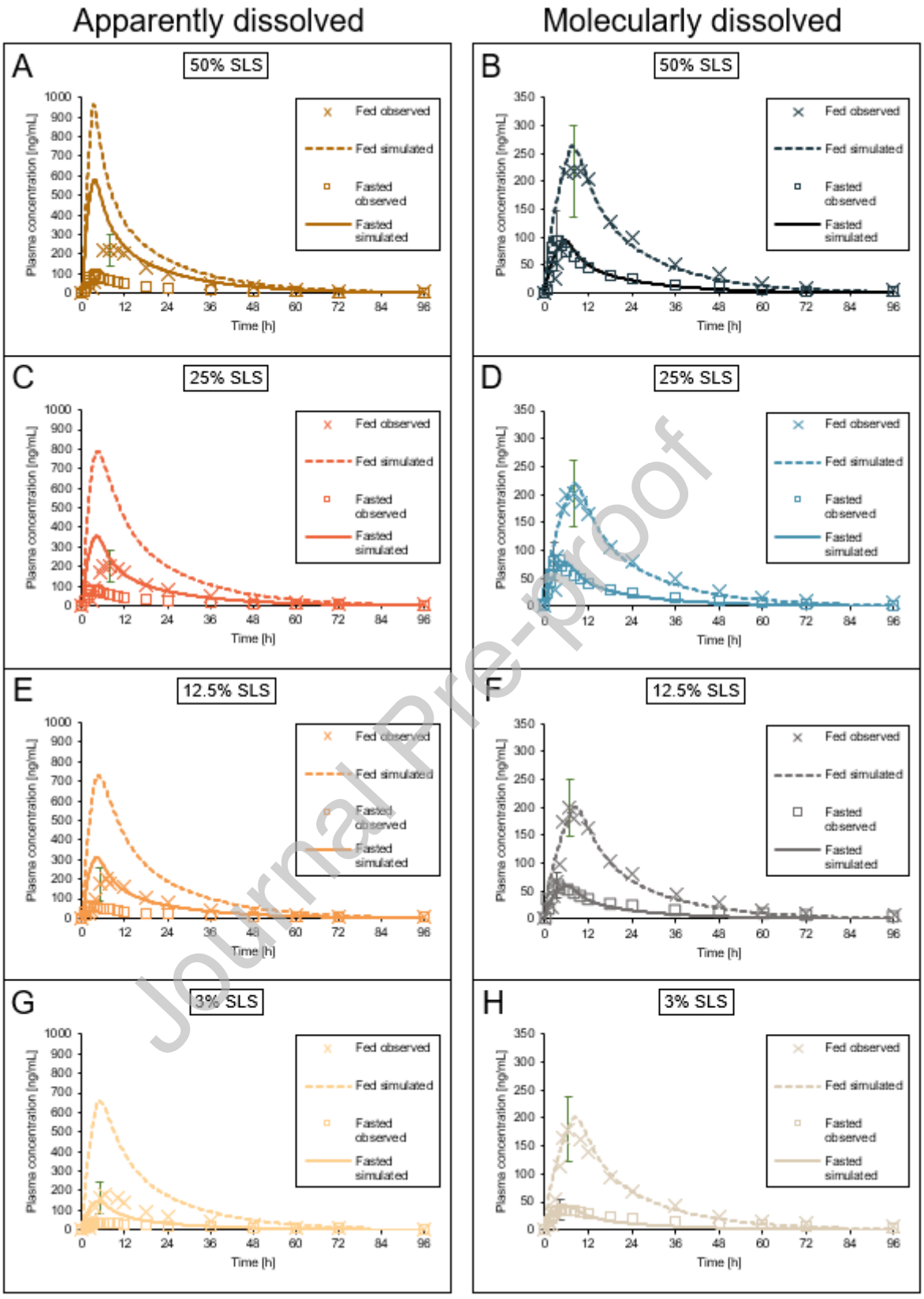


Figure 3: Simulated and observed systemic plasma concentration-time profiles of Alectinib formulations with 50% (A and B), 25% (C and D), 12.5% (E and F), and 3% SLS (G and H), respectively. GastroPlus™ simulations based on apparently dissolved drug concentrations are presented in A, C, E, and G. Figures B, D, F, and H present GastroPlus™ simulations based on the incorporation of molecularly dissolved drug concentrations derived from a small-scale

dissolution test. In vivo data of the fed and fasted state are depicted as crosses and hollow squares, respectively. GastroPlus™ fed and fasted state simulations are presented as dotted and solid lines, respectively.

