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Lipophilicity of kinetically labile metal complexes through the example of antidiabetic Zn(II) and VO(IV) compounds

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ABSTRACT

The lipophilic character of several carrier ligands of antidiabetic Zn(II) and VO(IV) metal complexes were characterized by the traditional saturation shake flask method based on *n*-octanol/water partitioning. The transfer of the neutral ligand species to the organic phase was followed by UV spectrophotometry at various pH and the partition and distribution coefficients were calculated with the help of the proton dissociation constants and the spectra of the individual ligand species. Partition and distribution coefficients of the Zn(II) and VO(IV) complexes were determined by analysis of the metal ion content of the aqueous phases before and after separation by ICP-AES, their UV spectra and the corresponding concentration distribution curves. Results revealed the fairly hydrophilic character both the carrier ligands and their neutral Zn(II) and VO(IV) complexes. A quasi-linear relationship was found between log *P* of the ligands and that of the metal species in the case of the ligands studied with the exception of the picolinates. Importance of the knowledge of the chemical speciation (*i.e.* stoichiometry and stability constants) was also highlighted for the characterization of the lipophilic character of the kinetically labile metal complexes.

1. Introduction

Lipophilicity is a crucial physicochemical property characterizing a drug as it has a fundamental effect on the pharmacokinetic profile. The lipophilic character of a compound can influence the ADME (absorption, distribution, metabolism, elimination) processes, the protein or receptor binding ability, the effectiveness of enzyme inhibition, *etc.*; therefore, it affects the activity, the toxicity, the bioaccumulation or the route of administration. The knowledge of the lipophilicity of a potential medicine can strongly contribute to the success of QSAR (quantitative structure–activity relationship) studies [1–4].

Lipophilicity of a compound is often expressed by the partition coefficient (*P*) of the neutral, non-ionized species, which is transferred into the organic phase and *P* shows the equilibrium concentration ratio of the solute between the two phases. *P* is pH-independent. While the distribution coefficient (*D*) represents the ratio of concentrations of all species (ionized and neutral) in the organic and aqueous phases, therefore is pH-dependent. (Usually the transfer of the neutral species to the organic phase takes place only.)

Proton dissociation constants (and stability constants in the case of metal complexes) create the relationship between *P* and *D* (see equations in the Section 2). The lipophilic character of drugs is mostly interesting at pH 7.40. However, their behavior at the pH of the gastro-intestinal absorption sites has also importance in the case of orally administered compounds [1,3].

Measurement of the *D* (or *P*) values is performed via the partitioning of the compound between isotropic solvents such as *n*-octanol and water. The most general method is the traditional saturation shake flask approach to separate followed by UV–vis spectrophotometry, and for metal complexes by ICP-AES (inductively coupled plasma atomic emission spectroscopy) (or ICP-mass spectrometry), atomic absorption spectroscopy, *etc.* to measure the concentration of the compounds in the two phases [2]. HPLC and other alternative chromatographic techniques such as the micellar liquid or microemulsion electrokinetic chromatography are also frequently used [1]. In addition, QSPR (quantitative

Abbreviations: 6-Mepic, 6-methylpicolinic acid; bicine, 2-(bis(2-hydroxyethyl)amino)acetic acid; CAPS, *N*-cyclohexyl-3-aminopropanesulfonic acid; CHES, *N*-cyclohexyl-2-aminoethanesulfonic acid; *D*, (*n*-octanol–water) distribution coefficient; dhp, 3-hydroxy-1,2-dimethyl-pyridinone, Deferiprone; dipic, 2,6-pyridine dicarboxylic acid; ε, molar absorptivity; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hpno, 2-hydroxypyridine-*N*-oxide; ICP-AES, inductively coupled plasma atomic emission spectroscopy; maltol, 3-hydroxy-2-methyl-4H-pyran-4-one; MES, 2-(*N*-morpholino)ethanesulfonic acid; mpno, 2-mercaptopyridine-*N*-oxide, pyrithione; *P*, (*n*-octanol–water) partition coefficient; pic, picolinic acid; Tris, *tris*(hydroxymethyl)methylamine.

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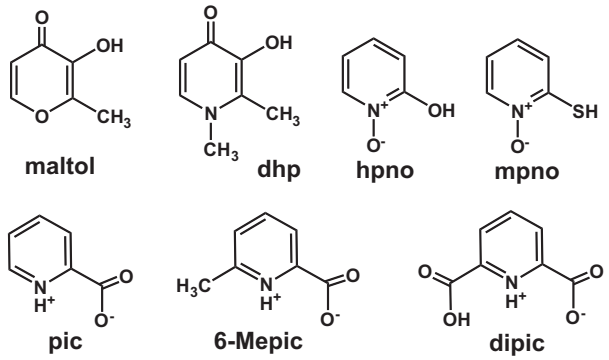


Scheme 1. Ligands used in this study in their neutral forms: maltol = 3-hydroxy-2-methyl-4H-pyran-4-one (HL); dhp = 3-hydroxy-1,2-dimethyl-pyridinone, deferiprone (HL); hpno = 2-hydroxypyridine-*N*-oxide (HL); mpno = 2-mercaptopyridine-*N*-oxide, Pyrithione (HL); pic = picolinic acid (HL); 6-Mepic = 6-methylpicolinic acid (HL); dipic = 2,6-pyridine dicarboxylic acid (H_2L).

structure–property relationship) modeling procedures or other *in silico* methods are applied to predict lipophilic character of a potential drug as well [2,5,6], but their possible use for more complicated systems *e.g.* metal complexes is fairly doubtful.

