Immobilized Artificial Membrane (IAM)-HPLC for Partition Studies of Neutral and Ionized Acids and Bases in Comparison with the Liposomal Partition System

Cornelia Ottiger and Heidi Wunderli-Allenspach[1,2]

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Purpose. To study the partitioning of model acids ((*RS*)-warfarin and salicylic acid), and bases (lidocaine, (*RS*)-propranolol and diazepam), with immobilized artificial membrane (IAM)-HPLC, as compared to partitioning in the standardized phosphatidylcholine liposome/buffer system.

Methods. The pH-dependent apparent partition coefficients D were calculated from capacity factors (k'_{IAM}) obtained by IAM-HPLC, using a 11-carboxylundecylphosphocholine column. For lipophilic compounds k'_{IAM}, values were determined with organic modifiers and extrapolation to 100% water phase (k'_{IAMw}) was optimized. Temperature dependence was explored (23 to 45 °C), and Gibbs free energy (ΔG), partial molar enthalpy (ΔH) and change in entropy (ΔS) were calculated. Equilibrium dialysis was used for the partitioning studies with the liposome/buffer system.

Results. For extrapolation of k'_{IAMw}, linear plots were obtained both with the respective dielectric constants and the mole fractions of the organic modifier. All tested compounds showed a similar pH-D diagram in both systems; however, significant differences were reproducibly found in the pH range of 5 to 8. In all cases, ΔG and ΔH were negative, whereas ΔS values were negative for acids and positive for bases.

Conclusions. In both partitioning systems, D values decreased significantly with the change from the neutral to the charged ionization state of the solute. The differences found under physiological conditions, i.e. around pH 7.4, were attributed to nonspecific interactions of the drug with the silica surface of the IAM column.

KEY WORDS: partitioning; IAM-HPLC; liposome; drug-lipid membrane interactions; acids; bases.

INTRODUCTION

The impact of the lipophilicity of drugs on their pharmacokinetic behavior and pharmacological activity has been recognized for a long time. In recent years the interest has focused on the important aspect of drug-membrane interactions (1,2). As a consequence, membrane-like systems have been developed for the determination of partition coefficients, for instance liposomes (3,4), and high performance liquid chromatography (HPLC) methods with many different packing materials. In 1989 a combination of an artificial lipid membrane and the fast analytical HPLC system was introduced (5). A membrane-like stationary phase was produced with a phosphatidylcholine analogue, which was synthesized and bound to the silica based matrix of a HPLC column (6). These immobilized artificial membrane (IAM) columns mimic the lipid environment of a fluid cell membrane on a solid matrix. For greater stability a single chain phosphatidylcholine that lacks the glycerol backbone (11-carboxylundecylphosphocholine) $^{\delta G}$IAM.PC$^{C10/C3}$ was synthesized and bound to the silica propylamine surface (6). As shown in Fig. 1 the free silica-propylamine matrix was endcapped with C_3- and C_{10}-alkyl chains (7). Partition studies by IAM-HPLC are attractive for several reasons. First, the method is very fast and thus permits a relatively high throughput. In addition, only small amounts of compounds are required and impurities as well as degradation products do not interfere with the procedure. With these advantages it is very important to evaluate the HPLC partition method with respect to its predictive potential for the *in vivo* situation and compare it to the traditional shake flask octanol/buffer system and the liposomal systems.

To measure lipophilicity with the HPLC method, the capacity factor log k′ is determined for the respective solutes. For lipophilic compounds, which tend to adhere to the column, binary mixtures of buffer with an organic modifier like methanol or acetonitrile are applied for elution. In this case the extrapolation has to be made to the capacity factor for a 100% aqueous phase (log k'_w) to determine the partition coefficient. Various characteristics of the binary mixtures, for instance volume % and solvatochromic solvent polarity, have been used for extrapolation, but not all of them are equally suited (8,9).

The retention mechanism for solutes on IAM columns has been explored by several groups. Studies on the temperature dependence of retention were performed and enthalpic and entropic parameters extracted from the van't Hoff plot for various stationary phases (10). In most cases, the retention of the tested solutes decreases with increasing temperature. The enthalpy parameter ΔH is therefore negative for the process of transfer for most solutes from the mobile to the stationary phase (11). In the same systems the Gibbs free energy ΔG values are negative for lipophilic compounds with $D > 1$, which means that in these cases retention is a favored process, i.e. the solute is preferentially transferred from a polar to a relatively nonpolar environment.

We studied the partition behavior of acids (salicylic acid, *(RS)*-warfarin) and bases (*(RS)*-propranolol, lidocaine, diazepam) in a broad pH range with IAM-HPLC and compared the results with the published data for the same compounds obtained with a phosphatidylcholine liposome/buffer system (12). For extrapolation of k'_{IAMw} values, special attention was paid to explore to what extent various characteristics of the binary mixture like volume %, density, refractive index, dielectric constant and molar fraction are suited. To clarify the thermodynamic behavior of those drugs on IAM-HPLC we studied their retention behavior in the temperature range of 23 to 45°C and calculated the relevant thermodynamic parameters like ΔG, ΔH and ΔS.

MATERIAL AND METHODS

Chemicals

(RS)-propranolol·HCl, lidocaine·HCl, diazepam, salicylic acid and *(RS)*-warfarin were from Sigma (St. Louis, MO, USA).

[1] Biopharmacy, Department of Pharmacy, ETH Zürich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland.

[2] To whom correspondence should be addressed. (e-mail: wunderli-allenspach@pharma.ethz.ch)

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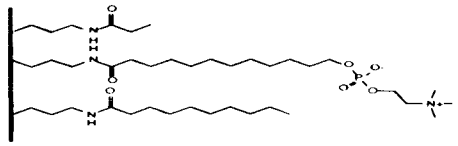

Fig. 1. Immobilized artificial membrane (IAM). 11-carboxylundecylphosphocholine on silica-propylamine matrix, end-capped with C_3-and C_{10}-alkyl chains.

