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Research paper

Drug solubility in biorelevant media in the context of an inhalation-based biopharmaceutics classification system (iBCS)

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ABSTRACT

An inhalation-based Biopharmaceutics Classification System for pulmonary drugs (iBCS) holds the perspective to allow for scientifically sound prediction of differences in the *in vivo* performance of orally inhaled drug products (OIDPs).

A set of nine drug substances were selected, that are administered via both the oral and pulmonary routes. Their solubility was determined in media representative for the oral (Fasted State Simulated Intestinal Fluid (FaSSIF)) and pulmonary (Alveofact medium and Simulated Lung Fluid (SLF)) routes of administration to confirm the need for a novel approach for inhaled drugs. The complexity of these media was then stepwise reduced with the purpose of understanding the contribution of their components to the solubilizing capacity of the media. A second reason for varying the complexity was to identify a medium that would allow robust but accurate dissolution testing. Hence, Hank's balanced salt solution (HBSS) as a medium used in many *in vitro* biological tests, non-buffered saline solution, and water were included.

For some drug substances (salbutamol sulfate, tobramycin, isoniazid, and tiotropium bromide), no significant differences were observed between the solubility in the media used. For other drugs, however, we observed either just small (rifampicin, budesonide, salmeterol) or unexpectedly large differences (beclomethasone dipropionate).

Based on the minimum theoretical solubility required for their common pulmonary dose in 10 ml of lung lining fluid, drug solubility was classified as either high or low. Two high solubility and two low solubility compounds were then selected for refined solubility testing in pulmonary relevant media by varying their content of phospholipids, surfactant proteins and other proteins.

The solubility of drug substances in simulated lung lining fluids was found to be dependent on the physico-chemical properties of the drug substance and the composition of the media. While a pulmonary dissolution medium that would fit all drugs could not be established, our approach may provide guidance for finding the most suitable dissolution medium for a given drug substance and better designing *in vitro* tests for predicting the *in vivo* performance of inhalable drug products.

Abbreviations: OIDP, orally inhaled drug product; DS, drug substance; giBCS, biopharmaceutics classification system for orally administered drug products; iBCS, inhalation-based biopharmaceutics classification system; PBPK models, physiologically based pharmacokinetic models; LLF, lung lining fluid; IgG, immunoglobulin G; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; HBSS, Hank's balanced salt solution; FaSSIF, Fasted state simulated intestinal fluid; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; SLF Alb, SLF containing only albumin from the protein fraction; SLF w/out proteins, SLF without any of the proteins; Alb in HBSS, albumin in HBSS: SLF without IgG, transferrin phospholipids and cholesterol.

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1. Introduction

The majority of OIDPs are intended for local action for treating diseases of the lung [1], but the inhalation route of administration is an attractive option for systemically acting drugs as well, with a few products having been marketed at least for a certain period of time (e.g., insulin, loxapine, levodopa, ergotamine), and many more being under investigation for this purpose [2].

Administering drug products via the lung presents multiple advantages: large surface area available for drug absorption, rapid onset of drug action and rapid clearance, absence of the first-pass metabolism with lower doses required and reduced systemic adverse effects. However, this route of administration also comes with its specific challenges. Different factors influence the extent of DS reaching the site of action. The dose being delivered to the lung is dependent on the drug formulation-device interaction, as well as individual handling of the device and pulmonary health status. Moreover, compared to the oral route of administration, it is much more complicated to directly observe the interaction and behavior of the drug formulation with the environment encountered after deposition at the pulmonary air–liquid interface.

Some OIDPs are administered as aerosols of solid particles, and the rate and extent of DS availability at the site of action in the lung depend on the dissolution or release rate of the DS from the formulation. The DS's absorption into the systemic circulation and other mechanisms contribute to the clearance from the deposition site (mucociliary clearance and macrophage uptake). Understanding the fate of drug formulation particles after deposition is a challenging task. Plasma concentration profiles are often evaluated, but in the case of locally acting drugs, they only reflect the rate at which DS leaves the site of action. This makes the classical bioavailability approach based on plasma concentrations not generally applicable to the pulmonary route of administration. *In vitro* dissolution testing may contribute to elucidating the fate of particles in the lung and be used as an *in vitro* performance test for predicting differences in the *in vivo* performance of drug products. In either case, however, media and methods for testing need to be established, which should be as simple and robust as possible, but must still be biorelevant to provide accurate predictions.

The development of orally administered drug products has greatly benefited from having standardized dissolution testing methods and a biopharmaceutics classification system (giBCS) in place. By considering the physicochemical properties of the DS, the characteristics of the route of administration, and the drug product's critical attributes, the giBCS places DS in one of 4 classes according to their aqueous solubilities (solubility of highest dosage strength or highest dose in 250 ml of dissolution medium of pH 1 to 6.8) and permeability through the intestinal epithelium. This helps guide formulators in what concerns the optimal drug product performance, depending on the class the DS belongs to. In some cases, *in vitro* dissolution testing results can be used as a waiver of *in vivo* bioavailability and bioequivalence studies [3].

The need to have a similar system for OIDPs has been intensely discussed in recent years. One of the first papers to mention the need for an iBCS "that will consider the impact of the unique physiology of the respiratory system on drug absorption" and lists "factors that potentially influence the absorption and how they can be integrated into" the iBCS, is the one published by Eixarch et al. [4]. In 2015, the AAPS/FDA/USP workshop held in Baltimore concluded that a classification system for OIDPs would be a useful tool for formulators and discovery chemists. The development of such a system was described by Hastedt et al. as "the first step in understanding the role of *in vitro* performance parameters as they affect *in vivo* product performance" [5].

The giBCS cannot be applied to OIDPs without modification since the characteristics of the route of administration and the desired properties of the drug product differ from the ones for oral drugs. In the development of iBCS, the principles of giBCS must be tailored to carefully consider factors relating to DS physicochemical properties in the context of lung biology (permeability through lung epithelium and solubility in lung lining fluid) and drug-device combination product attributes (delivered dose, aerodynamic particle size distribution of the formulation, dissolution/release rate).

Most recently, the foundational principles and a framework for iBCS were outlined by the PQRI iBCS working group. Their approach consisted of adapting the principles of the giBCS to the specifics of the inhalation route of administration. Model compounds with varying properties that cover the range of those of inhaled drug products were used with PBPK computational models for defining class boundaries for iBCS [6,7]. The group concluded that the implication of their findings "with respect to the design of an inhalation-based biopharmaceutical classification system and to the need for experimental methodologies to classify drugs needs to be further explored" [7].

In this context, the present study is proposing an approach to meaningful solubility testing with the purpose of contributing with methods and data towards the accurate classification of DS for pulmonary administration in the iBCS grid defined by Hastedt et al. and Bäckman et al. [6,7].

