ELSEVIER

International Journal of Pharmaceutics 113 (1995) 47–55

international journal of pharmaceutics

Lipophilicity of amphoteric molecules expressed by the true partition coefficient.

Krisztina Takács-Novák, Miklós Józan, György Szász *

Institute for Pharmaceutical Chemistry, Semmelweis University of Medicine, Högyes Endre u. 9, H-1092 Budapest, Hungary

Received 22 February 1994; modified version received 23 May 1994; accepted 31 May 1994

Abstract

The octanol/water partition coefficients of six amphoteric drugs were investigated. Nitrazepam, albendazole and sulfadimidine are ordinary ampholytes, while pyridoxine, niflumic acid and terbutaline belong to the zwitterionic amphoteric compounds. The pH-partition profile of compounds showed maximum (parabolic) curve. Analyses of the UV spectra in aqueous and octanol phases at different pH values after partitioning equilibrium had been achieved proved the transfer of the neutral species into the octanol phase. The true partition coefficients were calculated from log P_{app} values using macroprotonation constants for ordinary ampholytes and microprotonation constants in the case of zwitterionic molecules. The results emphasize that only the true partition coefficient closely represents the intrinsic lipophilicity of zwitterionic amphoteric compounds.

Keywords: pH-partition profile; Apparent-true partition coefficient relationship; Ordinary ampholyte; Zwitterionic ampholyte

1. Introduction

The lipophilicity of drugs plays an important role in their biological action. This property determines mainly the fate of drug in the body governing the absorption, distribution, storage and elimination processes. The octanol/water partition coefficient is a generally accepted physico-chemical parameter for characterization of lipophilicity. According to the Nernst law the true partition coefficient (log P) refers to the concentration ratio of unionized, monomer form of the drug in the two phases. For ionizable molecules the partition coefficient experimentally measured at a certain pH is an apparent value (log P_{app} or log D). The relationships between log P and log P_{app} are well known for acids and bases (Eq. 1 and 2) (Leo et al., 1971). However, in the case of ampholytes the relation is not so evidently defined.

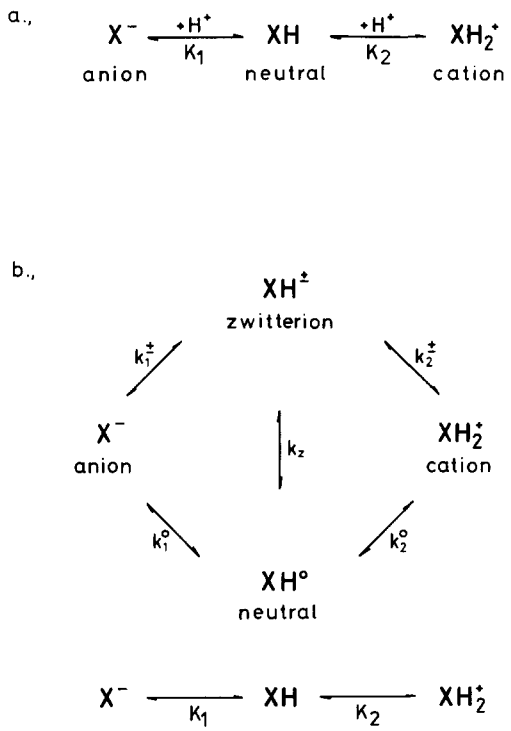
$$\log P = \log P_{app} + \log(1 + 10^{pH - pK_a}) \quad (1)$$

$$\log P = \log P_{app} + \log(1 + 10^{pK_a - pH}) \quad (2)$$

The amphoteric compounds can be classified into two main groups: ordinary amphoteric and zwitterionic amphoteric molecules (Albert and Serjeant, 1971). In the first group, where the

* Corresponding author.

SSDI 0378-5173(94)00176-6



Scheme 1. (a) Protonation of ordinary amphoteric molecules. (b) Macro- and microprotonation of zwitterionic amphoteric molecules.

protonation steps (Scheme 1a) are well separated from each other (generally Δ log $K > 4$) three species exist in solution: anion, neutral and cation. If specific ion-pair partitioning does not occur, only the neutral species of the three dissolves into the octanol phase. For such type of ampholytes the relationship between log P and log P_{app} is given by Eq. 3 (Asuero, 1988):

$$\log P = \log P_{app} + \log(1 + 10^{pH-pK_{a1}} + 10^{pK_{a2}-pH}) \quad (3)$$

where pK_{a1} and pK_{a2} are the macro- or stepwise protonation constants.

