4. WATER-MEMBRANE PARTITIONING AND DISTRIBUTION OF BUPIVACAINE AND KEToprofen in Bilayers in Dependency on pH and Electrostatics

4.1. Introduction

The pharmacokinetic and pharmacodynamic characteristics of a new drug molecule, or a well-established drug with a new indication or application form, are important and interesting in therapeutic product development. The hydrophilicity ↔ lipophilicity ratio often relates to the drug’s biological properties, as observed during Absorption, Distribution, Metabolism, and Excretion. Beyond ADME, the drug’s interaction with specific and unspecific targets (receptors, enzymes, proteins etc.) may also depend on relative hydrophilicity or lipophilicity of the active agent.

The widely used determination of 1-octanol/water partition coefficient, $P_{o/w}$, can yield useful information on the drug’s relative lipophilicity. However, 1-octanol is not a biological molecule. It is therefore much better and more relevant to study the drug’s partition coefficient between phospholipids and water, $P_{mem}$, which can be measured using lipid bilayer membrane vesicles. The membrane model simulates physiological drug surrounding more precisely than an isotropic solvent, such as 1-octanol. In vitro pharmacokinetic and pharmacodynamic properties are thus correlated better with an in vivo situation in which numerous membrane ↔ drug interactions are expected during ADME, e.g. the transcellular passage of an orally applied drug through lipid bilayers in the intestinal epithelium.

Beyond pharmacological aspects, galenic problems can also be tackled by studying bilayer membrane partition and distribution coefficient. Optimum loading of a drug on/into lipid vesicles is among others a major key in the development of good pharmaceutical products based on lipid bilayer vesicles (Langner et al. 1999; Cevc et al. 2001; 2004a; Allison 2007; Rother et al. 2007). Knowledge of $P_{mem}$ will thus provide a good basis in galenic research works.

In contrast to the common shake-flask method (Bouchard et al. 2002), which is used for $P_{o/w}$ but inapplicable to $P_{mem}$ determination, the drug membrane partition coefficient can be measured using different methods such as ultrafiltration (Austin et al. 1995), titration (Avdeef et al. 1998), dialysis (Kramer et al. 1998), immobilized artificial membrane (IAM)-HPLC (Ottiger et al. 1999), and the predictable quantitative structure activity relationship
simulations (QSAR) (Patel et al. 2001). Each of these methods has its merits, but \( pH \)-metric titration is the only one that allows determination of partition and distribution coefficients as a function of the drug dissociation.

The current commercial drug substances can be divided into three groups (Balon et al. 1999): bases (75 %), acids (20 %) and neutral molecules (5 %). The basic or acidic drugs often coexist in a charged and neutral form, dependent on the formulation or ambient \( pH \). Detailed \( pH \)-metric titrations by Avdeef (2003) showed that for the neutral molecules the partition coefficient in bilayer membranes is comparable with that in 1-octanol, \( P_{\text{mem}}^N \approx P_{\text{o/w}}^N \), while for the ionized drug form the partition coefficient is significantly higher in a membrane than in 1-octanol, \( P_{\text{mem}}^I \gg P_{\text{o/w}}^I \). Therefore, the resulting \( pH \) dependent drug distribution coefficient, \( D_{\text{mem}} \), is higher in the membrane at \( pH \) values where the ionized species predominates.

\[
\text{Ketoprofen} \quad \text{Bupivacaine}
\]

- A - B - C - D

Figure 25: Structures of the acid ketoprofen (left) and the base bupivacaine (right).
(A) chemical structure; (B) 3-D structure; (C) surface lipophilicity; (D) surface charge (Generated with HyperChem.)
Drug partitioning into membranes is also sensitive to electrostatic and polarity effects, and to interactions between the drug molecules and phospholipid bilayers (Cevc et al. 1987). This explains why different salt concentrations (Bauerle et al. 1991; Alcorn et al. 1993; Thomas et al. 1993; Austin et al. 1998) or different choice of phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine (Langner et al. 1995; Pedros et al. 1997; Takegami et al. 2005), influence $P_{mem}$ and $D_{mem}$.

I therefore studied partitioning and distribution of the basic local anaesthetic bupivacaine and of the acidic non-steroidal anti-inflammatory drug (NSAID) ketoprofen (Figure 25) into bilayers of soybean phosphatidylcholine. I measured the drug partitioning in this work in dependency on the bulk $pH$ but also explored the influence of salt concentration on equilibria. To the best of my knowledge, no bilayer membrane partitioning data have been published for these molecules to date, at least not considering electrostatic interactions between the drugs and the membrane in parallel. Such interactions are important, however, in designing drug containing ultradeformable bilayer membranes, with the intended use as vesicular carriers for transdermal drug delivery (Cevc et al. 2003b; Cevc 2004).