Numerous VO(IV) and Zn(II) complexes were prepared and tested both *in vitro* and *in vivo* against the most widespread metabolic diseases, *Diabetes mellitus* [7–10]; and one of them [$VO(maltol)_2$] has passed clinical phase I and II trials [10]. These metal complexes exhibit insulin-enhancing effect and their activity exceeds the efficacy of the simple inorganic salts such as $VOSO_4$, $ZnSO_4$ [7]. The main advantage of these VO(IV) and Zn(II) complexes over insulin is that they may be administered orally. Complex formation can provide the proper lipophilicity, which is prerequisite for the efficient penetration via the biomembranes; thus an improved gastro-intestinal absorption function is observed compared to their inorganic salts. The potential drug candidates are the neutral *bis*–ligand complexes of VO(IV) and Zn(II). Complexes with various coordination modes have been reported to display considerable insulin-enhancing properties [7–10]. Among the potent ligands 3-hydroxy-2-methyl-4H-pyran-4-one (maltol), 3-hydroxy-1,2-dimethyl-pyridinone (dhp), 2-hydroxypyridine-*N*-oxide (hpno), 2-mercaptopyridine-*N*-oxide (mpno), picolinic acid (pic), 6-methylpicolinic acid (6-Mepic) and 2,6-pyridine dicarboxylic acid (dipic) were chosen for the present study (see Scheme 1). It was found that the insulin-enhancing activity in terms of inhibition of free fatty acid release (IC_{50} values) of certain Zn(II) complexes were strongly correlated with the log *P* of the carrier ligands or the complexes [11,12].

The carrier ligands alone have biological interest as well. For example maltol is a naturally occurring compound used as a flavor enhancer, moreover, it is a strong chelating agent for hard metal ions (Fe(III), Al(III)) as well as dhp (also known as deferiprone), which is an oral drug against β-thalassemia as it binds Fe(III) very efficiently [13]. Pic is a catabolite of tryptophan; dipic is responsible for the heat resistance of the endospores [14]. Hpno exhibits hydroxamate function and is found to be efficient inhibitor for the mushroom tyrosinase [15]. Moreover, the Zn(II) complex of mpno (or pyrithione) inhibits the growth of fungi, bacteria and used as an active ingredient in anti-dandruff shampoos, in treatment of *Seborrhoeic dermatitis* [16].

Some lipophilicity data for the antidiabetic complexes of the carrier ligands (Scheme 1), mainly for the Zn(II) species, are already available in the literature [17–20], however, in these studies the metal complexes are considered as intact. Since the original integrity of the complexes may be changed in the aqueous phase, we reinvestigated the lipophilicity of these complexes paying attention to the actual speciation and study of the metal-free carrier ligands was involved as well.

Additionally, we are trying to draw attention to the most critical points of the traditional saturation shake flask approach, especially when it is used for kinetically labile metal complexes.

2. Experimental

2.1. Chemicals

Maltol, dhp, pic, 6-Mepic, dipic were commercially available products of *puriss* quality (Sigma–Aldrich); hpno and mpno were purchased from Tokyo Kasei Organic Chemicals (Japan). Doubled distilled Milli-Q water was used for sample preparations. The purity of the carrier ligands was checked and the exact concentrations of the stock solutions prepared were determined by potentiometric titrations using SUPERQUAD program for data evaluation [21].

2-(*N*-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxy-ethyl)-1-piperazineethanesulfonic acid (HEPES), *tris*(hydroxymethyl)methylamine (Tris), *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES), *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffers and *n*-octanol were obtained from Sigma–Aldrich, acetic acid, 37% HCl, KOH from Reanal (Hungary) and $ZnCl_2$, $VOSO_4$, conc. HNO_3 and 30% H_2O_2 were purchased from Merck (Suprapur).

A $ZnCl_2$ stock solution was made by dissolution of anhydrous $ZnCl_2$ in a known amount of HCl solution, and its concentration was determined by complexometry via ethylenediaminetetraacetate complexes. The V(IV)O stock solution was prepared as described in Ref. [22] and standardized for metal ion concentration by permanganate titration.

2.2. Determination of the distribution coefficients

D values of the carrier ligands (maltol, dhp, hpno, mpno, pic, 6-Mepic, dipic) and their zinc and vanadyl *bis*–ligand complexes were determined by the traditional shake flask method [1,2] in *n*-octanol/buffered aqueous solution at pH 1.5, 2.0 (HCl); 3.0 (CH_3COOH/CH_3COOK); 5.3; 6.0 (MES); 7.4 (HEPES); 8.0 (Tris); 9.0, 9.3, 9.5 (CHES) and 8.7, 9.5, 10.0, 10.5 (CAPS) at 25.0 ± 0.2 °C. Two parallel experiments were performed for each sample. All ligands were dissolved at 100 μM in the *n*-octanol pre-saturated aqueous solution of the buffer (0.01 M) without or with KCl (0.20 M). In the metal ion containing samples the ligand concentration was 200 μM and metal-to-ligand ratio 1:2 was applied at constant ionic strength (0.20 M KCl). The vanadium containing samples were completely deoxygenated by bubbling purified argon though them. The aqueous solutions and *n*-octanol with 1:1 phase ratio were gently mixed with 360° vertical rotation for 3 h to avoid the emulsion formation, and the mixtures were centrifuged at 5000 rpm for 3 min by a temperature controlled centrifuge (Sanyo) at 25 °C. After the separation of the two phases, the aqueous solution pH values were controlled and confirmed to be equal within ±0.02 unites to those of the starting buffered solution. UV spectra of the ligands or metal complexes in the aqueous phase were compared with those of the original aqueous solutions in the range 240–400 nm and metal ion content of the aqueous solutions was determined by ICP-AES.

Calculation of the *D* (phase ratio is equal to 1) and *P* values were based on the following equations:for dissociative ligands with one proton dissociation constant (K_a):

$$HL_{aq} \rightleftharpoons L^-_{aq} + H^+_{aq} \quad K_a = \frac{[L^-]_{aq}[H^+]_{aq}}{[HL]_{aq}} \tag{1}$$

$$c_{HL} = [HL]_{aq} + [L^-]_{aq} + [HL]_{n\text{-octanol}} \tag{2}$$

Table 1
Proton dissociation constants (pK_a of the carrier ligands studied; partition coefficient (P, log P) values of the neutral forms of the ligands (HL: maltol, dhp, hpno, mpno, pic, 6-Mepic and H_2L: dipic) with the measured and calculated $D_{7.40}$, log $D_{7.40}$ values together with molar fractions (x_{HL}) at physiological pH [t = 25 °C; I = 0.20 M (KCl)].

