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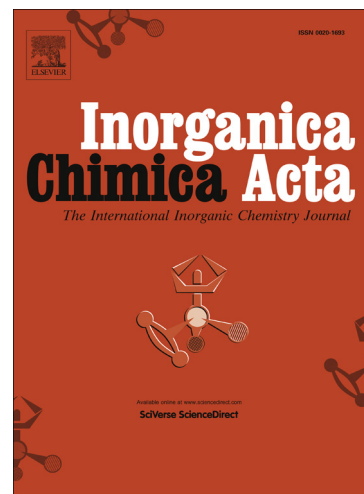
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Vanadate complexes of 3-hydroxy-1,2-dimethyl-pyridinone: speciation, structure and redox properties

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Abstract

Several articles were published about the vanadate-3-hydroxy-1,2-dimethyl-pyridinone (Hdhp) system, however, the results are contradictory and not complete: pH-potentiometry and ⁵¹V-NMR spectroscopy were used to clarify this complicated system. The eleven peaks in the spectra at different chemical shifts were assigned to ten stoichiometrically different compounds; four of them are new, never identified or assigned before. Besides the simple mono- (in two different protonation states) and bis complexes (in three different protonation states) a tris complex, three dinuclear and a trinuclear complex were found based on the ⁵¹V-NMR spectra measured at different pH values and various metal ion concentrations and metal-to-ligand ratios. As a joint evaluation of the two methods, overall stability constants were calculated for all species.

X-ray structure of the potassium salt of the bis complex, [V(V)O₂(dhp)₂]⁻ was also determined. The trans effect of the oxido-oxygens results in maltolato-type coordination of the ligand instead of the catecholate-like chelation.

The redox properties of [V(V)O₂(dhp)₂]⁻ and some other prodrug vanadium(V) bis complexes were investigated by spectrophotometry in aqueous solution via their reduction by glutathione (GSH) and

L-ascorbic acid (ASC) under strictly anaerobic conditions and by cyclic voltammetry at physiological pH. The reduction was found to be much faster by ASC in all cases as compared with GSH and the reaction rate of the reduction of $[V(V)O_2(dhp)_2]^-$ was prominently high most probably due to the formation of the significantly higher stability of the corresponding vanadium(IV) complex.

Keywords: vanadate complexes; 3-hydroxy-1,2-dimethyl-pyridinone; speciation; ^{51}V -NMR; redox properties

1. Introduction

Numerous vanadium(IV) and (V) complexes showed significant antidiabetic activity in preclinical *in vitro* and *in vivo* studies [1-4]. One of them the bis(ethylmaltolato)oxovanadium(IV) (BEOV) complex has entered into Phase IIa trial [5]. The active vanadium species exhibit *ca.* 30-70% of the activity of insulin *in vitro*, and generally the efficacy of the V(IV) salt and especially complexes exceeds the originally tested V(V) salts [5,6]. However the V(V)-compounds tend to be less toxic than V(IV)-complexes, and in general there is no significant correlation between vanadium oxidation state and the insulin-mimetic efficacy [3].

Up to now the most effective hypoglycemic drug candidates are the orally available charge-neutral bis complexes of V(IV) formed with bidentate ligands. The advantage of these metal complexes over the inorganic oxovanadium(IV) salts is their increased bioavailability and thus enhanced pharmaceutical efficacy. According to the stability of this type of complexes, they are usually not stable enough at the pH of the gastric juice resulting in unfavorable uptake, however, that can be bypassed by means of proper drug formulation such as encapsulation methods [7]. Based on the dosage range data of the clinical trials and the absorption properties of BEOV *ca.* 20 μM is estimated as the maximum concentration of vanadium attainable in the human blood serum during the treatment of diabetes mellitus [8]. This kind of vanadium complexes shows facile interconversion between the oxidation states (IV and V) and biologically relevant reducing agents such as L-ascorbic acid (ASC, $10-80 \times 10^{-6} \text{ mol dm}^{-3}$), cysteine ($33 \times 10^{-6} \text{ mol dm}^{-3}$), glutathione (GSH, $4 \times 10^{-6} \text{ mol dm}^{-3}$), uric acid ($200-400 \times 10^{-6} \text{ mol dm}^{-3}$), alpha-tocopherol ($20-30 \times 10^{-6} \text{ mol dm}^{-3}$) *etc.* and the dissolved oxygen ensure that both V(IV) and V(V) species are relevant under serum conditions [9].

It was pointed out in our previous works that vanadium in oxidation state IV and V is bound mostly to serum transferrin in the therapeutically relevant concentration range and the original carrier

ligand is displaced completely. The only exception among the bidentate drug candidate ligands is the 3-hydroxy-1,2-dimethyl-pyridinone (Hdhp) where the dissociation of the original complex is not complete in serum and the ligand tends to form V(IV)O-apotransferrin-dhp ternary complexes even at such concentration conditions [4,9,10]. However insulin-mimetic efficacy of the [V(IV)O(dhp)₂] complex looks ordinary [3,11,12].