The outcome of the present study will confirm observations of different authors which also measured and considered electrostatic effect on drug membrane distribution. Unfortunately, numerousness partition and distribution coefficient data published for various drugs and excipients to date neglect such electrostatic interactions. With the results of the following partition coefficient measurements, I tried to study drug partitioning as a function of bilayer’s charge density, surface potential, and polarity, finding out that these interactions are substantial when measuring and presenting $P_{N_{mem}}$, $P_{I_{mem}}$, and $D_{mem}$. 
4.2. Theoretical background

The dissociation constant, \( pK_a \), of an ionizable acid or base in water is defined as the negative common logarithm of the dissociation equilibrium, \( K_a \),

\[
pK_a = -\log K_a = -\log \frac{[H_2O^+][A^-]}{[AH]} \quad \text{for acids}
\]

\[
= -\log \frac{[H_2O^+][B]}{[BH^+]} \quad \text{for bases}
\]

with a pH dependent dissociation degree, \( \alpha \), defined as:

\[
\alpha = \frac{10^{pH-pK_a}}{10^{pH-pK_a} + 1}
\]

Any drug’s dissociation is influenced by polar and electrostatic effects involving the surrounding solvent as well as ions. Dissociation constant is therefore different in a lipid bilayer membrane, \( pK_{mem} \), and in water, \( pK_a \). pH-metric titration of an acid or base in lipid bilayer vesicles suspension therefore yields an effective dissociation constant for the molecules in contact with water and with lipid aggregates. The relationship is not a straight one however, but rather a result of four coupled equations of equilibrium, as is illustrated in Figure 26.

The resulting apparent dissociation constant, \( pK_{app} \), depends on partition coefficients of the charged and the neutral drug forms, \( P^I_{mem} \) and \( P^N_{mem} \) respectively, as well as on the \( pK_a \) and \( pK_{mem} \) values, with the apparent value being between the two.

![Figure 26: Schematic illustration of dissociation and partitioning of bases (i.e. cationic acids) and acids in a system consisting of an aqueous bulk phase and lipid bilayer membranes.](image-url)
The charge-specific partition coefficients for the neutral and for the ionized molecules can be calculated from the respective, directly measured, drug concentrations in the bilayer membrane, $c_{mem}$, and in water, $c_w$ (Miyazaki et al. 1992):

$$P^N_{mem} = \frac{c^N_{mem}}{c^N_w} \quad P^I_{mem} = \frac{c^I_{mem}}{c^I_w}$$  \hspace{1cm} (20)

The associated pH dependent, phenomenological drug distribution coefficient, $D_{mem}$, considers both the neutral and ionized molecules (Miyazaki et al. 1992):

$$D_{mem} = \frac{c^N_{mem} + c^I_{mem}}{c^N_w + c^I_w}$$  \hspace{1cm} (21)

Alternatively, by measuring the apparent dissociation constants, $pK_{app}(j)$, for at least two different lipid volume ratios $v_j = V_{lipid} / V_{total}$ (j = 1 and 2), the neutral and ionized drug’s partition coefficients in lipid bilayers can be calculated from the known dissociation constant in water, $pK_a$ (Avdeef 1992; Avdeef et al. 1998):

$$P^N_{mem} = \frac{v_2 \cdot 10^{[pK_{app}(2)-pK_a]} - v_1 \cdot 10^{[pK_{app}(1)-pK_a]} - (v_2 - v_1) \cdot 10^{[pK_{app}(1)+pK_{app}(2)-2pK_a]} \right]}{v_1v_2 \left[10^{[pK_{app}(1)-pK_a]} - 10^{[pK_{app}(2)-pK_a]} \right]}$$  \hspace{1cm} (22)

$$P^I_{mem} = \frac{v_1 \cdot 10^{[pK_{app}(2)-pK_a]} - v_2 \cdot 10^{[pK_{app}(1)-pK_a]} + v_2 - v_1 \right]}{v_1v_2 \left[10^{[pK_{app}(1)-pK_a]} - 10^{[pK_{app}(2)-pK_a]} \right]}$$  \hspace{1cm} (23)

Knowledge of both partition coefficients enables pH-dependent evaluation of the drug’s distribution including both charged and neutral molecular forms:

$$\log D_{mem} = \log \left(P^I_{mem} + P^N_{mem} \cdot 10^{s(pK_a-pH)} \right) - \log \left(1 + 10^{s(pK_a-pH)} \right)$$  \hspace{1cm} (24)

with the drug specific sign $s = +1$ for acidic and $s = -1$ for basic drug substances.