Super purity organic solvents for HPLC, namely methanol (MeOH), ethanol (EtOH) and acetonitrile (ACN) were purchased from Romil Chemicals (Cambridge, Loughborough, GB). All other chemicals were of analytical grade.

Determination of Capacity Factors k'_{IAM} by IAM-HPLC

Five test compounds, i.e., *(RS)*-propranolol·HCl, lidocaine·HCl, diazepam, *(RS)*-warfarin and salicylic acid were analyzed by HPLC with a Varian 9012 delivery system. As an immobilized artificial membrane surface we used the S5-300-IAM.PC.DD column (100 × 4.6 mm, 5 µm particle size, 100 Å pore size; Regis Technology, Morton Grove, IL, USA), which contains 11-carboxylundecylphosphocholine covalently bound to the silica material and is end-capped with C_3- and C_{10}-alkyl groups (Fig. 1). Between 0.8 and 4 nmoles of drug in 20 µl solution were injected. The column was eluted with a flow rate of 1.5 ml/min. Compounds were detected with a Varian 9050 UV detector at 289 nm for *(RS)*-propranolol·HCl, at 262 nm for lidocaine· HCl, at 312 nm for diazepam, at 307 nm for *(RS)*-warfarin and at 300 nm for salicylic acid.

Capacity factors were calculated as follows (10):

$$K'_{IAM} = \frac{t_r - t_0}{t_0} \qquad (1)$$

where t_r is the retention time of the drug and t_0 the dead time of the column, which is equal to the retention time of a substance which is not retained, i.e. buffer.

Extrapolation Method for the Determination of log k'_{IAMw} Values from Co-solvent Containing Eluent Systems

To optimize elution for lipophilic compounds, organic modifiers like MeOH, EtOH or ACN were mixed with standardized modified universal buffers (SMUBS) (13) with an adjusted ionic strength of 0.23 moles/l (4). Binary mixtures were prepared with volume ratios between 0 and 50% of organic modifier in buffer and adjusted to pH 5.4. Solutions were degassed in an ultrasonic water bath prior to use. Densities of solvent mixtures were measured at 25°C on a DMA 38 density meter (Anton PAAR KG, Graz, Austria), refractive indices also at 25°C on an RFM 90 automatic refractometer (Bellingham + Stanley, Turnbridge Wells, GB). Dielectric constants were taken from the literature (14,15,16,17). Mole fractions were calculated according to:

$$\text{mole fraction} = \frac{x_{org}}{(x_{org} + x_{H_2O})} \qquad (2)$$

where x_{org} is the number of moles of organic solvent and x_{H_2O} of water in the mixture.

The following runs were performed: Diazepam or *(RS)*-propranolol·HCl were dissolved in mixtures containing different volume % of organic modifier (see above) and SMUBS. The solutions were adjusted to pH 5.4 with NaOH or HCl. 20 µl of the probes, corresponding to 0.8 and 4 nmoles of drug, respectively, were injected on the IAM-HPLC column and eluted as described above. The whole procedure was performed at room temperature. Measurements were in triplicates and capacity factors were calculated according to Eq. (1). Log k'_{IAM} values determined for the respective compositions of eluent were plotted against various parameters like volume %, densities, refractive indices, dielectric constants or mole fractions.

pH-Dependent Partitioning of Drugs with IAM-HPLC

The pH-dependent partition behavior of acids and bases was studied. Experiments were performed at 37°C in the pH range of 2.5 to about 8.0. This restricted range was due to low column stability with eluents of pH < 2.5 and pH > 8.0, respectively. Lidocaine·HCl and salicylic acid were each dissolved in SMUBS in the pH range of 2.5 to 8.0, the range also used for elution. Aliquots corresponding to 4 nmoles were injected. For the more lipophilic compounds, i.e. *(RS)*-propranolol·HCl, diazepam and *(RS)*-warfarin, MeOH/SMUBS mixtures were applied at those pH values, where the retention times of the drug in pure buffer were expected to exceed 25 min. *(RS)*-propranolol·HCl (2 nmoles) was eluted with SMUBS in the pH range of 2.5 to 7, whereas for pH 7.5 to 8.0 MeOH/SMUBS mixtures with volume ratios of 20/80 to 40/60 (v/v) were used. Diazepam (2 nmoles) was eluted with SMUBS in the pH range of 2.5 to 3.5, and with MeOH/SMUBS mixtures of 10/90 to 35/65 (v/v) in the pH range of 4.0 to 8.0. *(RS)*-warfarin (2 nmoles) was eluted using MeOH/SMUBS mixtures with volume ratios of 20/80 to 40/60 in the pH range of 2.5 to 5.0, and with SMUBS in the pH range of 6.0 to 8.0. Runs were performed as described above with triplicates at each pH. Log k'_{IAMw} values were extrapolated from log k'_{IAM} versus mole fractions plots (see Results) to 100% aqueous phase.

The partition coefficients, K_{IAM}, were calculated with the capacity factor k'_{IAMw} (Eq. (1)), the mobile phase volume V_m and the stationary phase volume V_s (10):

$$K_{IAM} = \frac{V_m}{V_s} k'_{IAM} \qquad (3)$$

For a 100 × 4.6 mm column: 1.269×10^{-6} m^3 for V_m and 5.261×10^{-8} m^3 for V_s.