Several articles were published about the speciation of the vanadate-dhp system [13-16], although the conclusions are sometimes contradictory in the different publications and the final picture is still not complete.

In the first short publication pH-metric results were reported [13] and the following species, mainly mono and bis complexes in different protonation forms were identified: $[(H_2VO_4)(HL)]^- \equiv [VO_2(dhp)OH]^-$, $[H(H_2VO_4)(HL)] \equiv [VO(dhp)(OH)_2]$, $[(H_2VO_4)(HL)_2]^- \equiv [VO_2(dhp)_2]^-$, $[H(H_2VO_4)(HL)_2] \equiv [VO(OH)(dhp)_2]$, $[H_2(H_2VO_4)(HL)_2] \equiv [VO(dhp)_2]^+$. No distinction was made if protonation occurs on the vanadate side or the ligand side of the complex. Only two ⁵¹V-NMR peaks (mono complexes: $\delta(^{51}V) = -502$ ppm, bis complexes: $\delta(^{51}V) = -476$ ppm) were identified at the physiological pH. Based on cyclic voltammetric (CV) measurements the authors also concluded that the reduction of the vanadate complexes to oxovanadium(IV) is not reversible, but the irreversibility is less pronounced at pH 3 [13].

Later X-ray structure for a trinuclear complex $[(VO_2)_3(dhp)_3 \cdot H_2O]$ has been published [14]. In this cyclic compound, μ -oxygens form bridges between the vanadium centres, the three ligands and the vanadates are not equivalent due to an extra ligand-O-V bound.

The ⁵¹V-NMR spectral work in the same publication [14] is more detailed, but is still not complete: it reports unidentified species (C: $\delta(^{51}V) = -420 \text{ } \square \text{ } -405$ ppm; C': $\delta(^{51}V) = -350$ ppm). A peak at $\delta(^{51}V) = -489$ ppm was assigned improperly to a trinuclear complex $[(VO_2)_3(dhp)_3]$ instead of mononuclear one $[(VO_2)(dhp)]$. ¹H-NMR measurements for the mono and bis complexes have also been published [14], but no thermodynamic information was concluded from these measurements.

In the third article [15] stability and protonation constants were determined from the ⁵¹V-NMR data: the stability constants for the mono and bis complexes significantly differ from the earlier published values [13]. The authors determined one protonation constant (pK) for the mono and two others for the bis complexes by ⁵¹V-NMR spectroscopy. These processes were supposed to occur on the metal side. However, based on ¹H-NMR measurements further protonation processes for both the mono- and bis complexes were assumed which should occur on the ligand side [15].

From methanol-water solvent mixture a dinuclear dhp-vanadate-methyl-ester complex was $[(VO(OMe)(dhp))_2O]$ prepared, in which beside two oxygen donor atoms of each dhp ligand a μ -

oxygen also forms a bridge between the vanadium centres. ^1H and ^{51}V -NMR methods have been used to determine the equilibrium in different methanol-water mixtures.

Addition of methanol to the system makes the speciation even more complicated because the alcohol is able to react with the V-OH part of the complexes forming “esters”, and this process is not advantageous for understanding the feature of the basic V(V)-dhp system. Temperature dependence of ^{51}V -NMR spectra and 2D ^1H homonuclear NMR spectra was also measured and isomers of the dinuclear ($[\text{V}(\text{V})\text{O}(\text{OMe})(\text{dhp})_2\text{O}]$) and the bis complex $[\text{V}(\text{V})\text{O}(\text{OMe})(\text{dhp})_2]$ were detected [16].

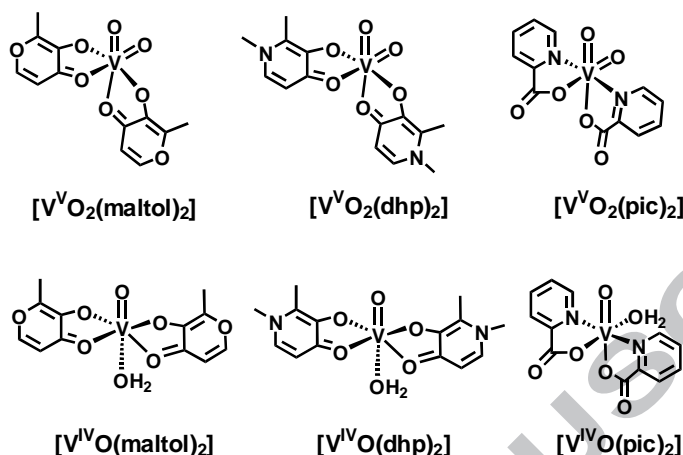
In order to clarify the speciation in the V(V)-dhp system and identify the composition and the stability of the complexes pH-metric and ^{51}V -NMR spectroscopic measurements were used in the present work.