	pK_1	pK_2	pK_3	P log P	P[a] log P	x_{HL}[b]	$D_{7.40}$[c] log $D_{7.40}$	$D_{7.40}$[d] log $D_{7.40}$
maltol	8.44[e]			1.34 ± 0.10 0.13	1.40 ± 0.09 0.15	0.92	1.25 ± 0.10 0.10	1.23 0.09
dhp	3.67[e]	9.77[e]		0.12 ± 0.02 −0.92	0.23 ± 0.01 −0.64	1.00	0.10 ± 0.05 −1.00	0.12 −0.92
hpno	5.78[f]			0.44 ± 0.01 −0.36	0.42 ± 0.01 −0.38	0.02	0.05 ± 0.01 −1.30	0.01 −2.00
mpno	4.49[f]			2.53 ± 0.22 0.40	2.49 ± 0.15 0.40	0.00	(0.22 ± 0.12) (−0.66)	0.0 <−2
pic	∼1.0[e]	5.19[e]		0.01 ± 0.03 −2.00	0.10 ± 0.10 −1.00	0.01	0.00 ± 0.03 <−2	0.0 <−2
6-mepic	∼1.2[e]	5.81[e]		0.02 ± 0.02 −1.70	0.06 ± 0.10 −1.22	0.03	0.00 ± 0.02 <−2	0.0 <−2
dipic	∼1.0[e]	2.08[e]	4.58[e]	2.58 ± 0.07 0.41	2.83 ± 0.08 0.45	0.00	0.00 ± 0.07 <−2	0.0 <−2

[a] Without KCl.
[b] Calculated at pH 7.40 (for dipic $x(H_2L)$).
[c] Measured values.
[d] Calculated from P values.
[e] Data taken from Ref. [24].
[f] Data taken from Ref. [17].

$$D = \frac{[HL]_{n\text{-octanol}}}{[HL]_{aq} \cdot (1 + K_a/[H^+])} = \frac{P}{(1 + K_a/[H^+])} \quad (3)$$

$$P = D \times \left(1 + \frac{K_a}{[H^+}\right) \quad \text{or} \quad P = \frac{D}{x_{HL}} \quad \text{where} \quad x: \quad \text{molar fraction} \quad (4)$$

for dissociative ligands with two or three proton dissociation constants (K_i):

$$P = D \times \left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]}\right) \quad \text{or} \quad P = \frac{D}{x_{HL}} \quad (5)$$

$$P = D \times \left(\frac{[H^+]}{K_1} + 1 + \frac{K_2}{[H^+]} \times \frac{K_3K_2}{[H^+]^2}\right) \quad \text{or} \quad P = \frac{D}{x_{HL}} \quad (6)$$

for the $[ML_2]$ complexes:

$$D_{[ML_2]} = \frac{[ML_2]_{n\text{-octanol}}}{[\text{total metal ion}]_{aq}} \quad (7)$$

$$P_{[ML_2]} = \frac{[ML_2]_{n\text{-octanol}}}{[ML_2]_{aq}} \quad \text{or} \quad P_{[ML_2]} = \frac{D_{[ML_2]}}{x_{[ML_2]}} \quad (8)$$

D of the carrier ligands was calculated as the mean of (Absorbance (original solution)/Absorbance (aqueous phase after separation) − 1) obtained at the region of λ_{max} ±10 nm. D of the metal complexes was calculated as Eq. (7) shows based on the ICP-AES measurements.

2.3. Spectrophotometric measurements

An Unicam Helios Alpha spectrophotometer was used to record the spectra in the region of 205–400 nm at 25.0 ± 0.1 °C. Path length was 1 cm.

UV spectrophotometric titrations were performed on maltol, dhp, hpno, mpno, pic, 6-Mepic ligands and their Zn(II) complexes and on the VO(IV) complexes of hpno and mpno in the pH range between 2 and 11. The ligand concentration was 80–160 μM at an ionic strength of 0.20 M (KCl) and 1:2 metal-to-ligand ratio was used. Samples were deoxygenated by bubbling argon. UV spectra of the individual ligand and complex species were calculated by the computer program PSEQUAD [23].

2.4. ICP-AES measurements

The zinc or vanadium content of the samples (original solution and the aqueous phase obtained after the separation) was determined by an ICP-AES Jobin-Yvon instrument. Prior to the analysis, the 2 ml samples were digested in an acid mixture containing 0.5 ml of conc. HNO_3 and 0.5 ml of 30% H_2O_2. The resulting 5.00 ml solutions were prepared by using Millipore MilliQ quality deionized water. The analysis was performed at 213.86 nm emission wavelength for Zn or at 292.40 nm for V using a Jobin-Yvon 240 all-argon spectrometer equipped with a Teflon V-groove nebulizer and a Gilson Minipuls III peristaltic pump. Two determinations on each solution were carried out with 2-point background correction, 4-point direct calibration and Gaussian spectral drift-compensation. Standard solutions for calibration were prepared from a 1 g l^{-1} commercial Zn(II) or VO(IV) stock solution (Merck) through dilution with Millipore MilliQ quality deionized water.

3. Results and discussion

3.1. Lipophilicity of the carrier ligands

Log D values of seven carrier ligands of antidiabetic metal complexes (Scheme 1) were determined at different pH values and partition coefficients of their neutral forms could be calculated with the help of the proton dissociation constants. Results are summarized in Table 1.

The classical shake flask approach was used and the transfer of the ligands from the aqueous environment to the n-octanol phase was followed by UV spectrophotometry. Here we call attention some general mistakes, which appear (see below) occasionally in the chemical literature, which have to be avoided.

Although, the definition of log D and log P is clear; their use is mixed up rather frequently in the literature. Usually log D is determined at pH 7.40 and used as an indication of the drug lipophilicity at the pH of blood plasma. It is well-known that the lipophilicity–pH profile curves can provide a deeper insight into the overall character of the compound in contrast to the one-point test. Therefore, log D values of the ligands were determined in a wider pH range, instead of the exclusive use of pH 7.40.

