Multilamellar Liposomes and Solid-Supported Lipid Membranes (TRANSIL): Screening of Lipid-Water Partitioning toward a High-Throughput Scale

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Purpose. Lipid-water partitioning of 187 pharmaceuticals has been assessed with solid-supported lipid membranes (TRANSIL) in microwell plates and with multilamellar liposomes for a data comparison. The high-throughput potential of the new approach was evaluated.

Methods. Drugs were incubated at pH 7.4 with egg yolk lecithin membranes either on a solid support (TRANSIL beads) or in the form of multilamellar liposomes. Phase separation of lipid and water phase was achieved by ultracentrifugation in case of liposomes or by a short filtration step in case of solid-supported lipid membranes.

Results. Lipid-water partitioning data of both approaches correlate well without systematic deviations in the investigated lipophilicity range. The solid-supported lipid membrane approach provides high-precision data in an automated microwell-plate setup. The lipid composition of the solid-supported lipid membranes was varied to study the influence of membrane change on lipid-water partitioning. In addition, pH-dependent measurements have been performed with minimal experimental effort.

Conclusions. Solid-supported lipid membranes represent a valuable tool to determine physiologically relevant lipid-water partitioning data of pharmaceuticals in an automated setup and is well suited for high-throughput data generation in lead optimization programs.

KEY WORDS: lipid-water partitioning, membrane affinity, automation, liposomes, solid-supported lipid membranes, membrane composition, pH dependence.

INTRODUCTION

Physicochemical parameters, such as solubility or lipid membrane affinity (MA), affect drug-receptor interactions (1) as well as the pharmacokinetic properties of a given drug (2,3). Therefore, early screening of pharmaceutically important physicochemical properties can significantly cut time and costs in the process of lead optimization. Computational approaches that can be performed without prior synthesis of the compound of interest try to model an array of parameters (octanol-water partitioning, polarity, ionization state, polar surface area, and hydrogen-bonding capacity) to yield a picture of passive absorption and distribution processes (4–7). Most of these fast computational methods are preliminarily useful for the assessment of large compound bases in early drug screening.

For the purpose of drug optimization where a differentiation between close analogues within a compound class is essential, experimental screening tools for pharmacokinetically relevant physicochemical parameters are needed. The large number of substances can only be handled in an automated setup.

The partition coefficient K_{ow} between an octanol and a water phase is widely used to assess lipophilicity (8,9). However, the model character of $\log K_{ow}$ (logP) for the interaction of a drug with a cell membrane is limited, and $\log K_{ow}$ often exhibits poor correlations with the logarithm of drug activities even in homologous series of drugs (10). It is well recognized that simple organic solvent-water systems are good models for solute-membrane partitioning only when no polar group interactions between the solute and the phospholipid bilayer occur (11). In addition, the pH dependent partitioning of charged molecules into membranes can differ markedly from that into octanol (12), because the lipid-water partitioning of ionized compounds is severely underestimated by the octanol-water system. Because most drugs are ionizable (>67%) (13), the lipid-water partitioning is clearly the parameter of higher pharmacologic relevance.

The "gold standard" to measure lipid-water partitioning at drug concentrations far below saturation is equilibrium dialysis of liposomes with radioactive tracers (14–16). However, the high experimental effort and long measuring times make it unsuitable for high-throughput screening. Therefore, a variety of faster methods have been developed.

The pH metric method relies on two-phase potentiometric titrations (17,18): The pK_a shifts in the presence of a liposomal phase in different concentrations can be used to calculate membrane-water partitioning of pharmaceuticals (18). A drawback of this approach is the substantial amount of drug needed to monitor the titration; therefore, saturation effects cannot fully be excluded. Another limitation of the method is that for compounds with low membrane-water partitioning, high amounts of lipid have to be used.

In the so-called immobilized artificial membrane (IAM) approach (19,20), lipids are covalently linked to a silica support (a chromatography column stationary phase) to mimic a membrane phase. Lipophilicities may be quantified via retention factors in high-pressure liquid chromatography (HPLC) setups (19–21). IAM partition coefficients of pharmaceuticals can be correlated to a variety of biologic parameters, *e.g.,* membrane permeability (22) or intestinal absorption (23). However, the covalently bound monolayer is not a model for a freely mobile, self-assembling membrane. It behaves more like a conventional stationary chromatography phase. Besides, despite endcapping of free silanols on the IAM surface, unspecific electrostatic interactions of the solid support with charged molecules are likely to falsify the results (24,25).