Equations (20) - (24) are valid so long as membrane properties are not affected significantly by the membrane bound drug. Practically speaking, this is true for high lipid/drug ratios or small partition coefficient $P^I_{mem}$. Otherwise, when an appreciable drug quantity is bound to the membrane, corrections must be made for the drug-induced changes in the membrane properties, especially for the membrane’s electrostatic potential. The influence of electrostatics on the drug partitioning must then be considered. This is typically done as a function of the membrane surface charge density, $\sigma$, the bulk ions concentration, $c_{w,i}$, and the polarity of the membrane/water interface, $\varepsilon_{mem}$.
The surface charge density can be estimated from the known dissociation degree, $\alpha$, of an acid or a base at the bilayer membrane surface (Austin et al. 1998):

$$\sigma = \frac{\alpha \cdot r_{drug} \cdot z \cdot e}{A_{drug} \cdot r_{drug} + A_I \cdot r_I}$$  \hspace{1cm} (25)

$z$ is the drug’s charge number and $e$ the elementary electronic charge. $A_{drug}$ and $A_I$ are the surface area of the drug and the bilayer forming lipid in the membrane, respectively ($A_I >> A_{drug}$ within this work). $r_{drug}$ and $r_I$ are the mole ratios of the former and of the latter.

The Debye screening length, $\lambda_D$, which gives the “thickness” $\lambda_D$ of the diffuse electric double layer near a charge, influences the surface potential as well, in dependency on ions, $i$, with bulk concentrations $c_{w,i}$ and the charge number $z_i$ (Cevc 1990):

$$\lambda_D = \frac{\varepsilon \cdot \varepsilon_0 \cdot k_B T}{N_A \cdot e^2 \cdot \sum_i z_i^2 \cdot c_{w,i}}$$  \hspace{1cm} (26)

$\varepsilon$ and $\varepsilon_0$ are the dielectric constant of the solution and the permittivity of free space, respectively. The remaining parameters are the thermal energy, $k_B T$, and the Avogadro number, $N_A$.

At 25 °C equation (26) simplifies for monovalent salts with total bulk salt concentration $c_{w,t}$ (Israelachvili 1992):

$$\lambda_D = 0.304 \cdot c_{w,t}^{-0.5}$$  \hspace{1cm} (27)

The surface potential, $\psi_{0,GC}$, is usually calculated within the framework of the Gouy-Chapman model (Gouy 1910; Chapman 1913):

$$\psi_{0,GC} = \frac{2 \cdot k_B T}{z \cdot e} \cdot \sinh \left[ \frac{z \cdot e \cdot \sigma \cdot \lambda_D}{2 \cdot \varepsilon \cdot \varepsilon_0 \cdot k_B T} \right]$$

$$= \frac{\sigma \cdot \lambda_D}{\varepsilon \cdot \varepsilon_0} \hspace{1cm} \text{for} \hspace{0.2cm} \psi_0 < 25 \text{ mV}$$  \hspace{1cm} (28)

Within a membrane interface, e.g. near a phospholipid bilayer membrane, the polarity changes. In terms of the dielectric constant, the difference is from $\varepsilon_{mem} \approx 35$ at the headgroup to $\varepsilon_{mem} \approx 2$ within the fatty acid chains or to $\varepsilon \approx 78$ in the “bulk” (Marsh 2001).
Therefore, the membrane surface potential $\psi_0$ has to be calculated based on equation (28)

$$
\psi_0 = \frac{2 \cdot k_B T}{z \cdot e^\sigma} \cdot \sinh^{-1} \left[ \frac{z \cdot e \cdot \lambda_D}{2 \cdot \varepsilon_{mem} \cdot \varepsilon_0 \cdot k_B T} \right]
$$

(29)

which only differs from the latter in the dielectric constant value.

In this work, I assumed the location of the charged bupivacaine and ketoprofen, i.e. the position of the surface charge, near the headgroup and fixed the polarity to $\varepsilon_{mem} = 30$.

Knowledge of electrostatic surface potential then allows calculation of the drug’s concentration at the membrane surface, based on equation (20), simply by:

$$
c_{mem}' = c_w' \cdot P_{mem}^I \cdot \exp \left[ \psi_0 \cdot k_B T \right]
$$

(30)

Coming back to the experimental determination and evaluation of partition coefficients via pH-metric titrations, it is clear that, under electrostatic consideration and influence, the apparent dissociation constant is a function of lipid volume ratio, $v_j$. $pK_{app}$ values determined for at least two different lipid volume ratios thus allow derivation of the partition coefficients $P_{mem}^N$ and $P_{mem}^I$ for the neutral and ionized drug forms, respectively:

$$
pK_{app} (v_j) = pK_a + s \cdot \log \left[ \frac{P_{mem}^N \cdot v_j + 1}{P_{mem}^I \cdot \exp \left[ \psi_0 \cdot k_B T \right] \cdot v_j + 1} \right]
$$

(31)

Equation (31) must be solved together, and consistent, with equations (25),(29), and (30), using least squares fitting procedure.

