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Research paper

Surface Activity Profiling (SAP): A potential means of predicting intestinal membrane permeability

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ABSTRACT

Early characterization of new drug substances intended for oral application includes not only physicochemical properties and stability but also the ability of the substance to permeate through the intestinal mucosa. In this work, a rapid screening method, surface activity profiling (SAP), is proposed as an alternative to animal studies and screening in cell cultures. Measurements are made with a multichannel tensiometer and require only 50 µl of stock solution for the complete permeability analysis. Correlation of SAP results with human absorption was demonstrated for marketed drugs and with absorption in rats for development compounds of Boehringer Ingelheim. Cross-laboratory results for marketed drugs showed excellent agreement. For early stage investigations of lead compounds, where only small amounts of the compound are available, the SAP method appears to be an effective and fast tool to accurately predict fa, provided the compound is amphiphilic.

1. Introduction

Today's lead candidates (LC) in drug development encompass a wide range of physical properties. Many exhibit lipophilic properties, since candidates are often selected according to their receptor affinity in *in vitro* experiments. However, excessive lipophilicity can lead to unfavorable biopharmaceutical properties, such as poor solubility in the gastrointestinal lumen, while hydrophilic compounds may exhibit poor permeability across the intestinal membrane, either of which can result in poor bioavailability after oral dosing. Such compounds are more challenging and carry a higher risk of failure during drug development.

Thus, there is a great need to characterize compounds according their biopharmaceutical properties early on. Since there are just a few milligrams of LC available at early stages of drug discovery, methodologies to assess possible membrane penetration behavior that are simple, quick and accurate are imperative. Over the last decades, scientists have developed several tools with varying degrees of complexity and predictive power to characterize the permeability of compounds: these include simple *in silico* descriptors such as logD and hydrogen bonding potential [1,2], artificial membrane experiments [3,4], Lipinski's rule of five [5], *in vitro* cell line studies [6–8], perfusion studies [9], and excised tissue studies [10,11]. Since it is necessary to assay many compounds within a short time with just a few milligrams of LC, the more complex methods, which in general require more substance, have drawbacks for the lead selection and optimization steps.

Petereit et al. (2010) published a method to predict membrane permeability in the brain with just a few milligrams of LC i.e., Surface Activity Profiling (SAP). Using this method, the relationship between compound concentration and surface pressure of the aqueous solution is established and the relationship can be interpreted in terms of blood-brain-barrier penetration [12]. Petereit et al. used the SAP method in order to predict the permeability in terms of fraction absorbed for a compound set with diverse physicochemical properties. The SAP datasets and the corresponding fraction absorbed values showed a good correlation with $r^2 = 0.74$ [13]. It is postulated that the method can also be used to assess the compound behavior during the permeation process through membranes in the gastrointestinal tract [13].

Analogous to the Petereit approach for the blood-brain-barrier, membranes in the gastrointestinal tract can be seen as physiological barriers consisting of a hydrophobic core region and hydrophilic surfaces. This amphiphilic barrier is better described as an anisotropic interfacial system than as an isotropic system (as for example the partitioning between octanol and water) for compounds passing through a membrane [14,15]. Compounds which exhibit amphiphilic properties are able to orientate themselves at the hydrophobic-hydrophilic membrane interface. The incorporation of a substance in this layer results in a change of the layer pressure. The area requirements of a molecule at the interface can be described with the cross-sectional area of the

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compound at the interface. The cross-sectional area correlates with the change of the layer pressure [12]. It has been demonstrated in the literature that both lipid mono- and bilayers exhibit equivalent changes in their layer pressures when an amphiphilic compound partitions into the interface. This makes it possible to utilize monolayer models to assess the partitioning process of compounds between bulk solution and membranes.

The charge of a molecule, as well as its hydrogen bonding potential, molecular size and lipophilicity are important for permeation of substances through membranes [16]. But all these parameters are also important for the orientation of compounds at interfaces or, in general, for surface activity in solutions [17]. The hydrophobic effect is the driving force for amphiphilic compounds to orientate themselves at hydrophilic-lipophilic interfaces in an anisotropic manner. Polarity differences between different phases can strongly influence the hydrophobic effect. The dielectric constant, which is a well-known parameter describing polarity, can be approximated as $\varepsilon = 2$ for the lipid core region of a membrane and as $\varepsilon = 1$ for air. Thus, both phases can be seen as similar according to their polarity compared to water, which has a dielectric constant of $\varepsilon = 80$. Based on this analogy, it has been postulated that amphiphilic compounds organize themselves at the air/water interface and at the luminal/membrane interface in a comparable way [18]. This approach has already been used to successfully correlate surface activity values of compounds in solution with the potential of compounds to interact with or to penetrate through biological membranes [17]. Further parameters that can be used for such correlations include the critical micelle concentration (CMC), interfacial air/water partitioning coefficient ($K_{AW}{}^{-1}$) and the area requirements of a molecule at the interface, the cross sectional area (AS), which are all surface activity parameters, which can be derived from the Gibb's adsorption isotherm [12,19]:

$$\Gamma = -\frac{d\gamma}{d\mu} \tag{1}$$

where Γ is the surface excess, dγ the change of the surface tension and dμ the change of the chemical potential. In highly diluted solutions dμ can be expressed as RT d lnc, where R is the ideal gas constant, c is the molar concentration of the surface active compound and T the temperature [17].

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln c} \tag{2}$$

In addition, the surface excess can be conveyed as

$$\Gamma = \frac{1}{N_A \times A_S} \tag{3}$$

where N_A is the Avogadro constant and A_S the cross-sectional area of a surface active molecule at the interface [18].

In experiments, the surface pressure (π) rather than the surface tension (γ) is measured

$$\pi = \gamma^0 - \gamma \tag{4}$$

where $\gamma^0$is the surface tension of the solvent and γ is the surface tension in presence of a surface active compound. If the surface pressure (π) is plotted versus ln c, it is not only possible to derive $d\pi/d\ln c$ with the help of the slope but also to determine the CMC and the $K_{AW}{}^{-1}$ of an amphiphilic substance (Fig. 1).