An alternative tool to quantify lipid-water partitioning are solid-supported lipid membranes (SSLM), which are commercially available under the trademark TRANSIL (26,27). In these systems, porous silica beads are covered by a unilamellar liposomal membrane that is noncovalently bound to the bead. Differential scanning calorimetry proves the fluid

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character of such supported lipid membranes (26). The SSLM permit a high-throughput determination of membrane affinity, as has been shown on a set of model compounds (28). To further evaluate the application range of SSLM, the new approach has now been applied to a larger series of pharmaceuticals of different substance classes. In this article, membrane affinity data of 187 drug candidates determined by SSLM are correlated with results from a liposomal method in which phase separation is achieved by ultracentrifugation (29). In addition, the influence of different lipid membrane compositions on lipid-water partitioning has been investigated: By varying the content of ionic lipid molecules (POPS (1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-L-serine])), the interaction with, *e.g.,* intestinal, nerve, or brain cell membranes may be modeled (30). The influence of pH on the membrane affinity of ionizable compounds has been assessed on a series of 10 acidic and 2 neutral compounds.

MATERIALS AND METHODS

Materials

The 787 drug candidates were supplied in coded form by Bayer AG, Pharma Research Center, Wuppertal, Germany. Further structural information about the compounds under investigation can be found at http://www.nimbus-biotech.com/lit/pharmres2001/structures.html.

All chemicals and solvents were purchased from Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany, except phosphate-buffered saline (PBS; Flow laboratories, Meckenheim, Germany). Lecithin (egg yolk phosphatidyl choline) was purchased from Lipoid KG (Ludwigshafen, Germany). SSLM silica beads (diameter 10 μm) coated with egg yolk phosphatidyl choline are available from NIMBUS Biotechnology GmbH (Leipzig, Germany) and distributed under the trade name TRANSIL. The exact lipid volume of the SSLM batches are given by the supplier via dry weight, lipid content, and lipid concentration. Lipid content ranges typically between 8 and 12 mg lipid/mL SSLM suspension (HPLC analysis). Multi-Screen-BV filter plates covered with a 1.2-μm Durapore Membrane (Millipore, Eschborn, Germany) were used for SSLM separation. Filter membranes consist of hydrophilic polyvinylidenfluoride (PVDF) with low protein-binding properties.

Estimation of Partitioning Behavior

Partitioning between octanol and water phase at pH 7.4 ($logD_{7.4}$) has been calculated with the ACD software logD Suite, version 3.5. An estimate of the membrane affinity was calculated by a QSAR approach (31), which uses the measured membrane affinity of about 4000 different compounds and the HQSAR method available in SIBYL (TRIPOS) for the prediction of the membrane affinity.

Determination of Membrane Affinity with Liposomes

Each drug candidate was dissolved in PBS (pH 7.4) to give a concentration of 3 mg/l. If necessary, small amounts of organic solvents (dimethylsulfoxide, methanol, acetonitrile <0.5%) were used as a modifier to facilitate solubilization. To reduce the number of measurements for one compound, the membrane affinity of the compound under investigation is estimated via the QSAR approach mentioned above. Only in cases in which this estimate is more than one order of magnitude away from the measured value, measurements with different amounts of lipid have to be carried out. The desired amount of lecithin was added in dry form to the dissolved drug and equilibrated over night under mild agitation. To avoid saturation phenomena, the lecithin liposomes have to contain <1% (w/w) of the compound under equilibrium conditions. After centrifugation (48500 *g* for 1 h) the supernatant is taken for HPLC analysis. As reference sample, the compound under investigation but without added lipid was treated the same way. The difference between both HPLC areas was used for the evaluation of the membrane affinity. Similar methods have been described for mixtures of lipids (32).

Determination of Membrane Affinity with SSLM (TRANSIL Batch Assay)

The batch assay relying on SSLM was performed according to (28).