The $pH$ dependent distribution of ionizable molecules in a lipid bilayer membrane is then given, based on equation (24), by:

$$
\log D_{mem} = \log \left[ P_{mem}^I \cdot \exp \left[ \psi_0 \cdot k_B T \right] + P_{mem}^N \cdot 10^{s(pK_a-pH)} \right] - \log \left[ 1 + 10^{s(pK_a-pH)} \right]
$$

(32)
4.3. Material and Methods

4.3.1. Materials

Soybean phosphatidylcholine (SPC) was chosen as the bilayer forming lipid and was purchased from Lipoid with the purity grade Lipoid S100 (Ludwigshafen, Germany). Bupivacaine (hydrochloride monohydrate) and ketoprofen were supplied by Heumann PCS (Feucht, Germany) and Bidachem (Fornovo S. Giovanni, Italy), respectively; both met the quality specifications of the European Pharmacopoeia (EP). Potassium chloride, sodium hydroxide, hydrochloric acid (all VWR, Darmstadt, Germany), and water for injection (Deltaselect, Dreieich, Germany) had EP quality as well.

4.3.2. Preparation of drug containing large unilamellar lipid bilayer vesicles and blank solutions

Large unilamellar vesicle suspensions with two different lipid concentrations (160 mg/g and 50 mg/g, assuming \( \rho = 1 \text{ g/ml} \)) were used in the study. The corresponding SPC quantity was interspersed into an aqueous solution of either bupivacaine or ketoprofen, always keeping the final drug concentration constant (\( c_d = 20 \text{ mM} \)). To determine the influence of electrostatics on the drugs partitioning, different amounts of potassium chloride were added to each suspension.

For better homogeneity, the suspensions were stirred for 2 h, and then extruded through a set of different track-etched poly-carbonate membranes (PCTE, GE Osmonics) with decreasing pore size (400 nm – 100 nm); a freeze-thaw cycle (180 min at -70 °C, 30 min at 40 °C) and multi-extrusion through PCTE membranes with decreasing pore sizes (400 nm – 80 nm) finished the vesicle preparation (Olson et al. 1979; Mayer et al. 1986; MacDonald et al. 1993). The resulting vesicle size and polydispersity were determined by the dynamic light scattering (DLS), as is described in chapter 2.2.4. This yielded an average vesicle diameter of \( d_{ves} = 100 \pm 15 \text{ nm} \) and polydispersity index of \( PDI < 0.15 \).

The drug free blank solutions without any lipid vesicle were prepared by dissolving the required potassium chloride quantities in water.

Directly before a \( pH \)-metric titration, the \( pH \) value of the vesicle suspension and its allocated blank solution were adjusted to the starting \( pH < 3 \) and \( pH > 11 \) for bupivacaine and ketoprofen titrations, respectively. This was done using adequate volumes of sodium hydroxide (10 M) or hydrochloric acid (4 M). The resulting final ionic strength, comprising
all ions (potassium chloride, sodium hydroxide, hydrochloric acid, and drug counter ions) was calculated to be in the range of $I_c = 35 \text{ mM}$ to 265 mM.

### 4.3.3. pH-metric determination of the apparent dissociation constants, membrane partition coefficients, and distribution coefficients

Determination of drug partition coefficients for the uncharged (neutral) molecule and for the charged (ionized) entity, as well as analysis of the $pH$-dependent distribution coefficient, are based on the pioneer work of Avdeef (1992; 1998).

According to the fundamental equations (22) and (23), the $pK_{app}$ of lipid vesicle suspensions with two different total lipid volume ratios, $v_j$, is needed to derive $P^N_{mem}$ and $P^I_{mem}$. As pointed out by Balon et al. (1999), the partition coefficients for the neutral and ionized drug forms are independent on the tested lipid ratios; in contrast, the membrane partitioning of a drug is typically temperature sensitive. Thus, all the measurements were done at $25 \pm 0.3 \, ^\circ \text{C}$. Blank solutions provided controls, and supported the calculation of the mol equivalent of the titrated drug substance.

The pH glass electrode DG111-SC was calibrated using the titrator DL67 and the LabX Pro titration software (all from Mettler-Toledo, Giessen, Germany). This was done daily before the measurements, which were done in triplicate for the drug vesicle suspension and its reference blank solution. For the ketoprofen containing suspensions, a “down”-titration (from high to low $pH$) was employed. For the oppositely charged bupivacaine, an “up”-titration in opposite direction was chosen (lower panels in Figure 27). The analytes were determined with two different titrants: sodium hydroxide (0.5 M) and hydrochloric acid (1 M) for bupivacaine and ketoprofen samples, respectively.