The focus of the present study is DS solubility in the context of the route of administration, especially by comparing the oral and the pulmonary routes. Our approach is graphically depicted in Fig. 1. The specific aims are three-fold:

Evaluation of solubility of selected DS for inhalation in pulmonary relevant media for iBCS classification.
Finding out to what extent the giBCS needs to be adjusted for the specific characteristics of the lung, by comparing solubility in biorelevant media representative for the two routes of administration.
Identification of biorelevant media capable of simulating the solubilizing capacity of the lung lining fluid (LLF) at adequate accuracy and robustness as required for developing predictive dissolution testing methods for OIDPs.

2. Materials and methods

2.1. Materials

IgG from human serum (reagent grade, ≥95 %), transferrin human (bioreagent, cell culture), albumin human (recombinant, expressed in rice, ≥96 %), Hank's balanced salt solution (modified, with sodium bicarbonate, without phenol red, liquid, sterile-filtered, suitable for cell culture), (+)-sodium L-ascorbate (crystalline, ≥98 %), uric acid sodium salt, cholesterol (≥99 %), glutathione (certified reference material), isoniazid (analytical standard, ≥99 %), salmeterol xinafoate (certified reference material), rifampicin (≥97 %), tobramycin (certified reference material), ciprofloxacin (≥98 %) and beclomethasone dipropionate (certified reference material) were purchased from Sigma Aldrich (Darmstadt, Germany). 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-rec-glycerol) (sodium salt) (DPPG) were purchased from Avanti Polar Lipids (Alabama, USA), FaSSIF powder from Biorelevant (London, United Kingdom), budesonide from eBioChem, and tiotropium bromide from MedChemExpress (NJ, USA). Salbutamol sulfate was generously gifted by Lusochimica (Rozzano, Italy) and Alveofact® was a kind gift from Lyomark Pharma (Oberhaching, Germany).

For the HPLC quantification methods, acetonitrile (gradient grade for liquid chromatography), methanol (gradient grade for liquid chromatography), chloroform (reagent grade), potassium dihydrogen phosphate (reagent grade) sodium dodecyl sulfate (≥85 %), ammonium acetate (reagent grade), glacial acetic acid (reagent grade), sodium chloride (reagent grade) from Merck (Darmstadt, Germany), trifluoroacetic acid (sequanal grade) from ThermoFisher Scientific Life Technologies (Darmstadt, Germany), sodium octanesulfonate (~98 %), disodium tetraborate decahydrate (reagent grade), phosphoric acid (≥85 %), triethylamine (≥99.5 %) from Sigma Aldrich (Darmstadt,

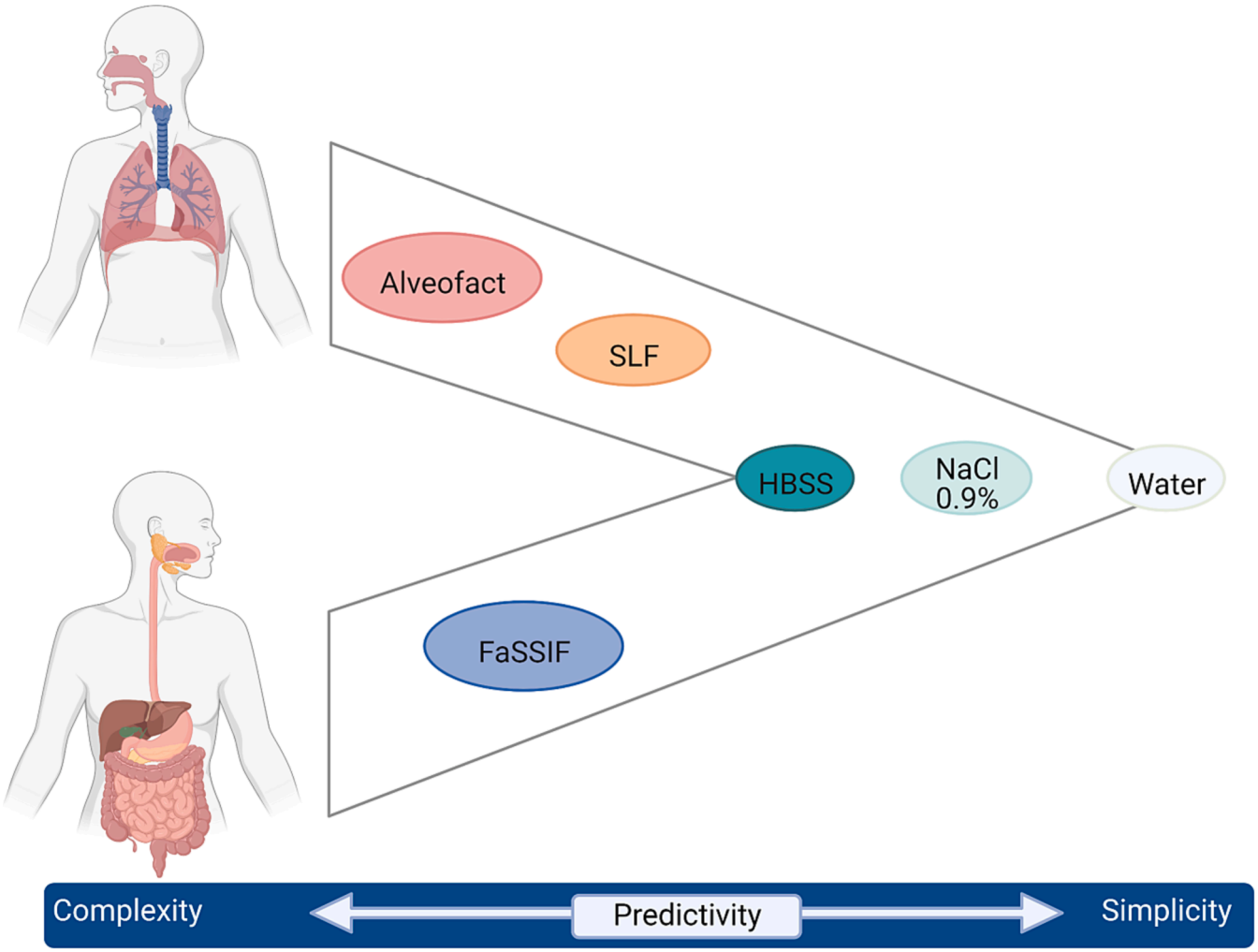


Fig. 1. For confirming the hypothesis that the BCS classification for oral drugs cannot be applied without modification to pulmonary administered drugs, the solubility of selected drug substances was measured in potentially biorelevant media for each of the two routes of administration. The complexity of media composition decreases gradually from left to right. The goal is finding biorelevant media that meet the need for predictivity with regards to the solubilizing capacity for the given route of administration so that it may be used for predictive *in vitro* dissolution testing. SLF = Simulated lung fluid; FaSSIF = Fasted state simulated intestinal fluid; HBSS = Hank's balanced salt solution. Created with Biorender.com.

Germany), sodium hydroxide (reagent grade) from VWR International (Leuven, Belgium) and sodium dihydrogen phosphate (99 %) from Acros Organics, (Geel, Belgium) were used.

The water used for all described experiments was purified using a Millipore Milli-Q Gradient A10 system (Merck, Darmstadt, Germany).

