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# Communications to the Editor

## Physicochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes

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Although biological activity is a key issue in developing a potent drug, other factors such as solubility, absorption, partitioning, or biodegredation are equally important. Unfortunately, the optimization process for these important properties is often situated late in the drug discovery/development process. Therefore the early incorporation of pharmacologically relevant physicochemical and biophysical compound properties into the process of lead optimization is an important issue today.

The gastrointestinal absorption of an orally administered drug is one of the key factors for its bioavailability. Several molecular properties have been recognized to govern the passive absorption process of a drug.[1] These include partition (log *P*) and distribution coefficients (log *D*), molecular weight, the ionization state, and the hydrogen-bonding capacity. Although these parameters have been shown to be useful in the prediction of passive permeation processes, restrictions in the availability of these parameters may hinder their usage. Additionally the use of octanol/water partition coefficients in the description of membrane permeation is controversial, due to the observed differences in octanol/water−membrane/water partition coefficients.[2−7]

In vitro assays, as well as tools based on physicochemical properties, are used in the prediction of in vivo absorption processes. The in vitro methods include simple artificial or biological membrane systems or assays based on biological cell layers,[8−16] e.g., Caco-2 cells.

Drug permeation of artificial[17−30] membranes, as well as biological cell layers, is mainly related to passive diffusion processes including paracellular and transcellular permeation.[9] However, an increasing number of carrier-mediated intestinal transported drugs have been found.[31] Unfortunately results from Caco-2 cells for actively transported compounds may incorrectly predict the extent of oral absorption, due to possible quantitatively under- or overexpressed active transporter system.[32] Caco-2 permeation studies are laboratory intensive and therefore currently are not suited for high-throughput measurements. Hence methods which can deliver data related to membrane permeation for large numbers of compounds (500−1000 per day) at an early stage of the discovery/development process are urgently needed and may help in the differentiation between active, paracellular, and transcellular absorption processes. This is the focus of the PAMPA, a simple method for prediction of transcellular drug absorption.

Pidgeon[33] and co-workers describe immobilized artificial membrane (IAM) columns as another tool in the study of drug−membrane interaction and the prediction of transcellular absorption. Although this approach seems promising, the throughput is limited by the analytic method, usually HPLC, and therefore currently not suited for HTS.

To validate PAMPA, a diverse set of well-described drugs has been selected from the literature. This set is mainly related to a publication by Artursson.[8] Some hydrophilic compounds had to be excluded, due to insufficient UV absorption. The selected set includes known passively absorbed compounds, as well as actively transported drugs. Cephalexin is known to be mainly absorbed by an active meachanism,[34] whereas polar compounds such as salicylic acid or theophyllin, with molecular weights below 200, can be assumed to be mainly absorbed via the paracellular route.

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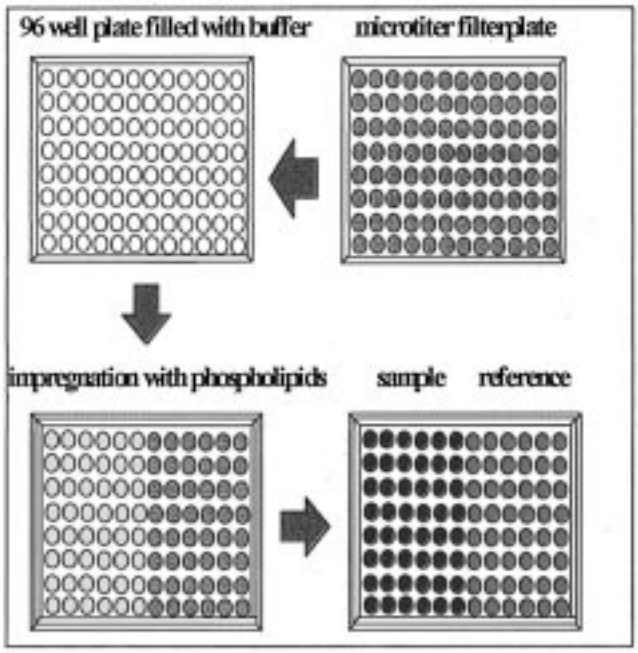