Temperature-Dependent Partitioning of Drugs with IAM-HPLC

The thermodynamic parameters (ΔG, ΔH, ΔS) were determined for the partitioning of acids and bases on the IAM-HPLC column. For this purpose, temperature dependent partition experiments were performed for the five test compounds under the conditions described above. In all cases, the column was eluted with SMUBS at pH 5.4 or 7.4 in the temperature range of 23°C to 45°C. The column was prewarmed in a column heater and all solutions in a water bath for about 15–30 min at the required temperature. After runs at 45°C, column stability was tested by repeating runs in the low temperature range (<30°C). Results were reproducible as compared to the ones

obtained before temperature shift. No indication for a detrimental effect of temperatures up to 45°C was found. This is also supported by the fact that in all tested cases a linear relationship was found for the van't Hoff plot.

The Gibbs free energy of transfer (ΔG) is composed of the partial molar enthalpy ΔH and the change in entropy ΔS. It represents the solute transfer from the mobile phase to the stationary phase and is related to K_{IAMw} as follows:

$$\Delta G = \Delta H - T\Delta S = -2.303\ RT \log K_{IAMw} \quad (4)$$

where R is the gas constant (8.314 $J°K^{-1}mole^{-1}$) and T the absolute temperature [°K]. Eq. (4) can be rearranged to result in the van't Hoff equation:

$$\log K_{IAMw} = -\frac{\Delta H}{2.303\ R}\frac{1}{T} + \frac{\Delta S}{2.303\ R} \quad (5)$$

i.e. from plots of log K_{IAMw} as a function of 1/T the thermodynamic parameters can be obtained.

RESULTS

Extrapolation of k'_{IAMw} from Co-Solvent Eluent Systems

To study the retention characteristics of lipophilic compounds on IAM-columns, different organic modifiers were tested and the conditions for the extrapolation of log k'_{IAMw} values were optimized. In a first step aliquots containing 0.8 nmoles diazepam or 4 nmoles *(RS)*-propranolol·HCl were injected on the IAM column. Drugs were eluted with binary mixtures containing between 0 and 50 volume % of MeOH in SMUBS pH 5.4 (see Methods). As expected, addition of organic solvent to the aqueous phase accelerated the elution of the compounds. The retention times decreased from about 20–22 min with 0–5% organic modifier to 0.9–1.5 min with 50% organic modifier. Capacity factors were calculated with Eq. 1. Log k'_{IAM} values were plotted against the respective amounts of organic modifier used expressed as volume %, densities, refractive indices, dielectric constants or mole fractions to establish optimal conditions for the extrapolation of k'_{IAMw} (Fig. 2). The plot log k'_{IAM} versus volume % shows a bending of the curve (Fig. 2A), and the same applies for densities and refractive indices (Fig. 2B and C). The plot log k'_{IAM} versus dielectric constants gave good linear correlations for both compounds with correlation coefficients of r = 0.99780 (n = 7) and r = 0.99966 (n = 6), respectively (Fig. 2D). Very good correlations were also found in plots of log k'_{IAM} versus mole fractions with correlation coefficients of r = −0.99908 (n = 7) and r = −0.99838 (n = 6) (Fig. 2E). Extrapolation with log k'_{IAM} versus mole fraction plots was hitherto used, although plots using the dielectric constants would be equally suited. To compare various organic modifiers the experiment was repeated with EtOH or ACN instead of MeOH. The slopes of the linear correlations with log k'_{IAM} versus mole fraction plots were calculated for *(RS)*-propranolol and diazepam (Table 1). For both drugs the steepness of the slopes increased from MeOH to EtOH to ACN. With all organic modifiers tested diazepam, a neutral molecule at pH 5.4, gave a steeper slope than *(RS)*-propranolol, which is positively charged at pH 5.4. The extrapolated log k'_{IAMw} values from the different co-solvent systems were very similar: 1.317 to 1.334 for *(RS)*-propranolol and 1.528 to 1.573 for diazepam. All following experiments were performed with MeOH/buffer mixtures if an organic modifier was needed.

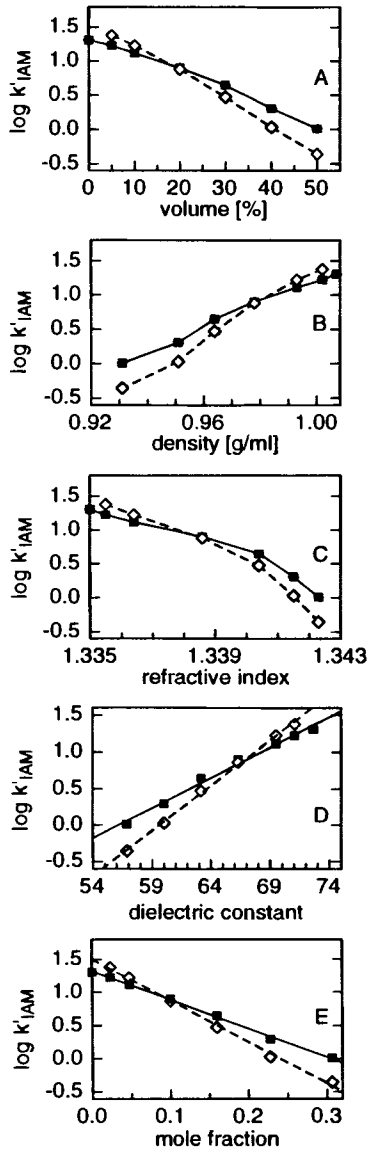

Fig. 2. Capacity factors log k'_{IAM} of drugs from IAM-HPLC as a function of the amount of organic modifier. Diazepam (◇) and *(RS)*-propranolol·HCl (■) were each solved in mixtures of MeOH/SMUBS at pH 5.4 and run on the IAM-HPLC column as described (see Methods). Log k'_{IAM} values were calculated and plotted against the amount of organic modifier expressed as: (A) volume %, (B) densities (1.007 g/ml for 100% water phase), (C) refractive indices (1.335 for 100% water phase), (D) dielectric constants (74.3 for 100% water phase at 37°C (15); corrected for the ionic strength according to (29)), and (E) mole fractions (see Methods).

