Ionization Constants and Distribution Coefficients of Phenothiazines and Calcium Channel Antagonists Determined by a pH-Metric Method and Correlation with Calculated Partition Coefficients

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Abstract □ The pH–metric technique was used to determine the ionization constants and distribution coefficients of 10 phenothiazines and five ionizable calcium channel antagonists. Because the studied compounds were poorly water soluble and quite lipophilic with partition coefficients in the range of 3.5 to 5.5, organic cosolvents had to be added for the determination of the ionization constants to avoid precipitation of the free bases. The effect of the cosolvents dioxane and methanol on the extrapolation to pure water was compared. For both cosolvents a very good agreement with accessible published ionization constants was obtained, however the slope of the regression line was much smaller for dioxane, yielding more reliable estimates according to the standard deviation of the extrapolated values. Thus, dioxane might be preferable to methanol as a cosolvent for the determination of ionization constants of sparingly water soluble bases. Also the *n*-octanol/water partition coefficients were determined and compared with published data and values calculated with the ClogP, ACD, and HINT programs. Although the obtained values were approximate in conformity with the published data, the calculated partition coefficients differed from the experimental ones considerably for the majority of the investigated compounds. Furthermore, the ion pair partitioning and the distribution coefficients at physiological pH 7.4 were determined. The pH-dependent distribution profiles showed the strong influence of the ionization constants and of the distribution of the ion pairs on the overall distribution. This result strongly suggests that greater use should be made of measured distribution coefficients in quantitative structure–activity relationship studies. The potentiometric method is a convenient way to determine the distribution properties of drug molecules at pH values relevant for the biological system under investigation.

Introduction

The knowledge of drug membrane interactions is important in the understanding of the biological action of many drugs.[1] Often the pharmacological behavior of drugs is related to their distribution or nonspecific binding in or to membranes.[2,3] *n*-Octanol/water is often used to model the distribution of a drug by measuring the partition coefficient in this system.[4] The partition coefficient, *P*, is most commonly defined for the uncharged form of an ionizable substance; in addition it can be also defined for the charged form of the substance. Often the neutral and charged forms of lipophilic molecules are able to partition into the organic phase in solvent/water mixtures.[5] The pH-dependent distribution profile enables the identification of the number of species present and is a powerful tool for comparing the properties of clusters of similar compounds.[6] Precisely and accurately determined values of the ionization constants, pK_a, and the partition coefficients of the neutral and charged species are necessary to obtain distribution coefficients. The conventional methods for measuring pK_a and/or log *P* values are UV spectroscopy, the shake-flask method,[7] HPLC,[8] and centrifugal partition chromatography,[9] respectively. A major drawback of these methods is that they are very time-consuming. An additional problem for these kinds of determination is often the very low solubility of pharmaceutical compounds. The acid−base titration in aqueous solution is an alternative for ionizable compounds.[10−15] To increase the solubility, a water-miscible cosolvent (methanol, dioxane, dimethyl sulfoxide) is added for the pK_a determination of sparingly water soluble compounds.

For phenothiazines used as neuroleptics, a good correlation between the determined log *P* values and a selected biological action has been reported.[16−20] Also, for calcium channel antagonists, the lipophilicity and interaction with membrane phospholipids seem to play a major role in their pharmacological activity.[2] In our efforts to characterize and better understand drug−membrane interactions and the distribution of membrane-active compounds, we first report in this paper the application of the pH−metric method to the determination of pK_a, log *P*, and log P_{ion} values for 10 structurely related phenothiazines and five calcium channel antagonists in *n*-octanol/water.

The aims of this work were severalfold: to validate the pH−metric method and to advance the exact analysis of pK_a values and the distribution in *n*-octanol/water of neutral and positively charged drugs; to study the influence of the cosolvents dioxane and methanol necessary in cases of poorly soluble compounds on the determined pK_a value; to compare experimental with calculated log *P* values to estimate the reliability of the latter ones; to establish the pH-dependent distribution profile of every sample; and to compare the application of either the log *P* values or apparent distribution coefficient (log *D*) values at physiological pH in quantitative structure−activity relationships (QSAR) studies (i.e., in attempting to predict the pharmaceutical potency or the in vivo absorption).

Experimental Section

Reagents—The pharmaceutical substances used are as follows: promethazine hydrochloride, chlorpromazine hydrochloride, triflupromazine hydrochloride, prochlorperazine dimaleate, trifluoperazine dihydrochloride, perphenazine, fendiline hydrochloride, and 1-octanol (HPLC grade) from Sigma Chemical Company, Germany; verapamil hydrochloride from Aldrich, Germany; methanol (HPLC grade) from Carl Roth GmbH, Karlsruhe, Germany; hydrochloric acid standardized ampules and potassium hydroxide

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standardized ampules from Riedel-de-Haen AG, Seelze, Germany; and potassium chloride (pro analysis) and dioxane from VEB Laborchemie, Apolda, GDR. The dioxane was distilled twice before use. The following drugs were generous gifts: levomepromazine hydrochloride from Bayer AG, Leverkusen, Germany; thioridazine hydrochloride and thiethylperazine dimaleate from Sandoz AG, Nürnberg, Germany; gallopamil hydrochloride from Knoll Deutschland GmbH, Ludwigshafen, Germany; amlodipine besylate from Pfizer GmbH, Karlsruhe, Germany; and nicardipine hydrochloride from Ciba-Geigy GmbH, Wehr, Germany. The water used was purified with an Elgastat Maxima, Elga Ltd., Bucks, UK.