**Figure 1.** Fundamentals of PAMPA. A 96-well microtiter plate completely filled with aqueous buffer solutions (pH 7.4/ 6.5) is covered with an microtiter filterplate in a sort of sandwich construction. The hydrophobic filter material (Durapore/ Millipore; pore size 0.22−0.45 $\mu$m) of the first 48 wells (sample) of the filterplate is impregnated with a 1−20% solution of lecithin in an organic solvent (dodecane, hexadecane, 1,9-decadiene). The filter surface of the remaining 48 wells (reference) is wetted with a small volume (4−5 $\mu$L) of a 50% (v/v) methanol/buffer solution. Transport studies were started by the transfer of 100−200 $\mu$L of a 250 or 500 $\mu$M stock solution on top of the filterplate in the sample and in the reference section, respectively. In general 0.05 M TRIS, pH 7.4, or 0.05 M phosphate, pH 6.5, buffers were used. The maximum DMSO content of the stock solutions was 5%.

Using a hydrophobic filter material as a support, the permeation of these compounds through a membrane, formed by a mixture of lecithin and an inert organic solvent, was measured. The obtained flux values were compared with known human absorption data.[8−16,34,35]

Thompson[36,37] and co-workers could show by electrochemical measurements that extremely stable bilayers, so-called micro-BLM (black lipid membranes), can be formed on supported filter material equal to that used in the PAMPA assay. Additionally photoinduced potential differences on filter-supported membranes formed from dodecan/lecithin and bacteriorhodopsin[38,39] gave further support for the formation of stable bilayers on filter support as used in PAMPA studies.

Although large variations in the phospholipid composition of mammalian membranes are described, egg lecithin mimics phospholipid composition of mammalian membranes[40] and therefore was chosen in the initial PAMPA experiments.

PAMPA is based on a 96-well microtiter plate technology (see Figure 1), completely artificial, without pores[36,37] and active transporter systems. It allows the measurement of hundreds of compounds a day. The main objective is the classification of passively transported compounds, focusing on the transcellular absorption route.

One precondition for the high throughput is a simple analytical method. Therefore the concentrations of the

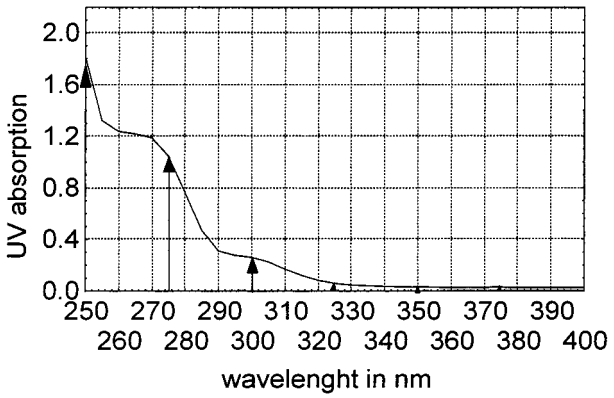


**Figure 2.** Schematic depiction of a parallel UV measurement using the example acetyl salicylic acid. The concentration of the solutions were determined by UV spectroscopy, using a microtiter plate reader Spectramax 250 (Molecular Devices). In general measurements were performed at 260, 280, 300, 320, 340, and 360 nm in parallel, without any optimization for the UV optimum. The permeation of a compound through the membrane layer is described by the percentage permeation (% flux). The flux values were calculated considering the UV absorption of the acceptor compartment after 15 h and that of a reference well with the same concentration containing no membrane barrier.