The bottom of 300-μL wells of a 96-microwell filter plate (Multi-Screen-BV) was wetted with a defined amount of 20 mM of phosphate buffer at pH 7.4. The drug of interest solubilized in phosphate buffer with 5% dimethyl sulfoxide (DMSO) was pipetted into the filter plate. Partitioning experiments were started in the microwell plates by addition of phosphate buffer (pH 7.4) and SSLM to a total volume of 300 μL. Because of the dilution, the actual modifier concentration in the SSLM-batch assay was <1%. This modifier concentration has been shown to cause neither a lipid leaching of the solid-supported lipid membrane system nor a systematic influence on membrane affinity values (data not shown).

Substrate concentration and the appropriate amount of SSLM depend on the expected MA of the compound and the detection window (in this case UV detection). Best results and error minimization are achieved at an average drug binding of 50% (see Calculation Example). During equilibrium conditions the lipid phase should contain <1% (mol/mol) of solute. Typically, 10–70 μM drug solutions were analyzed with 1–9 mM concentrations of SSLM.

The pipette routine during the SSLM batch assay permits a characterization of the membrane affinity of 48 different compounds in a 96-well plate because a reference value must be determined for each compound. After the last well is filled with the SSLM suspension, the plate is incubated for 2 min at room temperature to reach partitioning equilibrium. Longer equilibration times did not influence partitioning data (results not shown). The concentration of drug candidates were determined by UV detection HPLC as described previously (28). Therefore, the SSLM need to be separated from the solution. This was achieved by filtration aided by a short centrifugation step at 500 *g* for 10 min. For each compound, the amount of substance in the supernatant n_{water} and the reference value determined in the well without SSLM n_{total} were determined to calculate n_{lipid} ($n_{total} - n_{water}$).

To asses the effect of membrane charge on the partition coefficient, TRANSIL-egg-PC/POPS (silica beads coated with egg yolk phosphatidyl choline and phosphatidyl serine) with varying POPS content (4, 14, and 18%) were used.

The dependence of membrane partitioning behavior on

pH was investigated for 10 acidic and 2 neutral drugs (in coded form) that were analyzed for their membrane affinity at pH 7.4 and pH 5, by using adjusted PBS buffers. In addition, a complete membrane binding vs. pH profile (pH range 2–10) has been recorded for the acidic model compound warfarin (50–70 μM) in a SMUBS buffer system as described by Pauletti and Wunderli-Allenspach (33). The pH was measured at room temperature (RT) by using a Mettler Toledo InLab combination electrode.

Quantification of Membrane Affinity (TRANSIL Batch Assay)

The partition coefficient or membrane affinity is defined as

$$MA = \frac{c_{\text{lipid}}}{c_{\text{water}}} \tag{1}$$

where c_{lipid} is the concentration of the drug in the lipid phase, and c_{water} is the concentration in the aqueous phase. Every concentration can be expressed as a quotient of amount and volume ($c = n/V$). V_{water} and V_{lipid} are defined as water and lipid volume, respectively. n_{lipid} and n_{water} refer to the amount of drug solubilized in lipid and water phase. The amount of substance in the lipid phase can be calculated by $n_{\text{lipid}} = n_{\text{total}} - n_{\text{water}}$ (where n_{water} is the supernatant value and n_{total} is the reference value). Thus Eq. (1) can be rearranged to

$$MA = \frac{V_{\text{water}}}{V_{\text{lipid}}} \cdot \frac{n_{\text{lipid}}}{n_{\text{water}}} = \frac{V_{\text{water}}}{V_{\text{lipid}}} \cdot \frac{(n_{\text{total}} - n_{\text{water}})}{n_{\text{water}}} \tag{2}$$

n_{total} and n_{water} were detected via HPLC. V_{lipid} is known from the certificate of analysis. V_{water} can be deduced from volume V_{total} and the volume of the suspended beads ($V_{\text{support}} + V_{\text{lipid}}$):

$$V_{\text{water}} = V_{\text{total}} - (V_{\text{support}} + V_{\text{lipid}}) \tag{3}$$

In most cases, the volume of the beads is negligible (see Discussion). V_{water} can then be replaced by V_{total} to simplify the calculation

$$MA = \frac{V_{\text{water}}}{V_{\text{lipid}}} \cdot \frac{n_{\text{lipid}}}{n_{\text{water}}} = \frac{V_{\text{total}}}{V_{\text{lipid}}} \cdot \frac{(n_{\text{total}} - n_{\text{water}})}{n_{\text{water}}} \tag{4}$$