In the group of zwitterionic ampholytes, unambiguous definition of the partition coefficient cannot be found in the literature. In the most simple case of zwitterionic compounds, molecules with two proton-binding sites exist in four protonation forms in solution. Their protonation processes can be described by the equilibria shown in Scheme 1b. While the macroprotonation constants quantitate the overall basicity of the molecule, the relevant microprotonation constants describe the basicity of individual functional groups and determine the pH-dependent concentration of protonation microspecies (Noszál, 1989).

The partitioning behavior of zwitterionic drugs has been investigated by many authors and contradictory results have been published regarding the partitioning species. Colaizzi and Klink (1969) found the pH-partition profile of several tetracycline antibiotics to be maximum shape and concluded that tetracyclines transfer into octanol in zwitterionic form presumably resulting from an intramolecular type of ion-pair formation. Later, in a group of β-lactam antibiotics, the opposite behavior, minimum type log $P_{app} \sim$ pH curve was registered (Purich et al., 1973; Irwin et al., 1988). Minimum partitioning occurred in the isoelectric region, suggesting that the zwitterion did not partition to a significant extent and the anion was found to be the major partitioning species. The obvious contradiction has been solved based on the following assumption (Purich et al., 1973). Two general types of ampholytes capable of forming zwitterions should be considered, depending on whether or not significant amounts of uncharged (neutral, XH^0) species are present in the isoelectric region. Maximum partitioning into the lipid phase should occur if concentration of the two protonation isomers (neutral, XH^0 and zwitterion $XH^{\pm}$) is commensurable. Taylor and Cruickshank (1985) have drawn a similar conclusion in the case of the β-blocker sotalol, where the partitioning of the minor species, the uncharged one (estimated concentration at isoelectric point 4.2%) is assumed and the partitioning of the zwitterion is excluded. From the experimentally obtained log P_{app} value (-0.79), the log P of uncharged subspecies, designated as 'micro' partition coefficient was calculated (0.59). This term was used as a 'true' partition coefficient in our recently published paper (Takács-Novák et al., 1992) for the investigation of lipophilicity of antibacterial fluoroquinolones. Here again the neutral species is postulated as the only partitioning one based on theoretical considerations. A

general relationship was derived for zwitterionic drugs between log P and log P_{app} using microprotonation constants:

$$P = \frac{[XH^0]_o}{[XH^0]_w} \quad (4)$$

$$P_{app} = \frac{[XH^0]_o}{[X^-]_w + [XH^\pm]_w + [XH^0]_w + [XH_2^+]_w} \quad (5)$$

$$\log P = \log P_{app} + \log\left(1 + \frac{1}{k_1^0[H^+]} + \frac{k_2^0}{k_2^\pm} + k_2^0[H^+]\right) \quad (6)$$

The aim of the present work was to extend the pH-partition profile investigation for different types of amphoteric molecules and to determine simple experimental evidence of the above-cited assumption: the partitioning of the neutral species. Six molecules were selected for this study (see structures in Fig. 1). Nitrazepam, albendazole and sulfadimidine are ordinary ampholytes, while pyridoxine, niflumic acid and terbutaline belong to the zwitterionic type.

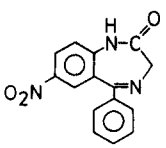
The apparent partition coefficients were measured by the shake-flask method over a wide pH range, and the UV spectra of partitioning compounds in both phases were analyzed.

2. Materials and methods

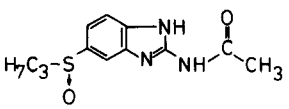
2.1. Materials

Samples of model compounds were generously supplied by Chinoin Pharm. Works (albendazole), Gedeon Richter Chemical Works (niflumic acid) and EGIS Pharm. Works (terbutaline) or purchased from Reanal Co. Budapest, Hungary (nitrazepam, sulfadimidine, pyridoxine) and used without further purification. The n-octanol was HPLC grade (Aldrich). Britton-Robinson buffer (acetic, phosphoric and boric acids, each at O.O4 M, and O.2 M sodium hydroxide) was used for the pH range 2–12, with the exception of pyridoxine and terbutaline where Sörensen buffer (potassium dihydrogen phosphate and disodium phosphate dihydrate, each at O.O67 M) was applied in order to avoid complex formation with borate. For pH O and 1, 1 M and O.1 M hydro-

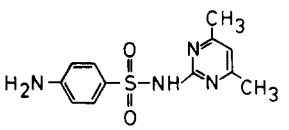
ORDINARY AMPHOLYTES:



nitrazepam

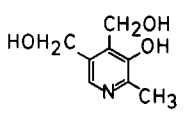


albendazole

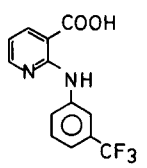


sulfadimidine

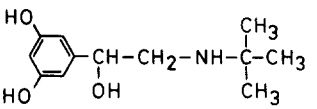
ZWITTERIONIC AMPHOLYTES:



pyridoxine



niflumic acid



terbutaline

Fig. 1. Structure of model compounds.

chloric acid served as aqueous phase.