During the determination of the log D values, external factors such as the ionic strength and the temperature have to be kept

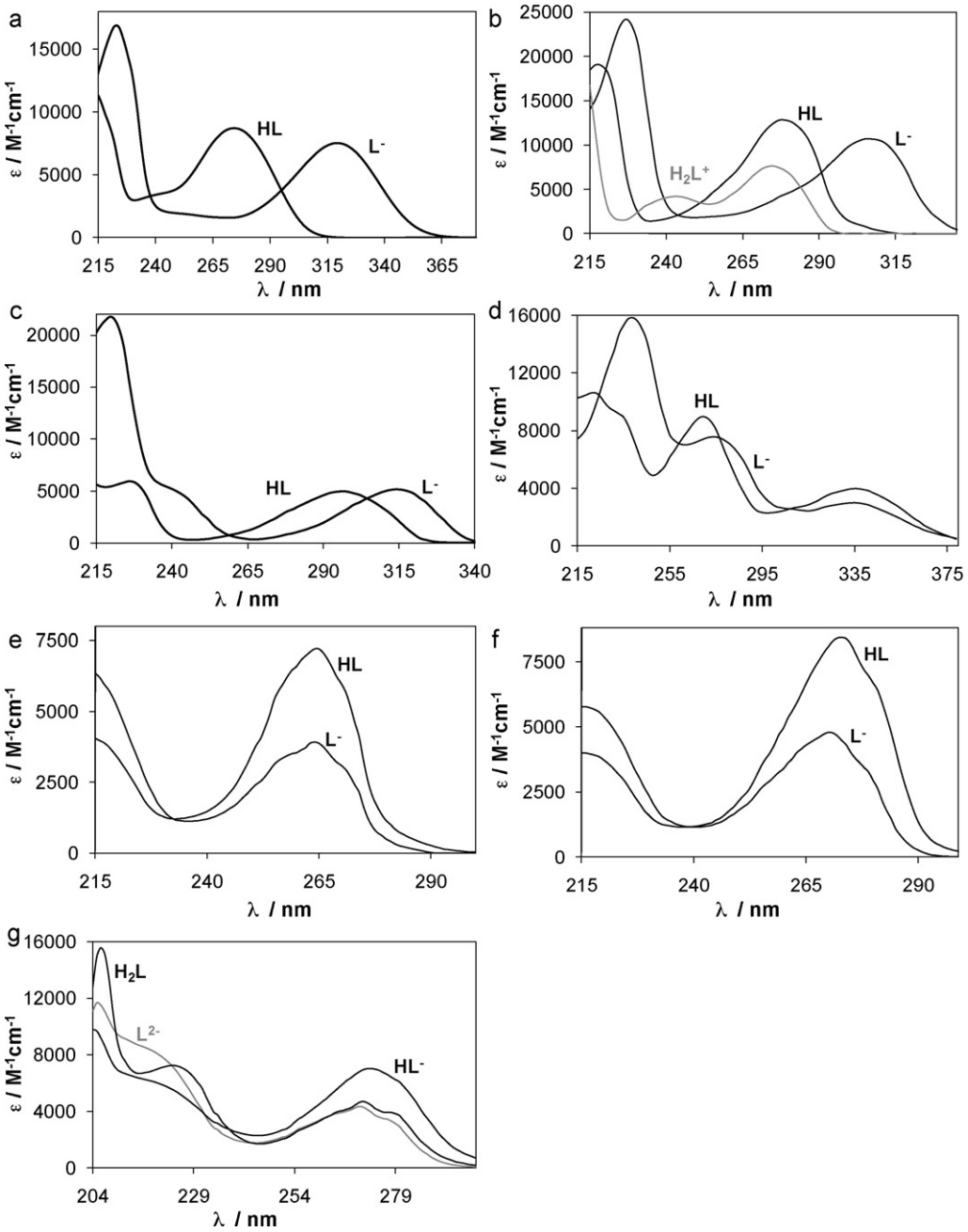


Fig. 1. Calculated UV absorbance spectra of individual ligand species in different protonated states: maltol (a); dhp (b); hpno (c); mpno (d); pic (e); 6-Mepic (f); dipic (g) {t = 25.0 °C, I = 0.20 M (KCl), in water}.

as constant (I = 0.20 M KCl, 25 °C) since they have influence on the pK_a and log D values. These experimental parameters were chosen because the proton dissociation constants are known under these conditions [17,24]. For comparison, log D and log P values were also determined without the presence of the salt (see Table 1). It can be concluded that the lipophilicity is not altered significantly in the presence or the absence of KCl in the case of these compounds.

It is also important to note that when UV–visible spectrophotometry is used for the determination of the concentrations in the two phases, only the spectra recorded for the aqueous solutions are comparable, since the spectral parameters may be significantly different of a compound in the *n*-octanol phase. In order to obtain an insight into the spectral character of the compounds in water, spectra of the individual ligand species in the different protonation states were calculated by deconvolution of UV spectra recorded at different pH values (see Fig. 1 and Table 2). Additionally, spectra of the *n*-octanol phase were also recorded and representative spectra of dhp in *n*-octanol obtained by separation at different pH values are shown in Fig. 2 (and for maltol in Fig. S1).

The similarity of these spectra shows that only one kind of ligand species is present in the organic solvent at the pH studied and it can be seen that the change of the absorbance is parallel to the formation of the neutral HL. Results revealed that the spectra recorded in the *n*-octanol were similar to those of the HL forms (H_2L in the case of dipic), but not identical with them; as the λ_{max} and ε values are changed (see Table 2) in the different solvents. The λ_{max} of maltol, dhp, hpno, mpno is increased, while that of the picolinates is slightly decreased in the organic phase. The red shift is significant in the case of mpno and hpno (see Fig. S2). (Based on these results the transfer of the ionized species to the *n*-octanol phase via ion-

Table 2
λ_{max} and ε (M^{-1} cm^{-1}) values for the ligand species determined by UV spectrophotometric titrations in water [t = 25 °C; I = 0.20 M (KCl)] and in n-octanol [t = 25 °C].