pH-Dependent Partitioning of Drugs with IAM-HPLC

The pH-dependent partition behavior of diazepam, *(RS)*-propranolol, lidocaine, *(RS)*-warfarin and salicylic acid was studied on the IAM-HPLC column. Runs were performed at pH values between 2.5 and 8.0. Higher pH values were not possible due to column instability. Capacity factors k'_{IAM} and

Table I. Extrapolation of log k'_{IAMw} Values of (*RS*)-Propranolol and Diazepam: Comparison of Organic Modifiers

	(*RS*)-propranolol		Diazepam	
	slope	log k'_{IAMw}	slope	log k'_{IAMw}
MeOH	−4.38 ± 0.01	1.32 ± 0.01	−6.58 ± 0.03	1.53 ± 0.01
EtOH	−6.81 ± 0.01	1.32 ± 0.01	−10.46 ± 0.01	1.57 ± 0.01
ACN	−11.15 ± 0.02	1.33 ± 0.01	−13.04 ± 0.01	1.53 ± 0.01

Note: Experiments with (*RS*)-propranolol and diazepam were performed as described. Log k'_{IAMw} values were extrapolated by linear regression of the plot log k'_{IAMw} versus mole fractions of organic modifier/SMUBS at pH 5.4. Values are listed with their standard deviations (n = 3).

partition coefficients K_{IAMw} were calculated according to Eq. 1 and 3 respectively, and compared with D values from the PhC-liposome/buffer system (12). Data are presented in Fig. 3. With diazepam (pK_a 3.3) a steep increase of K_{IAMw} values from 90 at pH 2.5, where diazepam is mainly protonated, to about 600 at pH 5, where diazepam is mainly neutral, was seen. K_{IAMw} values remained constant between pH 5 and pH 7. Above pH 7 a slight decrease to about 500 occurred. For comparison, membrane distribution coefficients ($D_{mem(EDS)}$) of about 1000 were found with PhC-liposomes in the equilibrium dialysis system (EDS) between pH 5.5 and 10.0. With the HPLC method stability of drugs plays no role for the retention times. Therefore, measurements with diazepam, which is unstable in acid environment, could also be performed at low pH on IAM-HPLC, in contrast to the liposome/buffer system in which the drug degraded during equilibrium dialysis.

With (*RS*)-propranolol a gradual increase of K_{IAMw} values from 350 to 700 between pH 3 and pH 7 was observed. This is in contrast to D vs. pH diagrams obtained with PhC-liposomes where a plateau of 577 had been found between pH 3 and pH 7. In this pH range (*RS*)-propranolol is mainly positively charged. Above pH 7 a steep increase up to 1220 around pH 8.5 was observed. Column instability precluded measurements in the interesting pH range around the pK_a of (*RS*)-propranolol, i.e. 9.24.

For lidocaine (pK_a 7.8) K_{IAMw} values of about 10 were found between pH 2.5 and 5 with IAM-HPLC; they were in the same range as the D values obtained with liposomes. The values gradually increased to a K_{IAMw} of about 25 at pH 7 and then to 72 at pH 8, similar to the values obtained in the liposome/buffer system. Again measurements in the pH range around the pK_a value were limited by column instability.

(*RS*)-warfarin, an acidic compound with a pK_a value of 5.0, yielded highest K_{IAMw} values, i.e. 2500, around pH 3, where it is in the neutral form. The corresponding value in the liposomal system was 2433. Between pH 4 and 5.5 a steep drop was seen to about 600 which was followed by a more gradual decrease to 130 at pH 8. With PhC-liposomes the steep drop was followed by a low plateau of 20 at pH values above pH 6.

For salicylic acid (pK_a 3.0) the K_{IAMw} value was highest, namely 170 at the lowest pH used, i.e. 2.5. A steep drop was observed between pH 2.5 and 4. As with (*RS*)-warfarin a shoulder was seen between pH 4 and 8 with decreasing K_{IAMw} values from 40 to 6. With liposomes D at pH 2.5 was about 220 and reached a plateau of about 8 around pH 5 which remained up to pH 10.

Thermodynamic Parameters for the Partitioning of the Test Compounds on IAM-HPLC Columns

To obtain the thermodynamic parameters of the test compounds on the IAM-HPLC column, their elution was followed in the temperature range of 23 to 45°C at pH 7.4 and 5.4. The respective capacity factors ln k'_{IAMw} were determined and the corresponding log K_{IAMw} values plotted versus the reciprocal of the temperatures 1/T according to van't Hoff (Eq. 5). At both pH values linear plots were obtained (Fig. 4). The capacity factors for all tested drugs decreased with increasing temperature. The thermodynamic parameters were calculated (Table II). In all cases the Gibbs free energy (ΔG) was negative with values between −5.7 and −17.3 kJ/mole at 37°C. The enthalpy (ΔH) values were negative for all drugs with values between −2.9 and −35.2 kJ/mole. For the entropy parameter ΔS values were negative for the acids and positive for the bases. The relevance of these findings will be discussed.