2.2. Methods

The apparent partition coefficients were measured using the shake-flask technique at 25.0 ± 0.1°C. Most of the experimental circumstances were described previously (Takács-Novák, 1992). In this work a shaking thermostat (Lauda, M2OS) was used to equilibrate the organic and aqueous phases, and a Hewlett-Packard 8452A, UV-Vis spectrophotometer was used for spectrum registration and absorption measurement.

Each log P_{app} value is an average of a minimum of six parallel measurements, the standard deviation being generally less than 0.04 log P unit (see Table 1).

The protonation macroconstants were determined by potentiometry (pyridoxine, terbutaline) or spectrophotometry using standard methods (Albert and Serjeant, 1971). The protonation microconstants were calculated from the protonation macroconstants and the experimentally determined k_z tautomerization microconstant. A detailed description of the method and microconstant values will be published elsewhere (Takács-Novák et al., 1994), similarly to the details of the deductive method for terbutaline (Takács-Novák et al., 1995). In the present paper only the macro- and microconstants used for the calculation of log P are involved.

3. Results and discussion

3.1. pH dependence of log P_{app}

The apparent partition coefficients of the examined compounds are summarized in Table 1. Each compound was measured at seven different pH values including the one proximate (±0.3 unit) to the isoelectric point.

The pH-partition profile of molecules (Fig. 2) shows a maximum type (parabolic) curve in all cases. As a common feature the compounds exhibit the highest lipophilicity at the isoelectric region independently of the absolute value of the lipophilicity and the pH of the isoelectric point. Athough these latter two parameters range over quite a wide interval, there is no difference in the partitioning behavior of the ordinary and zwitterionic compounds, which supports the assumption about partitioning of the neutral species in both groups. However, the shape of these curves is

Table 1
The apparent partition coefficients of model compounds

Nitrazepam		Albendazole		Sulfadimidine	
pH	log P_{app}	pH	log P_{app}	pH	log P_{app}
2.00	0.82 ± 0.02	1.00	−1.09 ± 0.04	1.00	−1.40 ± 0.02
3.00	1.36 ± 0.04	2.00	−0.10 ± 0.02	2.00	−0.45 ± 0.03
5.00	2.01 ± 0.04	3.40	1.07 ± 0.04	3.00	0.13 ± 0.03
7.15 [a]	2.07 ± 0.01	6.65 [a]	1.22 ± 0.01	5.00 [a]	0.15 ± 0.04
9.00	2.06 ± 0.03	7.40	1.23 ± 0.01	7.00	0.14 ± 0.02
11.00	1.50 ± 0.03	10.00	0.96 ± 0.02	8.00	−0.37 ± 0.03
12.00	0.60 ± 0.02	12.00	−0.69 ± 0.06	9.00	−1.09 ± 0.05
Pyridoxine		**Niflumic acid**		**Terbutaline**	
pH	log P_{app}	pH	log P_{app}	pH	log P_{app}
5.00	−0.98 ± 0.04	0	0.97 ± 0.09	7.00	−2.10 ± 0.17
6.00	−0.72 ± 0.03	1.00	2.21 ± 0.01	8.00	−1.02 ± 0.07
7.00 [a]	−0.73 ± 0.03	2.00	2.88 ± 0.04	9.00	−0.42 ± 0.04
8.00	−0.72 ± 0.03	3.60 [a]	2.95 ± 0.08	9.80 [a]	−0.36 ± 0.04
9.00	−0.84 ± 0.05	5.00	2.93 ± 0.04	10.50	−0.45 ± 0.05
9.20	−0.91 ± 0.04	6.00	2.21 ± 0.02	11.00	−0.63 ± 0.04
10.00	−1.16 ± 0.04	7.00	1.31 ± 0.02	12.00	−1.83 ± 0.02

[a] pH near to the isoelectric point.

very characteristic: for the ordinary ampholytes the curves have a flat plateau, while curves of zwitterionic compounds show a peaked parabolic shape.