	L^{-} [a]	HL [a]	H_2L^{+} [a]	HL (H_2L) [b]
maltol	318 nm (7521)	274 nm (8640)		278 nm (~8500)
	222 nm (16832)	214 nm (11710;sh[c])		
dhp	307 nm (10700)	279 nm (12802)	273 nm (7554)	283 nm (~11300)
	227 nm (24223)	217 nm (19060)	241 nm (4131)	
hpno	314 nm (5160)	296 nm (4950)		305 nm (~4900)
	240 nm (5203;sh)	226 nm (5931)		
mpno	335 nm (2990)	335 nm (3980)		352 nm (~1600)
	277 nm (7315)	269 nm (8968)		281 nm (~7500)
	237 nm (15733)	221 nm (10614;sh)		
		233 nm (9170;sh)		
pic	263 nm (3874)	263 nm (7094)		261 nm[d]
6-Mepic	270 nm (4794)	273 nm (8443)		271 nm[d]
	215 nm (5790)	215 nm (4000)		
dipic[e]	270 nm (4341)	273 nm (7030)	271 nm (4620)	269 nm (~3800)
	205 nm (11678)	204 nm (9780)	224 nm (7251)	
			206 nm (15588)	

[a] In water.
[b] In the n-octanol phase, for dipic data of H_2L species is shown.
[c] sh: shoulder band.
[d] ε Values could not be determined due to the low absorbance in the n-octanol phases.
[e] Charges of dipic species: L^{2-}; HL^{-}; H_2L.

pairing could be disregarded.) Thus, the spectra of the n-octanol phase cannot be used for the calculation of D, but they can help to identify the type of the transferred species to the organic solvent and at the same time the summed spectra of the organic and aqueous phases show well how the original equilibrium is shifted. This is demonstrated for maltol in Fig. S3 at a pH value, where the formation of the HL species is predominant (a), and at a higher pH where the deprotonated form is also present (b).

It is important to note that reliable spectra of mpno could be collected only at pH < 6, since new absorbance bands appeared in the UV spectrum at elevated pH following the deprotonation of the ligand (λ_{max} = 242, 280, 329 nm), and a new species could be detected also in the n-octanol phase after the partitioning (λ_{max} = 274, 326 nm, see Fig. S4). Most probably the oxidation of the deprotonated mpno takes place at pH > 6 resulting in the measurement of a non-relevant $D_{7.4}$ value (see Table 1).

Lipophilicity–pH profile curves for maltol and dhp as examples are depicted in Fig. 3 together with the pH-dependence of the molar fraction of the ligands. P is expected to be constant at any pH, but the results show that its determination at a pH where the molar fraction of the neutral species is quite low gives high uncertainty and an incorrect value. Thus, more reliable data can be achieved when the neutral species predominates, and these log P values are collected in Table 1. Log $D_{7.4}$ values were also calculated based on the log P values accepted and the molar fraction of ligand species HL (see Eqs. (4)–(6)).

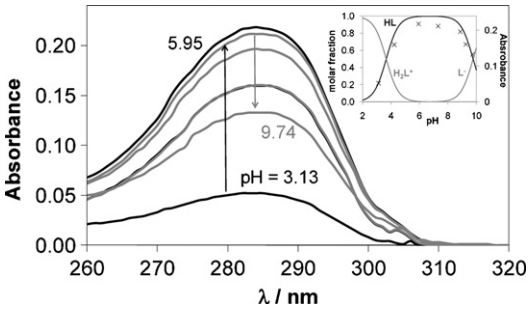


Fig. 2. UV absorption spectra of dhp in the n-octanol phase after the separation of the organic and aqueous phases at different pH {original sample composition: c_{ligand} = 100 μM; t = 25.0 °C, I = 0.20 M (KCl)}. Inset shows the pH-dependent absorbance values at 285 nm (×) measured in the n-octanol phase together with the molar fraction of dhp.

The choice of the type of the buffer and its concentration is also fundamental for these studies, since the buffer has to be chemically indifferent and its pK_a should be similar to the adjusted pH. The buffer solution has to provide high enough buffer capacity during the measurement as well; especially it is critical when Δ(pH–pK_a) is large and high number of proton release or binding is probable in the system. Mainly HEPES (pK_a = 7.55), Tris (pK_a = 8.08) or phosphate ($H_2PO_4^{-}$/HPO_4^{2-} pK_a = 7.20) buffers are used at physiological pH [25], but their pK_a is a bit far from pH 7.40. Phosphate can be a potent ligand for many metal ions (*e.g.* Al(III), Fe(III)), or it forms precipitate with numerous metal ions (*e.g.* Zn(II), Ca(II)). Tris can chelate Cu(II), Hg(II) or it binds other transition or alkaline earth metal ions weakly [26–29]. The use of

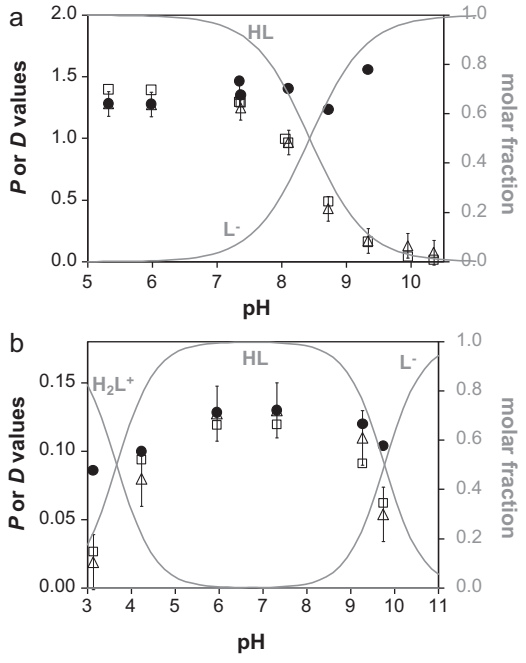
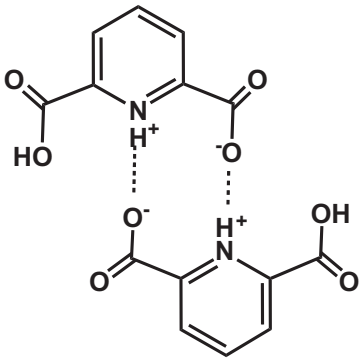
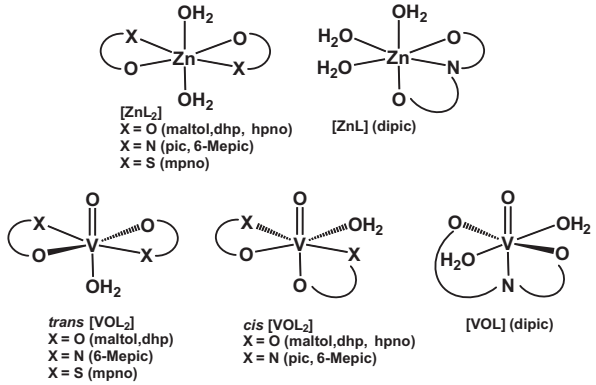