2.2. Selection of drug substances

A set of 9 DS has been chosen for saturation solubility testing in selected media. The goal was to include DS that are administered via both the oral and pulmonary routes, are diverse in chemical structure, lipophilicity, and solubility, and represent all four classes of the BCS established for orally administered drug products. The selected DS and their properties are listed in Table 1.

2.3. Quantification methods

Quantification was performed on an Agilent 1100 Series HPLC, equipped with a photodiode-array detector (Agilent Technologies, Santa Clara, California USA) using an XBridge Shield RP18, 5 µm, 150*4.6 mm column (Waters GmbH, Eschborn, Germany) for separation.

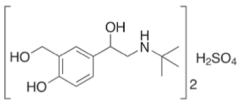
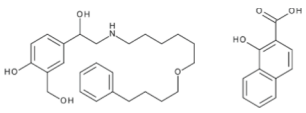
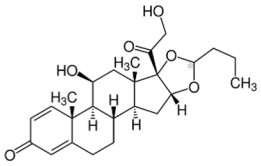
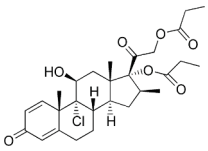
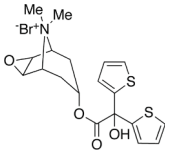
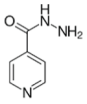
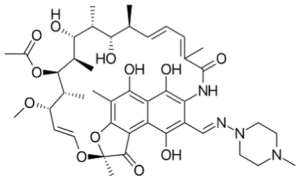
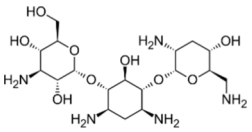
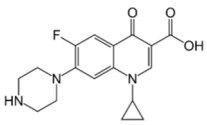
HPLC quantification methods were developed for salbutamol sulfate, budesonide, rifampicin, tiotropium bromide, and beclomethasone dipropionate. For the quantification of isoniazid and salmeterol, methods modified from USP were used [8,9]. For tobramycin and ciprofloxacin methods were adapted from Blanchaert et al., 2017 [10] and Torge et al., 2017 [11], respectively. All methods were validated for accuracy, linearity, specificity, repeatability and intermediate precision. The parameters of each method can be found in Table 2.

Additionally, a UV-spectroscopy method was developed for quantifying salmeterol in the Alveofact medium. The HPLC method applied for all other solubility experiments for salmeterol was shown to not meet the requirements for specificity in this medium. The concentrations were determined using a Perkin Elmer Spectrophotometer (PerkinElmer, Waltham Massachusetts, USA) at a wavelength of 251 nm, using a quartz cuvette (path length 10 mm).

2.4. Media preparation

The components and properties of the media are listed in Table 3. Saturation solubility of selected DS was measured in Alveofact medium and SLF – as the media being physiologically closest to the lung lining fluid, in parallel with FaSSIF – chosen as a representative medium of the GI tract. Native human lung surfactant was not available for inclusion in this study due to limitations posed by the complexity of the bronchoalveolar lavage procedure used for obtaining it. Alveofact® is a

Table 1
Selected drug substances for solubility study.

Drug class	Substance	Chemical structure	giBCS class	logP[c]	Administration		Water solubility[a]
					Oral	Pulmonary	
β agonists	Salbutamol sulfate		I [29]	0.4	✓	✓	Freely soluble
	Salmeterol xinafoate		II [30]	4.2	✓	✓	Practically insoluble
Glucocorticosteroids	Budesonide		II [31]	2.4	✓[b]	✓	Practically insoluble
	Beclomethasone dipropionate		NA	3.7	–	✓	Very slightly soluble
Muscarinic antagonists	Tiotropium bromide		III [30]	−1.8	–	✓	Sparingly soluble
Antibacterials	Isoniazid		I / III [29]	−0.7	✓	R&D	Freely soluble
	Rifampicin		II [29]	3.9	✓	R&D	Very slightly soluble
	Tobramycin		NA	−3.0	–	✓	Freely soluble
	Ciprofloxacin		IV [23]	−0.6	✓	R&D	Practically insoluble

[a] As described in the USP-NF reference table: Description and relative solubility [32].
[b] Oral administration for local action.
[c] Values from DrugBank Online (https://go.drugbank.com/, last accessed October 3rd, 2023).

Table 2
Parameters of HPLC quantification methods.

DS	Column	Mobile phase mode	Mobile phase	Flow rate	Temp.	Detection	Injection volume	Run time	R^2 (COD)
Salbutamol sulfate	XBridge Shield RP18, 5 µm, 150*4.6 mm	Isocratic	90 % water + 0.1 % TFA 10 % acetonitrile + 0.1 % TFA	1.5 ml/min	45 °C	276 nm	5 µl	3.5 min	1.00
Isoniazid[1]		Isocratic	95 % 0.1 M KH_2PO_4 pH 6.9 5 % methanol	1 ml/min	35 °C	262 nm	5 µl	5 min	1.00
Salmeterol xinafoate[2]		Isocratic	52 % acetonitrile, 24 % 0.1 M sodium dodecyl sulfate: 24 % 0.1 M ammonium acetate Adjusted to pH 3.8 with glacial acetic acid	2 ml/min	30 °C	278 nm	100 µl	8 min	1.00
Budesonide		Gradient	A: water + 0.1 % TFA B: acetonitrile + 0.1 % TFA 0 min 20 % B 8 min 100 % B 12 min 20 % B	0.5 ml/min	45 °C	244 nm	20 µl	18 min	1.00
Rifampicin		Gradient	A: water + 0.1 % TFA B: acetonitrile + 0.1 % TFA 0 min 40 % B 6 min 70 % B 7 min 40 % B	1 ml/min	30°c	336 nm	5 µl	10 min	1.00
Tiotropium bromide		Gradient	A: water + 0.1 % TFA B: acetonitrile + 0.1 % TFA 0 min 10 % B 10 min 50 % B 11 min 10 %B	1 ml/min	30 °C	235 nm	10 µl	15 min	1.00
Tobramycin[3]		Isocratic	1 g/L sodium octanesulfonate in a mixture of 20 % methanol, 20 % disodium tetraborate decahydrate buffer (0.1 M, pH 9) and 60 % water	1 ml/min	45 °C	210 nm	10 µl	8 min	0.99
Ciprofloxacin[4]		Isocratic	87 % 0.025 mol/L H_3PO_4 adjusted to pH 3 with triethylamine 13 % acetonitrile	1.5 ml/min	45 °C	278 nm	5 µl	6 min	1.00
Beclomethasone dipropionate		Gradient	A: water B: acetonitrile 0 min 65 % B 1.5 min 100 % B 2 min 65 % B	1.5 ml/min	45 °C	240 nm	50 µl	6 min	0.98

[1] Modified from USP [8]; [2] Modified from USP [9]; [3] Adapted from Blanchaert et al., 2017 [10]; [4] Adapted from Torge et al., 2017 [11]. All methods were validated for accuracy, linearity, specificity, repeatability and intermediate precision. DS = drug substance; COD = coefficient of determination.

natural lung surfactant preparation extracted from bovine lung, used for treating and preventing the development of respiratory distress syndrome in premature neonates. Alveofact® is composed of phospholipids and two of the four pulmonary-specific surfactant proteins: SP-B and SP-C, primarily associated with the surface tension-lowering properties of the surfactant [5,12].