Several surface tension measurement techniques are described in the literature [20–25]. Most of these methods are time-consuming and require large liquid volumes (≥10 ml) and are therefore unsuitable for use at the lead selection stage. For this reason, Kibron (Finland) developed a 96-well plate multichannel microtensiometer. The methodology is analogous to the Du Nouy Ring method but utilizes small needles, which are attached to microbalances, instead of rings. The balances measure small changes in the force needed to withdraw the needles out of the liquid phase. By measuring the individual forces, it is possible to calculate the surface tension, since the diameter of the probes is constant:

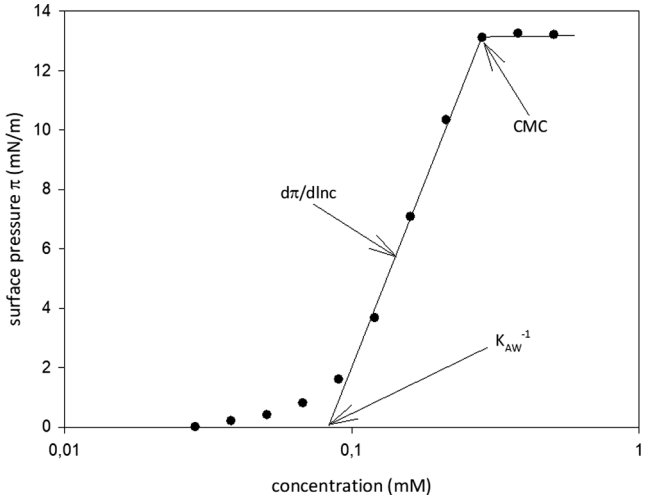

Fig. 1. Surface activity profile (SAP): increasing concentration of an amphiphilic substance results in higher surface pressure (π). $K_{AW}{}^{-1}$ is the air water partitioning coefficient, CMC is the critical micelle concentration, dπ/dlnc is the slope and describes the cross sectional area of the substance at the interface.

$$\gamma = \frac{F_{max}}{4\pi r} \tag{5}$$

where γ is the surface tension, $F_{max}$is the maximum force reached before contraction of the meniscus under the probe, π is a mathematical constant and r is the radius of the probe. The measurements are automated and only small amounts of liquids are required (50 µl per sample) [19,26].

In this work, we present a re-evaluation and refinement of the SAP method proposed by Petereit et al. for the prediction of fa to streamline the SAP measurements and to further improve correlations [13]. The refined approach has been validated in the laboratories of Boehringer Ingelheim (BI) with both marketed compounds as well as with compounds from past and current research programs. In addition, correlation with in-house apparent permeability (P_{app}) data measured with the parallel artificial membrane permeability assay (PAMPA) was evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were obtained from Sigma Aldrich (Seelze, Germany), methanol, methylenchloride and chloroform from VWR (Darmstadt, Germany). Simulated intestinal fluid pH 6.8 (SIFsp) was freshly prepared according to the 2009 USP using monobasic potassium phosphate, sodium hydroxide from Merck KGaA (Darmstadt, Germany) and Milli-Q water from Millipore (Schwalbach, Germany). Furosemide, ibuprofen, dipyridamole, dabigatran, flibanserin, ambroxol, talsaclidine, fenoterol, linagliptin, and pirenzepin were all provided by Boehringer Ingelheim (Biberach, Germany), risperidone, ridogrel and prucalopride were kindly provided by Janssen Pharmaceutica N.V. (Beerse, Belgium). Itraconazole, ketoconazole, fenofibrate, bromocriptine, carvedilol, felodipine, zafirlukast and valsartan were kindly provided by the OrBiTo project (http://www.orbitoproject.eu/). Theophylline was purchased from Sigma Aldrich (Seelze, Germany).

Compound sets are shown in Tables 1–3.

Table 1
Compound set 1: Marketed compounds studied by both Goethe University and Boehringer Ingelheim.

Compound	MW (g/mol)	pKa	logP	SAP category: highly soluble and amphiphilic (H) poorly soluble and amphiphilic (P) or non-amphiphilic (N)	fa	Reference for fa	Compound set
Furosemide	330.7	4.2 (acid)	2.0	H	0.61	[27]	1
Ibuprofen[a]	206.3	4.9 (acid)	3.8	H	1.0	[27]	1
Prucalopride	367.9	8.6 (base) 1.3(base)	1.3	H	0.93	[12]	1
Ridogrel	366.3	3.5 (acid) 4.3 (base)	3.2	P	1.0	[12]	1
Risperidone	410.5	8.8 (base)	2.6	P	1.0	[27]	1
Bromocriptine	654.6	9.7 (acid) 6.7 (base)	3.9	P	0.7	[28]	1
Carvedilol[a]	406.5	8.7 (base)	3.1	P	0.65	[27]	1
Felodipine	384.3	5.4 (base)	3.4	P	0.88	[27]	1
Fenofibrate[a]	360.8	–	5.3	P	0.70	[27]	1
Itraconazole	705.6	3.9 (base)	7.3	P	0.85	[27]	1
Ketoconazole	531.4	6.8 (base)	4.2	P	0.75	[3]	1
Valsartan	435.5	4.4 (acid)	5.3	H	0.55	[27]	1
Zafirlukast	575.7	4.29 (acid)	6.4	P	0.6	[27]	1
Theophylline[a]	180.2	7.82 (acid) 0.78(base)	−0.8	N	1.0	[27]	1

[a] Highlighted compounds were analyzed in the laboratories of both the Goethe University, Frankfurt Germany and Boehringer Ingelheim, Biberach Germany.

Table 2
Compound set 2 [29]: Marketed Compounds studied at Boehringer Ingelheim.

Compound	MW (g/mol)	pKa	logP ($logD_{pH\ 7.4}$)	SAP category: highly soluble and amphiphilic (H) poorly soluble and amphiphilic (P) or non-amphiphilic (N)	PAMPA permeability [$\times 10^{-9}$ cm/s] buf, pH 6.5	Reference for PAMPA	Compound set
Dipyridamol	456.0	6.4 (base) 0.8 (base)	4.1 (4.0)	P	2300	[29]	2
Dabigatran etexilat	723.9	6.7 (base) 4.0 (base)	3.8 (3.7)	P	170	[29]	2
Flibanserin	390.4	5.9 (base)	4.3	P	2300	[29]	2
Linagliptin	472.6	8.6 (base) 1.9 (base)	1.9 (0.4)	H	65	[29]	2
Pirenzepin	442.3	8.1 (base) 2.0 (base) 11.5 (acid)	−0.1 (−0.7)	H	32	[29]	2
Ambroxol	414.6	8.1 (base)	3.0 (2.2)	H	2800	[29]	2
Fenoterole	339.8	9.6 (base) 8.9 (acid) 9.7 (acid) 10.8 (acid)	n.d.[a] (0,9)	H	11	[29]	2
Talsaclidine	281.3	10.5 (base)	n.d.	H	2500	[29]	2

[a] Not applicable, because for zwitterionic compounds no log P can be measured. [29]; n.d. not determined.

Table 3
Compound set 3: Development compounds.