Practical Part of pH−Metric Method—The pK_a and the log *P* values were determined by potentiometric titration using a PCA 101 instrument from Sirius Analytical Instruments Ltd., Forrest Row, UK, at 25.0 ± 0.2 °C at an adjusted ionic strength of the aqueous phase of 0.15 M potassium chloride. Details of the method have been previously published elsewhere.[10−15] The pK_a and log *P* measurements were done in separate experiments, and the concentration of the drug titrated was between 0.08 and 0.15 mM. The lower pK_a values of the diprotic phenothiazines were determined in triplicate in pure aqueous 0.15 M KCl. To avoid precipitation of the neutral bases, certain amounts of cosolvents were added in case of the higher pK_a values. The concentration of the dioxane cosolvent varied between 15 and 40 wt % and, for the phenothiazines, 20−50 wt % methanol cosolvent was used additionally. The apparent pK_a values (p$_s K_a$) were measured in at least five different cosolvent/water mixtures. The log *P* determinations were replicated at least three times with different volume ratios of *n*-octanol/water. Because of the high partitioning of all samples into the organic phase, a reasonable volume ratio of 0.005 to 0.05 was applied (0.1 to 1.0 mL octanol in 20 mL total volume). For the dimaleate counterion present in the prochlorperazine and thiethylperazine salts, a pK_a of 5.78 and a log *P* of −0.47 were determined in the pH range between pH 2.5 and 10.5, which is in close agreement with the literature (pK_a = 5.80; log *P* = −0.4).[21]

Theoretical Part of pH−Metric Method—An iterative least squares refinement procedure was used to calculate the final values. Typically, the values of the goodness of fit (GOF) weighting scheme used for all refinements were in the range from 0.6 to 1.4 for pK_a measurements without cosolvent, log *P* titrations, and multi-set refinements. A GOF value of 1 means that on average the observed and calculated curve differ not more than one internal standard deviation of the device. The Yasuda−Shedlovsky procedure extrapolates the real aqueous pK_a from the obtained p$_s K_a$ values by plotting the p$_s K_a$ value and the logarithm of the water concentration against the reciprocal of the dielectric constant, ϵ, for the mixtures according to eq 1:[13]

$$p_s K_a + \log [H_2O] = a + b/\epsilon \quad (1)$$

A negative slope of the regression line is characteristic for basic substances. To investigate the reliability of the electrode calibration factors supplied, comparative titrations in dioxane and methanol cosolvent mixtures were performed.

The partition coefficient, log *P*, of the neutral bases was calculated according to eq 2:

$$\log P = \log(10^{(pK_a - p_o K_a)} - 1) - \log(r) \quad (2)$$

If only the partitioning of the neutral species into *n*-octanol was assumed, the calculated partition coefficients decreased with increasing amounts of *n*-octanol, indicating a significant contribution of the monoprotonated form to partitioning. Therefore, the partitioning of the monoprotonated form was taken into account in the fitting process. For the monoprotic bases, the partition coefficient of the ion, log P_{ion}, was calculated iteratively, correcting the apparent log *P* value obtained in different *n*-octanol/water ratios to a constant value. According to this procedure, only the partitioning of the monoprotonated form accounted for the distribution for the investigated diprotic phenothiazine derivatives. To investigate the influence of the uncertainty in the pK_a determination on the log *P* measurement, the log *P* values were calculated again at ± one standard deviation of the determined pK_a value. Only in this manner, by taking into account the errors of measurements and extrapolations, can a realistic estimate of the reliability of the log *P* results be obtained. Finally, the pH-dependent apparent distribution coefficients, log *D*, were calculated as pH − log *P* − log P_{ion} profile by the analysis software.

Table 1—Comparison of Measured pK_a Values with Published Data

drug	measured pK_a in dioxane[a]	in methanol[b]	in water[c]	published pK_a
promethazine	8.86 ± 0.01[d]	9.07 ± 0.08		9.11,[29] 9.10[30]
promazine	9.00 ± 0.05[d]	8.92 ± 0.09		9.28,[29] 9.40[16]
chlorpromazine	9.15 ± 0.09[d]	9.22 ± 0.13		9.30,[29] 9.30[16]
triflupromazine	8.95 ± 0.03[d]	9.07 ± 0.19		9.20,[30] 9.20[16]
levomepromazine	9.03 ± 0.09[d]	9.07 ± 0.16		9.19[29]
thioridazine	9.19 ± 0.06[d]	9.25 ± 0.07		9.50[30]
prochlorperazine	3.77 ± 0.16	3.73 ± 0.04	3.79 ± 0.01[d]	3.73,[31] 3.78[30]
	8.21 ± 0.13[d]	7.96 ± 0.09		8.10[31]
trifluoperazine	3.70 ± 0.11	3.95 ± 0.35	3.91 ± 0.01[d]	3.60,[32] 3.90[5]
	8.11 ± 0.06[d]	8.38 ± 0.13		8.10,[32] 8.10[5]
thiethylperazine	3.65 ± 0.02	3.81 ± 0.27	3.80 ± 0.01[d]	
	8.00 ± 0.09[d]	8.06 ± 0.06		8.12[29]
perphenazine	3.97 ± 0.09	3.98 ± 0.05	3.59 ± 0.01[d]	3.70[31]
	7.90 ± 0.09[d]	7.82 ± 0.06		7.80[31]
verapamil	8.68 ± 0.09[d]			8.60,[33] 8.92[30]
gallopamil	8.57 ± 0.06[d]	8.42 ± 0.01		
fendiline	9.04 ± 0.03[d]			
amlodipine	9.31 ± 0.10[d]			9.02[34]
nicardipine	7.28 ± 0.10[d]			7.20,[35] 7.33[36]