Vanadium may be assumed to enter the cells via the transferrin receptors, when V(IV), or via the phosphate or sulfate pathway, when V(V) [1]. In the intracellular medium V(V) species may suffer reduction by certain cell components and ASC and GSH are the most important and abundant cellular antioxidants ($0.01\text{--}0.02 \text{ mol dm}^{-3}$, $0.5\text{--}10 \text{ mmol dm}^{-3}$, respectively) and their role is frequently discussed [17,18]. It is noteworthy that GSH and its oxidized form glutathione disulfide (GSSG) are also able to form binary or ternary $\text{V}(\text{V})\text{O}_2^+$ and $\text{V}(\text{IV})\text{O}^{2+}$ complexes by the complete or partial substitution of the carrier ligand [19] and a similar behavior can not be completely ruled out either for ASC, however based on the conditions and stability ratios the original bis complexes should dominate in the solution before and after the reduction too.

The reaction rate of the reduction of the V(V) complexes basically depends on the type of the reducing agent and that of the coordinating ligand. The greater ability of the chelating ligand to stabilize the V(V) oxidation state, the stronger the tendency to promote the reduction. It was found *e.g.* that NADPH can reduce *in vitro* V(V) complexes having formation constant ($\log K'$) higher than 7 in the case of a series of amino acid, oligopeptide, aminopolycarboxylate ligands [1]. Simple thiols, as other possible reductants, are able to form stable complexes with V(V) at neutral or alkaline pH, however, they are oxidized by the metal ion under other conditions, such as low pH and high thiol excess [1].

B. Song *et al.* performed a detailed kinetic study of the reduction of $[\text{V}(\text{V})\text{O}_2(\text{maltol})_2]$ and $[\text{V}(\text{V})\text{O}_2(\text{ethylmaltol})_2]$ with ASC or GSH in aqueous solution monitored by UV-visible spectrophotometric, EPR and ^{51}V NMR techniques [17]. These V(V) complexes showed similar behavior; therefore, the replacement of the methyl group by the ethyl group in the ligand structure had little influence on the reaction rate. The reduction by GSH was found to be much slower than by ASC at physiologically relevant pH. First-order kinetics at large excess of GSH and ASC is

suggested and the observed first-order rate constants showed a linear relationship with the concentration of the reductants. An acid dependent mechanism was proposed from kinetic studies with varying pH and carrier ligand concentration.



Scheme 1. Bis-ligand vanadium(V/IV) complexes formed with maltol = 3-hydroxy-2-methyl-4H-pyran-4-one; dhp = 3-hydroxy-1,2-dimethyl-pyridinone and pic = picolinic acid.

The reaction mechanism, however, seems to be quite complicated due to the additional binary and ternary complex formation reactions with the reducing agents (*vide supra*). In this work we attempt to compare the effect of the dhp with that of various other carrier ligands on the reduction reaction rate when GSH and ASC are applied as reductants under strictly anaerobic conditions at pH 7.4 via monitoring the spectral changes in the near UV-visible range. Additionally, cyclic voltammetric investigations were performed on the direct redox processes of the vanadium complexes formed with maltol, dhp as (O,O) donor ligands and picolinic acid (pic) as an (N,O) binder (see Scheme 1 for the bis-ligand complexes).

2. Experimental Section

2.1. Chemicals

Maltol, dhp, pic, GSH, ASC, NaVO_3 , VOSO_4 , D_2O , $\text{Na}_3[\text{Fe}(\text{CN})_6]$ and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were commercially available products of *puriss* quality (Sigma-Aldrich). KCl, 37% HCl and KOH were purchased from Reanal (Hungary). Double distilled Milli-Q water was used for sample preparations. The exact concentrations of the ligand (except for pH-metry) and vanadate stock solutions were calculated based on their mass and the total volumes. Latter were prepared by dissolving NaVO_3 in known concentration KOH solution. The V(IV)O stock solution was prepared as described in ref. [20] and standardized for metal ion concentration by permanganate titration.

2.2 Preparation of the single crystal of $\text{K}[\text{VO}_2(\text{dhp})_2] \times 2\text{H}_2\text{O}$

First a 3 cm^3 $0.005 \text{ mol dm}^{-3}$ potassium-vanadate solution (K_2HVO_4) was prepared by solving V_2O_5 in known concentration KOH solution. Solid dhp was added, 1 : 2 metal-to-ligand ratio was set. In order for faster solubilization the solution was heated gently and the pH of the solution was set to 8.5 by addition of 6 mol dm^{-3} HCl. An open vessel containing this solution was placed in a bigger container containing acetone in order for slow solvent exchange. Single crystals were collected several weeks later.