SLF contains the most abundant components found in the human airways: proteins, phospholipids, cholesterol, and antioxidants, but it lacks pulmonary surfactant proteins [13,14]. This medium was developed as an artificial lung lining fluid simulant to address the need for "improving *in vitro* testing systems that reflect the interactions between inhaled drugs and the components of the respiratory tract lining fluid"

Table 3
Media composition and characteristics.

		Medium								
		Alveofact	SLF	FaSSIF	HBSS	NaCl 0.9 %	Water	SLF Alb	Alb in HBSS	SLF w/out protein
Isotonic		+	+	-	+	+	-	+	+	+
Buffered		pH 7.8	pH 7.4	pH 6.5	pH 7.4	-	-	pH 7.4	pH 7.4	pH 7.4
Surface tension mN/m	Measured value	NA	54	NA	69	NA	72	54	NA	69
	Literature value	NA	55 [17]	52 [33]	72 [34]	73 [35]	72	NA	NA	NA
Proteins		Pulmonary surfactant proteins SP-B and SP-C	Albumin IgG Transferrin	–	–	–	–	Albumin	Albumin	–
Surfactants		Pulmonary surfactant proteins SP-B and SP-C	DPPC DPPG Cholesterol	Bile salts Lecithin	–	–	–	DPPC DPPG Cholesterol	–	DPPC DPPG Cholesterol
Pulmonary surfactant proteins (SP-B, SP-C)		+	–	–	–	–	–	–	–	–

SLF = Simulated lung fluid; FaSSIF = Fasted state simulated intestinal fluid; HBSS = Hank's balanced salt solution; SLF Alb = SLF with only albumin from the protein fraction; Alb in HBSS = Albumin in Hank's balance salt solution; SLF w/out prot = SLF without proteins.

[14,15]. The composition and characterization regarding the physicochemical properties and stability of SLF are described in detail elsewhere [13,16,17]. For this study, SLF was prepared following the protocol described by Kumar et al. [18]: DPPG was suspended in chloroform containing 2 % methanol for a final concentration of 25 mg/ml. 1.92 ml of this solution were pipetted (Eppendorf Multipette - Eppendorf, Hamburg, Germany) in a round bottom volumetric flask together with 1 ml of 5 mg/ml DPPG in chloroform and 0.1 ml of 10 mg/ml cholesterol in chloroform. The resulting solution was gently stirred under a stream of nitrogen gas in a water bath at 55 °C until the solvents evaporated and a thin layer of lipids was produced at the base of the flask. 2 ml of HBSS, pre-warmed at 55 °C were added. The mixture was vortexed (Vortex-Genie 2 - Scientific Industries, Bohemia, New York, USA) for 30 min, followed by one hour of sonication in the water bath at 55 °C (Bandelin Sonorex RK100H ultrasonic bath – Bandelin electronic, Berlin, Germany). Next, the protein solutions were slowly added: 1 ml of 88 mg/ml Albumin, 2.6 ml of 10 mg/ml IgG and 1 ml of 15 mg/ml Transferrin, all dissolved in HBSS. Finally, HBBS was added to a total volume of 10 ml. The pH of the resulting solution was between 7.2 and 7.4.

The Alveofact medium was prepared to contain a phospholipid concentration equivalent to the one in SLF. 53 mg of Alveofact® powder consisting of 96 % phospholipids were weighed under controlled, low humidity conditions. Approximately 8 ml of HBSS were added, and the solution was stirred using a magnetic stirrer until solid particles were no longer visible (approx. one h). After the volume was brought to 10 ml, the pH of the resulting solution was between 7.7 and 7.8.

FaSSIF contains surfactants present in the gastrointestinal fluid it replicates, bile salts and phospholipids [19,20]. It was prepared according to the manufacturer's instructions [21]. First, a buffer solution was prepared by dissolving 42 mg sodium hydroxide, 395 mg sodium dihydrogen phosphate and 619 mg sodium chloride in approx. 90 ml water. After the pH was corrected to 6.5 (Metrohm 713 pH meter - Deutsche Metrohm, Filderstadt, Germany), the volume was adjusted to 100 ml. 224 mg of the FaSSIF powder were added to 50 ml of buffer and stirred until completely dissolved, followed by volume adjustment to 100 ml with buffer at room temperature. Before use, the resulting FaSSIF was left to stand for 2 h, during which it turned slightly opaque. NaCl 0,9 % was prepared by dissolving 9 g of sodium chloride in 1 L of purified water, and HBSS was used without further modification.

Media used to refine screening for relevance to the inhalation route (SLF Alb, Alb in HBSS, and SLF w/out proteins) was prepared according to the procedure for SLF. The steps related to components not included in each respective medium are omitted from the preparation protocol.

2.5. Surface tension measurement

For relative comparison, the surface tension of most of the media was measured using the static contact angle method based on optical systems. A LAUDA Surface Analyzer LSA60 (LAUDA Scientific, Lauda-Koenigshofen, Germany) was used to measure the contact angle on a Teflon surface.

2.6. Solubility testing method

Saturation solubility measurement was performed using a miniaturized shake flask method. An excess amount of DS was added to 0.5 or 1 ml of medium (depending on the DS solubility) in 1.5 ml Eppendorf tubes. The tubes were shaken at 700 rpm and 37 °C for 48 h using a Thermomixer Comfort (Eppendorf, Hamburg, Germany).

After 48 h, the solutions were centrifuged for 10 min at 13000 rpm and 37 °C (Centrifuge 5415R – Eppendorf, Hamburg, Germany). The supernatant was transferred to a 0.5 ml Eppendorf tube and centrifuged under the same conditions. A defined volume of this supernatant was transferred into a 1.5 ml tube and diluted with methanol for precipitating proteins and breaking micelles and lipid vesicles [13,22]. The precipitated proteins were separated by centrifugation. If needed, solutions were further diluted by adding defined volumes of appropriate solvents before quantification.

In order to ascertain that co-precipitation of DS did not occur at the methanol dilution step, randomly selected solutions were analysed. The precipitate was isolated and resolubilized before performing a second precipitation step. The resulting supernatant was analysed for DS content.

2.7. Statistics

All measurements were performed in triplicate (unless mentioned otherwise), with a blank sample processed in parallel. In cases where normalization of data was performed, results were normalized for the mean solubility of each respective DS in HBSS. Error bars represent the standard deviation.

The Kolmogorov-Smirnov test was applied to determine that the values in each group are normally distributed. One-way ANOVA followed by post-hoc Tukey test was performed for statistical significance.

For data acquisition and processing, Empower® 3 software (Waters, GmbH, Eschborn, Germany) and OriginPro (OriginLab Corporation, Northampton, Massachusetts, USA) were used.