These phenomena can be appropriately explained on the basis of the different dissociation ability of compounds. In the group of ordinary amphoterics the neutral (XH) form predominates over a wide pH range. In the group of zwitterionic amphoterics, due to overlapping protonation processes, the concentration of microspecies (that of the partitioning XH^0 as well) changes rapidly within a narrow pH range.

3.2. *Investigation of species partitioning into octanol*

Nitrazepam has been proved to be a good model for ordinary amphoteric molecules to study the partitioning species by a very simple spectrophotometric method. Fig. 3 shows the protonation scheme of the molecule and the pH-dependent distribution of the differently protonated forms. It is well known that characteristic UV spectra belong to these forms (Aboul-Enein et al., 198O).

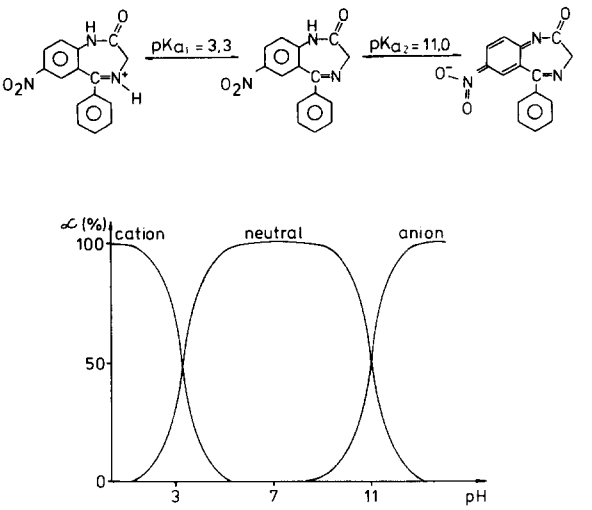


Fig. 3. The protonation of nitrazepam and distribution of its species.

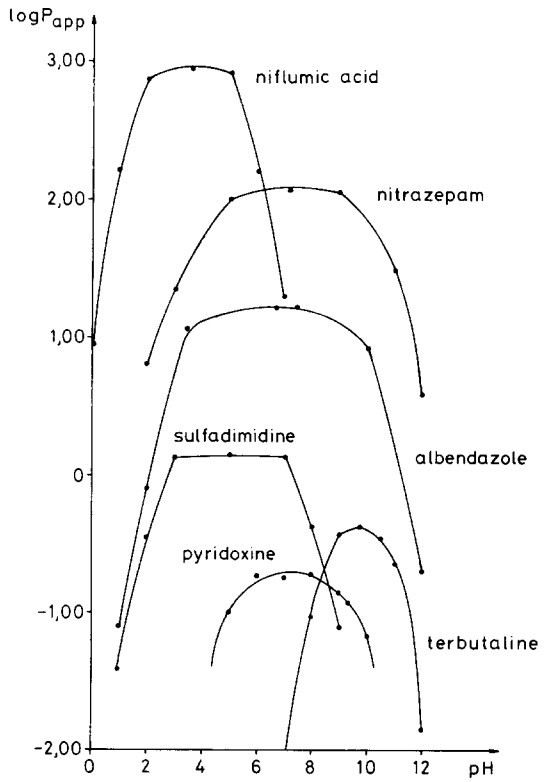


Fig. 2. The pH-partition profile of model compounds.

Upon the determination of log P_{app} values the UV spectra of nitrazepam were registered in both aqueous and octanol phases at different pH values, after partitioning equilibrium had been achieved. The three significant states are represented by Fig. 4. While in the aqueous phase the spectrum of the cation (pH 2), neutral (pH 7) and anion forms (pH 12) is registered, in the octanol phase the spectrum of the neutral form was obtained at all pH values.

Investigation of albendazole has led to identical results. The pK_a values are 3.37 and 9.93, the neutral form being present from pH 5.4 to 7.9. On structural bases the protonations cause only small shifts in the UV absorbance, therefore, this molecule is less suitable for demonstration.

In spite of our expectation, sulfadimidine produced different UV spectra in the two partitioning phases at the isoelectric point (see Fig. 5). This apparent contradiction can be solved considering the existence of uncharged sulfadimidine in two tautomeric (amido and imido) forms. As was established by Bult and Klasen (1978) in a com-

prehensive spectrometric study of sulfonamides, the amido form exists in less polar organic solvent and has a spectrum with one main band. In the spectrum of the imido form there are three well separated absorption maxima and this form is present in aqueous solution. In accordance with this, we found the appearance of the amido form of neutral sulfadimidine in octanol phase.