Fig. 3. Lipophilicity–pH profile curves: measured D values (△) of maltol (a) and dhp (b) at different pH with the calculated P values based on them (●). D values (□) calculated with the help of the accepted P value (see Table 1) and molar fractions of neutral HL species, together with the concentration distribution curves of the ligands (grey solid lines) {c_{ligand} = 100 μM; t = 25.0 °C, I = 0.20 M (KCl)}.



Scheme 2. Possible intermolecular interactions between the charge-neutral dipic molecules.



Scheme 3. Binding modes of the antidiabetic neutral Zn(II) and VO(IV) complexes ([ML_2] and [ML] when L = dipic) studied.

the 2-(bis(2-hydroxyethyl)amino)acetic acid (bicine) as a buffer in metal containing samples is suggested to be avoided due to its significant metal binding properties [30]. Moreover, certain buffers may behave as surfactants. Therefore, the pH values were always controlled of the aqueous phase before and after the separation in this study and the "innocence" of the buffer was also checked. It could be concluded that the use of the CHES buffer has to be avoided in the case of these ligands, since it seems to act as a surfactant resulting in much lower absorbance of the aqueous phase after partitioning, hence incorrect log *D* values, compared to those of other buffer such as CAPS, while the spectra of *n*-octanol phases are similar (see Fig. S5).

The intrinsic lipophilicity of the ligands (see log *P* values in Table 1) shows that the compounds studied are fairly hydrophilic, only the neutral forms of mpno and dipic represent somewhat higher lipophilicity. Replacement of the oxygen to sulfur donor results in a significantly increased lipophilic character as compared the log *P* value of mpno to that of hpno. It would be expected that the introduction of a methyl substituent at the pyridine ring of pic results in an increased log *P* value, but no change in the lipophilicity was measurable, most probably due to the zwitterionic form of both 6-Mepic and pic (see Scheme 1). However, the neutral form of dipic (H_2L) is also zwitterionic, but it displays a considerable tendency of dimerization in aqueous solutions via intermolecular hydrogen bonding (Scheme 2) [31], and this self-association may lead to its outstanding lipophilic character among the ligands studied. The apparent coefficients (log $D_{7.40}$, see Table 1) characterizing the lipophilicity at physiological pH are considerably low, only maltol and dhp show measurable transfer to *n*-octanol at this pH. The log $D_{7.40}$ values of maltol and dhp are in a reasonably good agreement with the literature data [19,20,32].

3.2. Lipophilicity of the antidiabetic Zn(II) and VO(IV) complexes

The antidiabetic Zn(II) and VO(IV) complexes are considered as kinetically labile species with moderate stability [9,17,24,33–35]. The thermodynamic equilibrium state in their aqueous solution is basically dependent on the stability of the species formed under the given circumstances. Based on the stability constants, it was pointed out that these neutral *bis*–ligand complexes of Zn(II) and VO(IV) (see Scheme 3) cannot keep their original integrity, VO(IV) species can partly hydrolyze, and Zn(II) complexes can partly disproportionate to *mono* and *tris* complexes even at the blood plasma pH depending on the total concentrations [34]. In order to obtain reliable log *P* values of the neutral species ([ML_2], or [ML] in the case of dipic) the actual solution speciation has to be considered.

The characterization of the lipophilic properties is fairly simple in the case of chemically intact metal species such as the kinetically inert complexes (*e.g.* Cr(III), Pt(II) complexes) [36,37]. Numerous examples can be also found in the literature for complexes with significantly high stability (*e.g.* [$Fe(III)L_3$], [$Ga(III)L_3$], [$Al(III)L_3$], when L = hydroxypyridinone) resulting in the predominant formation of a single species, and the transfer of the metal ion is measured [32,38–40]. On the contrary, studies on the log *P* values of moderately stable, kinetically labile metal complexes with the knowledge of the speciation are quite rare [41–43]. Mostly the actual form and the equilibrium concentration of the metal

Table 3
Partition coefficient (*P*, log *P*) values of the *bis*–ligand zinc and vanadyl complexes with their molar fractions (*x*)[a] at pH 7.40 and calculated $D_{7.40}$, log $D_{7.40}$ values [*t* = 25 °C; *I* = 0.20 M (KCl)].

	P[ZnL_2] Log *P*[ZnL_2]	*x*[ZnL_2]	$D_{7.40}$[ZnL_2] Log $D_{7.40}$[ZnL_2]	*P*[VOL_2] Log *P*[VOL_2]	*x*[VOL_2]	$D_{7.40}$[VOL_2] Log $D_{7.40}$[VOL_2]
maltol	0.50 ± 0.15 −0.30	0.25	0.13 −0.89	0.22 ± 0.15 −0.66	0.68	0.15 −0.82
dhp	0.09 ± 0.01 −1.05	0.37	0.03 −1.52	0.08 ± 0.04 −1.10	1.00	0.08 −1.10
hpno	0.15 ± 0.03 −0.82	0.40	0.06 −1.22	0.20 ± 0.02 −0.70	0.93	0.19 −0.72
mpno	4.92 ± 0.15 0.69	0.72	3.54 0.55	2.26 ± 0.20 0.35	0.28	0.63 −0.20
pic	0.20 ± 0.09 −0.70	0.43	0.09 −1.05	0.08 ± 0.01 −1.10	0.08	0.01 −2.00
6-Mepic	0.78 ± 0.06 −0.11	0.32	0.25 −0.60	1.94 ± 0.20 0.29	0.00	0.00 <−2
dipic[b]	0.51 ± 0.10 −0.29	0.04	0.02 −1.70	0.02 ± 0.01 −1.70	0.00	0.00 <−2

[a] Calculated at c_{ligand} = 200 μM; M:L = 1:2; pH 7.40 based on stability constants taken from Refs. [9,17,24,33–35].
[b] Values refer to the neutral [ML] species.

