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Studying the interactions of drugs and hydrophobic model membranes using contact angle goniometry

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ABSTRACT

This study demonstrates a method where contact angle goniometry combined with surface tension measurements is used to assess the interactions of drugs with the hydrophobic core of a biological membrane. To this end, self-assembled monolayers (SAMs) of two alkanethiol and one thiolipid on Au(1 1 1) surfaces are used as model membranes and their interaction with six β-blockers is studied. The Gibbs equation and the Langmuir adsorption isotherm are used to determine the partition coefficients for the adsorption of the drugs, which are compared to the octanol–water partition coefficients as well as the liposome–water partition coefficients. The ability of the different SAMs to serve as model membranes in partitioning of drugs is discussed.

1. Introduction

The interactions of drugs with biological membranes affect the delivery of drugs to the target sites within the body. Usually, a drug has to cross several membranes in order to enter the target location. Because of this, optimisation of the delivery of drugs requires understanding of the interactions of drugs with biological membranes. Understanding of these interactions is also of prime importance when predicting adsorption, distribution, metabolism, and excretion (ADME) properties of drugs already in the early phases of drug discovery process.

The most common physicochemical property used in the prediction of drug–membrane interactions is the lipophilicity of a drug, which is usually expressed as log *P*, the logarithm of the partition coefficient between two immiscible solvents. Traditionally, the partition coefficient has been determined using *n*-octanol and water. However, the ability of the octanol–water partition coefficient to describe drug partitioning has been questioned due to the major differences in the biophysical properties of octanol and phospholipid cell membrane. Because of this, alternative approaches, including both experimental and computational methods, have been developed.

Many of the popular experimental approaches used in drug partitioning utilise various model membranes, such as cell culture monolayers, artificial membranes, or liposomes. The most frequently used cell cultures for passive drug transport studies are Caco-2 cultures, which are derived from human colon carcinoma cells [1]. In Caco-2 cultures, the monolayers of the polarised cells, which mimic the function of the small intestinal villus epithelium, are grown on permeable filter supports and the transport of drugs trough the monolayer is measured. In the parallel artificial permeation assay (PAMPA), on the other hand, the two compartments are separated by a hydrophobic filter impregnated with an organic solution of lipid, which forms bilayer structures in the filter pores [2]. Even though both of these techniques are extensively used, both Caco-2 system and PAMPA seem to suffer from interlaboratory variability [3,4].

Because of their excellent biomimetic properties, liposomes have become a popular alternative in membrane partitioning studies. Liposome–water partition coefficients have been measured using various methods, including the distribution technique [5], equilibrium dialysis [6] potentiometric titration [7,8] and NMR-spectroscopy [9]. Despite the better biomimetic properties of liposomes, most of the approaches based on liposomes as model membranes are not very efficient to be used in large scale as they are very tedious and time-consuming. To overcome the problem in efficiency, automated methods for the rapid screening of drug compounds have been developed, where the biomimetic properties of liposomes have been combined with chromatographic techniques [10,11]. In addition to these, liposome–water partition coefficients can be measured using electrochemical methods. We have earlier presented a method, where the transfer of drugs encapsulated in liposomes was studied using square wave voltammetry at a water-

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1,2-dichloroethane interface created at the tip of a micropipette [12].

As an alternative to the lipid–water partition coefficient, Seelig and co-workers have proposed an approach based on the measurement of the Gibbs equation of a drug [13,14]. In this method, an air–water partition coefficient was determined by measuring the surface pressure of a drug monolayer at the air–water interface. Also, using three parameters derived from the adsorption isotherm, the minimum concentration at which surface activity is induced, the surface area of a molecule, and the critical micelle concentration (CMC), the ability of a drug to reach the central nervous system was predicted. Surface activity of drugs has been utilised also in the work of Suomalainen et al. [15], where a rapid method for the approximation of membrane partitioning based on the impact of a drug on the air–water interfacial tension was presented. In this method, surface tension measurements were carried out using a multichannel microtensiometer and the Gibbs equation was used to predict the passive uptake of drugs into the brain. In both of these approaches, partitioning of drugs at the air–water interface is assumed to be similar to the partitioning at the lipid–water interface, since partitioning into both interfaces is driven by the hydrophobic effect [15], and the dielectric constant for air is close to that for the hydrocarbon region of the lipid membrane [13]. As a result, the orientation of the nonpolar and polar moieties of a drug molecule at the air–water interface is comparable to that at the lipid–water interface.