DISCUSSION

Exploration of the pH-dependent partition behavior of a set of model compounds (acids and bases) on IAM columns revealed some interesting features which now will be discussed in comparison with data from the PhC liposome/buffer system also presented here. Plots of the respective log K_{IAMw} values and log $D_{mem(EDS)}$ values at pH 7.4 and pH 5.4 reveal a linear relationship (Fig. 5) with a correlation coefficient of r = 0.8983 (n = 5), a slope of 0.99 and an intercept of −0.11 for pH 7.4, and a correlation coefficient of 0.9865 (n = 5), a slope of 1.23 and an intercept of −0.62 for pH 5.4. This linearity is in agreement with previous studies on the partition behavior of 23 solutes with ^{ether}IAM.PC$^{C10/C3}$ surfaces as compared to DMPC liposomes (6, 19). A closer look at Fig. 5 reveals that certain drugs deviate from linearity, and that they are not the same at pH 7.4 and 5.4. At pH 7.4, it is the (*RS*)-warfarin that reduces the correlation coefficient, whereas at pH 5.4 the largest difference between the respective log K_{IAMw} values and log $D_{mem(EDS)}$ values is found for salicylic acid. More information about the characteristics of PhC liposomes and IAM columns are gained from closer analysis of the pH-partition-diagrams of the five model compounds, which is facilitated by plotting partition coefficients at a linear and a logarithmic scale (see Fig. 3). There are striking similarities between the pH-K_{IAMw}- and the pH-$D_{mem(EDS)}$-diagrams regarding the shape and heights of the curves, which is reflected in the plots of the respective log K_{IAMw} values and log $D_{mem(EDS)}$ values at pH 7.4 and 5.4. However, there are also systematic differences between the two curves. Instead of the plateau found for the D values of both the neutral and the ionized species in the liposomal system (for details see (12)), the K_{IAMw} values show a gradual decrease, for the protonated bases with decreasing pH values and for the deprotonated acids with increasing pH, as far as the respective pH ranges were accessible with IAM-HPLC. In general the affinity of a drug to a particular lipid membrane strongly depends on the ionization states of the drug and of the lipid components. As has been demonstrated with liposomes using (*RS*)-propranolol as a model compound, the pH-partition diagram for a certain solute follows the ionization curves of both

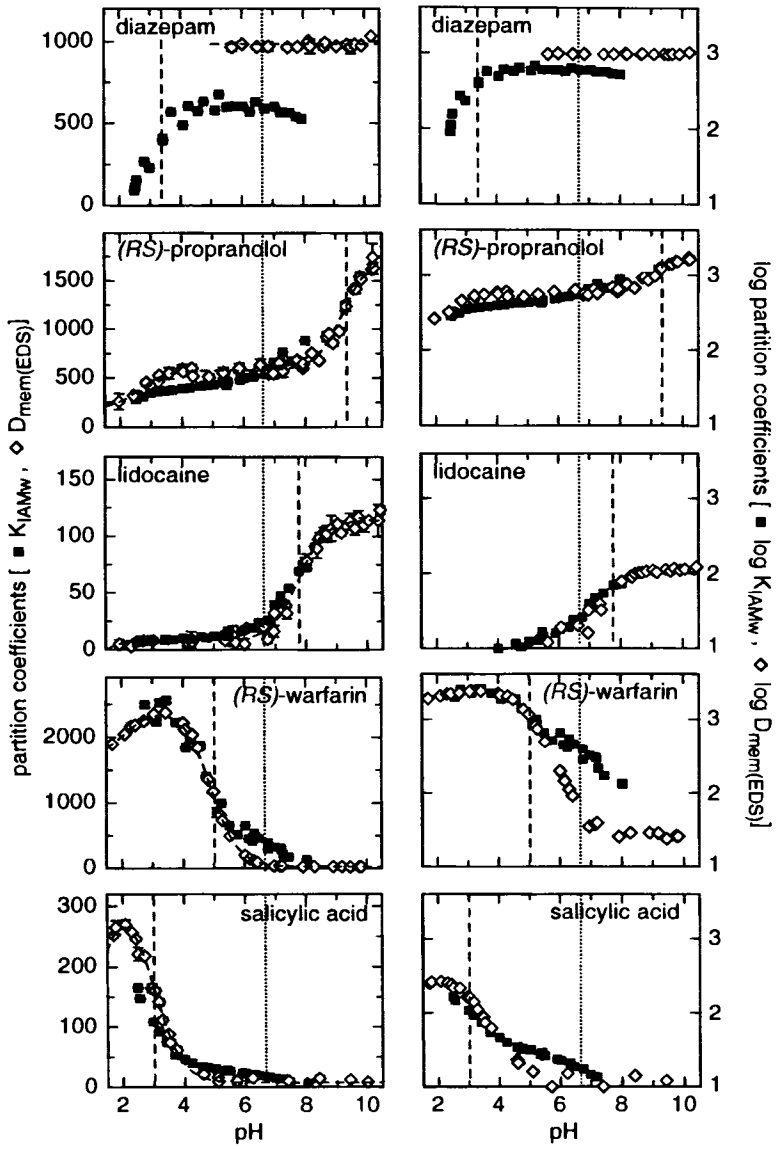

Fig. 3. Comparison of the partition-pH diagrams from IAM-HPLC (K_{IAMw}) and from PhC-liposomes in the equilibrium dialysis system ($D_{mem(EDS)}$). Partition-pH diagrams were determined as described (see Methods). Capacity factors from the MeOH/SMUBS mixtures were extrapolated to 100 % aqueous phase by mole fractions. K_{IAMw} values from the IAM-HPLC (■) were calculated by Eq. 3. The partition behavior was compared with the $D_{mem(EDS)}$ obtained from PhC-liposomes (◇) with the equilibrium dialysis system (12). For a detailed analysis partition coefficients are plotted on a linear scale (*left*) and on a logarithmic scale (*right*). (---) pK_a values of the respective drugs (for *(RS)*-propranolol see (4); other drugs see (18); (····) pK_a values of the presumed silanol groups.