Table 4
λ_{max} and ε ($M^{-1}\,cm^{-1}$) values for the Zn(II) complexes formed with the carrier ligands and for VO(IV) *bis*–ligand complexes of hpno and mpno determined by UV spectrophotometric titrations [t = 25 °C; I = 0.20 M (KCl)].

	$[ZnL_2]$	$[ZnL]^+$
maltol	308 nm (12.7×10^3)	310 nm (9.10×10^3)
dhp	295 nm (21.1×10^3)	297 nm (12.2×10^3)
hpno	298 nm (12.4×10^3)	304 nm (5.52×10^3)
mpno	307 nm (~8.2×10^3)	307 nm (~3.9×10^3)
pic	264 nm (9.30×10^3)	264 nm (5.44×10^3)
6-Mepic	272 nm (~9.1×10^3)	272 nm (5.10×10^3)
dipic[a]	269 nm (11.1×10^3)	268 nm (5.1×10^3)
	$[VOL_2]$	
hpno	308 nm (8.44×10^3)	
mpno	313 nm (~7.3×10^3)	

[a] Taken from ref. [24].

complexes are disregarded in water, supposing that they keep their original integrity (as they exist in the solid phase). This assumption often may be wrong and it can result in the determination of incorrect log *P* values.

The lipophilicity of some Zn(II) and VO(IV) *bis*–ligand antidiabetic complexes had been already investigated, but only at pH 7.40 without taking into consideration their speciation [17,18,20]. The transfer of the metal complexes to the organic phase was followed by UV spectrophotometry and/or in the case of some VO(IV) complexes by EPR [17,18,20]. The problem is that the neutral *bis*–ligand complexes are usually not predominant at the physiological pH and at 1:2 metal-to-ligand ratio (see the molar fractions in Table 3).

Thus, besides the metal complexes the transfer of the neutral ligand species formed by the dissociation/hydrolysis of the complexes from the aqueous phase to the *n*-octanol is also likely. Whereas, determination of log *D* values in a wider pH range and the application of UV spectrophotometry together with the measurement of the metal content of the different phases can provide a more complete view about the partitioning in these systems.

First of all, the spectra of the individual complex species formed in the Zn(II)–carrier ligand systems (see Scheme 1) and those of some VO(IV) species (hpno, mpno) were determined, and λ_{max} and ε values are collected in Table 4. It can be concluded that changes only in the ligand bands by the complexation can be followed in the UV spectra of the complexes under such conditions due to the lack of the d–d bands of Zn(II) complexes, and because the d–d transitions of the VO(IV) species have very low ε values [44,45]. The individual spectra of the neutral HL and $[ML_2]$ species show some differences but strongly overlap, which makes the deconvolution of the spectra, measured for the aqueous phase after the separation, rather difficult.

According to the spectrum recorded in the *n*-octanol phase of the Zn(II)–maltol system the transfer of both the neutral HL (λ_{max} ~ 278 nm) and $[ZnL_2]$ (as a shoulder at λ ~ 320 nm) species is clearly demonstrated in Fig. 4a by the comparison of the spectrum of HL in the organic phase (*c.f.* Fig. S3 or Fig. S1). The concentration distribution curves in Fig. 4b represent well the pH range of the formation of the *bis*–ligand complex. This can help in the choice of the proper pH region for the determination of a reliable log *D* value of this complex. Therefore, speciation curves for the Zn(II), VO(IV) complexes were analyzed in order to obtain the pH range where the formation of the *bis*–ligand complex is maximal, and the sensitivity of the vanadyl species or the mpno complexes to the oxidation was also taken into account prior to the partitioning. Separation was performed at different pH values (4–5 points) and ICP-AES was used to determine the metal content of the aqueous phases before and after the separation in order to obtain the log *D* values. Then log *P* parameters of the species $[ML_2]$ ([ML] for dipic) were calculated with the use of their molar fractions as Eqs. (7) and (8) show (see Table 3). Log $D_{7.40}$ values of these complexes were also calculated.

On the other hand, the different feature of the UV spectra recorded for the aqueous solutions obtained before and after the partitioning show how the original equilibrium is shifted (see Fig. 4a). Spectrum of the aqueous phase recorded following the separation could be simulated using the individual spectra of the species present (HL, L^-, $[ZnL]^+$, $[ZnL_2]$) and the concentration of Zn(II) based on the ICP-AES results. The simulated spectrum shows an acceptable fitting to the measured one. It can be seen that the metal-to-ligand ratio and the total concentrations of Zn(II) and maltol are changed in the aqueous solution as a result of the simultaneous partitioning of the metal complex and the carrier ligand.

Concentration distribution curves are shown for the VO(IV)–pic system together with the *D* values measured at various pH (see Fig. 5). It can be concluded that the *D* value obtained at pH ~ 5 is the most relevant for the *bis* VO(IV)–pic complex. At the same time, formation of a minor, neutral dinuclear species can increase the vanadium content of the *n*-octanol phase at higher pH resulting in a higher "apparent" *D*.

Log *P* values of the neutral complexes $[ML_2]$, [ML] (see Table 3) indicate quite hydrophilic character. As the ligand maltol has somewhat higher lipophilicity as compared to dhp, the Zn(II) and VO(IV) complexes of this ligand also possess higher log *P* values. The significantly more lipophilic mpno forms complexes with higher log *P* than hpno does in accordance with the findings in ref. [17]. As a consequence, a quasi-linear relationship can be found between the log *P* of the neutral ligands and that of their *bis*–ligand complexes in the case of maltol, dhp, hpno and mpno, as it is shown in Fig. 6.