This study demonstrates a novel method where partitioning of drugs is studied at the hydrocarbon–water interface. As in the studies of Fisher et al. [13] and Suomalainen et al. [15], the Gibbs equation is used to determine the partition coefficient for the adsorption of the drugs. However, instead of measuring the surface pressure at air–water interface and applying the Szyszkowski equation in order to determine the partition coefficient, contact angle data measured at the hydrocarbon–water interface is combined with surface tension measurements and the Langmuir adsorption isotherms are constructed. The hydrocarbon region of the lipid membrane is modelled using three different self-assembled monolayers (SAMs) on Au(1 1 1) surfaces, two of which are formed of alkanethiols of different chain lengths and one of the thiolipid 1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol. The partition coefficients of six β-blockers are determined and compared to the octanol–water partition coefficients as well as liposome–water partition coefficients. The ability of the different SAMs to serve as model membranes in partitioning of drugs is discussed.

2. Materials and methods

2.1. Materials

Au(1 1 1) surfaces made of borosilicate glass covered with a 1–4 nm layer of chromium and a 200–300 nm layer of gold were purchased from Arrandee (Werther, Germany). Decanethiol and octadecanethiol were from Lancaster (Morecambe, England) and 1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol sodium salt (DPPTE) was from Avanti Polar Lipids (Alabaster, AL, USA). Alprenolol hydrochloride, labetalol hydrochloride, metoprolol tartrate, nadolol, propranolol hydrochloride and timolol maleate were from Sigma–Aldrich (Steinheim, Germany). The physicochemical properties of the drugs are shown in Table 1 [16] and the chemical structures of the drugs and DPPTE in Fig. 1. Aqueous solutions were prepared using MQ-water (18.2 MΩ cm at 25 °C). All other chemicals were of the highest available commercial purity and were used as received.

Table 1
Physicochemical properties of the drugs [16].

Drug	Molecular weight ($g\,mol^{-1}$)	Dissociation constant (pK_a)	$\log P_{oct}$ [a]	$\log P_{oct}$ [b]
Alprenolol	249.36	9.65	3.10	2.59
Labetalol	328.41	7.4; 8.7	3.09	2.18
Metoprolol	267.37	9.7	1.88	1.20
Nadolol	309.41	9.39	0.71	0.23
Propranolol	259.35	9.45	3.56	2.75
Timolol	316.43	9.21	1.91	1.63

[a] Determined experimentally.
[b] Calculated using CLOGP version 3.54.

2.2. Preparation of monolayers on gold

Before the preparation of monolayers, the gold substrates were washed and sonicated in ethanol. After this, the substrates were immersed overnight in 1 mM solutions of decanethiol, octadecanethiol and DPPTE in ethanol. After the self-assembly of the monolayers, the substrates were rinsed extensively with ethanol and dried under a stream of nitrogen.

2.3. Surface tension and contact angle measurements

Surface tensions and static contact angles were measured using a computer controlled and video based contact angle and surface tension meter (CAM 200, KSV Instruments, Finland). Surface tensions were measured using the pendant drop method whereas goniometric technique was used for contact angle measurements. Surface tensions and contact angles were measured using four different concentrations of drugs, 1, 2, 5 and 10 mM. The contact angle and surface tension of water were also measured for comparison. In all experiments, drop size was 5 μl and 45 images of each drop were taken at 1 s intervals. Images were analysed using the standard procedures provided by the instrument software. Surface tension measurements were done at least in triplicate and contact angle measurements in duplicate. The reported values are means of the measured values. A schematic illustration of the experimental set-up in contact angle measurements and a typical image of a drop of drug solution on a SAM are shown in Fig. 2.

3. Results and discussion

3.1. Surface tension and contact angle measurements

Value of $72.8 \pm 0.3\,mN\,m^{-1}$ was measured for the surface tension of water. The surface tensions measured for the drug solutions at different concentrations are shown in Table 2. The data show that, as expected, the drugs have a tendency to lower the surface tension. The higher the concentration of the drugs, the more the surface tension is lowered. The surface active properties of β-blockers are well known [17,18] and especially the aggregation of propranolol hydrochloride has been studied more in detail [19–23].