Compound	MW (g/mol)	pKa	logP	SAP category: highly soluble and amphiphilic (H) poorly soluble and amphiphilic (P) or non-amphiphilic (N)	$fa*F_{gut}$ [a]	Compound set
A 2276	718	3.6 (base)8.0 (base)	2.7	H	0.3	3
A 2092	440	5.8 (base)	2.4	P	0.2	3
A 3028	634	10.4 (acid)6.5 (base)	2.6	P	0.3	3
A 1149	539	5.5 (base)10.2 (acid)	8.77	N	0.1	3
A 6197	443	4.0 (acid)3.5 (base)	3.6	P	0.5	3
A 0799	528	2.9 (base)8.6 (base)	3.9	H	0.7	3

[a] fa * F_{gut} obtained by rat studies performed at Boehringer-Ingelheim.

2.1.2. Software

For controlling the Delta-8 instrument as well as for calculating the CMC, the CMCeeker V3.00 software from Kibron (Helsinki, Finland) was used. For the profile analysis Microsoft Excel 2013 (GO)/Microsoft Excel 2010 (BI) (Washington, USA) and for the statistics, SigmaPlot 11.0 from Systat Software Inc. (Chicago, USA)(GO)/Origin Pro 8G from OriginLab Corporation (Massachusetts, USA) (BI) were used. For calculations of logP and pK_a values, Marvin Scatch 4.1 from ChemAxon Kft. (Budapest, Hungary) was used.

2.1.3. Instruments

The Delta-8 multichannel tensiometer from Kibron (Helsinki, Finland) was used for the SAP measurements. For the preparation of the micelle solution, a Rotapor R-114 from Büchi Labortechnik (Essen,

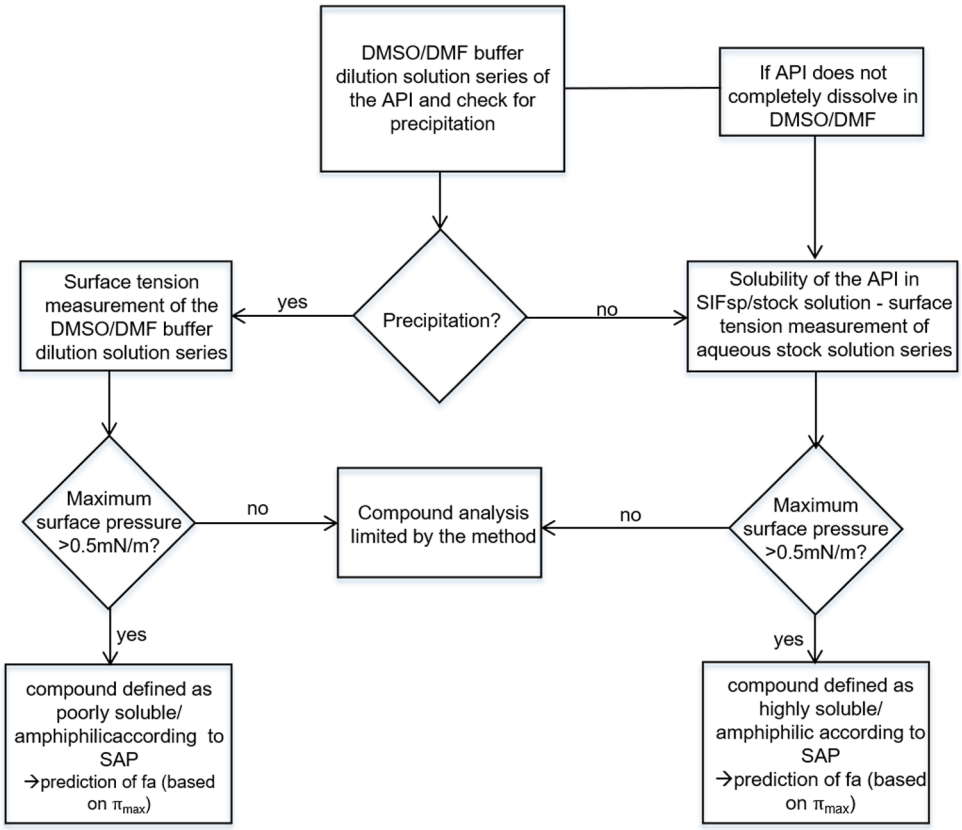

Fig. 2. Refined Workflow diagram for the SAP method based on the work of Petereit et al. The workflow diagram defines compounds as highly soluble amphiphilic, poorly soluble amphiphilic or non-amphiphilic. The measured π_{max} for the highly soluble and amphiphilic and the poorly soluble and amphiphilic compounds can be correlated with permeability data.

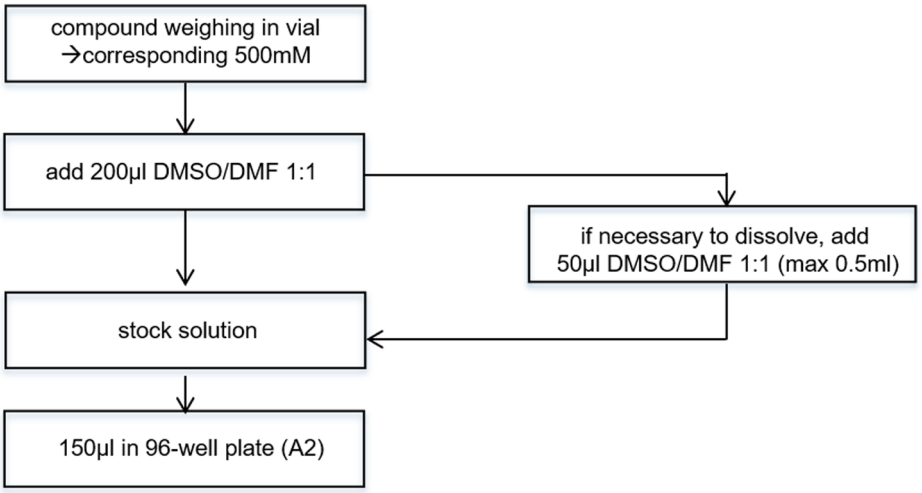

Fig. 3. Refined Workflow for preparation of compounds which are defined as poorly soluble and amphiphilic according to SAP.

Germany) and for quantifying the aqueous stock solutions, a UV-spectrophotometer U 3000 from Hitachi Ltd (Tokyo, Japan) (GO)/Agilent HP 8453 UV–VIS spectrophotometer (BI) was used.

2.2. Methods

2.2.1. PAMPA permeability

Permeability of compound sets 2 and 3 across the PAMPA membrane was determined according to Sieger et al [29].