[a] With cosolvent dioxane and Yasuda–Shedlovsky extrapolation. [b] With cosolvent methanol and Yasuda–Shedlovsky extrapolation. [c] In only aqueous solution. [d] pK_a value used for log *P* determination.

Calculation of log *P* Values—For comparative purposes, the partition coefficients in the *n*-octanol/water system were calculated with the programs ClogP,[22] ACD,[23] and HINT.[24] ClogP is based on the two-dimensional fragment method developed by Leo and Hansch[25] using fragment constants derived from differences in partition coefficient of substituted and unsubstituted compounds together with several correction terms that account for neighbor-group effects. The ACD program uses a similar algorithm, where the log *P* contribution values of atoms, fragments, and intramolecular interactions were derived from 3600 experimental log *P* values.[23] The calculated results are given with auxiliary certainty limits. HINT was developed as computational method for three-dimensional structures using hydrophobic atom constants. The lipophilicity contributions of hydrogens are constant and the lipophilicity values of the central atom(s) of a fragment are adjusted in a way that the sum of the atomic constants is equal to the fragment-constant value.[26] This program offers two methods for taking into account neighboring effects, either via bonds (e.g., according to the connectivity pattern) or through space, thus considering additionally the conformation of a molecule. The three-dimensional modeling was done with SYBYL.[27] The starting structures were extracted from the Cambridge Structural Database,[28] where entries for all compounds except gallopamil and nicardipine were found. The structures for these two compounds were built by modification of the X-ray structures of verapamil and amlodipine, respectively. The geometry optimization and the atom charge calculation of the neutral forms was performed with MOPAC 6 as implemented in SYBYL using the AM1 Hamiltonian and the keywords 'precise' and 'gnorm-0.2'. Both HINT log *P* values, with proximity effects via bonds, HINT-2D, and through space, HINT-3D, were calculated for the optimized structures.

Results and Discussion

The pK_a values measured in this study together with available published data are shown in Table 1. A very good agreement between potentiometrically measured and formerly reported pK_a values can be seen. The differences are mostly in the range of 0.1 to 0.2 units. This agreement can be regarded as very satisfactory because the published values have been determined in several laboratories and usually not all details about the conditions of the pK_a determination were given, so ionic strength and temperature might differ from our experimental conditions. The structures of all samples are shown in Figures 1 and 2.

Considering the statistics of the pK_a determinations, it can be concluded that the errors of pK_a values determined

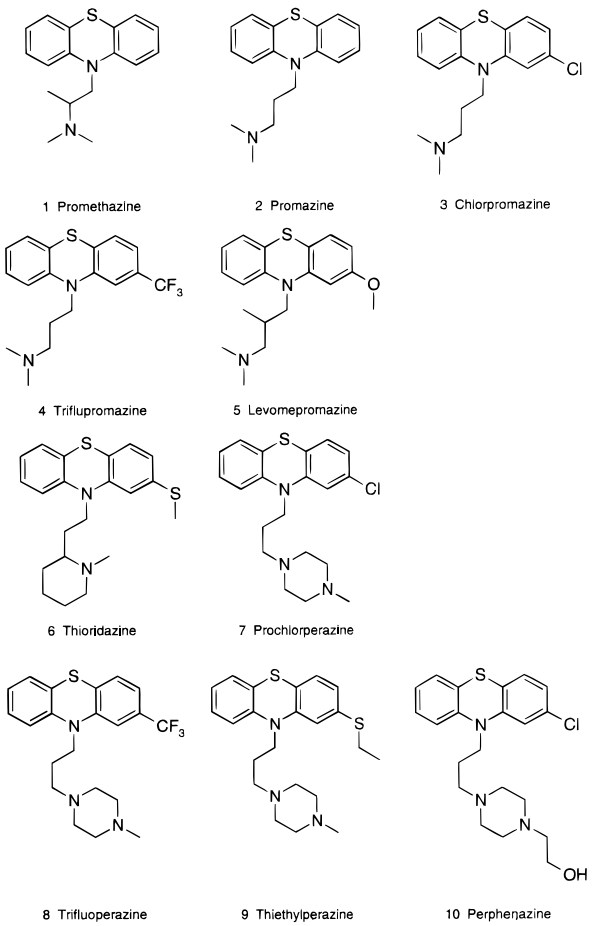

Figure 1—Structures of the phenothiazine derivatives investigated.