Contact angles of water on different self-assembled monolayers (SAMs) are shown in Table 3. Results suggest that the alkane thiol SAM with longer chain length is more hydrophobic than the one with shorter chain length, which is in good agreement with the literature [24]. However, the contact angle values for the octadecanethiol and decanethiol SAMs reported in this study deviate slightly from the contact angles reported earlier. This is most probably due to the fact that our values are static contact angles whereas the ones reported elsewhere are dynamic contact angles [24–26]. The static contact angle for octadecanethiol SAM is between the advancing and receding contact angle reported in the literature [24–26] and also for the decanethiol SAM the static contact angle is less than the advancing one [24]. In the light of the studies of Yang et al. [26] and Kwok et al. [29] our values seem to be quite low,

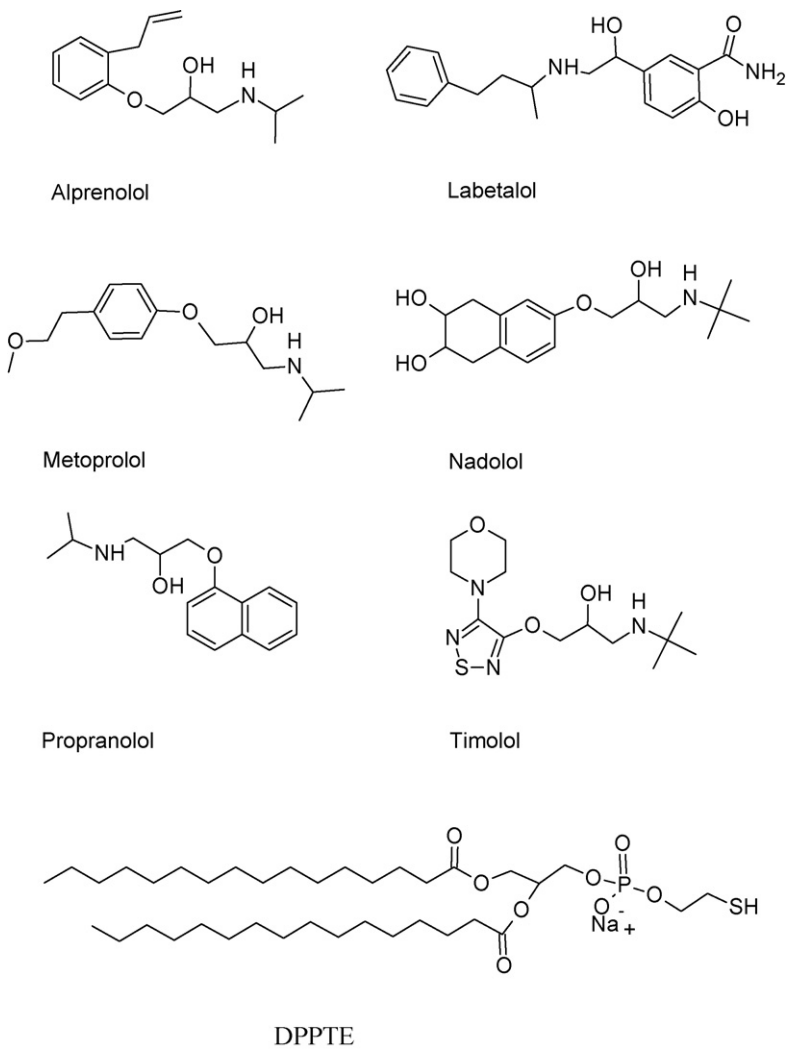

Fig. 1. Chemical structures of the drugs and DPPTE.

since the advancing contact angle measured using low velocities of the three-phase contact line should give values close to the static contact angle. However, Miyama et al. have reported that in general, the static contact angle is greater than the receding and less than the advancing contact angle [27], which is consistent with our results. Nevertheless, slight discrepancies between the measured values and the values reported in the literature could be anticipated as contact angle measurements can be affected by various factors, such as surface roughness and defects [26], ambient humidity [28], and the choice of method [29].