Pyridoxine was selected as a good model of zwitterionic amphoteric molecules, since it is known to exist as a zwitterion in aqueous medium as proved by spectroscopy (Lunn and Morton, 1952). The protonation scheme and the relevant

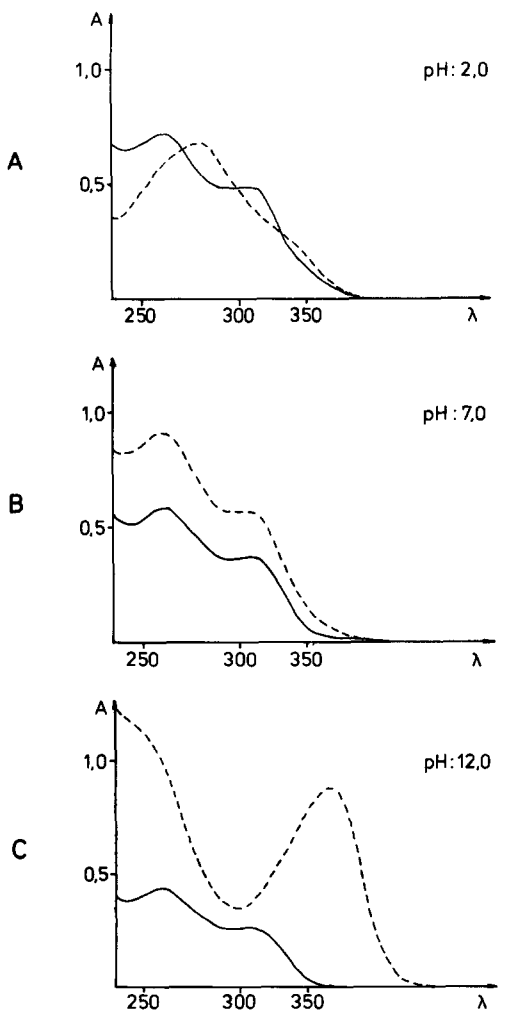


Fig. 4. UV spectra of nitrazepam in aqueous (broken line) and in octanol (continuous line) phases at different pH values.

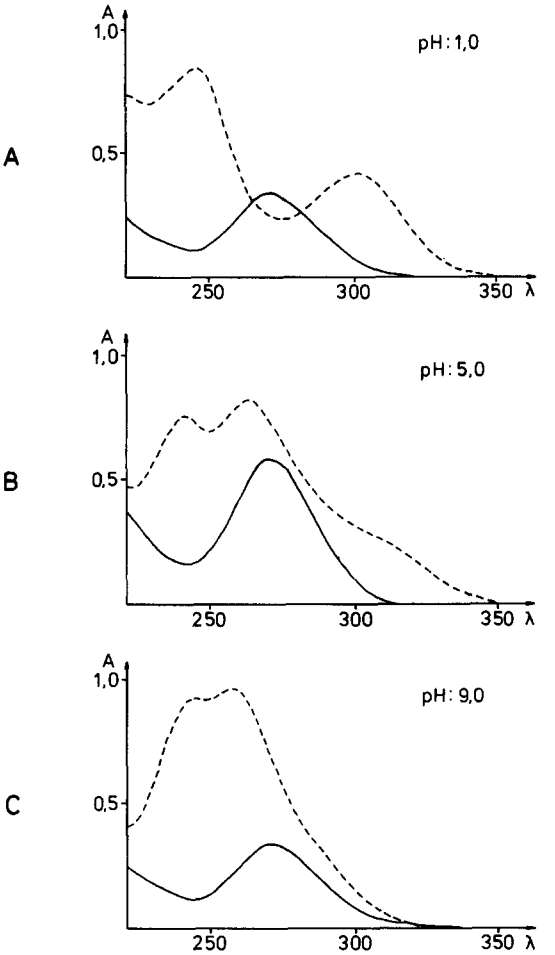


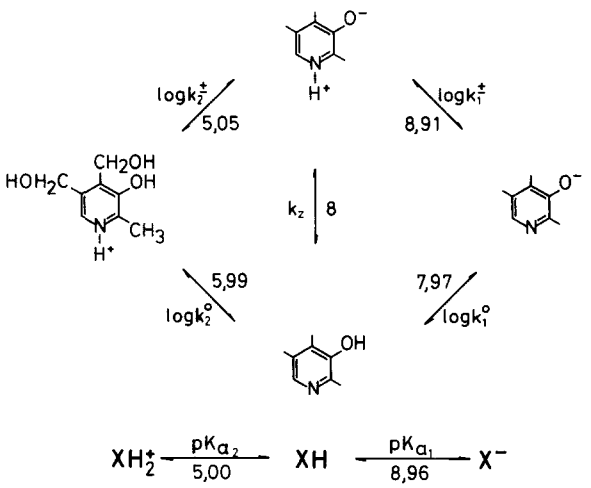
Fig. 5. UV spectra of sulfadimidine in aqueous (broken line) and in octanol (continuous line) phases at different pH values.