In all experiments, the $pH$ change per titrant addition was limited to $\Delta pH \leq 0.1$. This was done dynamically by the software. The $pH$ equilibration between the lipid vesicle exterior and interior was adjusted to be 1 - 2 min between each addition, as had been suggested in the literature (Pauletti et al. 1994).

To evaluate the $pK_{app}$ values, the ratio of the ionized drug molecules

$$r_{ionized} = \frac{c_i \cdot s(V_{ib} - V_{is})}{c_d \cdot V_{total}}$$

was first calculated. $c_i$ is the titrant concentration and $V_{ib}$ and $V_{is}$ are the titrant volumes used for titrating blank solution and vesicle suspension in dependency on $pH$, respectively. $c_d$ and $V_{total}$ are the total drug concentration and the measured volume, respectively.
The ratio of the ionized drug molecules as a function of pH yields an apparent dissociation constant, $pK_{app}$, read-off at the inflexion point of the sigmoidal curve given in Figure 27. At $pH = pK_{app}$, the ratio of the charged and neutral molecules is 1:1.

Figure 27: Illustration of pH-metric titrations for the determination of the apparent dissociation constant, $pK_{app}$, of bupivacaine (left panels) and ketoprofen (right panels) in bilayer vesicle suspensions. The lower panels show the measurements of the drug containing lipid vesicle suspensions (black dotted line) and the blank solutions (grey dotted line). The upper panels illustrate the ratio of ionized drug (black line) and its derivation (grey line) in dependency on pH.

With the $pK_{app}$ derived from the experimental data measured with two different lipid ratios, $v_j$, and further considering the known aqueous dissociation constants of bupivacaine, $pK_a = 8.09$ (Friberger et al. 1971)) or of ketoprofen, $pK_a = 4.36$ (Rafols et al. 1997), the various partition and distribution coefficients were derived in dependency on electrostatics with a least square fitting procedure involving equations (31) and (32). The results are given in the following section.
4.4. Results and discussion

4.4.1. Apparent dissociation equilibrium of bupivacaine and ketoprofen

pH-metric titrations of bupivacaine or ketoprofen containing bilayer membranes yielded different apparent dissociation equilibria in dependency on lipid/drug ratio and total ionic strength, $I_c$ (symbols in Figure 28).

![Figure 28: The apparent dissociation constant, $pK_{app}$, of bupivacaine and ketoprofen in bilayer membrane with different lipid/drug ratios.](image)

With decreasing lipid/drug ratio, the $pK_{app}$ shift is reduced due to lower lipid volume ratio, $v_j$, and due to electrostatic repulsion between the charged bilayer membrane and the drug molecules at the membrane/water interface.

The $pK_a$ shift between the bilayer membrane and water is empirically in the range of –1 to –1.5 units for bases (Miyazaki et al. 1992) and +2 to +3 units for acids (Austin et al. 1998). For the base bupivacaine, which has an intrinsic dissociation constant of $pK_a = 8.09$ in water, the apparent dissociation constant is shifted downwards to $pK_{app} = 7.15 – 7.33$. For ketoprofen the shift is upwards, from $pK_a = 4.36$ to $pK_{app} = 5.96 – 6.38$. Figure 28 suggests, that the magnitude of this effect depends, among others, on lipid/drug ratio, as is illustrated in Figure 26 for the four coupled equations of equilibrium. In addition, the data fitting curve based on equation (31) shows an electrostatic influence on $pK_{app}$ shift, which will get attention in the following section.

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4.4.2. Bilayer surface charge in dependency on pH

The fitted $pK_{\text{app}}$ curves given in Figure 28 highlight electrostatic effects on drug partitioning. They show, for example, that at low lipid/drug ratio the $pK_{\text{app}}$ shift increases with salt concentration. This is not surprising given that ions from the surrounding bulk solution not only affect the Debye screening length, $\lambda_D$; they moreover affect the surface charge density, $\sigma$, and the resulting surface potential, $\psi_0$ (cf. equations (25),(26), and (29)).

Figure 29 for bupivacaine and Figure 30 for ketoprofen furthermore illustrate that for constant drug concentration the drug dependent bilayer surface charge density decreases with increasing lipid concentration. This is due to enlarged lipid area in case of high total lipid concentration. High ionic strength, and the corresponding short screening length, reduce repulsion of the equally charged drug molecules from the membrane/water interface, where more such molecules reside, and increase $pK_{\text{app}}$ shift, as is shown in Figure 28.