the solute and the lipids (20,21,22). IAM columns have been designed with lipids which do not show any change in the ionization state over the pH range of about 2 to 8, the range in which the columns are stable. Therefore, the shape of the partition-pH curve of a particular solute on these columns should only be influenced by its ionization state. The occurrence of changes in the K_{IAMw} values despite the theoretically unchanged ionization state of solute and lipid indicates that an artefact is interfering with the drug-membrane interaction particularly in the range of pH 5 to 8, i.e. around the physiological pH. We ascribe the shoulder between pH 5.0 and 8.0 in the pH-partition diagram, which is most prominent with the acids, to non-specific interactions of the drugs with column material, for instance with the silica surface. Although the silica propylamine residues are end-capped with alkyl groups, some free silanol groups are always present (23). The pK_a value of silanol groups is found between 6.3 and 6.8 (24,25). The following interactions of acids and bases with silanol groups have been proposed (26):

[R-O$^-$ + HO-Si ⇆ R-O$^-$···H···O-Si-] and [R_3NH^+ + $^-$O-Si- ⇆ R_3NH^+ $^-$O-Si-]. Evidence for such an interaction comes from the titration of silicagel in 0.15 M KCl which

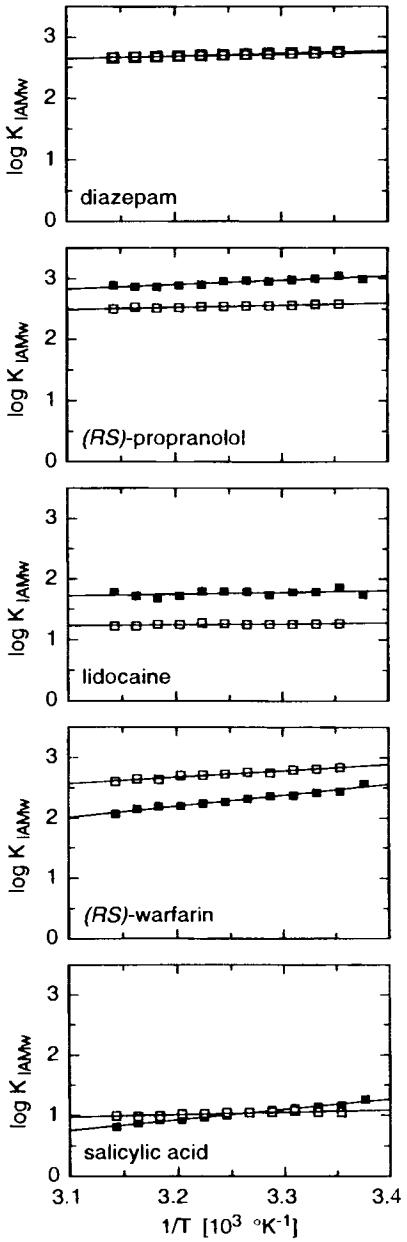

Fig. 4. Temperature-dependent partition coefficients log K_{IAMw} obtained from IAM-HPLC (van't Hoff plot). 20 µl corresponding to 2 to 4 nmoles of drug were injected on the IAM-HPLC column. Elution was performed with SMUBS at pH 5.4 (–) or 7.4 (■), respectively, in the temperature range of 23°C to 45°C with a flow rate of 1.5 ml/min.

revealed pK_a values of 5.70 ± 0.06 and 2.36 ± 0.13. Values are expected to be slightly higher in the apolar environment of the IAM column. This has been substantiated by the titration of free silanol groups in a lipid capped silicagel preparation in which pK_a values of 6.3 and 3.1 were found (Avdeef, personal communication). Electrostatic interaction of the drug with the silanol groups prolongs the retention times and thus increases K_{IAMw} values. This leads to an additional inflection point as a result of the additional dissociation function of the silanol groups. Partition values from IAM-columns in the pH range of 5 to 8 have to be validated and eventually confirmed by other methods. This non-specific binding limits the use of the IAM-columns for one point determinations of partition coefficients in the physiological pH range.

Table II. Thermodynamic Parameters of Drugs on IAM-HPLC Columns

	ΔH [kJ/mole]		ΔS [J/mole °K]		$\Delta G^{37°C}$ [kJ/mole]	
	pH 7.4	pH 5.4	pH 7.4	pH 5.4	pH 7.4	pH 5.4
diazepam	−8.6	−5.5	+23.9	+33.7	−16.0	−16.0
(RS) -propranolol	−13.9	−7.0	+11.0	+25.8	−17.3	−15.0
lidocaine	−5.5	−2.9	+15.9	+14.6	−10.4	−7.4
(RS) -warfarin	−35.2	−20.2	−70.7	−13.3	−13.3	−16.0
salicylic acid	−32.4	−7.0	−86.0	−3.2	−5.7	−6.0

Note: The thermodynamic parameters were determined from the van't Hoff plots shown in Fig. 4 by linear regression.

Comparison of the pH-partition diagrams determined by the IAM-column and the liposomal system, respectively, show another important feature. Due to the instability of IAM-columns at pH values lower than 2 and higher than 8 complete pH-partition diagrams, comprising K_{IAMw} values for the neutral as well as for the ionized species, can only be obtained for compounds with pK_a values between about 4 and 6, i.e. in a very restricted range. In contrast PhC liposomes have been shown to be stable between pH 2 and 11. The instability of the columns together with the above discussed artefact make the determination of log P values by IAM-HPLC a difficult task. It is clear from our results, however, that IAM columns have a great potential if more stable IAM surfaces can be developed which do not lead to artificial retention, presumably due to free silanol groups. The advantages of IAM-HPLC over the equilibrium dialysis liposomal system regarding time and resources are evident. It may be the method of choice for unstable solutes. A valid alternative to both methods has been introduced with the potentiometric titration method using liposomes as lipid phase (27). It is less work intensive than the equilibrium dialysis liposomal system, but also permits to determine P values for the neutral and the ionized molecules. In contrast, the octanol/buffer shake flask system does not represent a valid model for the *in vivo* fluid cell membranes, as only the hydrophobic contribution of the drug-membrane interaction can be studied (6).