2.3 Determination of structure of $\text{K}[\text{VO}_2(\text{dhp})_2] \cdot 2\text{H}_2\text{O}$

Crystals were mounted on a glass capillary with epoxy glue. Single crystal X-ray diffraction data were collected on an Enraf Nonius MACH3 diffractometer with graphite-monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 293 K. The structure was solved with the SIR-92 program [21] and refined by a full-matrix least-squares method using the SHELXL-97 package; [22] H atoms were located geometrically and treated isotropically with $U_{\text{iso}} = 1.2$ times the U_{eq} of parent atoms, with the exceptions of water and ammonium H atoms. These could be obtained from the difference electron density map treated isotropically, and restraints were applied to fix O–H lengths at 0.9. The publication material was prepared with the WINGX suite. [23]

2.4. Potentiometric measurements

The stability constants of V(V) complexes of the ligands were determined by pH-potentiometric titrations of 25.0 cm^3 samples; the ionic strength was 0.20 mol dm^{-3} (KCl). The concentrations of dhp were $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ and $4.0 \times 10^{-3} \text{ mol dm}^{-3}$, and the metal ion-to-ligand molar ratios were

0:1, 1:1, 1:2 and 1:4. pH was measured with an Orion 710A precision digital pH-meter equipped with a Metrohm 6.0234.100 semimicro combined glass electrode, and calibrated for hydrogen ion concentration according to Irving *et al.* [24] The pK_w calculated from strong acid–strong base titrations was 13.75 ± 0.01 . Back-titrations were performed with a HCl solution of known concentration (ca. 0.20 mol dm^{-3}). In all cases, the temperature was $25.0 \pm 0.1 \text{ }^\circ\text{C}$. Slow or irreversible processes (formation of decavanadates, redox reactions) were not observed. The reproducibility of the titration points included in the calculations was within 0.005 pH. The complex-formation equilibria studied are written in terms of the components H^+ , HVO_4^{2-} and dhp (L^-), which is the deprotonated form of the neutral ligand Hdhp :



Formation constants are denoted as $\beta_{p,q,r}$ and complexes have the notation (p,q,r). All calculations were performed with the aid of the PSEQUAD [25,26] or the pHCali [27] computer programs. Stability constants ($\log \beta$) of vanadates ($\text{H}^+/\text{V(V)}$) are taken from [28].

2.5 NMR measurements and data

^{51}V -NMR spectra were recorded on a Bruker Avance DRX500 spectrometer. The samples contained 10% (v/v) D_2O . The external reference was VOCl_3 for the ^{51}V -NMR measurements. Routine parameters were used for ^1H -NMR spectroscopy. A spectral window of 760 ppm, a 90° pulse angle and an acquisition time of 0.65 s with a relaxation delay of 0.5 s and a pulse width of 7 ms were applied in the ^{51}V -NMR measurements. Samples were freshly prepared and their pH was adjusted with concentrated HCl and KOH solutions. The ionic strength of the samples was

Table 1. The applied concentrations (in $10^{-3} \text{ mol dm}^{-3}$) and pH values and/or ranges during the ^{51}V -NMR spectroscopic measurements.

Series	c(V)	c(dhp)	c(V) : c(dhp)	pH
I	3.33	6.66	1 : 2	11.4-2.0
II	4	5	1 : 1.2	11.5-4.0
III	10	30	1 : 3	7.5-1.8
IV/a	10	10-20	1 : 1 - 1 : 2	4.45
IV/b	10	10-20	1 : 1 - 1 : 2	4.8
IV/c	10	10-20	1 : 1 - 1 : 2	5.4
V	0.5 - 20	0.5 - 20	1 : 1.1	4.8
VI/a	6	30	1 : 5	4.5-2.0
VI/b	6	6-60	1 : 1 - 1 : 10	2
VI/c	6	6-60	1 : 1 - 1 : 10	3.6
VII/a	1-16	2-32	1 : 2	3.6
II/b	1-16	2-32	1 : 2	5.4

0.20 mol dm⁻³ (KCl). The applied conditions for different measurement series are summarized in Table 1. Stability and acidity constants were determined with the aid of the computer program PSEQUAD [25,26] based on the integral values and chemical shifts.

2.6. UV-Vis spectrophotometric kinetic measurements

Data collection: A Hewlett Packard 8452A diode array spectrophotometer was used to record the UV-Vis spectra in the interval 200-800 nm at 25.0 ± 0.1 °C, the path length was 1 cm. A special, tightly closed tandem cuvette (Hellma Tandem Cell, 238-QS) was used and the reactants were separated until the reaction was triggered. Both isolated pockets of the cuvette were completely deoxygenated by bubbling a stream of argon for 10-10 min before mixing the reactants. Spectra were recorded before and then immediately after the mixing, and changes were followed till no further absorbance change was observed. One of the isolated pockets contained the reducing agent (GSH or ASC) and its concentration was in the range of 0.07-160×10⁻³ mol/dm³ and the other contained the V(V) complex, which was prepared *in situ* by the addition of at least 10 fold excess of the chelating ligand to the vanadate providing the predominant formation of the bis-ligand complex, which concentration was chosen as 2×10⁻⁴, 5×10⁻⁴ or 2.0×10⁻³ mol dm⁻³ in order to obtain measurable absorbance changes in the visible wavelength range and reaction rates. The pH of all the solutions was adjusted to 7.40 by 0.10 mol dm⁻³ HEPES buffer and an ionic strength of 0.2 mol dm⁻³ (KCl) was applied. All the stock solutions of the V(V) complexes and the reducing agents were freshly prepared every day. Prior to the study of the reduction of the V(V) complexes by GSH and ASC under the strictly anaerobic conditions, the effect of the oxygen on the UV spectra of GSH and ASC was followed at 2×10⁻⁴ and 2×10⁻³ mol dm⁻³ concentrations, respectively. The UV-Vis spectra of the V(IV)O and V(V) complexes at 1:10 metal-to-ligand ratio were also recorded at various concentrations of vanadium (2×10⁻⁵-2×10⁻³ mol dm⁻³). Altogether 4-10 kinetic runs were made for each system.