Pre-coated PAMPA were used for permeability assessment (Gentest© PAMPA plates from Corning, Wiesbaden, Germany). Compound concentration in donor compartments was 100 µM. Buffer pH values are adjusted either with citrate or with NaOH to 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, and 7.4. After an incubation time of 5 h, samples in receiver compartments were taken and measured by LC-MS/MS and compared to the concentration in the donor compartment prior to incubation. In-house data indicated a compound transfer under sink conditions during the complete incubation period, even for compounds with very high permeability (< 5% compound transfer from donor to receiver in 5 h). Under these conditions, the compound transfer can be considered linear during the incubation.

The permeability coefficiency (PAMPA) through the artificial membrane was calculated according to the following equation:

$$P_{app} = V_{rec}*C_{rec}/(A*C_{don}*t).$$

With A = area of the filter in cm^2, C_{don} = substance concentration in

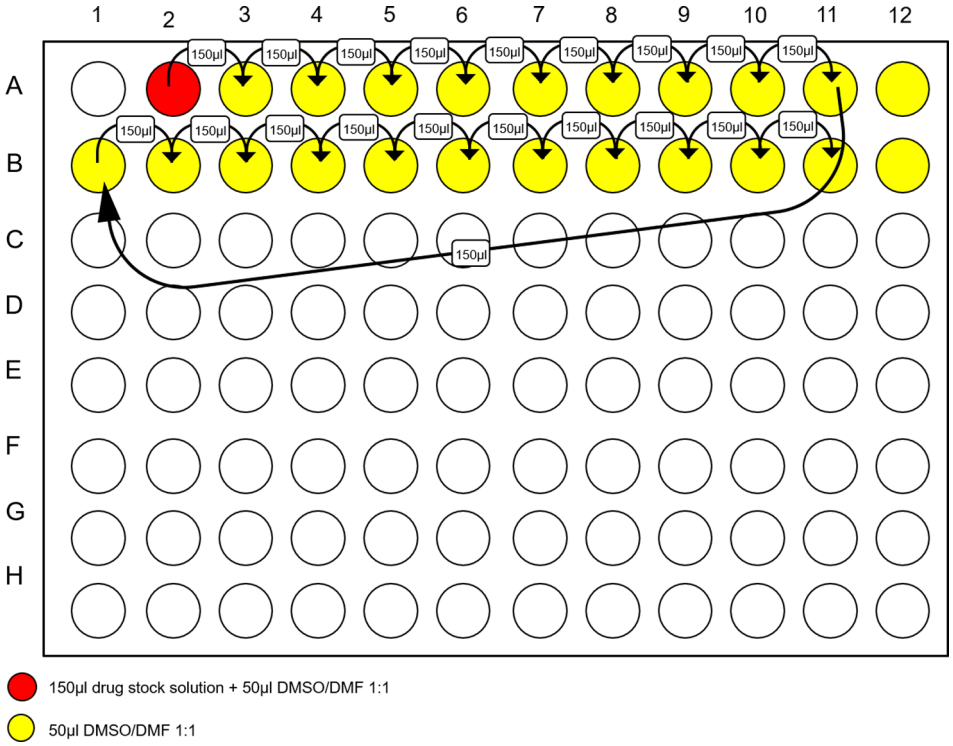

Fig. 4. Refined scheme for pipetting the 96-well plate for poorly soluble and amphiphilic compounds.

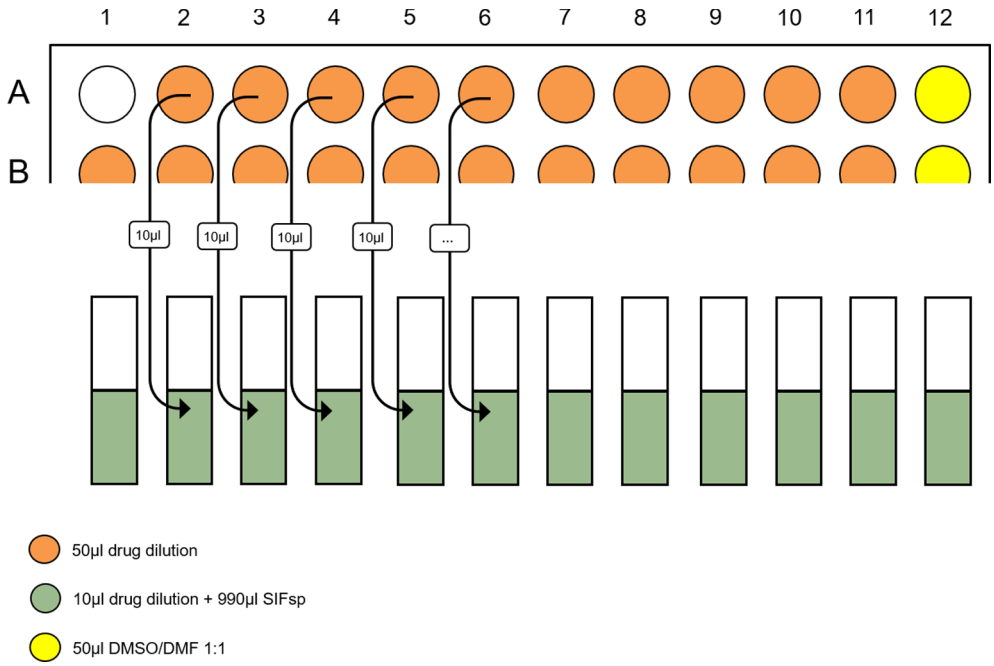

Fig. 5. Refined scheme for preparation of vials for poorly soluble and amphiphilic compounds.

the donor compartment at time t0 in µmol/mL, V_{rec} = volume of buffer in the receiver compartment in mL, C_{rec} = substance concentration in the receiver compartment at time t in µmol/mL, t = incubation time in sec.

2.2.2. Sample preparation for SAP measurements

2.2.2.1. Sample preparation: Workflow. If the surface pressure of a solution of the compound is high, it is assumed that the fully dissolved compound exhibits amphiphilic properties. But not every substance is highly soluble in aqueous media nor does every compound show amphiphilic properties. Hence, based on the work of Petereit et al. a workflow diagram was developed to overcome the limitations of poorly soluble or poorly amphiphilic compounds (Fig. 2) for surface activity profiling.