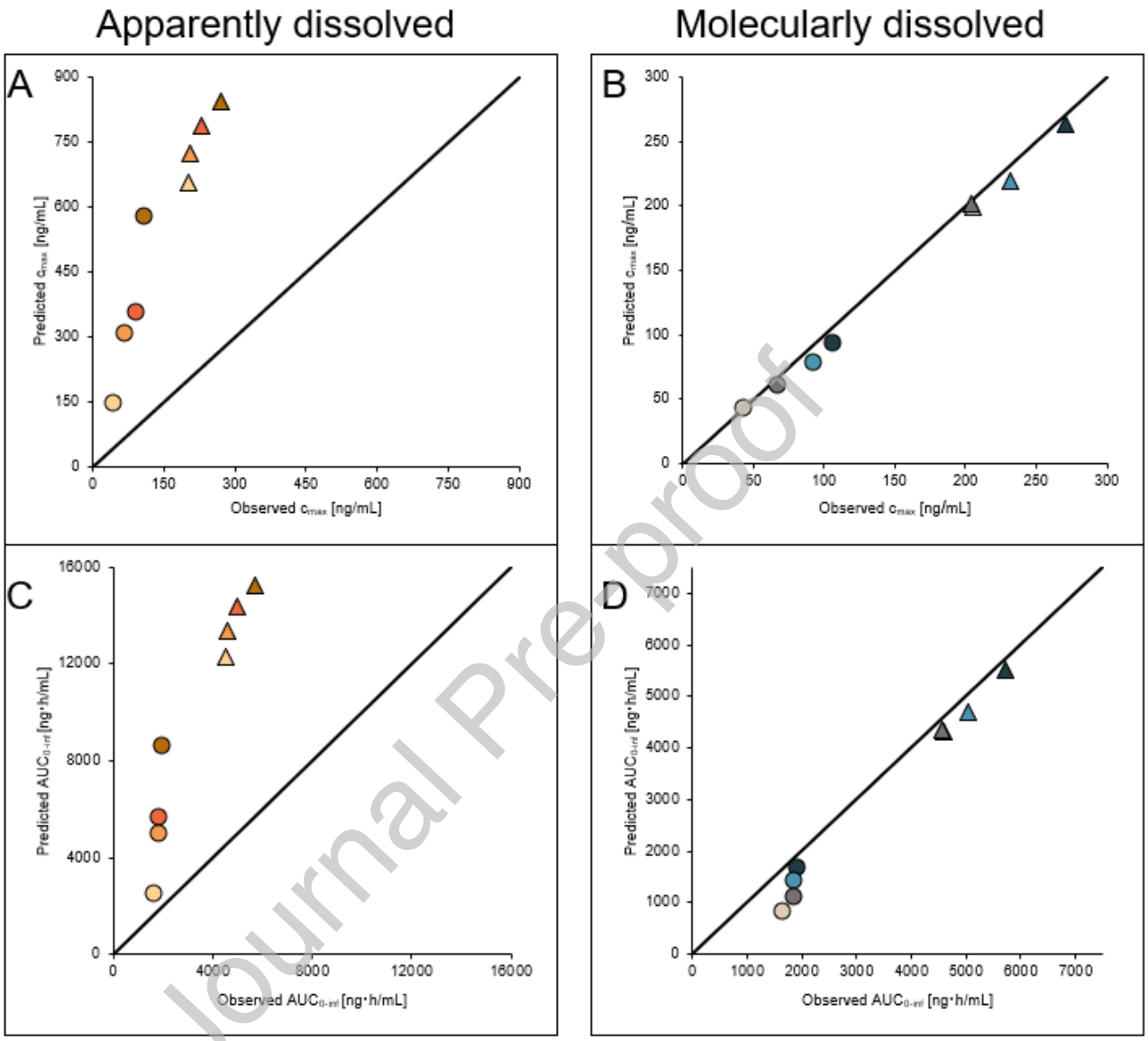


Figure 4: Predicted vs. observed pharmacokinetic parameter (AUC_{0-inf} and c_{max}) based on apparently dissolved drug concentrations (A, C) and molecularly dissolved drug (B, D) as input data. Fasted state and fed state conditions are presented as circles and triangles, respectively. The color coding refers to the Alectinib/SLS mixtures shown in Figure 1. The solid lines indicate a ratio of 1, corresponding to a perfect fit between observed and simulated PK parameters.