3. Results

3.1. Solubility of selected drug substances in the different media

The results of these measurements are provided in supplementary Table S1 and depicted in Figs. 2 and 3. Saturation solubility of selected DS was measured in Alveofact medium and SLF, in parallel with FaSSIF.

Next, components of the media were removed stepwise with the purpose of determining the degree of complexity necessary to still reflect the solubilizing capacity of the biorelevant medium. HBSS – a commercially available, chemically defined balanced salt solution, non-buffered saline (NaCl 0.9 %), and purified water were included as potentially the most simple, reproducible, robust, and cost-effective surrogate media for dissolution testing. It has, however, already been observed that different solubilizing capacities of the dissolution media lead to different dissolution profiles, which further complicates the task of comparing the various methods developed for *in vitro* dissolution testing of inhalation products.

Measured, as well as literature values for surface tension of the media included in the study are presented in Table 3. Surface tension was found to not be a strong indicator for the solubilizing capacity of the media.

For some of the DS included in the study, no relevant differences in solubility in different media were observed (See Fig. 2 and supplementary Table S1). This was the case for salbutamol sulfate (values obtained ranging from 263 mg/ml in Alveofact medium to 273 mg/ml in HBSS), tobramycin (between 519 mg/ml in FaSSIF and 558 mg/ml in saline solution), isoniazid (180 mg/ml in Alveofact solution to 203 mg/ml in saline solution), and tiotropium bromide (between 37 mg/ml in SLF and 41 mg/ml in FaSSIF) - mainly highly soluble DS. For rifampicin, a decrease of approximately 50 % was observed in the non-buffered saline solution (859 μg/ml) and water (754 μg/ml) compared to the more complex media (1714 μg/ml in SLF, 1722 μg/ml in FaSSIF, and 1831 μg/ml in HBSS, 1898 μg/ml in Alveofact solution). Similarly, ciprofloxacin had lower solubility in these simple media (136 μg/ml in saline solution and 100 μg/ml in water). However, for ciprofloxacin, the solubility was slightly increased in SLF (204 μg/ml) and FaSSIF (207 μg/ml) when compared to Alveofact solution (166 μg/ml). This is going against the trend noticed for the rest of DS, but it could be caused by the added influence of its pH-dependent solubility behaviour [23]. Salmeterol also showed an increase of solubility in FaSSIF (135 μg/ml) when compared to SLF (84 μg/ml), but the value obtained in Alveofact solution is almost double the former (264 μg/ml).

For the other drugs, a significant decrease in solubility was observed with reducing the compositional complexity and moving further away from simulated lung lining fluids (Alveofact medium and SLF) and towards the protein-lacking simulated intestinal fluid and the other less complex media. Solubility of budesonide decreased from 101 μg/ml in the Alveofact medium to 66 μg/ml in SLF, 43 μg/ml in FaSSIF and 26 μg/ml in HBSS. The values measured in saline solution and water were comparable to those in HBSS, at 25 μg/ml and 28 μg/ml, respectively.

The most pronounced differences were apparent for beclomethasone dipropionate, a poorly water-soluble DS, with values of approximately 0.02 μg/ml in water and saline solution. However, the value obtained in the Alveofact medium (30 μg/ml) was six times higher than in SLF (5 μg/ml), about nine times higher than in FaSSIF (3.5 μg/ml) and 60-fold the solubility in HBSS (0.45 μg/ml).

The DS in this study were categorized based on their dose as having either high (i.e., the entire dose is soluble in the LLF volume available, Fig. 2) or low solubility (i.e., drug solubility is lower than, or comparable to, the calculated theoretical minimum solubility, Fig. 3). The details of this concept are provided in the discussion part.

3.2. Refined screening in pulmonary relevant media

A refined screening in media relevant for the inhalation route was performed for selected DS: ciprofloxacin and beclomethasone dipropionate representing low solubility drugs, and salbutamol sulfate and budesonide representing high solubility drugs. Equilibrium solubility was determined in media chosen to allow the evaluation of different components' contribution to the solubilizing capacity. Starting from the most complex, Alveofact medium and SLF, components were gradually left out. Solubility was measured in SLF containing only albumin from

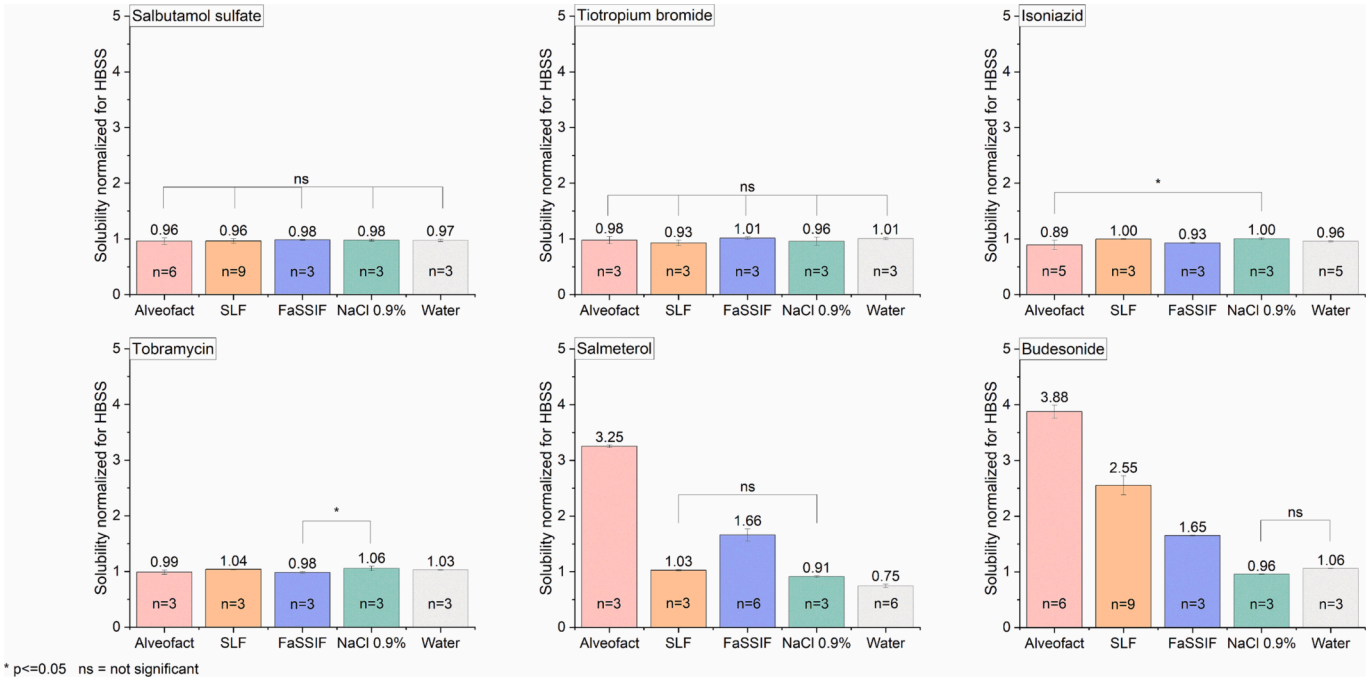


Fig. 2. Equilibrium solubility of drug substances identified as having high pulmonary solubility. Results have been normalized for each substance's respective solubility in Hank's balanced salt solution (HBSS). Error bars represent the standard deviation. For isoniazid and tobramycin, statistically significant differences between solubilities in different media are marked (* $p \leq 0.05$). For the other DS, statistically not significant differences are marked (ns); all other results are significantly different to each other. SLF = Simulated lung fluid; FaSSIF = Fasted state simulated intestinal fluid.