(a) First the kinetic solubility of a compound is determined by predissolving the substance in DMSO/DMF 1:1 (500 mM) and then diluting the stock solution in SIFsp. For safety reasons in the laboratories of Boehringer Ingelheim, only DMSO was used. If the compound shows precipitation in this step, the maximum surface

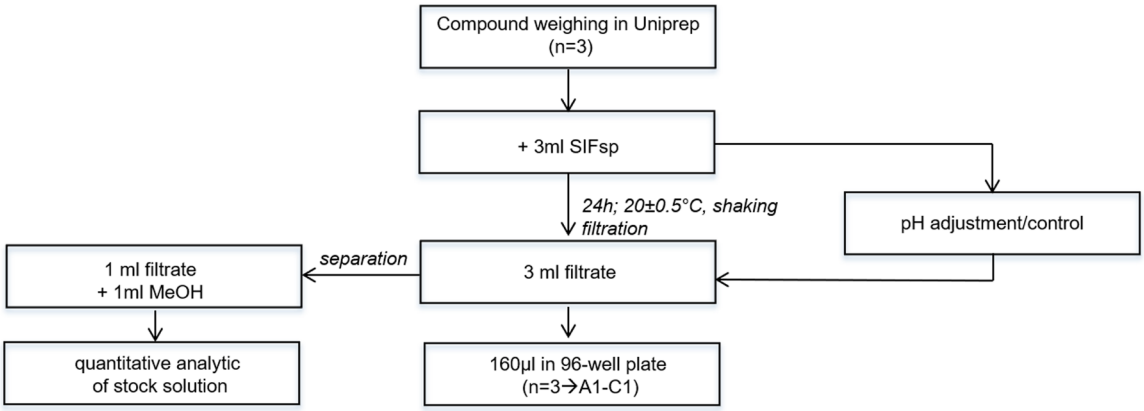

Fig. 6. Workflow for preparation of compounds which are defined as highly soluble and amphiphilic for SAP measurement.

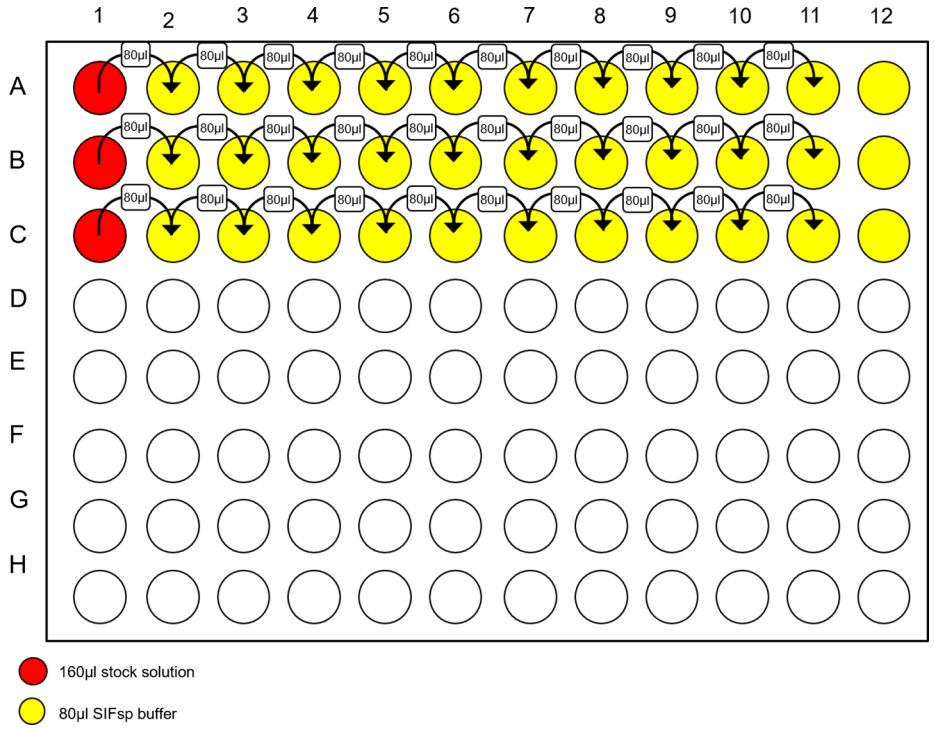

Fig. 7. Scheme for pipetting the 96-well plate with a highly soluble and amphiphilic compound.

pressure of the DMSO/DMF/buffer dilution series is determined. If the π_{max} is above 0.5 mN/m, then the compound can be defined as poorly soluble and amphiphilic. To accurately check for possible precipitation after diluting the stock solution in SIFsp, a UV-spectrophotometer is used with a wavelength-scan from 800 to 400 nm. Then, π_{max} can be correlated with fraction dose absorbed.

(b) If either the compound does not dissolve in the mixture of DMSO/DMF or shows any precipitation, the equilibrium aqueous solubility in SIFsp pH 6.8 is determined. If the maximum surface pressure of this solution is higher than 0.5 mN/m, the compound can be defined as highly soluble and amphiphilic according to the SAP and π_{max} can be correlated with fraction dose absorbed.

(c) If in both cases the measured π_{max} is below 0.5 mN/m, the compound can be classified as non-amphiphilic according to the SAP methodology and no correlation with fraction dose absorbed is possible.

2.2.2.2. Refined workflow for poorly soluble amphiphilic compounds. The compound is weighed into a glass vial in an amount corresponding to 500 mM and pre-dissolved in 200 µl of organic solvent (1:1 mixture of DMSO and DMF). If dissolution is not complete, even after sonification, a further 50 µl solvent is added and so on (Fig. 3).

50 µl of the solvent is filled in each of the wells A2 to A12 and B1 to B12 of a 96-well plate (polypropylene). 150 µl of the drug stock solution is then transferred into well A2. 150 µl is then consequently transferred from one well to another (A2 → A11) and then continued from A11 → B1 → B11. Well A12 and B12 are filled with pure solvent as reference (Fig. 4).

10 µl of each dilution + 10 µl of the stock solution is then transferred to separate 1.5 ml vials. 990 µl of SIFsp is added to the vials and consequently mixed (Fig. 5).

Any vials with significant precipitation are excluded from further investigations. Then 3 × 50 µl of each of the remaining vials is pipetted on the Delta-8 plate, 50 µl in each well. The residual of 850 µl in the vials are analyzed via UV-spectrometer with wavelength scan from 800 to 200 nm to detect possible precipitation in the range of 800–400 nm. Vials where the drug is fully dissolved are used to check whether the compound shows absorption > 400 nm, which would lead to a false positive precipitation evaluation. Before the plates are analyzed with the Delta-8 instrument, a 10 min equilibrium time is required.

Table 4
Ex perimental setups for non-GLP studies performed at Boehringer-Ingelheim in male & female rats.