Table 2
The surface tensions measured for the drug solutions at different concentrations. The subscripts of γ are concentrations in mM.

Drug	γ_1 (mN m^{-1})	γ_2 (mN m^{-1})	γ_5 (mN m^{-1})	γ_{10} (mN m^{-1})
Alprenolol	72.1 ± 0.2	72.1 ± 0.2	71.0 ± 0.1	69.1 ± 0.1
Labetalol	72.6 ± 0.1	72.1 ± 0.1	71.3 ± 0.1	70.0 ± 0.1
Metoprolol	72.0 ± 0.2	70.6 ± 0.1	67.6 ± 0.2	65.4 ± 0.1
Nadolol	70.0 ± 0.1	67.3 ± 0.2	62.5 ± 0.1	58.8 ± 0.2
Propranolol	72.8 ± 0.2	72.4 ± 0.4	71.9 ± 0.1	70.6 ± 0.1
Timolol	72.3 ± 0.1	72.1 ± 0.1	71.6 ± 0.2	70.9 ± 0.1

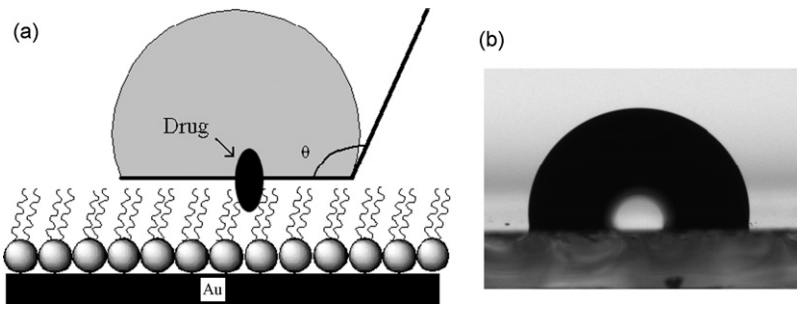

Fig. 2. A schematic illustration of the experimental set-up in contact angle measurements (a) and an image of a drop of 1 mM propranolol solution on DPPTE SAM (b).

Table 3
Water contact angles on different surfaces.

Surface	Water contact angle (°)
Decanethiol	103.4 ± 0.3
Octadecanethiol	106.7 ± 0.5
DPPTE	102.8 ± 0.3

Interestingly, the contact angle of water on DPPTE SAM is of the same magnitude as that on the decanethiol SAM, even though the length of the hydrocarbon chain in DPPTE (Fig. 1) is closer to that of octadecanethiol. The result suggests that the hydrocarbon chain packing in DPPTE SAMs is different from that in alkanethiol SAMs. Further support for the structural differences was obtained from the contact angles measured using the β-blocker solutions at four different concentrations. When the contact angle data combined with surface tension measurements was fitted to a model based on the Gibbs equation and the Langmuir adsorption isotherm, the differences in the partitioning of the drugs in the three different SAMs was revealed.

3.2. Gibbs equation

Partitioning of β-blockers into the interface can be described by the Gibbs equation, which relates the surface excess Γ to the chemical potential μ of the drug:

$$\Gamma = -\left(\frac{\partial \gamma_{SL}}{\partial \mu}\right) \quad (1)$$

where R is the gas constant, 8.314 J mol^{-1} K^{-1}, T is temperature and γ_{SL} is the solid–liquid interfacial tension. Since the solid–liquid interfacial tension cannot be obtained directly from the contact angle measurements, the Young equation can be used to rewrite the Gibbs equation in terms of the contact angle θ and the liquid–vapour interfacial tension γ_{LV}. The Young equation represents the mechanical equilibrium of a drop under the action of the three interfacial tensions:

$$\gamma_{SL} + \gamma_{LV} \cos\theta = \gamma_{SV} \quad (2)$$

where γ_{SV} is the solid–vapour interfacial tension. Substituting γ_{SL} into the Gibbs equation gives

$$\Gamma = \frac{\partial(\gamma_{LV} \cos\theta)}{\partial \mu} \quad (3)$$

if γ_{SV} is assumed constant. Thus, the surface excess can be determined from the graph of $\gamma_{LV} \cos\theta$ versus μ by taking the derivative at each data point. The chemical potential of the drug can be approximated by the infinite dilution limit as