For the elution from the IAM column buffer is only used for hydrophilic drugs. However, for lipophilic compounds different organic modifier-buffer mixtures have to be applied. As already indicated previously (9), linearization of the log k'_{IAM} versus volume % plot, as often used in reversed-phase HPLC, is not adequate. This statement has been confirmed in our study and can be extended to the plots of log k'_{IAM} versus density and log k'_{IAM} versus refractive index. Only the plots with log k'_{IAM} versus mole fractions or dielectric constants of the mixtures gave a linear correlation. The linear dependence between the capacity factor and the dielectric constant demonstrates that the relative polarities between solute and solvent are linearly related.

As expected no significant difference was found between k'_w values extrapolated from experiments with MeOH, EtOH or ACN as organic modifier. Comparison of the slope S for *(RS)*-propranolol and diazepam at pH 5.4 with the three organic modifiers was made. The steepest slope was obtained with

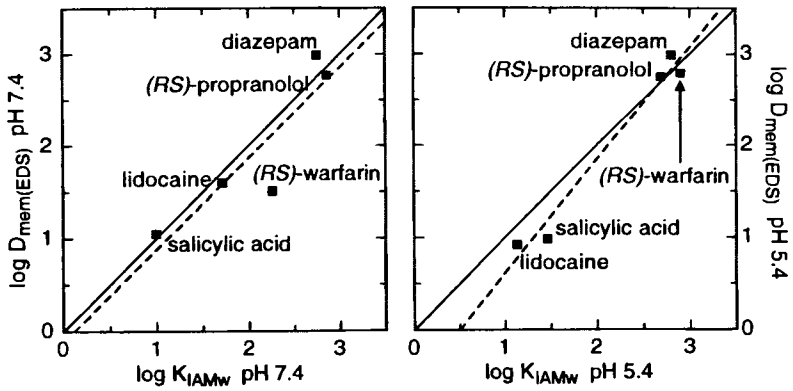

Fig. 5. Comparison of lipophilicity parameters determined with IAM-HPLC and the liposome/buffer system, respectively. (——) diagonal where log K_{IAMw} equals log $D_{mem(EDS)}$; (· · ·) linear regression through the plotted data.

ACN, which then was followed by EtOH and MeOH. The polarity scale of solvents is thus MeOH > EtOH > ACN. Because MeOH is a weaker organic modifier than EtOH and ACN, the same change in the amount of organic modifier will affect the hydrophobic expulsion process less extensively than with EtOH and ACN (28). With all three modifiers used diazepam, which is neutral at pH 5.4, shows a steeper slope than *(RS)*-propranolol, which at the same pH is positively charged.

Studies on the temperature-dependent partition behavior revealed linear van't Hoff plots for the five model compounds at pH 7.4 as well as at pH 5.4. With increasing temperature decreasing K_{IAMw} were observed which means that the partial molar enthalpy ΔH was negative in all studied cases. This corresponds to the findings of Ong and Pidgeon (10) with the same column material, who studied the partitioning of a set of phenol derivatives as well as of various β-blockers. The change in entropy ΔS was negative for the two acids and positive for the three bases. The calculated Gibbs free energies of transfer were negative at 37°C ($\Delta G^{37°C}$) reflecting partition coefficients $K_{IAMw} > 1$. No clear picture could be gained regarding the respective impact of entropy and enthalpy for the partitioning of the tested compounds. At pH 5.4 and 7.4 all molecules except for *(RS)*-warfarin and lidocaine are either fully ionized (salicylic acid and *(RS)*-propranolol) or neutral (diazepam). *(RS)*-warfarin is fully ionized at pH 7.4 and to about 70% ionized at pH 5.4, whereas lidocaine is fully ionized at pH 5.4 and to about 70% ionized at pH 7.4. Similar thermodynamic parameters at pH 7.4 and pH 5.4 would be expected for the compounds which are fully ionized or neutral at both pH values. The differences in the calculated numbers are an indication for the presence of an artefact on the column, which gives rise to differences in drug-column interactions at pH 5.4 and 7.4 respectively. This precludes further thermodynamic analysis of the studied drug-lipid membrane interactions on IAM columns.

NOTATIONS

D	Apparent partition coefficient, distribution coefficient
$D_{mem(ES)}$	Apparent partition coefficient determined by equilibrium dialysis in the liposomal system
IAM	Immobilized artificial membrane
k'_{IAM}	Capacity factor
k'_{IAMw}	Capacity factor in 100% water phase
K_{IAM}	Apparent partition coefficient determined by IAM-HPLC
P	True partition coefficient
PhC	Phosphatidylcholine
SMUBS	Standardized modified universal buffer system