6 Discussion, Part 2: PBBM

Results of the PBBM based on apparently dissolved drug concentrations demonstrated an immense overprediction of systemic exposure of all formulations in both fasted and fed state (Figures 3 and 4). The overprediction was highest for the 50% SLS formulation, which is in line with the results of the in vitro-in vivo correlation shown in Figure 2, where the 50% formulation fell outside the linear correlation. As mentioned above, the in vitro data demonstrated that SLS mainly

increased the solubilization capacity, while the molecularly dissolved drug did not increase in a proportional manner.

Predictions based on molecularly dissolved (free) drug were able to accurately predict the effect of SLS on oral Alectinib exposure under both, fasted and fed conditions. These results once more strengthen the importance of discriminating between different “dissolved” states (i.e., solubilized, complexed, colloid-associated, molecularly dissolved) when predicting oral absorption from enabling drug formulations. While the effect may be less relevant for well soluble compounds, it was shown that the importance increases when a major fraction of the drug is assumed to be present in different colloidal states (as expected for ASDs, cyclodextrin formulations, and surfactant containing formulations). This assumption is underlined by a previous publication [34], which has compared the influence of molecularly and apparently dissolved drug on the PBBM predictions of supersaturating Posaconazole formulations. A major overprediction was found for an ASD-based tablet, where a substantial amount of Posaconazole was released in a colloidal state, as confirmed by in vitro microdialysis and nanofiltration sampling [18]. This colloidal state acted as a reservoir which replenished the molecularly dissolved drug fraction. However, for the crystalline suspension - , the predictions were less affected by the discrimination between molecularly and apparently dissolved drug. It was assumed that only minor contents of the surfactant (Polysorbate 80) were present in the formulation and thus, the additional solubilization effects were low.

Here, besides the improved in vitro to in vivo correlation based on the molecularly dissolved drug fraction, which may be used as a tool for early formulation ranking, the use of PBBM provides a powerful tool to link the solubilization behavior to the systemic exposure profiles. This study further highlights the need for an accurate in vitro characterization of oral formulations to fully understand the mechanism of drug release and absorption before integrating this knowledge into a PBBM.

7 Conclusion

The present work demonstrates the influence of molecularly vs. apparently dissolved drug fractions on the oral absorption from enabling drug formulations. Small-scale dissolution tests of Alectinib mixtures with increasing SLS contents resulted in a good in vitro-in vivo correlation when using the molecularly dissolved drug measured by microdialysis. Moreover, the dissolution data served as a valuable basis for incorporation into a PBBM. If apparently dissolved drug concentrations measured by classical sampling methods were implemented into the PBBM a clear overprediction of the oral exposure and of the effect of SLS in both, fed and fasted state was found. In contrast, when implementing molecularly dissolved drug concentrations (assessed after microdialysis sampling) as input data, an excellent agreement with the observed plasma concentration-time profiles in fed and fasted state were obtained. Thus, besides the successful prospective absorption modeling, the present dataset contributes to a further mechanistic understanding of drug absorption processes from surfactant-based drug formulations.

8 Acknowledgements

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955756.

Declarations of interest

C. Stillhart, N. Parrott and J. Petrig Schaffland are employees of F. Hoffmann-La Roche AG. F. Holzem is a visiting scientist at F. Hoffmann-La Roche AG.

References

1. Fong SY, Martins SM, Brandl M, Bauer-Brandl A. Solid Phospholipid Dispersions for Oral Delivery of Poorly Soluble Drugs: Investigation Into Celecoxib Incorporation and Solubility-In Vitro Permeability Enhancement. J Pharm Sci. 2016;105(3):1113-23.

2. Fong SY, Bauer-Brandl A, Brandl M. Oral bioavailability enhancement through supersaturation: an update and meta-analysis. Expert Opin Drug Deliv. 2017;14(3):403-26.