API	Radiolabeling	i.v. dose (μmol/kg)	p.o. dose (μmol/kg)	p.o. dosage form	Strain and gender of rats
A2276	[^{14}C]	1	1	Solution	Han Wistar (male)
A2092	[^{14}C]	5	10	Solution	Han Wistar (male)
A3028	[^{14}C]	10	10	Solution	Han Wistar (male)
A1149	no radiolabel	27.2 (based on active moiety)	22.8	Suspension of prodrug (analysis of active moiety)	Boehringer Ingelheim rats [Chbb:THOM](only males for Fa analysis)
A6197	[^{14}C]	9.5	9.5	Solution	Boehringer Ingelheim rats (only females for Fa analysis) [Chbb:THOM]
A0799	[^{14}C]	2.1	214.3	Suspension	Boehringer Ingelheim rats (only males for Fa analysis) [Chbb:THOM]

2.2.2.3. Preparation of highly soluble, amphiphilic compounds. If either no precipitation is observed after diluting the DMSO/DMF stock solution in SIFsp or the compound does not completely dissolve in DMSO/DMF, the aqueous saturation solubility is determined.

An excess amount of compound beyond its expected solubility is weighed into a Uniprep (n = 3) and 3 ml of SIFsp are added. The Uniprep is then shaken for 24 h at 20 ± 0.5 °C on a mechanical shaker (Polymax 1040 orbital shaker, Heidolph Instruments, Schwabach, Germany). If necessary, pH adjustment is performed after 4 h with a final pH control before filtering after 24 h. 1 ml filtrate is appropriately diluted with 1 ml methanol or acetonitrile and then quantified via UV spectroscopy. The workflow is shown in Fig. 6.

Another 160 μl of the filtrate is transferred onto a 96-well plate (A1-C1). In the remaining wells 80 μl pure SIFsp buffer are added (A2 → A12; B2 → B12; C2 → C12).

Then 80 μl of the dilution are pipetted from one well to another (A1 → A11; B2 → B11; C2 → C11) and each time carefully mixed by repeated charging and syringing (10 ×). The last column (A12–C12) with pure buffer is used as reference (Fig. 7).

Then 50 μl of each well is transferred on the final 96-well plate for the Delta8-measurement. Before the plates are analyzed with the Delta-8 instrument, a 10 min equilibrium time is required.

2.2.3. Rat studies

All studies in rats were non-GLP studies performed at Boehringer-Ingelheim in male & female rats of different strains and suppliers. The p.o. and i.v. doses for each drug candidate and the type of API are shown in Table 4, along with whether the p.o. dose was given as a suspension or a solution. All i.v. doses were administered as solutions. Blood samples were collected in EDTA micro tubes at pre-defined time points (see Table 4).

For the calculation of the observed *fa* in rats, the following equations were used:

$$CL_{blood} = \frac{CL_{plasma}}{Cb/Cp}$$

where CL is the clearance and Cb/Cp the blood plasma ratio.

$$CL_{blood\_hep} = CL_{blood}*(1 - fe, u, urine)$$

where fe, u, $urine$ is the fraction excreted unchanged in urine,

$$F_{hep} = 1 - \frac{CL_{blood_{hep}}}{Q_{hep}}$$

F is the bioavailability and Q_{hep} the liver blood flow [ml/min/kg].

$$fa*F_{gut} = \frac{F}{F_{hep}}$$

2.3. Statistical methods

The π_{max} values are presented as the arithmetic mean with the standard deviation. The experiments are performed at least in triplicate ($n \geq 3$). The π_{max} values were compared using analysis of variance (ANOVA) with $p < 0.05$ considered significant.

3. Results and discussion

To evaluate the interlaboratory robustness of the SAP method, four test compounds with differing physicochemical properties (ibuprofen, carvedilol, fenofibrate and theophylline) were analyzed in the laboratories of BI and Goethe University. It should be mentioned that due to environment, health and safety (EHS) restrictions, the DMSO/DMF solvent was changed to DMSO at BI. All four compounds were successfully classified by both laboratories into the same classes according to the SAP method (Table 5); i.e. Ibuprofen as highly soluble and

Table 5
fa predictions for poorly soluble/amphiphilic and highly soluble/amphiphilic compounds by the laboratories of Goethe and BI - direct comparison.

Compound	SAP class	π_{max} (mN/m) (Goethe)	π_{max} (mN/m) (BI)	predicted *fa* (Goethe)	predicted *fa* (BI)	literature *fa*
Ibuprofen	H	30.4 (± 0.9)	27.0 (± 0.3)	0.93	0.90	1.0
Carvedilol	P	5.3 (± 0.1)	7.6 (± 0.1)	0.60	0.67	0.65
Fenofibrate	P	6.3 (± 0.4)	2.9 (± 0.1)	0.64	0.49	0.7
Theophylline	N	0.5 (± 0.4)	0.82 (± 0.3)	–	–	1.0

Table 6
fa predictions for poorly soluble/amphiphilic and highly soluble/amphiphilic compounds by the revised SAP method.

Compound	π_{max} (mN/m)	Predicted *fa* by the SAP method	Literature *fa*	SAP category: highly soluble/amphiphilic (H) or poorly soluble/amphiphilic (P)
Bromocriptine	5.8 (± 0.1)	0.62	0.70–0.95	P
Carvedilol	5.3 (± 0.1)	0.60	0.65	P
Felodipine	13.1 (± 0.7)	0.77	0.88	P
Fenofibrate	6.3 (± 0.4)	0.64	0.70	P
Itraconazole	3.4 (± 0.3)	0.52	0.85	P
Ketoconazole	17.6 (± 0.3)	0.82	0.75	P
Ridogrel	18.1 (± 0.4)	0.83	1.00	P
Risperidone	10.5 (± 0.6)	0.73	1.00	P
Zafirlukast	12.3 (± 0.4)	0.76	0.60	P
Furosemid	5.7 (± 0.2)	0.61	0.50	H
Ibuprofen	30.4 ± (0.9)	0.93	1.00	H
Prucalopride	22.9 (± 0.8)	0.87	0.93	H
Valsartan	16.6 (± 1.1)	0.81	0.55	H

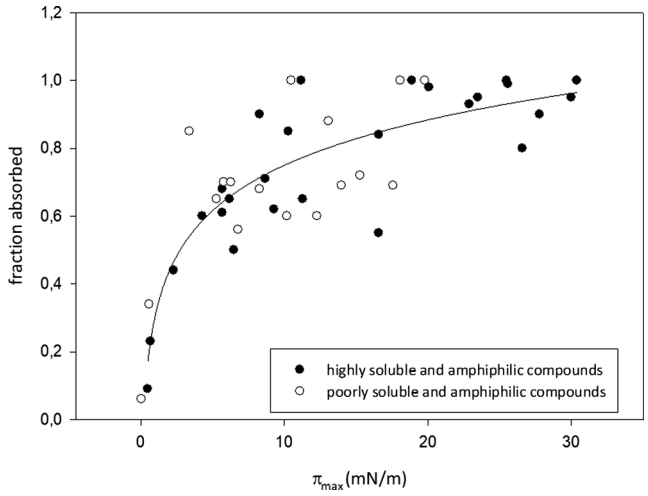

Fig. 8. Correlation of π_{max} and *fa* for both highly and poorly soluble and amphiphilic compounds of compound set 1 and data from Petereit et al.. A correlation coefficient of $r^2 = 0.73$ can be calculated.