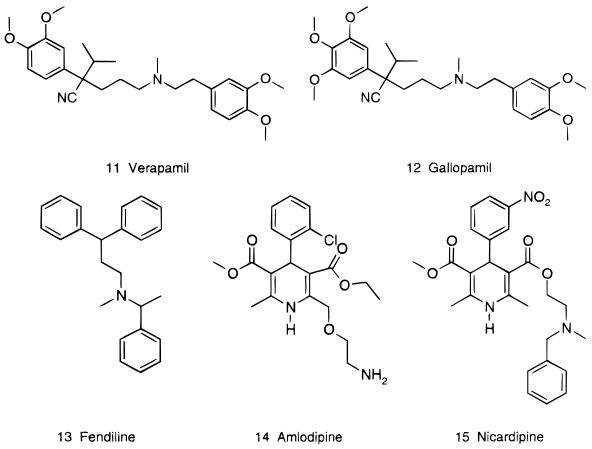

Figure 2—Structures of the calcium channel antagonists investigated.

in purely aqueous 0.15 M KCl seem to be negligible (standard deviations of about ±0.01). The quality of the Yasuda−Shedlovsky extrapolation depended on the number of titrations introduced, the amount and kind of cosolvent, and the quality of the electrode parameter validation at certain cosolvent concentrations. The comparison of the pK_a values obtained from either dioxane or methanol cosolvent mixtures showed generally good agreement, with differences in the range of <0.1 to 0.2 units. For all compounds, except promethazine, gallopamil, and the highest pK_a values of prochlorpromazine and trifluoperazine, the confidence intervals of the pK_a values overlap; therefore, they are statistically identical. The results obtained with dioxane seemed more reliable because lower concentrations were needed to keep the free bases in

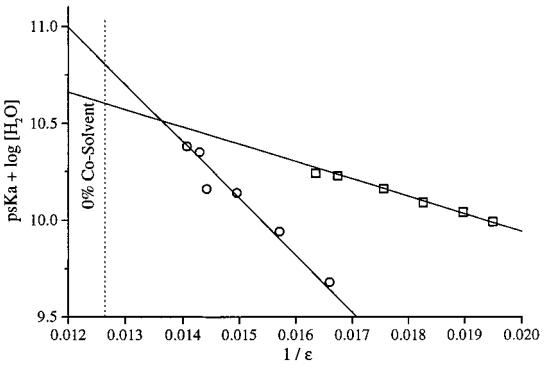

Figure 3—Yasuda–Shedlovsky extrapolation: cosolvent dioxane (□); pK_a of promethazine; extrapolation equation: $p_sK_a + \log [H_2O] = 11{,}745 - 89{,}6/\epsilon$; p$K_a = 8.86 \pm 0.01$ cosolvent methanol (○); pK_a of promethazine; extrapolation equation: $p_sK_a + \log [H_2O] = 14{,}528 - 291{,}9/\epsilon$; p$K_a = 9.07 \pm 0.08$.

Table 2—Comparison of Measured log *P* Values with Published Data

drug	measured log *P*	− limit[a]	+ limit[a]	GOF	published log *P*[b]
promethazine	4.51	4.47	4.55	1.15	4.30,[37] 4.75,[29] 4.81[30]
promazine	4.57	4.53	4.62	1.66	4.40,[37] 4.55,[30] 4.64[29]
chlorpromazine	5.10	5.02	5.21	1.03	5.00,[38] 5.16,[37] 5.35[30]
triflupromazine	5.17	5.14	5.25	1.02	5.03,[30] 5.19,[29] 5.30[37]
levomepromazine	4.89	4.84	5.02	0.71	4.40,[37] 4.86[29]
thioridazine	5.32	5.28	5.37	0.85	5.80,[37] 5.90[31]
prochlorperazine	4.79	4.66	4.91	0.78	3.00,[18] 4.60[30]
trifluoperazine	5.10	4.98	5.19	1.07	4.90,[37] 5.03,[30] 5.07[29]
thiethylperazine	4.82	4.70	4.90	0.75	4.60,[37] 5.18,[29] 5.41[30]
perphenazine	4.25	4.00	4.29	1.38	3.70,[18] 4.19,[37] 4.20[30]
verapamil	3.74	3.67	3.83	0.85	3.79,[30] 4.80[39]
gallopamil	3.71	3.65	3.77	0.68	
fendiline	4.89	4.84	4.91	0.93	
amlodipine	3.17	3.06	3.27	1.10	3.15,[40] 3.30[30]
nicardipine	4.65	4.53	4.74	0.60	3.82,[41] 4.96[30]

[a] The ± limits of log *P* based on ± one standard deviation of the corresponding pK_a values. [b] All published values were obtained by shake-flask method.

solution and the slope of this extrapolation line was less steep (Figure 3). In other words, the influence of the dielectric constant to the sum pK_a + log[H_2O] was less pronounced.

This result is also reflected in the lower standard deviations of pK_a values determined in dioxane/water compared with methanol/water mixtures. A comparison by linear regression yielded eq 3:

$$pK_a(\text{methanol}) = 0.14(\pm 0.14) + 0.99(\pm 0.02)\, pK_a(\text{dioxane}) \quad (3)$$

$$n = 15 \qquad SD = 0.15 \qquad r = 0.99 \qquad F = 3104.0$$

Intercept and slope of the regression line do not deviate significantly from their ideal values. Thus, the values obtained in both systems are comparable, confirming that the quality of the electrode parameter validation made by Sirius Analytical Instruments Ltd. is sufficient if the pK_a lies in the investigated pH range (3 to 10).