Calculation Example

For error minimization, the membrane affinity should be measured with binding values of 20–80%. Optimal assay conditions are met at 50% binding. If a preliminary rating of membrane affinity exists, this can be used for optimization of the corresponding pipette routine in a SSLM batch assay. An expected membrane affinity (*e.g.,* MA = 316 or logMA of 2.5) can be used to precalculate the amount of lipid at which 50% binding should occur [Eq. (2)]. If $n_{\text{water}} = n_{\text{lipid}} = \frac{1}{2} n_{\text{total}}$, then Eq. (2) simplifies to

$$V_{\text{lipid}} = \frac{V_{\text{water}}}{MA} \cdot \frac{(n_{\text{total}} - n_{\text{water}})}{n_{\text{water}}} = \frac{V_{\text{water}}}{MA} \tag{5}$$

From this, the corresponding volume of solid-supported lipid membranes (V_{SSLM}) is obtained by Eq. (5):

$$V_{\text{lipid}} = V_{\text{SSLM}} \cdot c_{\text{lip,Sus}} \tag{6}$$

The actual lipid content $c_{\text{lip,Sus}}$ [$\mu L/mL_{\text{Sus}}$] of each SSLM preparation is given by the supplier in the certificate of analysis (*e.g.,* 10.6 $\mu L/mL_{\text{suspension}}$). Combination of Eqs. (2) and (5) allows a direct calculation of a starting value of V_{SSLM} from an expected membrane affinity (6):

$$V_{\text{SSLM}} = \frac{V_{\text{water}}}{c_{\text{lip,Sus}} \cdot MA} \tag{7}$$

In a corresponding experiment (V_{SSLM} = 89,50 μL) the well would be filled with 50 μL of the drug solution, 90 μL of SSLM, and 160 μL of buffer to complete the total volume of the well.

Where no preliminary estimates of membrane affinity data exist, the SSLM batch assay was routinely performed with 100 μL of SSLM. If the value of the binding ratio was not within 20–80%, the experiment was repeated with adjusted assay parameters.

RESULTS

A large number of drug candidates were analyzed for their membrane affinity at physiologic pH (pH = 7.4). Because phosphatidyl choline (PC) is a major representative of biologic membranes, egg PC was chosen as lipid model.

Membrane water partitioning of a given drug is determined by electrostatic, polar, and steric interactions. In case of small nonionic molecules, octanol should be a sufficient surrogate for biologic membranes mimicking their polarity (18,34). Therefore, we tried to correlate membrane affinity data with K_{ow} values (logD 7.4: calculated with ACD software version 3.5). In Fig. 1, the calculated octanol water partitioning of 91 neutral compounds is plotted as a function of their log MA as determined with liposomes. As expected for neutral compounds (23), partitioning into biomembranes is higher than into octanol. There is no evident correlation between the two data sets; the values are broadly scattered around the logD = logMA-1 line (R^2 = 0.54). However, a linear relationship between octanol partitioning and membrane affinity as proposed by Avdeef *et al.* (18) for neutral drugs can neither be confirmed nor rejected.

The situation is even more complex with acids and bases (Fig. 2). The data scatter more than for the neutral compounds (R^2 = 0.24). As has often been observed, octanol significantly underestimates membrane affinity of charged molecules (14,18,23). Electrostatic interactions of charged

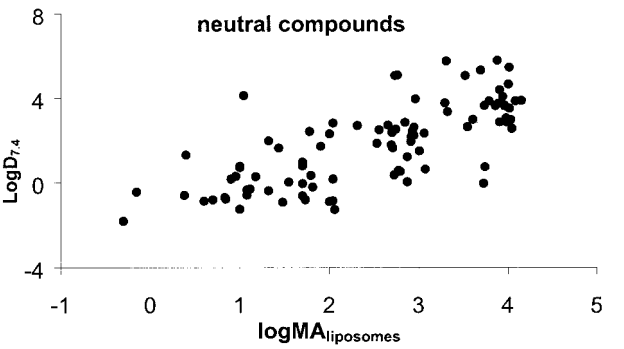

Fig. 1. Octanol-water partitioning (calculated from ACD logD Suite v 3.5) vs. liposome-water partitioning at pH 7.4 for a series of 91 neutral drugs.