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REFERENCES

1. L. Herbette, A. M. Katz, and J. M. Sturtevant. Comparisons of the interaction of propranolol and timolol with model and biological membrane systems. *Mol. Pharmacol.* **24**:259–269 (1983).
2. R. P. Mason, D. G. Rhodes, and L. G. Herbette. Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. *J. Med. Chem.* **34**:869–877 (1991).
3. G. V. Betageri and J. A. Rogers. Thermodynamics of partitioning of β-blockers in the *n*-octanol-buffer and liposome systems. *Int. J. Pharm.* **36**:165–173 (1987).
4. G. M. Pauletti and H. Wunderli-Allenspach. Partition coefficients in vitro: artificial membranes as a standardized distribution model. *Eur. J. Pharm. Sci.* **1**:273–282 (1994).
5. C. Pidgeon and U. V. Venkataram. Immobilized artificial membrane chromatography: supports composed of membrane lipids. *Anal. Biochem.* **176**:36–47 (1989).
6. S. Ong, H. Liu, X. Qiu, G. Bhat, and C. Pidgeon. Membrane partition coefficients chromatographically measured using immobilized artificial membrane surfaces. *Anal. Chem.* **67**:755–762 (1995).
7. D. Rhee, R. Markovich, W. G. Chae, X. Qiu, and C. Pidgeon. Chromatographic surfaces prepared from lyso phosphatidylcholine ligands. *Anal. Chim. Acta* **297**:377–386 (1994).
8. J. J. Michels and J. G. Dorsey. Retention in reversed-phase liquid chromatography: solvatochromic investigation of homologous alcohol-water binary mobile phases. *J. Chromatogr.* **457**:85–98 (1988).
9. J. G. Dorsey and M. G. Khaledi. Hydrophobicity estimations by reversed-phase liquid chromatography. Implications for biological partitioning processes. *J. Chromatogr. A* **656**:485–499 (1993).
10. S. Ong and C. Pidgeon. Thermodynamics of solute partitioning

into immobilized artificial membranes. *Anal. Chem.* **67**:2119–2128 (1995).
11. L. A. Cole and J. G. Dorsey. Temperature dependence of retention in reversed-phase liquid chromatography. 1. Stationary-phase considerations. *Anal. Chem.* **64**:1317–1323 (1992).
12. C. Ottiger and H. Wunderli-Allenspach. Partition behaviour of acids and bases in a phosphatidylcholine liposome-buffer equilibrium dialysis system. *Eur. J. Pharm. Sci.* **5**:223–231 (1997).
13. T. Teorell and E. Stenhagen. Ein Universalpuffer für den pH-Bereich 2.0 bis 12.0. *Biochem. Z.* **299**:416–419 (1938).
14. H. H. Landolt and R. L. Börnstein. *Elektrische Eigenschaften I.* Band 2, Springer Verlag, Stuttgart, 1960.
15. P. S. Albright and L. J. Gosting. Dielectric constants of the methanol-water system from 5 to 55°. *J. Am. Chem. Soc.* **68**:1061–1063 (1946).
16. G. Kortüm, S. D. Gokhale and H. Wilski. Über Leitfähigkeitsmessungen an Tetraäthylammoniumjodid in Lösungsmittelgemischen. *Z. Physik. Chem., Neue Folge* **4**:286–296 (1955).
17. H. S. Harned and B. B. Owen. *The physical chemistry of electrolytic solutions.* Reinhold Publishing Corporation, New York, 1958.
18. K. Hartke, H. Hartke, E. Mutschler, G. Ruecker and M. Wichtl. *DAB 10: Deutsches Arzneibuch, Kommentar.* Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1991.
19. C. Pidgeon, S. Ong, H. Choi, and H. Liu. Preparation of mixed ligand immobilized artificial membranes for predicting drug binding to membranes. *Anal. Chem.* **66**:2701–2709 (1994).
20. S. D. Krämer and H. Wunderli-Allenspach. The pH-dependence in the partitioning behaviour of (*RS*)-[^{3}H]Propranolol between MDCK cell lipid vesicles and buffer. *Pharm. Res.* **13**:1851–1855 (1996).
21. S. D. Krämer, C. Jakits-Deiser, and H. Wunderli-Allenspach. Free fatty acids cause pH-dependent changes in drug-lipid membrane interactions around physiological pH. *Pharm. Res.* **14**:827–832 (1997).
22. S. D. Krämer, A. Braun, C. Jakits-Deiser, and H. Wunderli-Allenspach. Towards the predictability of drug-lipid membrane interactions: the pH-dependent affinity of propranolol to phosphatidylinositol containing liposomes. *Pharm. Res.* **15**:739–744 (1998).
23. R. J. Markovich, X. Qiu, D. E. Nichols, C. Pidgeon, B. Invergo, and F. M. Alvarez. Silica subsurface amine effect on the chemical stability and chromatographic properties of end-capped immobilized artificial membrane surfaces. *Anal. Chem.* **63**:1851–1860 (1991).
24. P. Schindler and H. R. Kamber. Die Acidität von Silanolgruppen. *Helv. Chim. Acta* **51**:1781–1786 (1968).
25. Y. Guo, G. A. Imahori, and L. A. Colon. Hydrolytically stable amino-silica glass coating material for manipulation of the electroosmotic flow in capillary electrophoresis. *J. Chromatogr. A* **744**:17–29 (1996).
26. J. W. Dolan and L. R. Snyder. *Troubleshooting LC systems: a comprehensive approach to troubleshooting LC equipment and separations.* Humana Press, Clifton, NJ, 1989.
27. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, and K. Y. Tam. pH-metric logP 10. Determination of liposomal membrane-water partition coefficients of ionizable drugs. *Pharm. Res.* **15**:209–215 (1998).
28. F. Barbato, M. I. La Rotonda, and F. Quaglia. Interactions of nonsteroidal antiinflammatory drugs with phospholipids: Comparison between octanol/buffer partition coefficients and chromatographic indexes on immobilized artificial membranes. *J. Pharm. Sci.* **86**:225–229 (1997).
29. J. B. Hasted, D. M. Ritson, and C. H. Collie. Dielectric properties of aqueous ionic solutions. Parts I and II. *J. Chem. Physics* **16**:1–21 (1948).