$$d\mu = RTd \ln(c/c^*) \quad (4)$$

In Eq. (4) c is the concentration of the drug and c^* is 1.0 mol dm^{-3}. As an example of determining the surface excess using the experimental data, Fig. 3 shows the plot of $\gamma_{LV} \cos\theta$ versus $\mu - \mu^0$ for propranolol on octadecanethiol surface. The actual value of μ^0 is not significant because only the derivative is used in the calculations. Data for the other two surfaces and the five drugs produced similar plots.

3.3. Langmuir isotherm

Using the Gibbs equation, the surface excesses of the drugs at each concentration were determined. To evaluate the partition coefficients, the Langmuir adsorption isotherm was used. The Langmuir isotherm describes the equilibrium between the drug molecules in the aqueous solution and those partitioned at the surface: D + S ⇄ DS. The adsorption coefficient for the equilibrium is

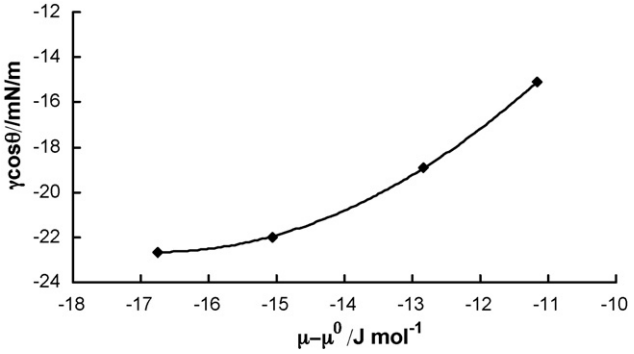

Fig. 3. $\gamma_{LV} \cos\theta$ as a function of μ for propranolol on octadecanethiol surface. Solid line is a quadratic equation fitted to the data.

K = [DS]/([D][S]), where [DS] is the concentration of the drug at the surface, [D] is the concentration of the drug in the aqueous phase, and [S] is the concentration of unoccupied sites at the surface. The adsorption coefficient for the partitioning of the drugs can be determined by fitting the Langmuir isotherm to the data, as the surface excess at each drug concentration is known:

$$\Gamma = \frac{\Gamma_{max} Kc}{1 + Kc} \quad (5)$$

In Eq. (5), Γ_{max} is the maximum surface excess and c is the concentration of the drug. Adsorption isotherms for propranolol and alprenolol are presented in Fig. 4. Fig. 4 shows the typical adsorption behaviour of all β-blockers on decanethiol and octadecanethiol SAMs. The maximum surface excesses for all drugs except for timolol were greater on decanethiol surface compared to octadecanethiol surface. On the DPPTE SAM, however, no such trend was

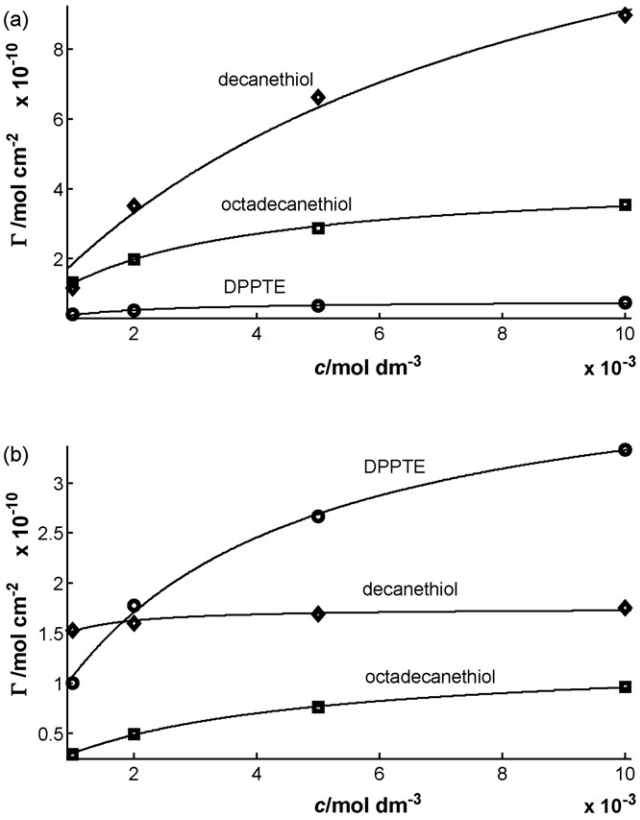

Fig. 4. Langmuir isotherms for propranolol (a) and alprenolol (b) on decanethiol (♦), octadecanethiol (■), and DPPTE (●) SAMs.