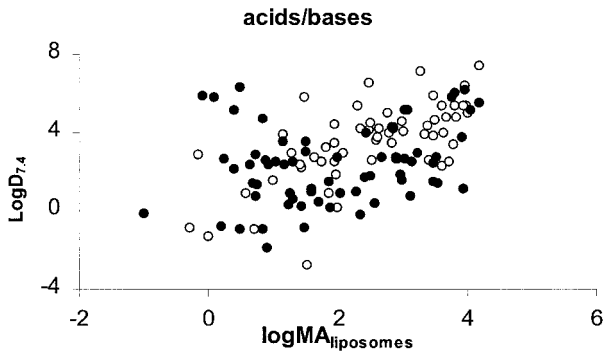

Fig. 2. Octanol-water partitioning (calculated from ACD logD Suite v 3.5) vs. liposome-water partitioning at pH 7.4 for a series of 96 drugs with either acid (○) or basic (●).

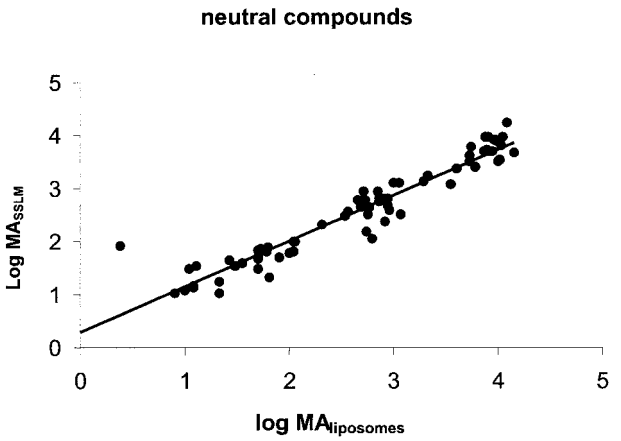

Fig. 3. Lipid-water partitioning at pH 7.4: SSLM vs. liposomal approach for a series of 91 neutral drugs ($\log MA_{SSLM} = 0.86 \log MA_{liposomes} + 0.29$; $R^2 = 0.92$); the solid line is the least square fit to the data.

molecules with the zwitter ionic PC head group are likely to occur (18,24). A lot of drugs can be polarized, and some drugs are able to penetrate into the membrane with hydrophobic structure elements while exposing their charge to the water/lipid interface. All these interactions cannot be mimicked by a simple octanol phase. Therefore, in Fig. 2 for acids and bases no correlation between the octanol-water and membrane-water partitioning behavior can be seen.

In consequence, physicochemical data of higher physiological relevance are usually obtained by analyzing the membrane affinity of pharmaceuticals. To meet the industrial need for high throughput, we compared two membrane assays for their reliability and efficiency to quantify membrane-water partitioning.

In the first approach, the lipid was presented to the drug in the form of multilamellar liposomes. To avoid saturation phenomena, the lipid-drug concentration ratio was controlled: at equilibrium conditions the lipid membrane should contain <1% (w/w) of the drug compound. Lipid-water phase separation was achieved by ultracentrifugation.

The second approach uses SSLM with an egg PC surface for the partitioning studies. Equilibrium is reached within the mixing time. Because of the density shift caused by the support, the solid-supported lipid membrane system allows for a very easy phase separation of lipid and water phase either by a short centrifugation or a filtration step.

Figure 3 shows a comparison of membrane affinity data for nonionic drug molecules obtained with these two different approaches. The correlation is $R^2 = 0.92$. Both experimental systems deliver congruent membrane affinity data; however, because of the complex structure of the multilamellar liposomes, the liposomal approach needs time-consuming incubation times (12 h) to reach equilbrium, whereas in the SSLM system partitioning is completed on a very short timescale (<2 min). In addition, the SSLM filtration step is faster than the ultracentrifugation phase separation of the liposomal approach. Therefore, the quantification of membrane partitioning is greatly accelerated when solid supported lipid bilayers are used.

The supported lipid membranes are composed of porous silica particles that have been completely coated with a fluid lipid bilayer. Because the pK_a of silica is around 6.8 (35), the support exposes negative charges at pH 7.4. One might ask whether this silica surface charge is completely shielded. Residual silica charge at the surface would falsify membrane affinity data of charged molecules. To test this, we compared the partitioning data of the two approaches for 96 drug molecules with either basic or acidic character. The results are shown in Fig. 4. For both substance classes, the data were fitted independently. Within the experimental error, the membrane affinity data of the two experimental setups correlate in the same way for acids and bases. There is no attractive force between the support and drugs of basic character and no repulsive effect to deprotonated acids. Scattering in the correlation plot is presumably due to the standardized HPLC quantification methods for all compounds under investigation, which was different in the two approaches.