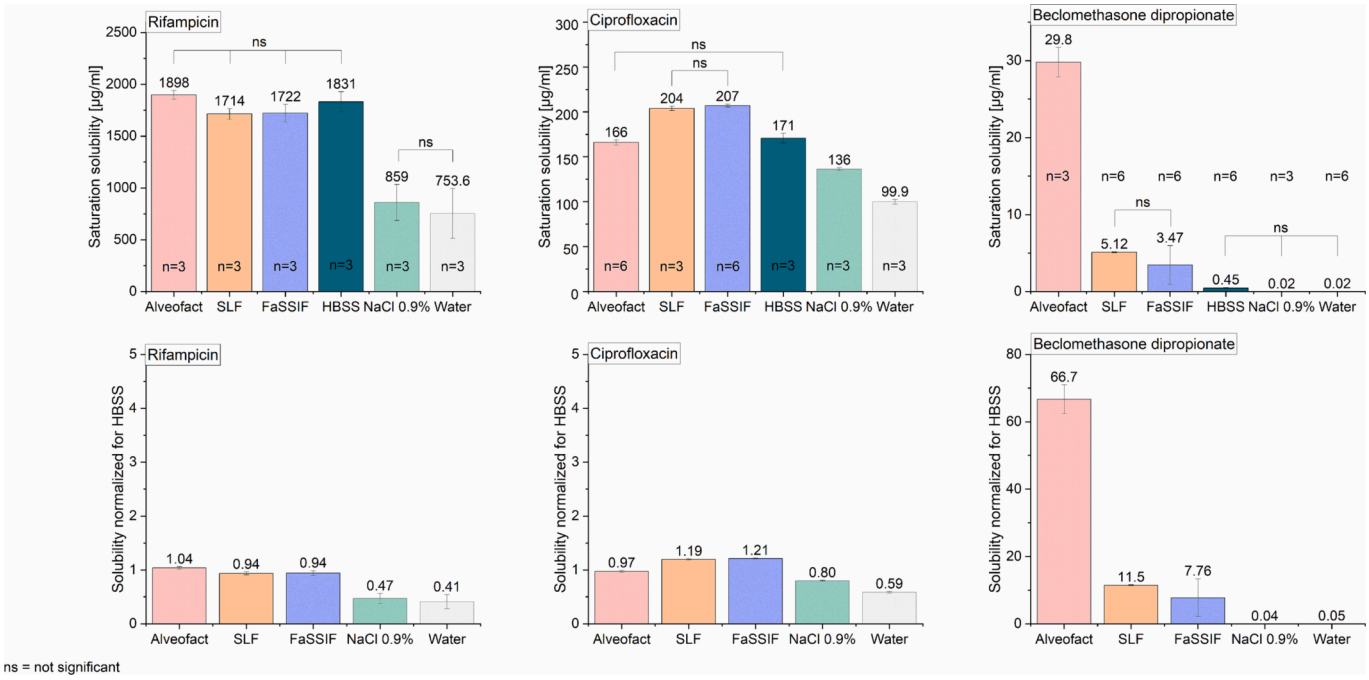


Fig. 3. Drug substances identified as having low pulmonary solubility. Upper panel – absolute values, lower panel – results normalized for solubility in Hank's balanced salt solution. Error bars represent the standard deviation. The statistically not significant differences in solubility are marked (ns), all other results being significantly different to each other ($p \leq 0.05$, upper panel). SLF = Simulated lung fluid; FaSSIF = Fasted state simulated intestinal fluid; HBSS = Hank's balanced salt solution.

the protein fraction – without IgG and transferrin (SLF Alb), SLF without any of the proteins – only phospholipids and cholesterol in HBSS (SLF w/out proteins) and albumin in HBSS – SLF without IgG, transferrin phospholipids and cholesterol (Alb in HBSS).

The results of the refined screening in pulmonary relevant media are provided in supplementary Table S2 and depicted in Fig. 4. Salbutamol sulfate showed no statistically significant differences in solubility in the media used, with values ranging between 263 mg/ml in Alveofact medium and 279 mg/ml in Alb in HBSS. For ciprofloxacin, excluding IgG and transferrin from the SLF composition led to a solubility decrease comparable to the one caused by leaving out these two proteins as well as all the lipids (169 µg/ml in SLF Alb, 180 µg/ml in Alb in HBSS, compared to 204 µg/ml in SLF). When only the lipids were included in the medium composition, ciprofloxacin's solubility decreased even further (141 µg/ml in SLF w/out proteins), reaching values lower than in HBSS (171 µg/ml). Beclomethasone dipropionate and budesonide showed similar behaviour with a significantly higher solubility in the Alveofact medium compared to the rest of the media and no significant difference between the values obtained in SLF and SLF Alb for each respective drug (29.8 µg/ml in Alveofact medium to 5.12 µg/ml in SLF and 5.66 µg/ml in SLF Alb for beclomethasone dipropionate and 100.6 µg/ml in Alveofact medium to 66.3 µg/ml in SLF and 67.7 µg/ml in SLF Alb for budesonide). Further decrease in solubility was observed for these two drugs with the removal of more components from the medium composition (beclomethasone with 2.66 µg/ml in Alb in HBSS and 0.46 µg/ml in SLF w/out proteins, and budesonide: 59.6 µg/ml in Alb in HBSS and 29.7 µg/ml in SLF w/out proteins).

4. Discussion

Any BCS must consider the critical attributes that dictate the rate and extent of DS absorption after administration [6]. Solubility and permeability of the DS in the context of the given route of administration, as well as the drug product and formulation characteristics influencing the dose administered and dissolution process, are the fundamental principles considered in the development of giBCS that can be adapted for application to the pulmonary route of administration [6]. While the two classification systems are based on common principles, it is essential that in the development of iBCS the key attributes are evaluated in the context of the lung as a route of administration [5–7]. The giBCS solubility classification is based on DS solubilities in buffer solutions simulating the conditions the drug product encounters in the GI tract after oral administration. The main considered variable of this physiological environment is the pH value, and these buffers are commonly used as dissolution media for *in vitro* dissolution testing as well. In the case of pulmonary delivery, the composition of the LLF varies from proximal to distal regions in the lung, but the pH value is not that much variable. Moreover, for oral drug products, the composition and volume of fluid available are altered by drug administration, but the same is not likely to hold for orally inhaled drug products, at least in the case of DPIs and pMDIs. Media that closely mimic the relevant biological fluids representative of the GI environment exist but are not part of the BCS. Our results indicate that for pulmonary administered drugs, it is rather necessary to perform solubility measurements in media mimicking the LLF to allow for an accurate solubility classification in iBCS.