Table 4
Maximum surface excess of the β-blockers on the different SAMs.

Drug	$\Gamma_{max,\ octadecanethiol} \times 10^{10}$ (mol cm^{-2})	$\Gamma_{max,\ decanethiol} \times 10^{10}$ (mol cm^{-2})	$\Gamma_{max,\ DPPTE} \times 10^{10}$ (mol cm^{-2})
Alprenolol	1.3 ± 0.1	1.8 ± 0.1	4.4 ± 0.7
Labetalol	4.2 ± 0.5	5 ± 4	2.5 ± 0.4
Metoprolol	5 ± 3	4.9 ± 0.6	9 ± 6
Nadolol	11 ± 23	11 ± 1	15 ± 22
Propranolol	4.3 ± 0.5	16 ± 14	0.8 ± 0.1
Timolol	7 ± 9	6.4 ± 0.9	4 ± 2

observed among the studied β-blockers. For propranolol (Fig. 4a), labetalol and timolol, the surface excess on DPPTE SAM was much lower than the surface excesses on the alkanethiol SAMs whereas for alprenolol (Fig. 4b), metoprolol and nadolol, the maximum surface excess on DPPTE was higher compared to the ones on alkanethiol SAMs. The maximum surface excesses for all the β-blockers with 95% confidence limits are summarised in Table 4.

Higher surface excesses of the drugs on the decanethiol SAMs compared to octadecanethiol SAMs can be attributed to the structural difference of SAMs. Even though in both of the monolayers the molecules are packed hexagonally with a tilting angle of 30–35° from the surface normal, it has been shown that alkyl thiols with longer chain lengths form more ordered monolayers [30,31]. This has been explained by the presence of a relatively higher percentage of gauche conformations in shorter chains. Also, the tilt angle of shorter chains is slightly larger than that of longer chains, because of which the molecules of shorter chains are in a more highly strained chainpacking configuration [32]. The strained packing and the weaker interaction between the shorter hydrocarbon chains results in less ordered monolayers, into which the drugs can partition more readily.

Lower surface excesses of propranolol, labetalol and timolol on DPPTE SAMs than on the alkanethiol SAMs may be due to denser packing of the hydrocarbon chains of DPPTE compared to the chains in the alkanethiol SAMs. As there are two hydrocarbon chains in a DPPTE molecule, it is likely that the hydrocarbon chains are very densely packed when a monolayer is formed. Thus, steric hindrance may prevent propranolol with the naphthalene moiety and labetalol with the two benzene rings from penetrating the DPPTE monolayer. Drugs with less bulky structure, on the other hand, are able to interact with the monolayer. In addition, the hydrophilic