3. Kuentz M, Holm R, Kronseder C, Saal C, Griffin BT. Rational Selection of Bio-Enabling Oral Drug Formulations - A PEARRL Commentary. J Pharm Sci. 2021;110(5):1921-30.

4. Buckley ST, Frank KJ, Fricker G, Brandl M. Biopharmaceutical classification of poorly soluble drugs with respect to "enabling formulations". Eur J Pharm Sci. 2013;50(1):8-16.

5. Levy G, Reuning R. Effect of Complex Formation on Drug Absorption I: Complexes of Salicylic Acid wih absorbable and nonabsorbable sompounds. J Pharm Sci. 1964;53:1471-5.

6. Frank KJ, Westedt U, Rosenblatt KM, Holig P, Rosenberg J, Magerlein M, et al. Impact of FaSSIF on the solubility and dissolution-/permeation rate of a poorly water-soluble compound. Eur J Pharm Sci. 2012;47(1):16-20.

7. Frank KJ, Westedt U, Rosenblatt KM, Holig P, Rosenberg J, Magerlein M, et al. What is the mechanism behind increased permeation rate of a poorly soluble drug from aqueous dispersions of an amorphous solid dispersion? J Pharm Sci. 2014;103(6):1779-86.

8. Nunes PD, Ferreira AF, Pinto JF, Bauer-Brandl A, Brandl M, Henriques J, et al. In vitro Dissolution/Permeation tools for amorphous solid dispersions bioavailability forecasting II: Comparison and mechanistic insights. Eur J Pharm Sci. 2023:106513.

9. Fischer SM, Brandl M, Fricker G. Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers. Eur J Pharm Biopharm. 2011;79(2):416-22.

10. Fischer SM, Flaten GE, Hagesaether E, Fricker G, Brandl M. In-vitro permeability of poorly water soluble drugs in the phospholipid vesicle-based permeation assay: the influence of nonionic surfactants. J Pharm Pharmacol. 2011;63(8):1022-30.

11. Bauer-Brandl A, Brandl M. Solubility and Supersaturation. In: Saal C, Nair A, editors. Solubility in Pharmaceutical Chemistry: De Gruyter; 2019.

12. Kanzer J, Hupfeld S, Vasskog T, Tho I, Holig P, Magerlein M, et al. In situ formation of nanoparticles upon dispersion of melt extrudate formulations in aqueous medium assessed by asymmetrical flow field-flow fractionation. J Pharm Biomed Anal. 2010;53(3):359-65.

13. Frank KJ, Westedt U, Rosenblatt KM, Holig P, Rosenberg J, Magerlein M, et al. The amorphous solid dispersion of the poorly soluble ABT-102 forms nano/microparticulate structures in aqueous medium: impact on solubility. Int J Nanomedicine. 2012;7:5757-68.

14. Hinna AH, Hupfeld S, Kuntsche J, Brandl M. The use of asymmetrical flow field-flow fractionation with on-line detection in the study of drug retention within liposomal nanocarriers and drug transfer kinetics. J Pharm Biomed Anal. 2016;124:157-63.

15. Nunes PD, Pinto JF, Henriques J, Paiva AM. Insights into the Release Mechanisms of ITZ:HPMCAS Amorphous Solid Dispersions: The Role of Drug-Rich Colloids. Mol Pharm. 2022;19(1):51-66.

16. Frank KJ, Rosenblatt KM, Westedt U, Holig P, Rosenberg J, Magerlein M, et al. Amorphous solid dispersion enhances permeation of poorly soluble ABT-102: true supersaturation vs. apparent solubility enhancement. Int J Pharm. 2012;437(1-2):288-93.

17. Eriksen JB, Christiansen JJ, Bauer-Brandl A, Ruponen M, Rautio J, Brandl M. In-vitro dynamic dissolution/bioconversion/permeation of fosamprenavir using a novel tool with an artificial biomimetic permeation barrier and microdialysis-sampling. Eur J Pharm Sci. 2023;181:106366.

18. Holzem FL, Schaffland JP, Brandl M, Bauer-Brandl A, Stillhart C. Microdialysis and nanofiltration allow to distinguish molecularly dissolved from colloid- associated drug concentrations during biomimetic dissolution testing of supersaturating formulations. Eur J Pharm Sci. 2022:106166.