Data processing: During the calculations the absorbance (A)-time (t) curves were fitted and analyzed at 360, 390, 410 and 430 nm. $(A_0 - A_{\text{final}}) \times e^{(-a \times t)} + A_{\text{final}}$ equation was used where A_0 , A_{final} and “a” parameters were refined and accepted at the minimal value of the weighted sum of squared residuals (difference between the measured and calculated absorbance values) at each chosen wavelength. Then observed rate constants (k_{obs}) of the redox reaction were obtained from the data points of both the experimental and fitting simulated absorbance-time curves as the slope of the $\ln(A/A_0)$ vs. t plots.

2.7. Cyclic voltammetry

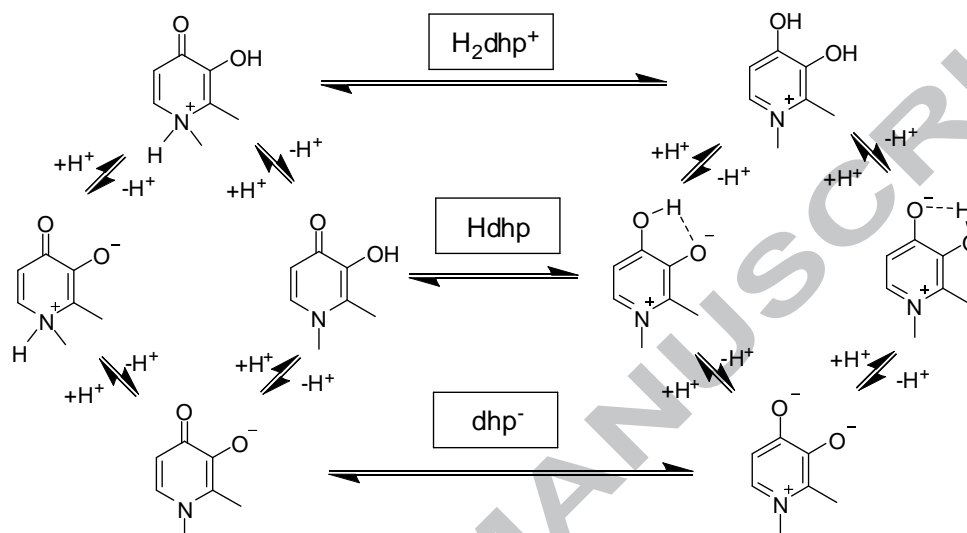
Data collection: Cyclic voltammograms of the V(V) complexes in aqueous solution containing 0.01 mol dm^{-3} V(V) and 0.02 mol/dm^3 ligand were determined at $25.0 \pm 0.1 \text{ }^\circ\text{C}$ at pH 7.40 adjusted by HCl, KOH solutions. The two fold excess of the ligand already can provide the predominant formation of the bis-ligand V complexes in this concentration range [9]. Ionic strength was 0.06 mol dm^{-3} (KCl) similarly to the studies performed by D. Crans *et al.* [29]. Measurements were performed on a conventional three-electrode system under nitrogen atmosphere and a PC Controlled Electrochemical Measurement System (EF 451). Samples were purged for 10-15 min with argon before recording the cyclic voltammograms. Platinum electrode was used as the working and auxiliary electrode and Ag/AgCl (in 1 mol dm^{-3} KCl) as reference electrode. Electrochemical potentials were converted into the normal hydrogen electrode (NHE) scale by adding 0.222 V. The electrochemical system was calibrated with an aqueous solution of $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($E_{1/2} = +0.386 \text{ V}$ vs. NHE). Redox potentials were obtained at 10 mV/s scan rate in the range of -0.80 to $+1.00 \text{ V}$.

Data processing: Characteristic values of the voltammograms such as E_a , E_c as the peak potentials and i_a , i_c as the anodic and cathodic currents were measured graphically.

3. Results and Discussion

3.1 The acid-base properties of the ligand dhp

The ligand dhp (see Scheme 2) have at least two resonance structures in its fully deprotonation state (L^-). One structure is the „normal” maltolate type; the other is the catecholate type,



Scheme 2. The protonation microequilibria of the ligand dhp.

Table 2 Acidity constants (pK_a) of dhp ($\equiv L$) and overall stability constants ($\log \beta_{p,q,r}(3SD)$) of dhp complexes formed with V(V) in aqueous solution determined by different ways and compared with literature values. Data refer to HVO_4^{2-} as component at $T = 298$ K, $I = 0.2$ mol dm^{-3} (KCl).