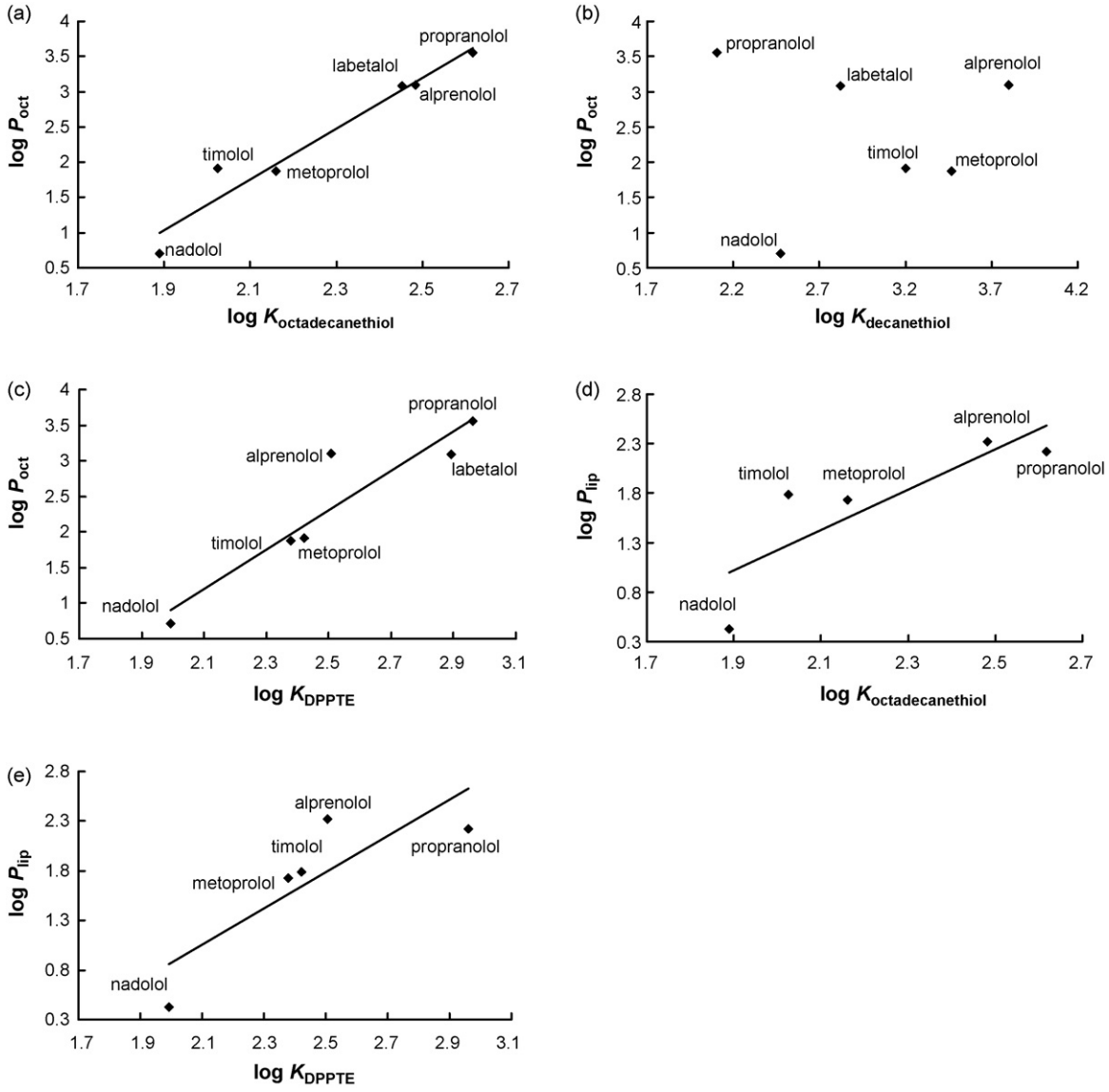

Fig. 5. Correlation of $\log K_{octadecanethiol}$ with $\log P_{oct}$ (a), $\log K_{decanethiol}$ with $\log P_{oct}$ (b), $\log K_{DPPTE}$ with $\log P_{oct}$ (c), $\log K_{octadecanethiol}$ with $\log P_{lip}$ (d), and $\log K_{DPPTE}$ with $\log P_{lip}$ (e).

part of the DPPTE molecule may enhance the interaction with the hydrophilic parts of the drugs.

3.4. Comparison of log K and log P_{oct}

The adsorption coefficient K can be compared to the traditional octanol–water partition coefficient P_{oct}, as both of the coefficients describe the equilibrium between the phase mimicking the hydrocarbon region of the membrane and the aqueous phase. When the logarithm of K is compared to the logarithm P_{oct}, it can be seen that $\log K_{octadecanethiol}$ correlates very well with $\log P_{oct}$ (Fig. 5a, $R^2 = 0.95$), whereas no correlation is observed between $\log K_{decanethiol}$ and $\log P_{oct}$ (Fig. 5b). The difference is probably due to the less-ordered structure of the decanethiol SAM compared to the octadecanethiol SAM. As the lipid molecules in the phospholipid bilayer of the biological membranes are relatively tightly packed and well-ordered [33], the results suggest that the ability of the decanethiol surface to serve as a model membrane is not as good as that of the octadecanethiol SAM.