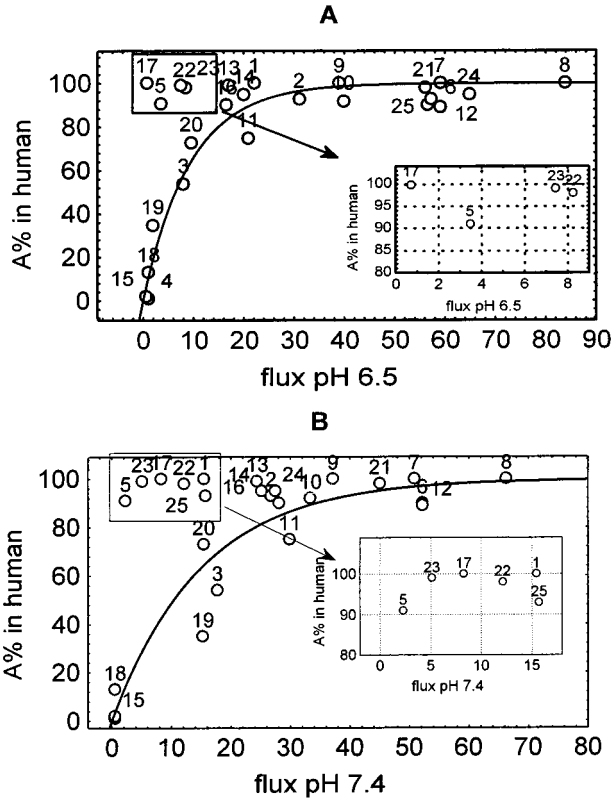


**Figure 3.** PAMPA flux at different pH values versus human absorption data (see Table 1 for measurement results). Insets describe compounds which are actively transported or polar compounds with low molecular weight, which can assumed to be absorbed by the paracellular route in human: (A) pH 6.5; (A) pH 7.4.

compounds were determined by simultaneous UV measurements at six different wavelengths using a 96-well microplate photometer. A reference solution defining equilibrium conditions was used as an internal standard (see Figure 2). Influences of pH changes, phospholipid composition, solubility, or effects of surfactants, like bile

**Table 1.** Combined Measurement Results[a]

| | compound | A% | c1 | logD | flux pH 6.5 | c2 | flux pH 7.4 | c3 | c4 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | acetylsalicylic acid | 100 | h | −2.57 | 22 | m | 15 | m | m |
| 2 | alprenolol | 93 | h | 1.00 | 31 | h | 27 | h | h |
| 3 | atenolol | 54 | m | −2.14 | 8 | 18 | m | m | |
| 4 | ceftriaxone | 1 | l | −1.23 | < 5 | l | < 5 | l | l |
| 5 | cephalexin | 91 | h | −1.00 | < 5 | l | | | l |
| 6 | chloramphenicol | 90 | h | 1.00 | 57 | h | 52 | h | h |
| 7 | corticosterone | 100 | h | 1.89 | 59 | h | 51 | h | h |
| 8 | coumarin | 100 | h | 1.39 | 84 | h | 66 | h | h |
| 9 | dexamethason | 100 | h | 1.74 | 39 | h | 37 | h | h |
| 10 | diltiazem | 92 | h | 2.22 | 40 | h | 33 | h | h |
| 11 | guanabenz | 75 | h | 1.67 | 21 | m | 30 | h | h |
| 12 | hydrocortison | 89 | h | 1.53 | 59 | h | 52 | h | h |
| 13 | imipramine | 99 | h | 2.52 | 17 | m | 24 | m | m |
| 14 | metoprolol | 95 | h | 0.07 | 20 | m | 25 | h | h |
| 15 | olsalazine | 2 | l | −4.5 | < 5 | l | < 5 | l | l |
| 16 | propranolol | 90 | h | 1.54 | 17 | m | 28 | h | h |
| 17 | salicylic acid | 100 | h | −2.14 | | | 8 | m | m |
| 18 | sulfasalazine | 13 | l | −0.13 | < 5 | l | < 5 | l | l |
| 19 | sulpiride | 35 | m | −1.15 | < 5 | l | 15 | m | m |
| 20 | terbutaline | 73 | h | −1.4 | 10 | m | 15 | m | m |
| 21 | testosterone | 98 | h | 3.31 | 56 | h | 45 | h | h |
| 22 | theophylline | 98 | h | −0.02 | 8 | m | 12 | m | m |
| 23 | tiacrilast | 99 | h | −1.05 | 7 | m | 5 | l | m |
| 24 | verapamil | 95 | h | 1.91 | 65 | h | 28 | h | h |
| 25 | warfarin | 93 | h | 0.12 | 57 | h | 16 | m | h |