Many natural membranes are negatively charged, including pharmacologically important systems such as cell membranes of the brain or lung (30). Therefore, we investigated the influence of the membrane charge on the membrane affinity of neutral and charged molecules. The lipid composition on the surface of solid-supported lipid bilayers can easily be varied and fine-tuned to specific conditions. Phosphatidyl serine was chosen as a physiologically relevant negatively charged phospholipid and presented in three concentrations

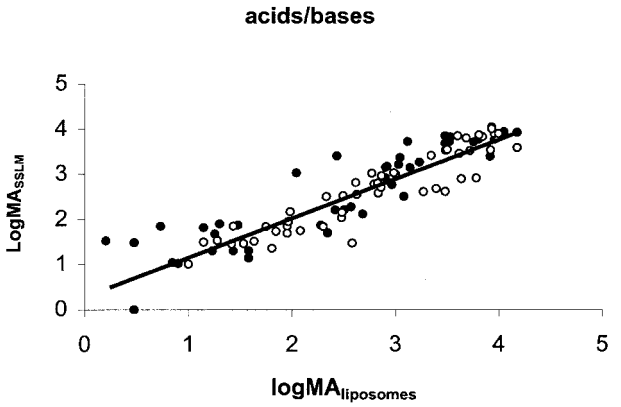

Fig. 4. Lipid-water partitioning at pH 7.4: SSLM vs. liposomal approach for a series of 96 drugs with either acid (○) or basic (●) character ($\log MA_{SSLM} = 0.85 \log MA_{liposomes} + 0.38$; $R^2 = 0.83$); the solid line is the least square fit to the data.

(4, 14, and 18%) in a two-component membrane otherwise consisting of (neutral) PC. The results are illustrated for a neutral, an acidic, and a basic compound in Fig. 5. The neutral compound Nifedipine binds with a constant membrane affinity of about 3.5 to all presented bilayers. In the acidic compound BAY X-1005, an increased phosphatidyl serine content decreases membrane affinity due to electrostatic repulsion effects. At the same time, the lipophilicity values of the positively charged compound BAY 11-7849 were shifted by attractive interactions. It is obvious that alterations of membrane composition influence membrane affinities especially of ionizable drugs. SSLM provide an elegant approach to quantify this phenomenon.

Membrane affinity of ionizable drugs is furthermore a function of pH: the relation between pK_a and pH determines the fraction of neutral and charged drug molecules, each of which has a distinct lipid-water distribution ratio ($MA_{neutral}$: $MA_{charged}$). SSLM show a pH stability that is only limited by the stability of the support material and the lipids themselves. Therefore, the described SSLM approach can be used to monitor the pH dependence of membrane binding. Extreme pH values become accessible because of the short incubation time during the TRANSIL batch assay.

Figure 6 shows the results for the acidic drug warfarin [pK_a = 4.90 (18)]. Measurements have been performed in various pH intervals by using a SMUBS buffer system (33) in the pH range of 2–10. log $MA_{neutral}$ and $logMA_{charged}$ as well as the pH range of transition were consistent within the experimental error with the corresponding values of an equilibrium dialysis approach (15), indicating, *e.g.,* no lipid leaching effects at low pH.

In addition, membrane affinity of a series of 10 different acidic and 2 neutral drugs (each compound in encoded form) has been analyzed at pH 5.0 and 7.4 with the SSLM system. Data are shown in Table I. As expected, membrane affinity of neutral compounds does not depend on pH. However, in acids, the fraction of ionized species increases with pH, resulting in a reduced membrane affinity.

DISCUSSION

The enlarged number of potential drugs reaching research and development in pharmaceutical industry because of the technologic improvements in the last decade (genomics, automated synthesis, and high-throughput screening) makes it all the more important to screen physicochemical parameters also on a high-throughput scale. Reliable and effective membrane bilayer models are needed to obtain membrane affinity data of physiologic relevance within a short expense of time. The liposomal approach as well as the assay relying on solid-supported lipid bilayers provide suitable setups to quantify membrane affinity. However, liposomes have to be freshly prepared before each experiment. Besides aggregation and fusion problems, phase separation is tedious: 1 h of ultracentrifugation is applied to separate the multilamellar liposomes from the water phase.