![Figure 29: Surface charge density, $\sigma$, of bupivacaine containing bilayer membranes in dependency on the bulk pH, ionic strength, $I_c$, or the drug/lipid ratio. Bupivacaine, a tertiary amine base, is positively charged at low pH and neutral at high pH. With increasing pH, the drug dependent surface charge density therefore changes. Decreasing lipid/drug ratio and increasing $I_c$ both results in higher surface charge densities, due to weakened electrostatic repulsions.](image-url)
Figure 30: Surface charge density, $\sigma$, of ketoprofen containing bilayer membranes in dependency on the bulk pH, ionic strength, $I_c$, or the drug/lipid ratio.

Ketoprofen, a propionic acid derivative, is negatively charged at high pH and neutral at low pH. With increasing pH, the drug dependent surface charge density therefore changes. Decreasing lipid/drug ratio and increasing $I_c$ both result in higher surface charge densities, due to weakened electrostatic repulsions.

The deviation from sigmoidal curve progression, which is seen in Figure 29 and Figure 30 at low ionic strengths, highlights a phenomenon. Theoretically, in absence of any repulsion, the membrane bound drug molecules should change from neutral form into the charged one according to dissociation equation, i.e. sigmoidally with decreasing or increasing pH for bupivacaine or ketoprofen suspensions, respectively. In reality, the pH-induced changes of bilayer surface charge density can actually force some of the charged drug molecules out of the bilayer, thus reducing the final surface charge density. The propensity for this is higher at low salt concentration, when electrostatic interactions are less screened.

Electrostatic surface potential is therefore one reason why charged and neutral drugs show different membrane partitioning in dependency on the surrounding salt concentration, an observation, which will be presented and discussed in the following section.
4.4.3. Bilayer membrane partition and distribution in dependency on charge state and electrostatics

The widely used 1-octanol/water partition coefficient ($P_{N\ o/w}$) of neutral molecules is similar to the value measured with phospholipid bilayer membranes in water ($P_{N\ mem}^N$) (Avdeef et al. 1998). For the charged molecules, however, the partition coefficients $P_{I\ o/w}$ and $P_{I\ mem}$ are totally different for the most part (Miyazaki et al. 1992; Austin et al. 1995; Avdeef et al. 1998). The reason is that partitioning of amphiphilic acidic or basic drugs from water into a membrane depends, among others, on electrostatic effects, on the drug’s charge state, and on polarity. For example:

The propionic acid derivative ketoprofen in neutral form possesses an 1-octanol/water partition coefficient $\log P_{N\ o/w} = 3.12$ (La Rotonda et al. 1983). This is in good accord with $\log P_{N\ mem}^N = 3.27$ found in this study. For the ionized ketoprofen the partition values in octanol and membrane differ significantly: $\log P_{I\ o/w} = -0.95$ (Avdeef 2003) and $\log P_{I\ mem}^I = 0.15 - 1.18$, in dependency on ionic strength.

Bupivacaine, a tertiary amine base, has a similar $\log P_{N\ o/w} = 3.38$ (Razak et al. 2001) but ten times lower partition coefficient in the neutral form in a bilayer: $\log P_{N\ mem}^N = 2.43$. However, the partitioning of the positively charged bupivacaine is significantly higher compared to ionized ketoprofen, particularly at low salt concentrations: $\log P_{I\ mem}^I = 1.23 - 1.58$. Unfortunately, to the best of my knowledge, there are no other comparable literature data for the partitioning of charged bupivacaine in 1-octanol/water.

<table>
<thead>
<tr>
<th>$I_c$ [mM]</th>
<th>$\log P_{N\ o/w}$</th>
<th>$\log P_{N\ mem}^N$</th>
<th>$\log P_{I\ o/w}$</th>
<th>$\log P_{I\ mem}^I$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen</td>
<td>35</td>
<td>3.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00</td>
<td>-0.95&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>85</td>
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<td>3.19</td>
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<tr>
<td></td>
<td>235</td>
<td></td>
<td>3.27</td>
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</tr>
<tr>
<td>Bupivacaine</td>
<td>35</td>
<td>3.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.08</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td></td>
<td>2.17</td>
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</tr>
<tr>
<td></td>
<td>265</td>
<td></td>
<td>2.43</td>
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Table 8: Partition coefficients of neutral (N) and ionized (I) ketoprofen and bupivacaine in a 1-octanol/water, $P_{o/w}$, or bilayer membrane/water, $P_{mem}$, system in dependency on the ionic strength, $I_c$.

<sup>a</sup>(La Rotonda et al. 1983), <sup>b</sup>(Avdeef 2003), <sup>c</sup>(Razak et al. 2001)
Since both ketoprofen and bupivacaine have a similar log $P_{o/w}^{N}$ value but different partition coefficients for the neutral as well as the ionized molecular form (cf. Figure 31), these observations are interesting and form the basis for a deeper theoretical discussion at molecular level.