[a] A%: human Absorption Values.[8−16, 35] c1: classification human absorption. log D: distribution coefficients (pH 7.4 octanol/water).[8,35,43] c2: classification permeation at pH 6.5. c3: classification pH 7.4. c4: combined classification pH 7.4 and 6.5. (c2 and c3). Classification scale: l, low; m, medium; h, high. Flux measurement were performed in triplicate. Standard deviations in all cases were less than 5% related to the flux value.

acids,[41] on transport processes can easily be examined by our novel parallel system.

Graphs of Caco-2 permeation rates depicted against human absorption rates usually show a very steep slope.[9,34] Our results reflect a similar situation. Figure 3 shows the typical hyperbolical function with a steep slope in the range 0−25% PAMPA flux. This steep slope can complicate the prediction of human absorption rates for compounds with lower passive permeation ability. Nevertheless our measurements permit a simple classification between compounds having low, intermediate, and high human absorption probabilities. A further reduction in membrane thickness and increase in concentration, currently under investigation, might allow a more precise classification of compounds with lower permeation rates. Permeation of our membrane layer is strongly dependent on the pH, especially if the examined compounds have p$K_a$ values near the pH of the buffer used in the flux measurements. This indicates the importance in the consideration of different pH values in permeation assays used in human absorption prediction. Warfarin for example, a weak acid with a known p$K_a$[42] of 5.0, shows an increase of more than 30% in permeation if the pH is changed from 7.4 to 6.5 under our measurement conditions.

According to a modified classification scale described by Amidon,[34] we can separate the examined drugs into three groups. Well-absorbed compounds (human absorption, 70−100%; PAMPA flux, 25−100%), intermediate-absorbed compounds (human absorption, 30−70%; PAMPA flux, 5−25%), and compounds with low absorption (human absorption, 0−30%; PAMPA flux, <5%). Using this classification scheme, nearly 80% of the compounds would be correctly predicted in their in vivo absorption ability (Table 1), considering our flux values at different pH (see Table 1/c4). Problems occur in the group of polar compounds (MW < 250) mainly transported via the paracellular pathway as well as in case of actively transported compounds like cephalexin (see Figure 3). A combination of our method with CACO-2 cell measurements would seem to be favorable in cases where no information on the preferred transportation route is available and the active transport systems in Caco-2 cells are sufficiently expressed.

**Conclusion.** The parallel artificial membrane permeation assay (PAMPA) permits the fast determination of artificial membrane permeation properties of drugs, related to transcellular in vivo absorption processes. PAMPA shows trends in the ability of a compound to permeate membranes by passive diffusion. In addition, PAMPA can deliver information on the lipophilicity, the ionization state, and the solubility of a compound without time-consuming single substance measurements. The greatest potential of PAMPA lies in the screening of large compound libraries. Therefore PAMPA with its various possibilities can support a more parallel multidimensional optimization in lead finding, considering biological activity and parameters more closely related to bioavailibility at the same time.

**Supporting Information Available:** Figures showing the PAMPA sandwich construction, flux studies at different pH values, and the time dependency of flux through PAMPA membranes (4 pages). Ordering information is given on any current masthead page.

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