Interestingly, $\log K_{DPPTE}$ correlates fairly well with $\log P_{oct}$ (Fig. 5c, $R^2 = 0.86$), even though the maximum surface excess of the drugs determined at DPPTE SAMs are somewhat different from those determined at the alkanethiol SAMs. Compared to the octadecanethiol SAM, the correlation of $\log K_{DPPTE}$ with $\log P_{oct}$ is slightly lower. The structural properties of the SAMs may account for this difference. The isotropic hydrocarbon phase in the octadecanethiol SAM is very similar to the octanol phase, whereas the DPPTE SAM is more anisotropic due to the phosphorus moiety of the DPPTE molecule. Because of this, it is interesting to compare the $\log K$ values not only to the $\log P_{oct}$ values, but also to the $\log P_{lip}$ values, the liposome–water partition coefficients.

3.5. Comparison of log K and log P_{lip}

The adsorption coefficients were compared to the liposome–water partition coefficients. The $\log P_{lip}$ values for metoprolol, nadolol, propranolol and timolol were measured using an electrochemical method [12] and $\log P_{lip}$ for alprenolol was taken from the literature [34]. Unfortunately, $\log P_{lip}$ for labetalol was not available. Comparison of $\log K_{octadecanethiol}$ and $\log P_{lip}$ is shown in Fig. 5d ($R^2 = 0.69$), and that of $\log K_{DPPTE}$ and $\log P_{lip}$ in Fig. 5e ($R^2 = 0.70$). $\log K_{decanethiol}$ and $\log P_{lip}$ were also compared, but again, no correlation was found between $\log K_{decanethiol}$ and $\log P_{lip}$. The lack of correlation is probably due to the less-ordered structure of the decanethiol monolayer as discussed above. As can be seen from Fig. 5d and e, there is no difference in the correlation of $\log K_{octadecanethiol}$ and $\log K_{DPPTE}$. Both $\log K_{octadecanethiol}$ and $\log K_{DPPTE}$ correlate better with $\log P_{oct}$ than with $\log P_{lip}$. Poor correlation with $\log P_{lip}$ can be explained by the anisotropy of the liposomal membrane. Due to the excellent biomimetic properties of liposomes, $\log P_{lip}$ describe well the whole partitioning process of the drug into the membrane, which includes multiple barriers for the drugs. In addition to the hydrocarbon core of the membrane, the two polar headgroup interfaces act as diffusion barriers. Because of the simplicity of the model membranes used in this study, the $\log K$ values mainly describe the barrier properties of the hydrocarbon core of the bilayer membrane and should not be interpreted as mimicking the partitioning process as a whole. Moreover, good correlation with the traditional $\log P_{oct}$ supports the interpretation that the method presented in this paper gives insight into the interaction of drugs with the hydrophobic core of the biological membrane.

4. Conclusions

The purpose of this study was to demonstrate how surface tension and contact angle measurements on different SAMs can be used to assess drug–membrane interactions. When data obtained using the pendant drop method and contact angle goniometry were fitted to the Gibbs equation and the Langmuir isotherm, adsorption coefficient K could be determined. It was found that $\log K$ determined on the octadecanethiol and DPPTE SAMs correlated very well with $\log P_{oct}$ and fairly well with $\log P_{lip}$ whereas no correlation was found between $\log K$ on the decanethiol surface and $\log P_{oct}$ or $\log P_{lip}$. It was concluded that the ability of the decanethiol SAM to model the hydrophobic core of the membrane is not as good as that of the octadecanethiol SAM or DPPTE SAM due to its less ordered structure. Also, comparison of the relative magnitudes of the surface excesses of the drugs on the alkanethiol SAMs versus those on DPPTE SAMs revealed that it is not only hydrophobicity, but also the conformational effects that are decisive factors in drug–membrane interactions. Because of the simplicity of the contact angle and surface tension measurements, the approach presented in this paper can easily be applied to other biomimetic surfaces as well when studying drug–membrane interactions.

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