Species	<i>p</i>	<i>q</i>	<i>r</i>	pH-metry	^{51}V -NMR (integral)	^{51}V -NMR (δ)	$\log \beta$ (final) ^a	Ref. [13] ^b	Ref. [15] ^c
[VO ₂ L]	1	1	3	27.84(2)	28.07(13)		28.02(4)	28.11	27.84
[VO ₂ L(OH)] ⁻	1	1	2	22.02(6)	22.41(7)		22.28(3)	22.39	21.96
[(VO ₂ L) ₂ OH]	2	2	5	52.98(8)	53.2(2)		53.03(9)		
[(VO ₂ L) ₃]	3	3	9	88.08(17)	87.5(5)		87.3(3)		
[(VO ₂ L)O(VOL ₂)] ⁻	2	3	6	-	66.2(2)		66.01(11)		
[VOL ₂] ⁺	1	2	5	43.70(8)	43.63(16)		43.55(8)	45.46	43.28
[VO(OH)L ₂]	1	2	4	41.12(4)	40.96(14)		40.95(5)	42.22	40.38
[VO ₂ L ₂] ⁻	1	2	3	35.48(2)	35.57(10)		35.42(4)	36.71	34.76
[(VOL ₂) ₂ O] ²⁻	2	4	8	-	83.9(3)		83.82(11)		
[V(OH)L ₃] ⁺	1	3	6	-	56.55(15)		56.51(7)		
 pK [VO ₂ L]				5.82	5.66	5.87(3)	5.74	5.72	5.88
pK [VOL ₂] ⁻				2.58	2.67	2.59(5)	2.60	3.24	2.9
pK [VO(OH)L ₂]				5.64	5.39	5.70(5)	5.53	5.51	5.62

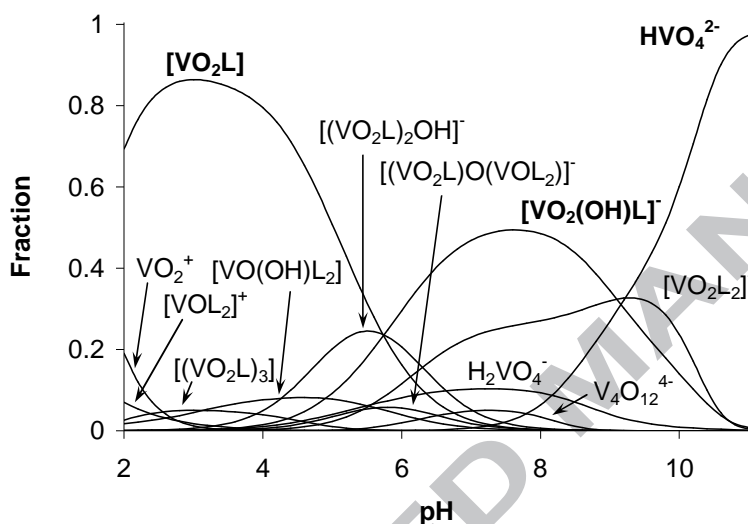
^a A joint evaluation of the pH-metric and 1H -NMR data were used.

^b $\{I = 0.15$ mol dm^{-3} (NaCl) $\}$

^c Recalculated values based on conditional constants. $\{I = 0.15$ mol dm^{-3} (NaCl) $\}$

with aromatic ring and a positively charged tertiary ammine. The first protonation ($\log K = 9.76$ [30]) similarly to the ligand maltol ($\log K = 8.44$ [31]) definitely should occur on one of the oxygen atoms. A hydrogen-bond is formed in this way between the two oxygen atoms, but the partial negative charge of the second oxygen and thus the basicity of the ligand increases as compared to maltol. The two resonance structures are still „available” in the monoprotonated form (LH). These structures of the ligand at the fully protonated state (LH₂⁺) becomes “real” isomers: based on ¹H-NMR titrations [30] the protonation ($\log K = 3.70$ [30]) takes place mainly on the oxygen atom rather than the ring nitrogen.

(a)



(b)

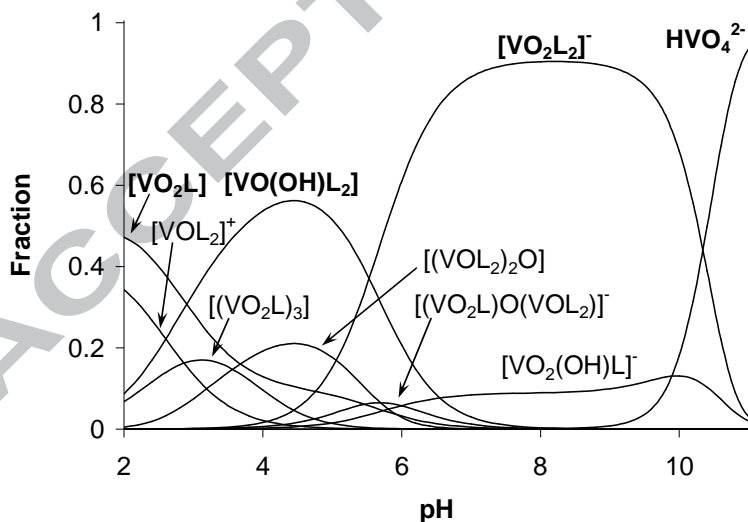


Fig. 1 Speciation curves of the complexes formed in the V(V) - dhp system at a metal-to-ligand ratio of **(a)** 1:1.1 and **(b)** 1:3. $\{c_{V(V)} = 4 \times 10^{-3} \text{ mol dm}^{-3} \text{ } I = 0.20 \text{ mol dm}^{-3} \text{ (KCl) and } T = 298 \text{ K.}\}$