On the other hand, the methyl group in the 6-Mepic molecule results in an increased lipophilic character of its complexes compared with those of pic, while this effect was not reflected in the log *P* values of the neutral ligands (see above). On the contrary, the

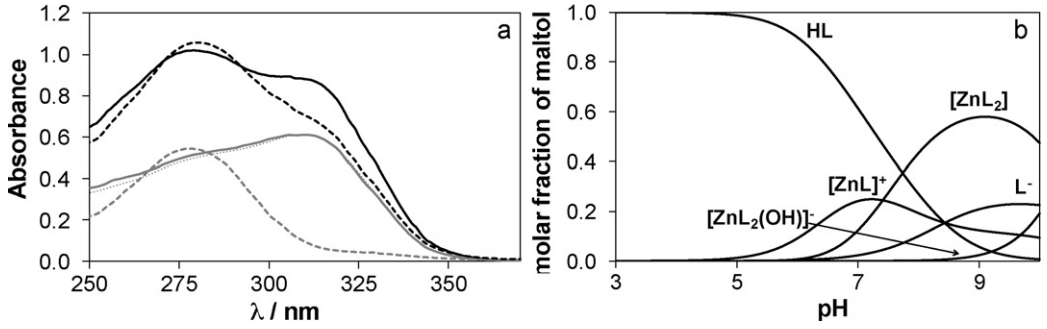


Fig. 4. UV absorption spectra of Zn(II)–maltol containing samples recorded for the original solution (black solid line), in the aqueous phase (grey solid line) and in the *n*-octanol phase (grey dashed line) following the separation together with their summed spectrum (black dashed line). Spectrum of the aqueous phase was simulated (grey thin and dashed line) based on the individual spectra of all species involved and the Zn(II) concentration determined by ICP-AES {original sample composition: c_{ligand} = 215 μM; M:L = 1:2.3; t = 25.0 °C, pH = 7.39 (HEPES), I = 0.20 M (KCl); aqueous phase after separation: c_{ligand} = 123 μM; M:L = 1:1.4}. (a) Concentration distribution curves for the Zn(II)–maltol system at the original sample composition (b).

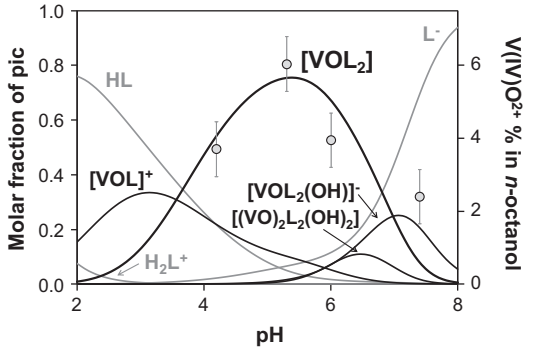


Fig. 5. Concentration distribution curves for the VO(IV)–pic system together with the distribution (%) of the vanadium in the *n*-octanol phase obtained from the ICP-AES results at chosen pH values (○) {c_{pic} = 200 μM; VO(IV):pic = 1:2; t = 25.0 °C, I = 0.20 M (KCl)}.

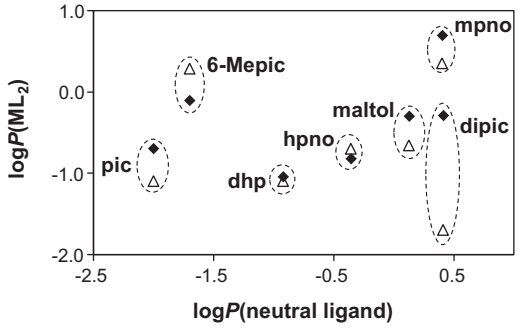


Fig. 6. Correlation diagram for the *bis*–ligand Zn(II) and VO(IV) complexes and their carrier ligands between log *P* vales of [ML_2] species and those of the neutral ligands (HL: pic, 6-Mepic, dhp, hpno, maltol, mpno; H_2L: dipic); Zn: ♦; VO: △..

complexes of dipic are more hydrophilic as it is expected on the basis of the log *P* value of the neutral ligand. Therefore, the values of pic, 6-Mepic, dipic fall well off the correlation in this diagram (Fig. 6) due to the different structure of the metal-free and complexed ligands in solution. It is noteworthy that the log *P* values of the Zn(II) and VO(IV) complex of a certain ligand do not differ significantly, but the species [ZnL] was found to be clearly more lipophilic than [VOL] in the case of dipic. The log $D_{7.40}$ values of the neutral metal complexes (see Table 3) depend on both the intrinsic lipophilicity (log *P*) and their molar fraction at pH 7.40. For this reason, the vanadyl complexes of the picolinates represent *ca.* zero *D* value at the blood plasma pH.

4. Conclusions

The lipophilic character of various ligands, which form neutral *bis*–ligand Zn(II) and VO(IV) complexes with considerable insulin-enhancing effect was studied and their log *P* and log $D_{7.4}$ values were determined. It was pointed out that the knowledge of the spectra of the individual ligand species besides the protonation processes and the lipophilicity–pH profile curves can help in the determination of the more reliable and correct log *P* and log $D_{7.4}$ parameters. The neutral forms of these carrier ligands were found to be quite hydrophilic (log *P* < 0.13), only mpno and dipic exhibit increased lipophilicity (log *P* = 0.40; 0.41 respectively).

The neutral Zn(II) and VO(IV) complexes of ligands such as maltol, dhp, hpno, pic, 6-Mepic, dipic exhibit fairly low log *P* values, but the metal complexes of mpno (pyrithione) are much more lipophilic (log *P* = 0.69 (Zn(II)); 0.35 (VO(IV)). Taking into account the speciation under the given circumstances (based on the stoichiometry and the stability constants of complexes formed) is indispensable for the determination of the log *P* and log $D_{7.4}$ parameters. Log $D_{7.40}$ values of the complexes are also relatively low, and complex [$Zn(mpno)_2$] represents the highest lipophilicity at the blood plasma pH.

Results show the importance of the accurate knowledge of the chemical speciation in solution under the given circumstances (pH, concentration) in order to obtain more trustable log *P* values of a ligand or metal complex, especially in the case of kinetically labile species with moderate stability. All the findings also draw the attention to the fact that a ligand's lipophilicity does not always correlate to that of its metal complex, which may be important in QSAR studies. Therefore, in many cases the actual measurement cannot be always replaced by prediction of log *P* values of complexes based on the lipophilic character of the chelating ligand.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2010.12.025.

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