According to our data, some components in the LLF affect the solubility of some of the DS investigated. For salbutamol sulfate, tobramycin, isoniazid and tiotropium bromide, changes in media composition did not influence the solubility values, while for the other DS, considerable differences were observed. The most significant differences in solubility between the media tested were observed for budesonide, salmeterol, and beclomethasone dipropionate, especially when comparing the Alveofact medium with less complex media. For salbutamol sulfate and budesonide, our results confirm those published by Radivojev et al. [22]. For beclomethasone dipropionate, comparable results were previously obtained for solubility in SLF as well as increased values when evaluated in Survanta®, an alternative surfactant preparation of bovine origin [13,24].

The refined screening in media relevant for the pulmonary environment revealed that the presence of proteins, especially lung surfactant proteins, contributes the most to the solubilizing capacity of the media. The lung surfactant proteins are present only in the Alveofact

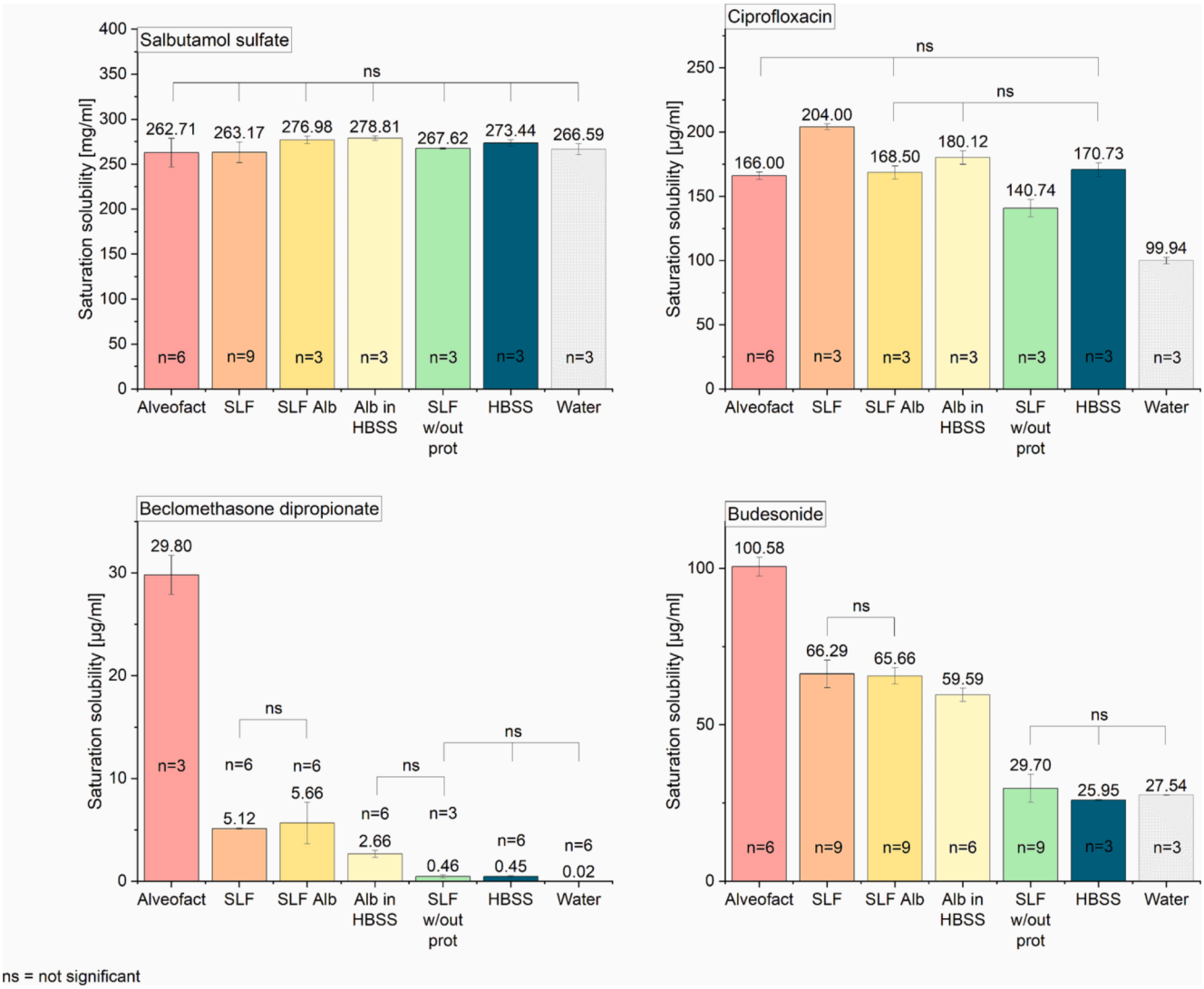


Fig. 4. Refined screening in media relevant for the lung environment. Error bars represent the standard deviation. The statistically not significant differences in solubility are marked (ns), all other results being significantly different to each other ($p \leq 0.05$). SLF = Simulated lung fluid; SLF Alb = SLF with only albumin from the protein fraction; Alb in HBSS = Albumin in Hank's balance salt solution; SLF w/out prot = SLF without proteins; HBSS = Hank's balanced salt solution.

medium. When proteins were completely left out of the SLF composition, the solubility of tested drugs was not significantly different from solubility in plain HBSS, even for the DS for which medium composition was proven to have an influence on solubility. Albumin is the main contributor to the solubilizing capacity of SLF. Since a slight decrease in solubility was observed in albumin in HBSS compared to the SLF with only albumin from the protein fraction (SLF Alb), it is possible that albumin together with the phospholipids act in a synergic way towards increasing solubility. The presence of IgG and transferrin did not influence the solubility of beclomethasone dipropionate and budesonide, but they seem to contribute to slightly increasing the solubility of ciprofloxacin. Leaving out the antioxidants from the original SLF composition was confirmed to have no influence on the solubilizing capacity by measuring solubility in medium with and without antioxidants (results not shown).