constants, together with the microspeciation, are shown in Fig. 6. The protonation of the molecule has a very significant influence on the UV spectrum. The cation ⇄ zwitterion equilibrium results in an intense bathochromic shift (from 291 to 324 nm) over the pH range 3–7, the zwitterion ⇄ anion equilibrium leading to a smaller shift to lower wavelength (from 324 to 312 nm) at pH above 7. In organic solvents with low dielectric constant the spectrum of the neutral pyridoxine was assigned and characterized by one maximum at 289–292 nm (Metzler and Snell, 1955).

Our results are in complete accordance with the above findings. As visible from Fig. 7, in the aqueous phase at the pH of the isoelectric point,

the spectrum of the zwitterion was registered while that characteristic for the neutral species was obtained in the organic phase. This can be considered as very simple but exact experimental evidence for the partitioning of the neutral species even when it is the minor component.

The same conclusion could be drawn from the investigation of niflumic acid. Our previous study on its acid-base properties revealed the predominance of the zwitterionic form in aqueous solution (log $k_1^0 = 3.88$, log $k_1^{\pm} = 5.12$, log $k_2^0 = 3.37$, log $k_2^{\pm} = 2.13$, unpublished results). Analyses of the spectra in the partitioning phases at pH values of 0, 3.6 and 7 showed significantly different spectra for the cation, zwitterion and anion, while identical spectra were obtained in octanol phases



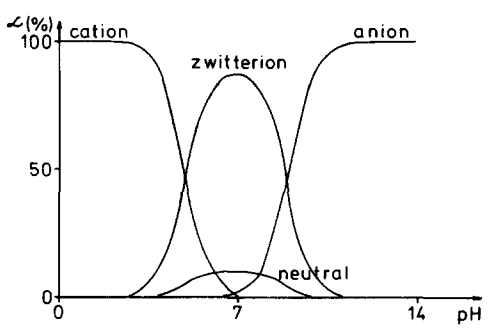


Fig. 6. Protonation of pyridoxine and distribution of its microspecies (protonation macro- and microconstants were taken from work of Metzler and Snell, 1955).

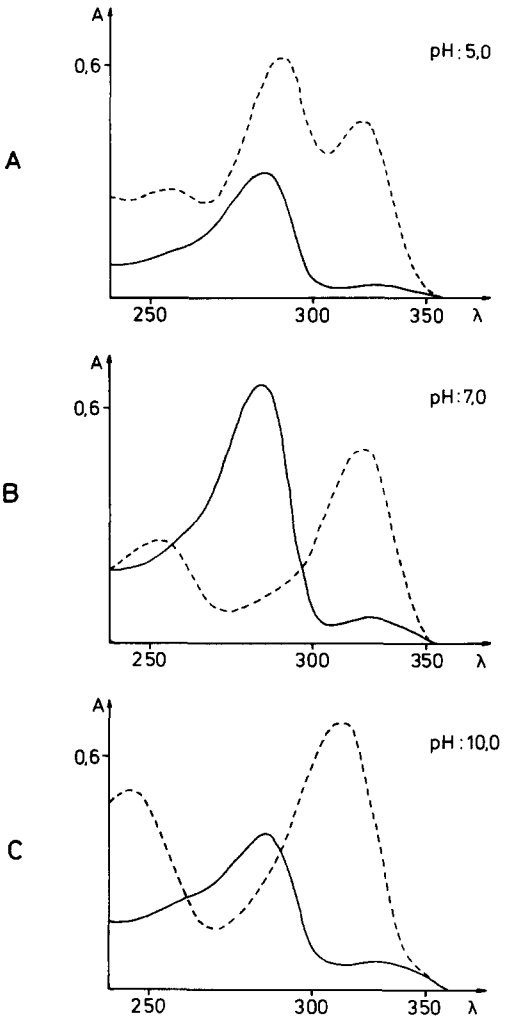


Fig. 7. UV spectra of pyridoxine in aqueous (broken line) and in octanol (continuous line) phases at different pH values.