The log *P* results are shown in Table 2 together with estimated errors for the log *P* values. The given error estimations are based on the uncertainty in the pK_a determination and not on the much lower ones that result from the fitting procedure that assumes a perfect determination of pK_a. The range of the log *P* uncertainty reflected directly the limits of the pK_a determination.

For the investigated compounds, a good agreement with available experimental log *P* values from the literature was

Table 3—Comparison of Measured log *P* Values and Calculated Values with the ClogP, ACD, and HINT Software

no.	drug	PCA 101[a]	ClogP	ACD	HINT-2D[b]	HINT-3D[c]
1	promethazine	4.51 ± 0.04	4.73	4.69 ± 0.26	4.63	3.89
2	promazine	4.57 ± 0.05	4.55	4.63 ± 0.25	4.39	4.00
3	chlorpromazine	5.10 ± 0.11	5.29	5.36 ± 0.27	4.98	4.60
4	triflupromazine	5.17 ± 0.08	5.63	5.70 ± 0.37	4.79	4.40
5	levomepromazine	4.89 ± 0.13	4.81	5.05 ± 0.27	4.76	4.27
6	thioridazine	5.32 ± 0.05	6.95	6.13 ± 0.38	5.90	5.78
7	prochlorperazine	4.79 ± 0.13	6.16	4.76 ± 0.39	5.83	4.21
8	trifluoperazine	5.10 ± 0.12	6.49	5.11 ± 0.41	5.64	4.04
9	thiethylperazine	4.82 ± 0.12	6.31	5.05 ± 0.41	6.41	4.76
10	perphenazine	4.15 ± 0.15	5.58	4.49 ± 0.42	5.14	3.04
11	verapamil	3.74 ± 0.09	3.79	5.03 ± 0.39	5.29	4.88
12	gallopamil	3.71 ± 0.06	3.22	4.73 ± 0.40	5.59	4.93
13	fendiline	4.88 ± 0.04	6.10	6.55 ± 0.34	5.43	5.43
14	amlodipine	3.17 ± 0.10	2.78	3.72 ± 0.62	1.85	−1.14
15	nicardipine	4.65 ± 0.12	4.30	5.22 ± 0.62	3.53	0.86

[a] Values with the estimated error range. [b] Polar proximity 'via bond' and hydrogen treatment 'all'. [c] Polar proximity 'through space' and hydrogen treatment 'all'.

found. Two major exceptions are evident: in this study a log *P* value of 5.32 was determined for thioridazine in comparison with published values of 5.8 and 5.9. The source for this difference is not clear but it should be kept in mind that the determination of such high log *P* values by the shake-flask method is very difficult. This difficulty is also substantiated by the large variation of log *P* values reported in the literature for promethazine, levomepromazine, thiethylperazine, perphenazine, verapamil, and nicardipine (Table 2). For prochlorperazine, the log *P* value determined in this study was considerably higher than that published in ref 18. A comparison of this log *P* with values from other sources show that the values published in this article are much lower than expected and probably in error. This conclusion is also supported by comparison with log *P* values of other closely related phenothiazines, such as trifluoperazine.

As at least one of the reference values is in approximate agreement with the log *P* values determined by potentiometric titration, the applied two-step method for the determination of log *P* values of poorly water soluble compounds can be regarded as reliable.

The measured log *P* values were also compared with the values calculated by the ClogP, ACD, and HINT software (Table 3) to estimate the ability of the programs to predict log *P* values without experimental work.

Mannhold and Dross regarded a calculated partition coefficient as acceptable if the difference between experiment and calculation was lower than ±0.5 log *P* units.[42] In general, this wide range can be accepted in QSAR studies only if a large variance in log *P* among the compounds investigated exists. We used more stringent reliability measurements; first, the estimated error interval of the experimental values, and second, the range of ±0.3 units of the calculated values because this is the generally accepted error in log *P* values determined by the shake-flask method.

Visual comparison of experimental and calculated log *P* values showed that according to either of these criteria, none of the programs was able to calculate log *P* values accurately (Figure 4). The 'best' results were obtained with the ACD software where the standard errors given by the software overlapped with the experimental values in 10 cases. Comparing the calculated with experimental log *P* values, including their estimated error intervals, overlap was observed in only two cases for the ACD, three for the ClogP, one for HINT-2D, and in no case for the HINT-3D software. If the less stringent criterion of ±0.3 log *P*

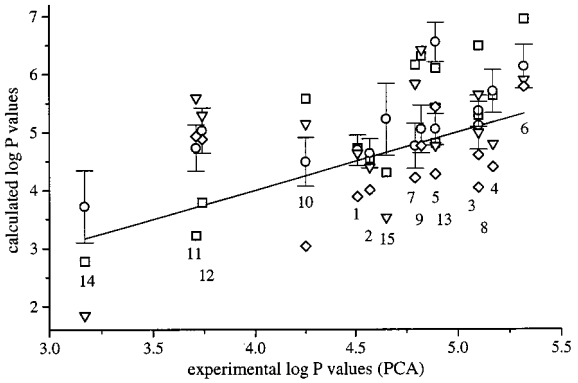

Figure 4—Prediction of measured log *P* values for the tested drugs; reference PCA (straight line), ClogP (□), ACD (○); (with error bars, values printed in Table 3), HINT-2D (▽), HINT-3D (◇).

deviation of the calculated values was applied, the success rate of the software did not increase much: seven coincidences in the case of ACD followed by five for ClogP, four for HINT-2D, and only one for HINT-3D.