SSLM exhibit a homogeneous unilamellar bilayer surface to which drugs can distribute (26,27). The SSLM beads are stable on a timescale of months; they are resistant to shear stress and therefore, can be pipetted without any problems (27,28). Separation of lipid and water phase after the partitioning experiment is quite easy to achieve, because the silica support shifts the density of the SSLM.

However, any influence of the surface of a support must be excluded. At physiologic pH (7.4), the silica support sur-

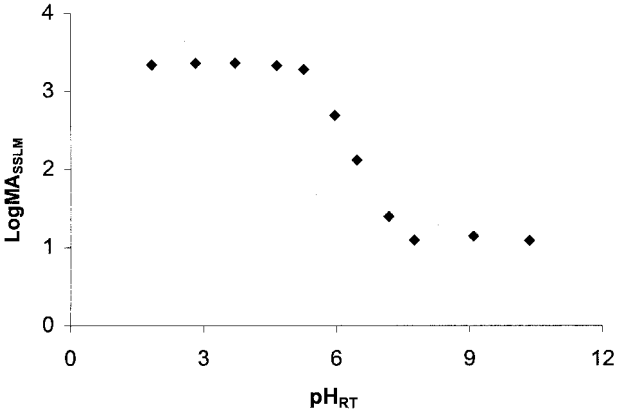

Fig. 6. pH-dependent membrane binding of warfarin in a phosphatidyl choline SSLM system. The partition profile was determined as described in the experimental part: SSLM beads (4.5 mM) were incubated with 50–70 μM warfarin at different buffer conditions; concentration of warfarin (total and unbound) was measured by UV-HPLC; logMA was calculated according to Eq. (4).

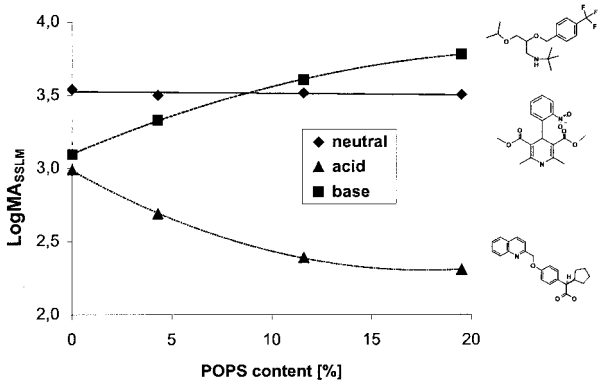

Fig. 5. Influence of membrane charge on membrane affinity: log-MA_{SSLM} of a neutral, an acid, and a basic drug vs. phosphatidyl serine content of a phosphatidyl choline membrane.

Table I. pH-Dependence of Membrane Binding: Membrane Affinities of 2 Neutral and 10 Acidic Drugs at pH 7.4 and pH 5.0 (SSLM Approach)

Code No.	pK_a	$logMA_{SSLM}$ (pH 7.4)	$logMA_{SSLM}$ (pH 5.0)
Neutral 1	—	3.7	3.7
Neutral 2	—	3.0	3.0
Acid 1	4.4	2.6	3.2
Acid 2	4.0	3.8	4.3
Acid 3	3.7	3.8	4.3
Acid 4	3.3	3.0	3.2
Acid 5	4.0	3.6	4.0
Acid 6	4.0	2.8	3.1
Acid 7	4.0	1.7	2.2
Acid 8	4.0	1.8	2.1
Acid 9	4.9	2.2	3.0
Acid 10	3.6	2.0	2.5

face is partially ionized because of its intrinsic pK_a (6.8). This surface charge of the support has to be completely shielded by the lipid bilayer coating to avoid unspecific attraction of positively charged drugs or repulsion of anions. The fact that membrane affinity data from the liposomal approach correlate well with those from the SSLM batch for both acids and bases proves that the unspecific electrostatic surface effects are indeed shielded. The fact that pH profiles may be recorded also shows the stability of the SSLM beads.