For comparative purposes, a minimum theoretical solubility was computed for each DS (see Table 4.) based on their common pulmonary dose and the value of 10 ml total LLF volume, conservatively set in the most recent publication defining the principles and framework of iBCS [6]. This is the solubility a DS would need for the entire dose to be dissolved in the available LLF. It represents the concentration of the initial dose and is part of the equation of the dose number (Do), one of the dimensionless numbers derived by Amidon et al. for assessing drug dissolution, alongside the dissolution number and absorption number [3,5]. The values obtained for solubility in the Alveofact medium were used for computing the dose number for the DS included in this study. If $Do < 1$, the delivered dose is assumed to dissolve completely, while for DS with $Do > 1$, absorption is expected to be limited by the dissolution process [5,7]. The total administered dose was considered for this computation. Lung dose and its deposition pattern (central to peripheral ratio), the influence of the surface of drug formulation particles, the large lung surface (reported values up to 180 m^2 [25]) in relation to the small volume of LLF (15–70 ml [26]) as well as the complexity of their *in vivo* interaction were not taken into account. After the particles' deposition in the lung, absorption in the lung tissue and into the systemic circulation takes place concomitantly with the dissolution process, but like with the giBCS, this was not further considered. Still, this simplification allows for an understanding of the dimension of the difference between the solubility needed for a DS in ideal conditions (entire volume of medium available for dissolving the administered dose) and the measured solubility in the physiologically closest to LLF medium available. The pulmonary solubility of the DS in this study was categorized as either high (Fig. 2, $Do < 1$ i.e., the entire dose is soluble in the LLF volume available) or low (Fig. 3, $Do > 1$ i.e., drug solubility is lower

Table 4
Classification of drug substances for inhalation in iBCS based on solubility in biorelevant medium, in comparison to giBCS classification.

	Drug Substance	Common Pulmonary dose	Theoretical minimum solubility (for 10 ml LLF volume [6])	Solubility			Pulmonary Do	Pulmonary solubility	Oral solubility according to giBCS
				Alveofact	SLF	FaSSIF			
1	Salbutamol sulfate	200 μg	20 μg/ml	263 mg/ml	263 mg/ml	268 mg/ml	$0.08*10^{-3}$	High	High [29]
2	Isoniazid	80 mg (R&D) [36]	8 mg/ml	180 mg/ml	201 mg/ml	187 μg/ml	0.04	High	High [29,37]
3	Salmeterol	50 μg	5 μg/ml	264 μg/ml	84 μg/ml	135 μg/ml	0.02	High	Low [30]
4	Budesonide	400 μg	40 μg/ml	101 μg/ml	66 μg/ml	43 μg/ml	0.40	High	Low [31]
5	Rifampicin	50 mg (R&D) [38]	5000 μg/ml	1898 μg/ml	1714 μg/ml	1722 μg/ml	2.63	Low	Low [39]
6	Tiotropium bromide	18 μg[a]	1.8 μg/ml	39 mg/ml	37 mg/ml	41 mg/ml	$0.05*10^{-3}$	High	High [30]
7	Tobramycin	112 mg	11 mg/ml	523 mg/ml	550 mg/ml	519 mg/ml	0.02	High	NA
8	Ciprofloxacin	32.5 mg (R&D) [40]	3250 μg/ml	166 μg/ml	204 μg/ml	207 μg/ml	1.96	Low	Low [23]
9	Beclomethasone dipropionate	400 μg	40 μg/ml	30 μg/ml	5.1 μg/ml	3.5 μg/ml	1.33	Low	NA

LLF = lung lining fluid; SLF = Simulated lung fluid; FaSSIF = Fasted state simulated intestinal fluid; Do = dose number.
[a] Expressed as tiotropium free base.

than, or comparable to the calculated theoretical minimum solubility).

The increased solubility measured in media relevant for the pulmonary route indicates that drugs that are classified as having low solubility in the giBCS will not necessarily belong to the same solubility class in the iBCS. From our set of DS, fewer drugs appear to have low solubility when administered by inhalation compared to oral administration (see Table 4), supporting the hypothesis that data obtained with methods relevant for the respective route of administration are essential for accurate classification in the iBCS.

With respect to *in vitro* dissolution testing method development, the goal should be to identify surrogate, less complex media that comply with the analytical reagent quality standards while at the same time meeting the need for predictivity concerning the solubilizing capacity of the respective medium. While for some DS, a medium as simple as water could be considered biorelevant, this was not the case for all. Water could potentially be used as dissolution medium in method development for OIDPs containing DS for which it meets the predictivity criteria with regards to solubilizing capacity. However, excipients present in the drug formulation may alter the dissolution properties expected based on DS solubility experiments, and this should be carefully considered in the experimental design. In order to produce meaningful results, the level of complexity needed for a dissolution medium will have to be identified on a case-by-case basis. Obtaining information about DS behaviour in complex media simulating the lung lining fluid is essential for developing meaningful OIDP dissolution testing methods, and for better understanding how *in vitro* performance testing of inhalable powders could allow for prediction of their *in vivo* performance.

Besides the importance for iBCS development, dissolution medium selection, and inherently *in vitro* dissolution testing method development, the results of the present studies are also likely to be relevant for novel formulation development. Albumin and pulmonary surfactant are being researched as potential carriers for pulmonary drug delivery [14,27]. The potential role and effects of using pulmonary surfactant in drug delivery are summarized in a review paper by Hidalgo et al. [28]. In addition to its roles as an additional therapeutic agent in cases of pulmonary surfactant dysfunction or excipient contributing to drug transport in the lung [28], pulmonary surfactant co-administration could also benefit drug dissolution in the lung environment by further increasing solubility. The contribution of albumin to the solubilizing capacity of the SLF is similarly an important finding, considering that its concentration in the lung lining fluid was found to be predominantly increased in the aged lung, healthy smokers, and COPD patients compared to the values observed for the lungs of healthy young individuals. In the COPD lung, increased SP-B concentrations were also found [14]. This could lead to an unexpectedly increased pulmonary drug dissolution and bioavailability as a consequence of a pathological process.

5. Conclusion

The present study was undertaken as another step towards classifying inhaled DS in the iBCS grid. The solubility of DS in different, potentially biorelevant media was evaluated, having in mind the importance of media selection for iBCS and *in vitro* dissolution testing method development. Most of the data available in the literature regarding drug solubility is not specific to the lung environment. Our results showed that depending on the DS physicochemical properties, the presence of lung lining fluid components could significantly contribute to increased solubility. Evaluating solubility in different media will allow for a more accurate iBCS classification of DS for pulmonary administration.

A complete classification of inhaled drugs in the iBCS will also require the evaluation of the permeability of DS for pulmonary administration through appropriate lung epithelial cell models. Still, however, *in vitro* dissolution testing is a key tool for the application of BCS, for which in the case of OIDPs no standardized methods are yet available. A next step in this direction would be identifying suitable dissolution media capable of simulating the solubilizing capacity of the lung lining fluid. For the moment, this has to be done on a case-by-case basis for each DS but appears to be necessary for meaningful dissolution testing as well as for comparing different setups employed in the context of OIDPs.

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CRediT authorship contribution statement

Andreea Floroiu: Conceptualization, Investigation, Visualization, Writing – original draft. **Brigitta Loretz:** Conceptualization, Visualization, Writing – review & editing. **Johannes Krämer:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Claus-Michael Lehr:** Conceptualization, Supervision, Writing – review &

editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Andreea Floroiu reports support, drug substances, or supplies were provided by Lusochimica (Rozzano, Italy), Lyomark Pharma (Oberhaching, Germany) and LAUDA Scientific (Lauda-Koenigshofen, Germany). Andreea Floroiu reports financial support was provided by Eurofins PHAST Development (Konstanz, Germany). Brigitta Loretz, Johannes Krämer and Claus-Michael Lehr declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.].

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2024.114206.

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