For structurally related derivatives, the picture seemed to be less disappointing because all programs except HINT-3D predicted the log *P* values of promazine-type phenothiazines quite well. However, ClogP and HINT-2D highly overestimated the lipophilicity of the piperazine substituent (Table 3). The presence of a piperazine ring did not increase the partition coefficient very much compared with the corresponding promazine derivatives. Instead, the second polar nitrogen appeared to reduce the tendency of drug enrichment in *n*-octanol.[5]

A quantitative comparison of the quality of log *P* predictions was carried out by linear regression. Because we were interested in the calculation ability of the programs, the experimentally determined log *P* value was the dependent variable. This situation corresponds to the condition frequently encountered in a QSAR analysis; that is some log *P* values are known and one wants to calculate the missing ones. It also ensures that the standard deviations of estimation can be compared directly.

For all phenothiazines ($n = 10$) covering a log *P* range of about one log unit, the following results were obtained:

$$\log P_{PCA} = 3.62 + 0.21 \log P_{ClogP}$$
$$SD = 0.30 \quad r = 0.53 \quad F = 3.5$$

$$\log P_{PCA} = 1.84 + 0.59 \log P_{ACD}$$
$$SD = 0.15 \quad r = 0.91 \quad F = 29.1$$

$$\log P_{PCA} = 4.02 + 0.16 \log P_{HINT.2D}$$
$$SD = 0.31 \quad r = 0.34 \quad F = 1.0$$

$$\log P_{PCA} = 3.19 + 0.39 \log P_{HINT.3D}$$
$$SD = 0.21 \quad r = 0.81 \quad F = 14.8$$

As can be seen, only the equations using ACD or HINT-3D as the calculation method are significant at all. In all cases, the slopes are considerably lower than one and the intercepts are much higher than zero. According to these criteria and the statistical parameters, only the ACD program yielded reliable estimates. Inspection of the residuals showed, that in no case was an 'outlier' based on statistical reasoning present, meaning that the low correlations were due to the scatter in the calculated data. The exclusion of thioridazine from the regression, because our log *P* value differed by 0.5 from published data, even worsened the results (data not shown). Only for ACD were the statistics virtually unchanged; however, the intercept dropped to 1.17 and the slope increased slightly to 0.73.

To study the influence of the overestimation of the lipophilicity of the piperazine-containing compounds, the regressions for the two subsets of phenothiazines with alkyl amino or piperazine side chains were derived.

Phenothiazines with alkyl amino side chain ($n = 5$):

$$\log P_{PCA} = 1.79 + 0.61 \log P_{ClogP}$$
$$SD = 0.15 \quad r = 0.91 \quad F = 13.8$$

$$\log P_{PCA} = 1.58 + 0.64 \log P_{ACD}$$
$$SD = 0.09 \quad r = 0.97 \quad F = 45.1$$

$$\log P_{PCA} = -0.39 + 1.11 \log P_{HINT.2D}$$
$$SD = 0.20 \quad r = 0.81 \quad F = 5.7$$

$$\log P_{PCA} = 0.71 + 0.98 \log P_{HINT.3D}$$
$$SD = 0.11 \quad r = 0.94 \quad F = 24.6$$

Phenothiazines with piperazine ring ($n = 4$):

$$\log P_{PCA} = -0.73 + 0.89 \log P_{ClogP}$$
$$SD = 0.07 \quad r = 0.99 \quad F = 87.5$$

$$\log P_{PCA} = -0.83 + 1.15 \log P_{ACD}$$
$$SD = 0.17 \quad r = 0.92 \quad F = 11.6$$

$$\log P_{PCA} = 2.54 + 0.38 \log P_{HINT.2D}$$
$$SD = 0.36 \quad r = 0.57 \quad F = 0.9$$

$$\log P_{PCA} = 3.29 + 0.36 \log P_{HINT.3D}$$
$$SD = 0.30 \quad r = 0.73 \quad F = 2.2$$

It should be mentioned that the variance of log *P* in the subsets is only slightly lower than for all phenothiazines and that therefore the statistical parameters are comparable. The fit of the alkyl amino compounds was improved in all cases. The best results were obtained with the ACD program and especially with HINT-3D. A comparison of the slopes and intercepts of the equations shows that they differ considerably between the subsets. This difference clearly marks that even such small structural changes such as the replacement of an alkyl amino by a piperazine group are not well handled by the log *P* calculation programs. For the perazine-type phenothiazines, the best fit was obtained with ClogP.