One simplification made in the above calculation is that the volume of the beads is negligible compared with that of the aqueous phase. This is acceptable for lipophilic compounds. Hydrophilic drugs, however, need substantial amounts of SSLMs to achieve a detectable binding effect: these compounds were incubated with 200 μL of SSLM (*i.e.,* $V_{support}$ = 28.6 μL; V_{lipid} = 2.1 μL) in a complete volume of 300 μL. In consequence, the support should here be regarded as a third phase besides lipid and water. This phase simulates a higher concentration of unbound drug than would be found in a real lipid-water two-phase system. Therefore, we introduced a correction factor f defined as the ratio between the water volume V_{water} and V_{total}:

$$f = \frac{V_{water}}{V_{total}} \tag{8}$$

When f is introduced in Eq. 2, we are able to express the dependence of logMA from volume effects caused by the support in the SSLM system:

$$\begin{aligned} \log MA &= \log\left(\frac{V_{total}\cdot f}{V_{lipid}}\cdot\frac{((c_{total}\cdot V_{total})-(c_{water}\cdot V_{total}\cdot f))}{c_{water}\cdot V_{total}\cdot f}\right) \\ &= \log\left(\frac{V_{total}}{V_{lipid}}\cdot\frac{(c_{total}-(c_{water}\cdot f))}{c_{water}}\right) \end{aligned} \tag{9}$$

Figure 7 shows a schematic illustration of this dependence: for lipophilic compounds small amounts of SSLM are sufficient to obtain 50% lipid binding; therefore, the influence of f can be neglected. However, the more hydrophilic a compound, the larger is the necessary amount of SSLM to detect membrane binding. For drugs with logMA < 2.3, the correction factor f leads to an increase of the real value for membrane affinity when 50% binding should be achieved. When measurements are performed at 20% binding conditions, introduction of f markedly influences membrane affinity values <1.8. In any case, the consideration of volume effects [*i.e.,* the calculation of membrane affinities according to Eq. (9)] enlarges the "reliable" logMA range for the SSLM approach.

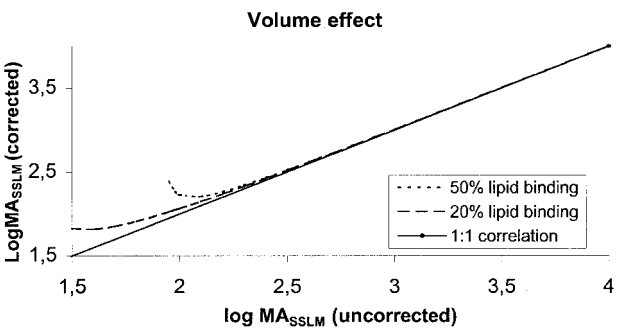

Fig. 7. Schematic illustration of the volume effect during the SSLM approach: $logMA_{SSLM}$ (corrected) vs. $logMA_{SSLM}$ (uncorrected).

By applying Eq. 9 to hydrophilic compounds, an underestimation of membrane affinity can be avoided.

A high lipid-drug ratio is necessary to avoid saturation phenomena at the membrane. Presenting the lipid phase on the surface of a solid support limits the amount of lipid that can be incorporated in a definite volume. Therefore, a remaining drawback of the SSLM approach is the lipophilicity window that can be covered. The commercially available TRANSIL contains 8–12 mg lipid/mL SSLM and allows screening membrane affinities >1.5 with the described batch assay, unless the condition 50% binding is given up (leading to a larger experimental error). For compounds that are more hydrophilic, several SSLM preparations with increased lipid content (up to 40 mg lipid/mL SSLM) are being tested at the moment.

CONCLUSION

One hundred eighty seven pharmaceuticals (of neutral and charged character) have been assessed for membrane affinity with two different approaches, both of them focusing on pharmaceutical industries' need for high-throughput screening. SSLM are a valuable tool for the determination of membrane affinity able to reach this HTS goal. The lipid phase on the beads is stable and can be pipetted without any problems into microwell plates. ph-dependent recording of membrane affinities can be performed without systematic difficulties. Lipophilicity values for neutrals, acids, and bases are congruent with data from the conventional liposomal assay, this excluding any unspecific surface effects of the support. The lipid composition of SSLM beads is highly variable and can be fine-tuned, *e.g.,* to investigate the influence of membrane charge on membrane affinity. The SSLM assay can be performed with high accuracy; errors by the support volume can be excluded by introducing a volume correction factor in the calculation of logMA. The development of new SSLM materials with higher lipid content should help to widen the accessible lipophilicity range.

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