Uncharged ketoprofen partitions into a bilayer membrane more than uncharged bupivacaine. In contrast, positively charged bupivacaine is more bilayer-affine than negatively charged ketoprofen, at least at low ionic strengths (Figure 31). These observations are consistent with the results of Avdeef et al. (1998) for other NSAIDs or local anaesthetics, and can be explained with different location of the charged and neutral molecular forms in the membrane-water interface (cf. Figure 32).

Ionized amphiphiles, like the positively charged bupivacaine and the negatively charged ketoprofen, are located closer to the bulk. Both drugs displace water of lipid hydration, as was shown for other charged amphiphiles by Hogberg et al. (2007). The drugs may moreover interact with the negatively charged phosphate group (bupivacaine) or the positively charged trimethylammonium group (ketoprofen). However, the drugs orientation is likely to be different for the two tested drugs, owing to more central position of the bupivacaine’s charge
in comparison with ketoprofen. This makes the ionized ketoprofen more sensitive to electrostatic effects, as is shown in Figure 31.

The neutral drug molecules are inserted deeper in the water-membrane interface, near the fatty acid ester group. Ketoprofen can then bind via hydrogen bonds to the fatty acid carbonyl group, finally resulting in an increased $P^N_{mem}$ (Avdeef et al. 1998; Hogberg et al. 2007). The following section supports this conclusion and provides deeper insight into the electrostatic and polarity induced contributions to the drug’s partitioning into lipid membranes.

**Figure 32:** Schematic illustration of ketoprofen and bupivacaine location within a phosphatidylcholine membrane in dependency on their ionization. (only a monolayer is presented). The upper graph shows charged ketoprofen (left molecule) and charged bupivacaine (right molecule) incorporated in the upper part of lipid headgroup region. The lower graph illustrates the position of the neutral drugs in the deeper lipid monolayer regions. (Generated with ACD/3D-viewer.)
4.4.4. Influence of electrostatics and polarity on membrane dissociation

The dissociation equilibrium of acids and bases in bilayer vesicle suspensions is shifted by polar and electrostatic effects compared to the bulk \( pK_a \) (Cevec et al. 1987):

\[
pK_{app} = pK_a + \Delta pK_a^{el} \pm \Delta pK_a^{pol}
\]

(34)

\( \Delta pK_a^{el} \) is the electrostatic contribution and \( \Delta pK_a^{pol} \) is the polarity dependent contribution to the shift.

The electrostatic shift is given by:

\[
\Delta pK_a^{el} = \frac{z \cdot e \cdot \psi_0}{2.3 \cdot k_B T}
\]

(35)

The value of \( \Delta pK_a^{pol} \) depends on interfacial dielectric constant, \( \varepsilon_{mem} \), at the drug binding site. Its sign depends on the change in total number of charges on dissociation (Fernandez et al. 1977; Cevec et al. 1981). In other words, interaction of zwitterionic phosphatidylcholine membranes with the molecular acid ketoprofen AH should cause a positive polarity induced shift, due to increase of total charge from (+ -) to (+ - -). For dissociation of the cationic acid bupivacaine BH\(^+\) the reverse should be true, owing to total charge decrease from (+ - +) to (+ -).

The values for \( \Delta pK_a^{el} \) and \( \Delta pK_a^{pol} \) for different salt concentrations at lipid/drug ratio of 10 mol/mol were calculated from the measured \( pK_{app} \) values and the determined surface potential \( \psi_0 \) (cf. equations (29)) using equations (34) and (35).

<table>
<thead>
<tr>
<th>( I_c ) [mM]</th>
<th>( \psi_0 ) [mV]</th>
<th>( \Delta pK_a^{el} )</th>
<th>( \Delta pK_a^{pol} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>61</td>
<td>1.03</td>
<td>-1.95</td>
</tr>
<tr>
<td>65</td>
<td>55</td>
<td>0.93</td>
<td>-1.79</td>
</tr>
<tr>
<td>265</td>
<td>37</td>
<td>0.63</td>
<td>-1.52</td>
</tr>
<tr>
<td>35</td>
<td>-36</td>
<td>0.61</td>
<td>1.41</td>
</tr>
<tr>
<td>85</td>
<td>-54</td>
<td>0.91</td>
<td>0.93</td>
</tr>
<tr>
<td>235</td>
<td>-38</td>
<td>0.64</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Table 9: Surface potential \( \psi_0 \) and the derived values of the electrostatic and polarity induced contribution to the \( pK_a \) shift for bupivacaine and ketoprofen in phosphatidylcholine bilayer membranes at different ionic strengths with a lipid/drug ratio of 10 mol/mol.