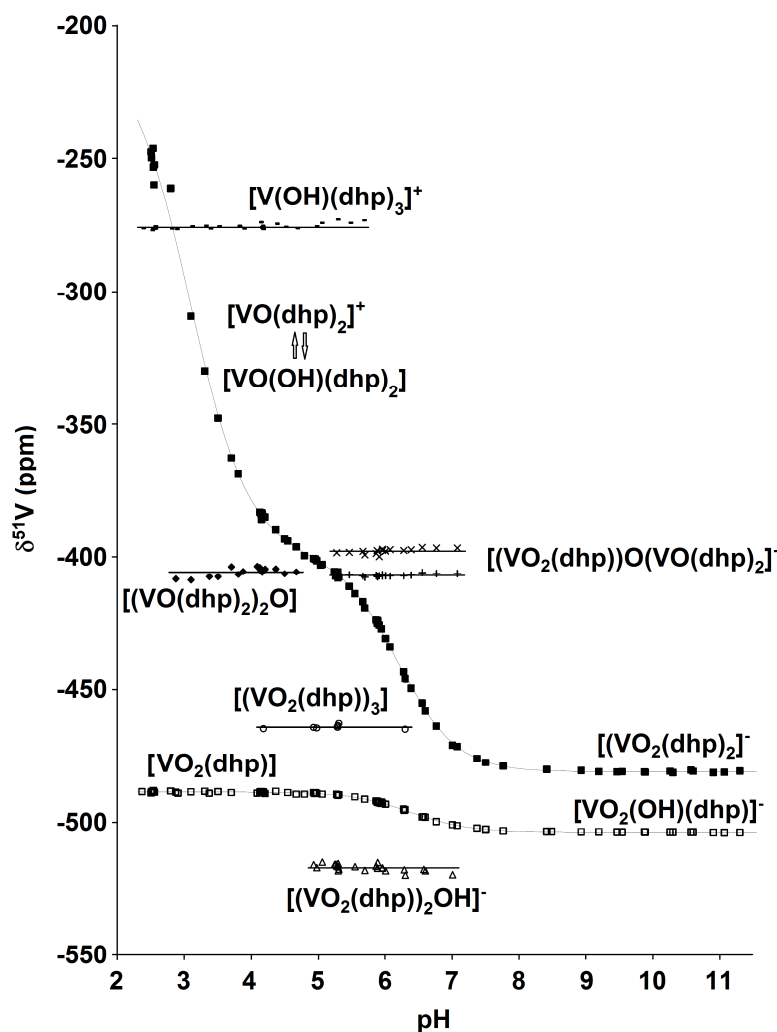


Fig. 2 The ^{51}V -NMR chemical shifts in the V(V)-dhp system as a function of pH. { $I = 0.2 \text{ mol dm}^{-3}$ (KCl), $T = 298 \text{ K}$ } The signs represent the measured points; the lines are theoretically fitted curves.

Table 3 ^{51}V -NMR chemical shifts ($\delta(^{51}\text{V})$ ppm) of V(V) complexes formed with dhp ($\equiv \text{L}$) in aqueous solution at $T = 298 \text{ K}$ and $I = 0.2 \text{ mol dm}^{-3}$ (KCl).

Species	p	q	r	$\delta(^{51}\text{V})$ [ppm]
$[\text{VO}_2\text{L}]$	1	1	3	-489
$[\text{VO}_2\text{L}(\text{OH})]^-$	1	1	2	-504
$[(\text{VO}_2\text{L})_2\text{OH}]^-$	2	2	5	-518
$[(\text{VO}_2\text{L})_3]$	3	3	9	-466
$[(\text{VO}_2\text{L})\text{O}(\text{VOL}_2)]^-$	2	3	6	-400; -408
$[\text{VOL}_2]^+$	1	2	5	-209(6) ^a
$[\text{VO}(\text{OH})\text{L}_2]$	1	2	4	-399(2) ^a
$[\text{VO}_2\text{L}_2]^-$	1	2	3	-481
$[(\text{VOL}_2)_2\text{O}]^{2-}$	2	4	8	-406
$[\text{V}(\text{OH})\text{L}_3]^+$	1	3	6	-276

^a Estimated from calculation of the pK_a of the bis complex ($[\text{VO}_2\text{L}_2]$) based on $\delta(^{51}\text{V})$.

3.2 Vanadate complexes of dhp

The pH-metric titration of the basic solutions of the V(V)-dhp system could be evaluated throughout the whole pH range studied (2.0–11.5). The stability constants calculated from pH-metry and/or ^{51}V -NMR for the V(V) complexes of dhp are listed in Table 2. Two representative speciation curves, one at equimolar ligand-to-metal ratio and one at ligand excess are depicted in Fig. 1. Table 3 contains the ^{51}V chemical shifts assigned to the different complexes formed in solution; their changes as a function of pH in the different experimental series were collected in Fig. 2.