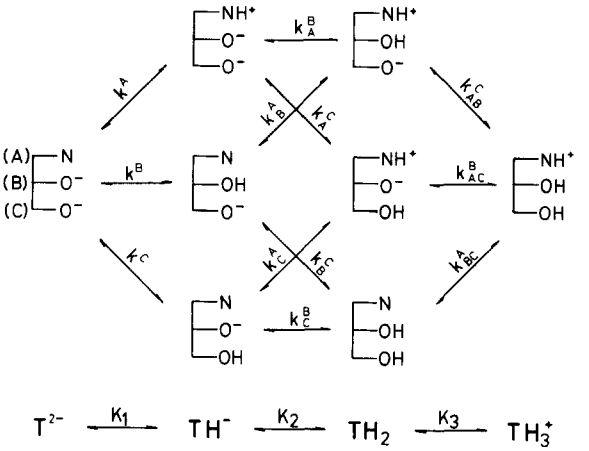
at any pH (Fig. 8). It was identified as that of the neutral species by comparison with spectra in dioxane and cyclohexane and with spectra of its O-CH_3 ester and N-CH_3 pyridinium salt derivatives.

In the case of terbutaline, UV spectroscopy is less suitable for the demonstration of partitioning of the neutral form because here the uncharged species has the same spectrum as that of the cation form. The protonation of the amino group does not cause a shift in bands due to its distance from the chromophore. Of course, the spectrum of the zwitterion at the isoelectric point and the

dianion form at pH 12 in the aqueous phase are different from that of the neutral one obtained in octanol (spectra not shown).

3.3. Intrinsic lipophilicity of amphoteric molecules

The above findings support the applicability of Eq. 3 and 6 for the calculation of the true partition coefficient of ordinary and zwitterionic ampholytes, respectively. Eq. 6 has been derived for zwitterionic compounds with two proton-binding sites. However, one of our model compounds, namely terbutaline, has three ionizable functional groups. In such a case, eight differently protonated microspecies exist in solution and 12 microconstants are necessary to describe the system (see Scheme 2). On the bases of the spectroscopic evidence that the neutral microspecies dissolves into octanol, a similar relation can be derived between log P and log P_{app} for trifunctional compounds:



Scheme 2. Protonation of terbutaline (N, O, O) represents the three protonation sites of the molecule without indication of the relevant valency of nitrogen).

$$\log P = \log P_{app} + \log\left(1 + \frac{1}{k_C^B k^C [H^+]^2} + \frac{k_{BC}^A}{k_{AC}^B k_A^C [H^+]} + \frac{1}{k_C^B [H^+]} + \frac{1}{k_C^B [H^+]} + \frac{k_{BC}^A}{k_{AB}^C} + \frac{k_{BC}^A}{k_{AC}^B} + k_{BC}^A [H^+]\right) \quad (7)$$

Microconstants necessary to calculate the log P value are: log $k^C = 10.54$, log $k_C^B = \log k_A^C = 10.08$, log $k_B^C = 9.33$, log $k_{BC}^A = 9.58$ and log $k_{AC}^B = \log k_{AB}^C = 8.87$ (Takács-Novák et al., 1995).

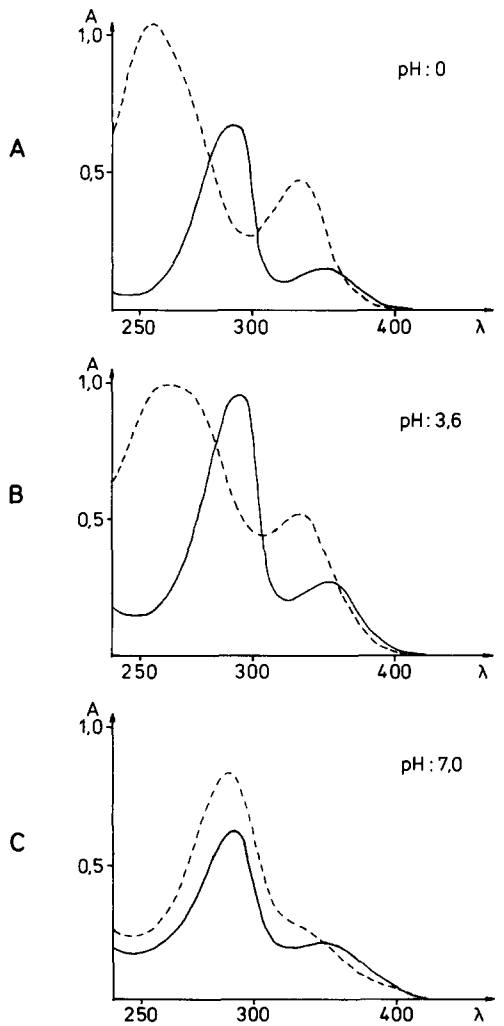


Fig. 8. UV spectra of niflumic acid in aqueous (broken line) and in octanol (continuous line) phases at different pH values.