The data for the investigated calcium channel antagonists ($n = 5$) comprised structurally diverse compounds and covered a slightly larger log *P* range of 1.7 units. For this subset, the following equations were obtained:

$$\log P_{PCA} = 1.99 + 0.51 \log P_{ClogP}$$
$$SD = 0.35 \quad r = 0.91 \quad F = 14.2$$

$$\log P_{PCA} = 0.81 + 0.64 \log P_{ACD}$$
$$SD = 0.35 \quad r = 0.91 \quad F = 14.0$$

$$\log P_{PCA} = 3.25 + 0.18 \log P_{HINT.2D}$$
$$SD = 0.75 \quad r = 0.41 \quad F = 0.6$$

$$\log P_{PCA} = 3.76 + 0.09 \log P_{HINT.3D}$$
$$SD = 0.77 \quad r = 0.37 \quad F = 0.5$$

As expected for a more diverse data set, the correlation decreased and the standard deviation increased. But again, no outliers were present so the regressions were not unduly influenced. As with the phenothiazines, high intercepts and low slopes were observed, pointing to a general overestimation of lipophilicity by the programs. Combining all investigated drugs ($n = 15$) yielded the following equations:

$$\log P_{PCA} = 2.40 + 0.43 \log P_{ClogP}$$
$$SD = 0.33 \quad r = 0.85 \quad F = 34.7$$

$$\log P_{PCA} = 1.33 + 0.64 \log P_{ACD}$$
$$SD = 0.45 \quad r = 0.71 \quad F = 13.2$$

$$\log P_{PCA} = 3.14 + 0.29 \log P_{HINT.2D}$$
$$SD = 0.54 \quad r = 0.52 \quad F = 5.1$$

$$\log P_{PCA} = 3.85 + 0.19 \log P_{HINT.3D}$$
$$SD = 0.53 \quad r = 0.55 \quad F = 5.5$$

Although the whole data set had a larger variance in log *P* (about 2.2 log units), the relationship between experimental and calculated log *P* values did not improve. It can be concluded that none of the programs used can calculate log *P* values for the investigated compounds with satisfying accuracy at all.

The ACD program performed best for phenothiazines, but did not yield satisfactory results for the other drugs investigated, and was followed next by the ClogP program. Phenothiazines with a piperazine or a piperidine ring in the side chain deviated the most; their lipophilicity was greatly overestimated by the programs. The HINT methodology, especially when taking into account the three-dimensional structure gave the least correlation between measured and calculated log *P* values for the studied derivatives. This result is mainly due to large errors for two calcium channel antagonists of the dihydropyridine type whose lipophilicity was greatly underestimated. HINT was also the only program that incorrectly assigned a chloro-substituent, which is a larger lipophilicity contribution in comparison with a trifluoromethyl group, at the same position in case of the phenothiazines (nos. 2 & 6 versus 3 & 7) and also overestimated the contribution of a 3,4,5-trimethoxyphenyl group compared with its 3,4-dimethoxyphenyl counterpart for the pair gallopamil and verapamil. These results emphasize the need for a further improvement in the accuracy of prediction of log *P* by calculation.

Additionally we focused our attention on the partition coefficients of the ions, log P_{ion}, and on the distribution coefficients at pH 7.4, log *D*, a quantity relevant for the distribution behavior of drugs under physiological conditions. Depending on the quality and quantity of the hydrophobic parts of a compound, a charged drug molecule can enter the organic phase. For example the ionized form of phenothiazines participated in hydrophobic interaction when its charge was suitably neutralized by appropriate anions.[5] Most of drugs analyzed were monoprotic bases that could be present as the neutral and ionic species in the organic phase. For phenothiazines with two basic nitrogens that were doubly protonated at low pH, only the neutral and the monoprotonated components were observed in the organic phase. Table 4 summarizes the partition and distribution coefficients together with published data.

A big advantage of the pH−metric method is that the distribution profile over the entire pH range can be obtained from the titration curves.[44] The calculated log *D* at pH 7.4 for promethazine, chlorpromazine, thioridazine, trifluoperazine, thiethylperazine, and perphenazine based on the measured log *P* and log P_{ion} corresponded excellently to values obtained earlier by the shake-flask method. On average, the distribution coefficients, log *D*, of bases containing two basic nitrogens were higher than those with only one basic nitrogen. This result was the opposite order in relation to the log *P* values. One reason for this discrepancy could be identified clearly; that is, because one

Table 4—Comparison of pH-dependent Distribution in *n*-Octanol/Water

drug	log *P*	log P_{ion}	log *D* pH 7.4	log D_{lit} pH 7.4
promethazine	4.51	1.38 ± 0.03	2.95	2.79,[37] 2.88[17]
promazine	4.57	2.08 ± 0.02	2.93	2.50,[37] 2,55[17]
chlorpromazine	5.10	1.52 ± 0.02	3.26	3.26,[16] 3.39[38]
triflupromazine	5.17	1.87 ± 0.03	3.52	3.39,[16] 3.70[37]
levomepromazine	4.89	1.76 ± 0.03	3.17	2.62[37]
thioridazine	5.32	2.08 ± 0.02	3.45	3.51[37]
prochlorperazine	4.79	1.47 ± 0.08	3.83	
trifluoperazine	5.10	2.26 ± 0.02	4.23	4.36[37]
thiethylperazine	4.82	1.92 ± 0.03	4.04	3.82[37]
perphenazine	4.25	1.50 ± 0.06	3.56	3.65[37]
verapamil	3.74	1.10 ± 0.09	2.37	1.83[43]
gallopamil	3.71	1.29 ± 0.06	2.45	
fendiline	4.89	2.14 ± 0.12	3.19	
amlodipine	3.17	1.06 ± 0.03	1.41	1.11[40]
nicardipine	4.65	1.39 ± 0.10	4.33	