19. Fong SYK, Poulsen J, Brandl M, Bauer-Brandl A. A novel microdialysis-dissolution/permeation system for testing oral dosage forms: A proof-of-concept study. Eur J Pharm Sci. 2017;96:154-63.

20. Shah KB, Patel PG, Khairuzzaman A, Bellantone RA. An improved method for the characterization of supersaturation and precipitation of poorly soluble drugs using pulsatile microdialysis (PMD). Int J Pharm. 2014;468(1-2):64-74.

21. Heimbach T, Suarez-Sharp S, Kakhi M, Holmstock N, Olivares-Morales A, Pepin X, et al. Dissolution and Translational Modeling Strategies Toward Establishing an In Vitro-In Vivo Link-a Workshop Summary Report. AAPS J. 2019;21(2):29.

22. Kostewicz ES, Aarons L, Bergstrand M, Bolger MB, Galetin A, Hatley O, et al. PBPK models for the prediction of in vivo performance of oral dosage forms. Eur J Pharm Sci. 2014;57:300-21.

23. Kesisoglou F, Chung J, van Asperen J, Heimbach T. Physiologically Based Absorption Modeling to Impact Biopharmaceutics and Formulation Strategies in Drug Development-Industry Case Studies. J Pharm Sci. 2016;105(9):2723-34.

24. Stillhart C, Pepin X, Tistaert C, Good D, Van Den Bergh A, Parrott N, et al. PBPK Absorption Modeling: Establishing the In Vitro-In Vivo Link-Industry Perspective. AAPS J. 2019;21(2):19.

25. Kesisoglou F, Mitra A. Application of Absorption Modeling in Rational Design of Drug Product Under Quality-by-Design Paradigm. AAPS J. 2015;17(5):1224-36.

26. Jamei M, Abrahamsson B, Brown J, Bevernage J, Bolger MB, Heimbach T, et al. Current status and future opportunities for incorporation of dissolution data in PBPK modeling for pharmaceutical development and regulatory applications: OrBiTo consortium commentary. Eur J Pharm Biopharm. 2020;155:55-68.

27. Mitra A, Zhu W, Kesisoglou F. Physiologically Based Absorption Modeling for Amorphous Solid Dispersion Formulations. Mol Pharm. 2016;13(9):3206-15.

28. Morcos PN, Parrott N, Banken L, Timpe C, Lindenberg M, Guerini E, et al. Effect of the Wetting Agent Sodium Lauryl Sulfate on the Pharmacokinetics of Alectinib: Results From a Bioequivalence Study in Healthy Subjects. Clin Pharmacol Drug Dev. 2017;6(3):266-79.

29. Parrott NJ, Yu LJ, Takano R, Nakamura M, Morcos PN. Physiologically Based Absorption Modeling to Explore the Impact of Food and Gastric pH Changes on the Pharmacokinetics of Alectinib. AAPS J. 2016;18(6):1464-74.

30. Holzem FL, Jensen IH, Schaffland JP, Stillhart C, Brandl M, Bauer-Brandl A. Combining in vitro dissolution/permeation with microdialysis sampling: Capabilities and limitations for biopharmaceutical assessments of supersaturating drug formulations. European Journal of Pharmaceutical Sciences. 2023.

31. Sironi D, Rosenberg J, Bauer-Brandl A, Brandl M. Dynamic dissolution-/permeation-testing of nano- and microparticle formulations of fenofibrate. Eur J Pharm Sci. 2017;96:20-7.

32. Madelung P, Ostergaard J, Bertelsen P, Jorgensen EV, Jacobsen J, Mullertz A. Impact of sodium dodecyl sulphate on the dissolution of poorly soluble drug into biorelevant medium from drug-surfactant discs. Int J Pharm. 2014;467(1-2):1-8.

33. Vinarov Z, Katev V, Burdzhiev N, Tcholakova S, Denkov N. Effect of Surfactant-Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media. Mol Pharm. 2018;15(12):5741-53.

34. Holzem FL, Schaffland JP, Brandl M, Bauer-Brandl A, Stillhart C. Using molecularly dissolved drug concentrations in PBBMs improves the prediction of oral absorption from supersaturating formulations. European Journal of Pharmaceutical Sciences. 2024:106703.