Table 2
Intrinsic lipophilicity of model compounds expressed by the true log P value

Compound	log P	
Nitrazepam	1.96 [a] ±0.13	$n = 5$
Albendazole	1.27 [a] ±0.04	$n = 5$
Sulfadiminide	0.19 [a] ±0.09	$n = 5$
Pyridoxine	0.33 [b] ±0.08	$n = 5$
Niflumic acid	4.43 [b] ±0.14	$n = 5$
Terbutaline	0.90 [c] ±0.02	$n = 3$

Based on: [a] Eq. 3, [b] Eq. 6, [c] Eq. 7.

The log P values of the model compounds were calculated using Eq. 3, 6 and 7 (Table 2). These are averaged values obtained from five log P_{app} values omitting data gained at the two extreme pH values. For terbutaline we could average only three data because above pH 10 the log P values increased continually, indicating ion-pair partitioning of the compound in this region.

On comparing the data in Table 1 and 2, it becomes evident that in the group of ordinary ampholytes there is no greater difference than the experimental error between log P_{app} (at the pH of the isoelectric point) and log P values. However, in the case of zwitterionic compounds the true partition coefficients are than one order of magnitude greater than the log P_{app} values, since the concentration of neutral species is low, relative to the other ones existing in the aqueous phase.

This serves to underline our previous statement (Takács-Novák et al., 1992) that only the true partition coefficient closely represents the intrinsic lipophilicity of amphoteric compounds and these are the data suitable for application in QSAR studies.

References

Aboul-Enein, H.Y., Jado, A.I. and Lautfy, M.A., *Analytical Profiles of Drug Substances,* Vol. 9, Academic Press, New York, 198O pp. 487–517.

Albert, A. and Serjeant, E.P., *Determination of Ionization Constants,* Chapman and Hall, London, 1971.

Asuero, A.G., Evaluation of partition coefficient and acidity constants of amphoteric substances by graphic differentiation of distribution ratio against pH curve. *Int. J. Pharm.,* 45 (1988) 157–159.

Bult, A. and Klasen, H.B., A spectrometric study of the tautomeric forms of some sulfanilamide derivatives in different media. *Pharm. Weekbl.,* (1978) 665–672.

Colaizzi, L.J. and Klink, P.R., pH-partition behavior of tetracyclines. *J. Pharm. Sci.,* 58 (1969) 1184–1189.

Irwin, W.P., Quigley, J.M. and Timoney, R.F., 1-Octanol-water partition coefficients of the anionic and zwitterionic species of diprotic zwitterionic cephalosporin antibiotics. *Int. J. Pharm.,* 43 (1988) 187–188.

Leo, A., Hansch, C. and Etkins, D., Partition coefficients and their uses. *Chem. Rev.,* 71 (1971) 525–615.

Lunn, A.K. and Morton, R.A., Ultra-violet absorption spectra of pyridoxine and related compounds. *Analyst,* 77 (1952) 718–731.

Metzler, D.E. and Snell, E.E., Spectra and ionization constants of the vitamin B_6 group and related 3-hydroxypyridine derivatives. *J. Am. Chem. Soc.,* 77 (1955) 2431–2437.

Noszál, B., Acid-base properties of bioligands. *Biocoordination Chemistry, Coordination Equilibria in Biologically Active Systems,* Ellis-Horwood, Chichester, 1989, pp. 18–55.

Purich, E.D., Colaizzi, L.J. and Poust, R.J., pH-partition behavior of amino acid-like β lactam antibiotics. *J. Pharm. Sci.,* 62 (1973) 545–549.

Takács-Novák, K., Józan M., Hermecz, I. and Szász, G., Lipophilicity of antibacterial fluoroquinolones. *Int. J. Pharm.,* 79 (1992) 89–96.

Takács-Novák, K., Noszál, B., Tökés-Kövesdi, M. and Szász, G., The molecular and submolecular acid-base properties of terbutaline. *J. Pharm. Pharmacol.,* (1995) submitted.

Takács-Novák, K., Tőkés-Kövesdi, M., Szász, G. and Noszál, B., Investigation of acid-base macro- and microequilibria of amphoteric drugs. *Magy. Kém. Folyóirat,* (1994) in press.

Taylor, P.J. and Cruickshank, J.M., Distribution coefficients of atenolol and sotalol. *J. Pharm. Pharmacol.,* 37 (1985) 143–144.