Table 7
fa predictions for poorly soluble/amphiphilic and highly soluble/amphiphilic pipeline compounds as well as for one non-amphiphilic compound.

API Code	SAP class	π_{max} (mN/m) (BI)	predicted *fa* (BI)	observed fa*F_{gut} (BI)[a]
A 2276	H	15.4 (± 0.6)	0.8	0.3
A 2092	P	0.95 (± 0.2)	0.3	0.2
A 3028	P	11.5 (± 0.04)	0.7	0.3
A 1149	N	0.52 (± 0.1)	0.2	0.1
A 6197	P	14.3 (± 0.3)	0.8	0.5
A 0799	H	37.0 (± 0.3)	1.0	0.7

[a] fa * F_{gut} obtained by rat studies performed at Boehringer-Ingelheim.

amphiphilic (H) carvedilol and fenofibrate as poorly soluble and amphiphilic (P) and theophylline as non-amphiphilic (N) (candidate with inappropriate surface activity according to the SAP method, no prediction of fa is possible with the current method). For the prediction of fa, the original equation of Petereit et al. was used

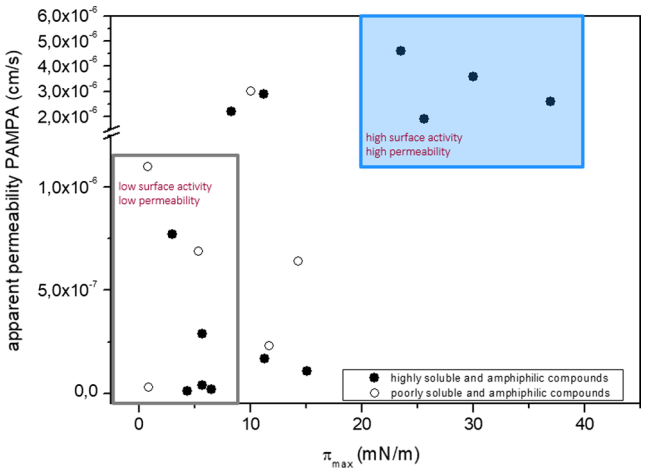

Fig. 9. Correlation of π_{max} with PAMPA data of BI for amphiphilic compounds.

(fa = 29.03 + 18.6 * ln(π_{max})) [13]. The π_{max} values for ibuprofen from both labs are in good agreement (30.4 ± 0.9 mN/m and 26.7 mN/m). The predicted *fa* of 0.93 and 0.90 are very close to the literature value of 1.0. The π_{max} values for carvedilol and fenofibrate differed somewhat more between the labs (5.3 ± 0.1 mN/m and 7.8 mN/m for carvedilol; 6.3 ± 0.4 mN/m and 2.5 mN/m for fenofibrate). However, the predicted *fa* for both compounds are quite similar with 0.60 and 0.67 for carvedilol and 0.64 and 0.46 for fenofibrate. The interlaboratory differences could be due to varying room temperatures and the placement of the instrument. The effect of changing the organic solvent from DMSO/DMF to DMSO appeared to be minor, since results were similar between the two laboratories.

The modified workflow diagram (Fig. 2) was then used to measure π_{max} for the complete compound set 1, predict *fa* and to compare the results with literature values. The results are shown in Table 6. It can be seen that the predictions for the four highly soluble and amphiphilic compounds according to the SAP are overall closer to the literature fa values than for the nine poorly soluble compounds. Most of the predictions of the poorly soluble compounds are underpredictions (exceptions are ketoconazole and zafirlukast), however, still close to the reported literature fa values. This might be due to solubility limitation

of the compounds. A potential way of mitigating the underprediction would be to increase the amount of organic solvent used to enhance the solubility of those compounds. However, the 1% organic solvent (DMSO/DMF) concentration used in the present study is already a compromise between solubility enhancement of the compounds on the one hand and the intrinsic influence of the solvent on the surface tension on the other hand.

Next, the measured π_{max} values for compound set 1 were correlated with *fa*, together with the π_{max} values for the highly soluble/amphiphilic and poorly soluble/amphiphilic compounds from the work of Petereit [13]. The results are shown in Fig. 8. A generally good overall correlation of $r^2 = 0.73$ was calculated when the proposed workflow diagram was used (with the original sample preparation procedure of Petereit, a correlation of only $r^2 = 0.6$ can be calculated (data not shown)).

Furthermore, 6 BI development compounds from the OrBiTo database were measured and predicted *fa* based on π_{max} was correlated with observed *fa* ($fa*F_{gut}$) from rat studies. The results are shown in Table 7. Five compounds were classified as amphiphilic, two of which were found to be highly soluble. Three compounds were classified as poorly soluble and one compound was classified as non-amphiphilic. The predicted *fa* with $fa*F_{gut}$ revealed a generally good correlation ($r^2 = 0.67$).

The correlation of π_{max} with apparent permeability obtained with the PAMPA model was evaluated.

SAP results from set 2 and set 3 were correlated with apparent permeability across PAMPA membranes (Fig. 9). This figure only includes compounds classified as amphiphilic ($\pi_{max} > 0.5$ mN/m).

Seven compounds with π_{max} values between 0 and 10 mN/m also revealed poor permeability across PAMPA membranes. By contrast, for compounds with π_{max} values between 20 and 40 mN/m, the permeability across PAMPA membranes was high.

Surprisingly, for compounds with intermediate π_{max} values the permeability could not be predicted as accurately as for the compounds with extreme π_{max} values. This could be due to overlapping effects and that for these compounds amphiphilicity isn't the only driving force for permeation. Lack of sensitivity of the method was also postulated as an explanation, but was ruled out by the robustness, as shown in 3.1.

4. Conclusion

For early stage investigations of LCs, where only small amounts of the compound are available, the SAP method appears to be an effective and fast tool to get a first estimate of fa, provided the compound is amphiphilic. Only small amounts of the LC stock solution are needed (50 µl) to be analyzed via the Delta-8 instrument and no further analytics such as HPLC analysis are necessary. Despite its simplicity, the method is able to predict fa for a broad range of compounds including highly and poorly soluble amphiphilic compounds. The refined SAP method for poorly soluble and highly soluble amphiphilic compounds predicts *fa* values within 35% of literature *fa* values.