Table 9 reveals that the polarity induced shift, which is an indicator of charged molecules location in the membrane-water interface (Fernandez et al. 1977), is different for bupivacaine.
and ketoprofen. For ketoprofen $\Delta pK_a^{pol}$ is in the range of 0.9 to 1.4, which is in good accord with the values reported for other amphiphiles: $\pm 1.1$ (Fernandez et al. 1977). The polarity induced shift for bupivacaine is negative and somewhat larger, $\Delta pK_a^{pol} = -1.5 - -2.0$, consistent with general expectations and the assumedly deeper insertion of this drug into the interface.

Fernandez and Fromherz (1977) published polarity induced shifts as a function of different dioxin-water mixtures representing various dielectric constants. Knowledge of the latter information on dielectric profile near a phosphatidylcholine bilayer membrane (Marsh 2001) enables semi-quantitative estimation of the drug’s location in the membrane-water interface. The location of ketoprofen is then concluded to be between the trimethylammonium and phosphate groups, having polarity of $\epsilon_{mem} \approx 28 – 35$. Bupivacaine is located somewhat deeper in the membrane, more precisely, between the phosphate and the fatty acid carbonyl groups ($\epsilon_{mem} \approx 20 – 28$). This observation is consistent with the discussion in section 4.4.3, where the higher $P_{mem}^{l}$ value for bupivacaine prompted me to assume its location more inside the membrane compared to ketoprofen.

4.5. Conclusion and outlook

Knowledge of the dissociation equilibria of basic and acidic drug molecules, such as bupivacaine and ketoprofen, and defined information on their partition and distribution behaviour in bio-mimetic phosphatidylcholine membranes are important and useful for solving pharmacological or galenic questions related to these drugs.

Primarily with regard to the development of modern pharmaceutical forms based on bilayer membrane vesicles, the partition and distribution behaviour in dependency on pH, electrostatics, and polarity will provide a good starting point for optimising drug loading, improving formulation stability, and simulating in vivo behaviour. The pH and salt concentration dependent effects studied herein are being used in the development of new medicinal products based on Transfersome® technology (Cevc et al. 2003b; Cevc 2004). Especially the combination of bupivacaine or ketoprofen with such ultra adaptable, highly fluctuating lipid vesicles, e.g. based on polyoxyethylene (20) oleyl ether saturated phosphatidylcholine bilayer membranes, could generate new products for the local and carrier mediated treatment of neuropathic pain or inflammation in near future.

Furthermore, membranes comprising phosphatidylcholine, which is the most common lipid in living cells (Yorek 1993), are suitable models to study biologically relevant partitioning of
new drugs or novel drug applications. Knowledge of the charged and uncharged drug distribution into such bio-mimetic membrane can thus provide a good starting point for optimisation of drug action, e.g. for potentiating local anaesthetics action or reducing adverse side effects of NSAIDs.

Local anaesthetics of the bupivacaine type, both in neutral and ionized form, bind to the 6th transmembrane segment of the α-IV subunit in the voltage-gated sodium ion channel (Ragsdale et al. 1994). The resulting channel blocking hinders action potential building and nerve pulse transmission (Chernoff et al. 1990). However, before interacting with the sodium channel, a local anaesthetic must first diffuse, predominantly in its neutral form, through the nerve cell membrane (Hogberg et al. 2007). This allows the drug to reach its binding site, which is only accessible from the cell interior. An uncharged local anaesthetic unbinds much faster from the channel, resulting in accelerated recovery from the nerve block (Schwarz et al. 1977). This relationship could be a prospective challenge in development of new or optimised anaesthetics comprising both: a high membrane partitioning to reach the site of action and a prolonged binding to the latter.

Partition coefficient measurements should therefore be used as a starting point for solving such pharmacological questions. To be more reliable, such future studies should be performed with a negatively charged membrane similar to that in nerve cell, e.g. composed of the zwitterionic phosphatidylcholines and the negatively charged phospholipids (Inouye et al. 1988).

Ketoprofen belongs to the group of non-selective cyclooxygenase COX-I and -II inhibitors, which are broadly used to treat any kind of mild to moderate pain and inflammation. Like the other plentifully administered non-selective NSAIDs, ketoprofen can induce gastrointestinal injuries due to COX-1 inhibition and to hindrance of the cytoprotective prostaglandin expression in the gastrointestinal tract. The scientific evidence of this theory is inconsistent, however, e.g. different drug potencies in COX-1 inhibition at comparable “COX-1 induced” adverse side effects were reported (Lichtenberger 2001). The non-selective NSAID-caused gastrointestinal injuries can therefore also be explained by chemical reaction with and destabilisation of the protective phospholipid lining of the mucus gel layer (Lichtenberger et al. 1995; Giraud et al. 1999). Therefore, knowledge of ketoprofen’s and any other NSAIDs partitioning and distribution into lipid bilayers could support further development of “safe” NSAIDs.