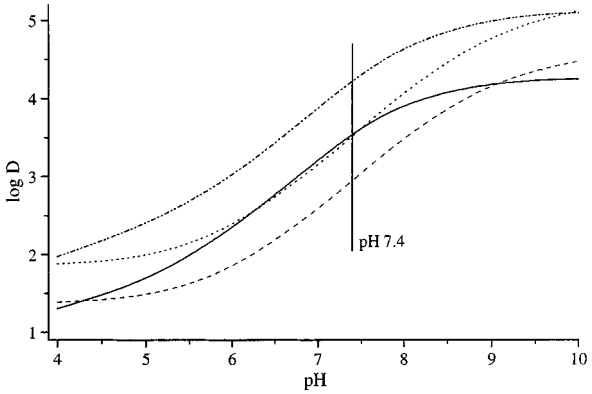

Figure 5—The pH-dependent distribution profile of four phenothiazines; promethazine (dashed line), triflupromazine (dotted line), trifluoperazine (dash/dotted line), and perphenazine (solid line).

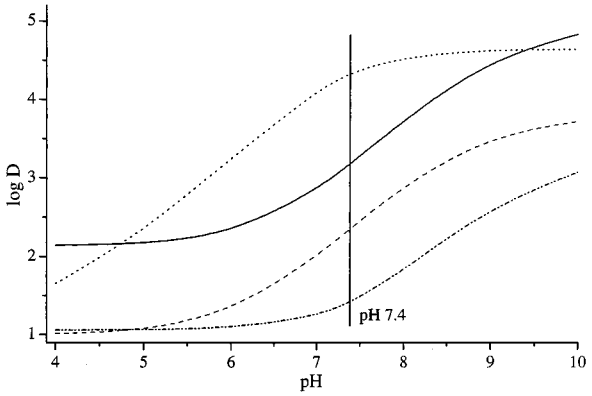

Figure 6—The pH-dependent distribution profile of four calcium channel antagonists; verapamil (dashed line), fendiline (solid line), amlodipine (dash/dotted line), nicardipine (dotted line).

pK_a value of the amino group of the piperazine ring is close to the physiological pH, the degree of protonation is relatively low compared with the monobasic drugs. The changes in lipophilicity of several drugs inspected that occurred during small changes in pH around 7.4 (Figures 5 and 6) were remarkable.

The pH-dependent distribution profiles of trifluoperazine and perphenazine shifted significantly to the curves of triflupromazine and promethazine (Figure 5). The order of the drugs (high to low values of log *P*) changed from triflupromazine = trifluoperazine > promethazine = perphenazine to trifluoperazine > perphenazine = triflupromazine > promethazine (high to low values of log *D* at pH 7.4). The phenothiazines containing a piperazine ring were more lipophilic at pH 7.4 than those with a dimethyl amino group. This result is in agreement with the pharmacological potency of the phenothiazine drugs because a replacement of the dimethyl amino group by a piperazine ring led to an increase of the neuroleptic power.

In Figure 6 the quite different pH-dependent distribution profiles of the structurally heterogeneous calcium channel antagonists verapamil, fendiline, amlodipine, and nicardipine are shown as a further example. The order of log *D* values at pH 7.4 (high to low values) was nicardipine > fendiline > verapamil > amlodipine. Fendiline and nicardipine had nearly the same log *P* values, but differed in their pK_a values. Therefore, the partitioning of nicardipine shifted to higher log *D* values over the whole pH range. As a consequence, the partitioning behavior became even more different at pH 7.4. This result illustrates that the choice of either of the partition or distribution coefficient can have a significant influence on the results of QSAR studies. The distribution coefficient should become more valuable than log *P* for many applications.

Conclusion

The pH−metric method is advantageous for the determination of pK_a values of ionizable compounds. The quality of the Yasuda−Shedlovsky extrapolation depends on the number of titrations performed and the concentrations and kind of cosolvent used. The pK_a values calculated from either dioxane or methanol cosolvent mixtures were in very good agreement in general, but the extrapolated pK_a was much less influenced by the cosolvent concentration in the case of dioxane. The log *P* measurements provided reliable and accurate partition coefficients of the neutral species and also of the corresponding ion. The pH-dependent partition profile can be obtained over the whole pH range investigated and should provide a better descriptor than log *P* for relationships between structure and distribution at physiological pH because it includes the contribution of the charged species to overall distribution. Although the studied data set is small, it can be concluded that the accuracy of log *P* calculation methods is not satisfactory for general use in QSAR analysis, because satisfying correlations were obtained only for selected subsets. None of the investigated programs showed a general superior performance. Therefore, calculation methods for log *P* without any experimental verification should be used with care.

A drawback of the popular *n*-octanol/water model system for the determination of the lipophilicity of drugs is, that it does not take into account specific ionic and hydrophobic interactions that can occur at an ordered interface like a membrane. Therefore, further studies to characterize and compare the distribution behavior in water/phospholipid systems are currently under way.

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