For non-amphiphilic compounds, on the other hand, the SAP in its current form cannot be used to reliably predict *fa*. For these compounds, alternative methods to predict *fa* should be applied.

For the BI compounds, the refined SAP method could be applied to predict high or low permeability. Except for one outlier, the refined SAP method predicts *fa* values within 35% of *fa* values in rats.

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References

[1] C.A. Bergström, M. Strafford, L. Lazorova, A. Avdeef, K. Luthman, P. Artursson, Absorption classification of oral drugs based on molecular surface properties, J. Med. Chem. 46 (2003) 558–570.
[2] M.H. Abraham, A. Ibrahim, A.M. Zissimos, Y.H. Zhao, J. Comer, D.P. Reynolds, Application of hydrogen bonding calculations in property based drug design, Drug Discovery Today 7 (2002) 1056–1063.
[3] C. Zhu, L. Jiang, T.-M. Chen, K.-K. Hwang, A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential, Eur. J. Med. Chem. 37 (2002) 399–407.
[4] K. Sugano, N. Takata, M. Machida, K. Saitoh, K. Terada, Prediction of passive intestinal absorption using bio-mimetic artificial membrane permeation assay and the paracellular pathway model, Int. J. Pharm. 241 (2002) 241–251.
[5] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 64 (2012) 4–17.
[6] J.D. Irvine, L. Takahashi, K. Lockhart, J. Cheong, J.W. Tolan, H. Selick, J.R. Grove, MDCK (Madin–Darby canine kidney) cells: a tool for membrane permeability screening, J. Pharm. Sci. 88 (1999) 28–33.
[7] H.H. Usansky, P.J. Sinko, Estimating human drug oral absorption kinetics from Caco-2 permeability using an absorption-disposition model: model development and evaluation and derivation of analytical solutions for ka and Fa, J. Pharmacol. Exp. Ther. 314 (1, 2011) (2005) 391–399.
[8] S. Tavelin, J. Taipalensuu, F. Hallböök, K.-S. Vellonen, V. Moore, P. Artursson, An improved cell culture model based on 2/4/A1 cell monolayers for studies of intestinal drug transport: characterization of transport routes, Pharm. Res. 20 (2003) 373–381.
[9] H. Lennernäs, Animal data: the contributions of the Ussing Chamber and perfusion systems to predicting human oral drug delivery in vivo, Adv. Drug Deliv. Rev. 59 (2007) 1103–1120.
[10] L.-P. Ruan, S. Chen, B.-Y. Yu, D.-N. Zhu, G. Cordell, S. Qiu, Prediction of human absorption of natural compounds by the non-everted rat intestinal sac model, Eur. J. Med. Chem. 41 (2006) 605–610.
[11] B. Levet-Trafit, M.-S. Gruyer, M. Marjanovic, R. Chou, Estimation of oral drug absorption in man based on intestine permeability in rats, Life Sci. 58 (1996) PL359–PL363.
[12] A.C. Petereit, K. Swinney, J. Mensch, C. Mackie, S. Stokbroekx, M. Brewster, J.B. Dressman, Prediction of blood–brain barrier penetration of poorly soluble drug candidates using surface activity profiling, Eur. J. Pharm. Biopharm. 75 (2010) 405–410.
[13] A.C. Petereit, Prediction of biological membrane penetration of poorly soluble drugs using surface activity profiling, in: Institute of Pharmaceutical Technology, Goethe University, Frankfurt am Main, 2011.
[14] D. Bassolino-Klimas, H.E. Alper, T.R. Stouch, Solute diffusion in lipid bilayer membranes: an atomic level study by molecular dynamics simulation, Biochemistry 32 (1993) 12624–12637.
[15] G.D. Eytan, R. Regev, G. Oren, Y.G. Assaraf, The role of passive transbilayer drug movement in multidrug resistance and its modulation, J. Biol. Chem. 271 (1996) 12897–12902.
[16] G.M. Pauletti, F.W. Okumu, R.T. Borchardt, Effect of size and charge on the passive diffusion of peptides across Caco-2 cell monolayers via the paracellular pathway, Pharm. Res. 14 (1997) 164–168.
[17] A. Seelig, R. Gottschlich, R.M. Devant, A method to determine the ability of drugs to diffuse through the blood-brain barrier, Proc. Natl. Acad. Sci. 91 (1994) 68–72.
[18] G. Gerebtzoff, X. Li-Blatter, H. Fischer, A. Frentzel, A. Seelig, Halogenation of drugs enhances membrane binding and permeation, ChemBioChem 5 (2004) 676–684.
[19] P. Suomalainen, C. Johans, T. Söderlund, P.K. Kinnunen, Surface activity profiling of drugs applied to the prediction of blood-brain barrier permeability, J. Med. Chem. 47 (2004) 1783–1788.
[20] N. Wu, J. Dai, F.J. Micale, Dynamic surface tension measurement with a dynamic Wilhelmy plate technique, J. Colloid Interface Sci. 215 (1999) 258–269.
[21] J. Eastoe, J. Dalton, Dynamic surface tension and adsorption mechanisms of surfactants at the air–water interface, Adv. Colloid Interface Sci. 85 (2000) 103–144.
[22] V. Fainerman, R. Miller, Maximum bubble pressure tensiometry—an analysis of experimental constraints, Adv. Colloid Interface Sci. 108 (2004) 287–301.
[23] V. Fainerman, R. Miller, Dynamic surface tension measurements in the sub-millisecond range, J. Colloid Interface Sci. 175 (1995) 118–121.
[24] P. Nouy, Concerning the ring method for measuring surface tension, Science (New York, NY) 65 (1927) 160.
[25] L.D. NoüY, The ring method in changing surface tension, Science (New York, NY) 67 (1928) 607-607.
[26] M. Kansy, F. Senner, K. Gubernator, Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes, J. Med. Chem. 41 (1998) 1007–1010.
[27] M.V. Varma, R.S. Obach, C. Rotter, H.R. Miller, G. Chang, S.J. Steyn, A. El-Kattan, M.D. Troutman, Physicochemical space for optimum oral bioavailability: contribution of human intestinal absorption and first-pass elimination, J. Med. Chem. 53 (2010) 1098–1108.
[28] G. Maurer, E. Schreier, S. Delaborde, R. Nufer, A. Shukla, Fate and disposition of bromocriptine in animals and man. II: Absorption, elimination and metabolism, Eur. J. Drug Metab. Pharmacokinet. 8 (1983) 51–62.
[29] P. Sieger, Y. Cui, S. Scheuerer, pH-dependent solubility and permeability profiles: a useful tool for prediction of oral bioavailability, Eur. J. Pharm. Sci. 105 (2017) 82–90.