3.2.1 The mono and bis complexes

It is seen in Fig. 2 that these complexes are formed practically in the whole pH range. Formation of such vanadate complexes is usual for bidentate ligands [32], the stability constants determined (Table 1) and $\text{p}K$ values are quite similar from one set of values recalculated from literature data [15]. The protonation equilibrium of the mono complex $([\text{V}(\text{V})\text{O}_2(\text{dhp})(\text{OH})]^- + \text{H}^+ = [\text{V}(\text{V})\text{O}_2(\text{dhp})(\text{H}_2\text{O})])$ is also usual in such complexes [32]. The main difference for example from the vanadate-maltol system is that in the two step protonation process of the bis complex: the second step is normally missing and the first step is usually occurs at $\text{pH} < 3$ [32]. However, the dhp is able to coordinate in a catecholate way (*vide infra*) and the increased charge on the central vanadate causes also increased partial charge on the oxido-oxygens, which finally leads to their double protonation equilibria.

Second protonation step for the mono and third for the bis complex were suggested based on ^1H -NMR measurements [15], the authors assign these processes to the ligand ring N [15]. However, this can be ruled out based on our pH-metric results and because no protonated complexes were published with any other metal complexes of dhp [30,33,34]. The observed spectral changes [15] in the ^1H -NMR spectra must due to the protonation on the vanadate side of the complexes.

A representative ^{51}V -NMR spectrum series is depicted in Fig. 3, where in the acidic pH region it is easy to observe the movement of the signals of both the mono and bis-complexes to the higher chemical shift values.

3.2.2 The $[(\text{VO}_2(\text{dhp}))_2\text{OH}]^-$ species

This complex was observed earlier in the ^{51}V -NMR spectra ($\delta = -518$ ppm [14,15]). It was suggested to be dinuclear [15] but the protonation state was not identified. The ^{51}V -NMR signal of this complex was observed in the pH range 4.0-6.5 as it can be seen in Fig. 2. The protonation

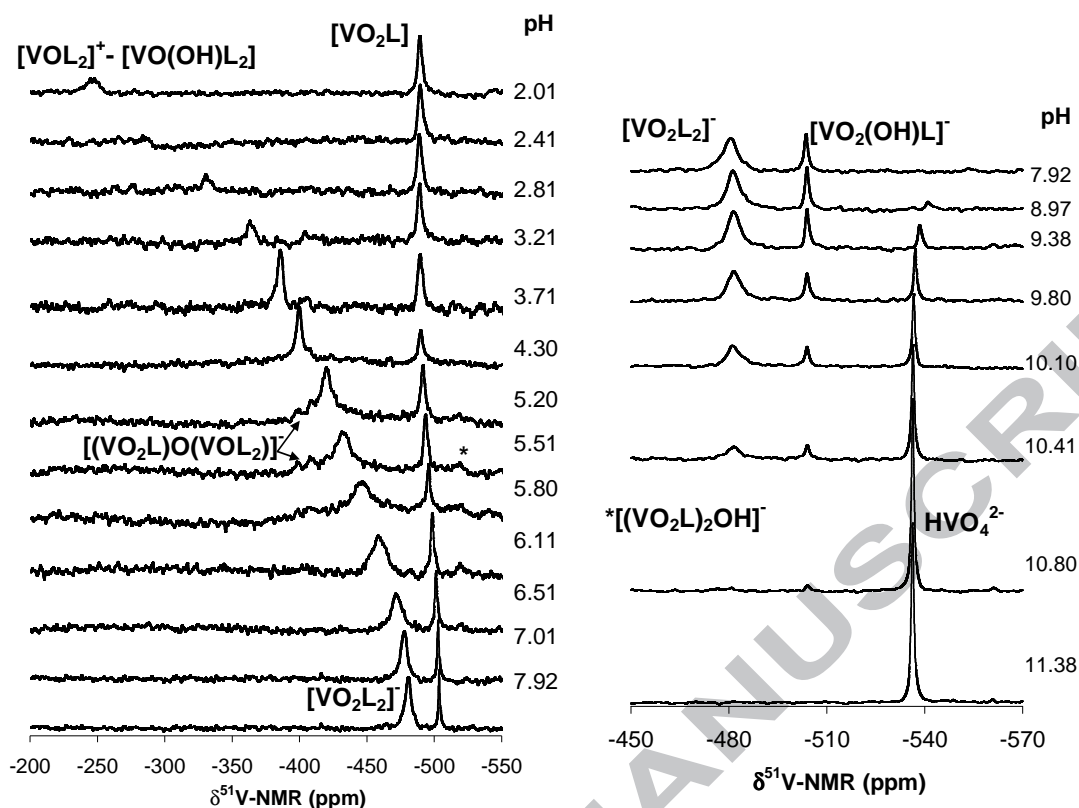


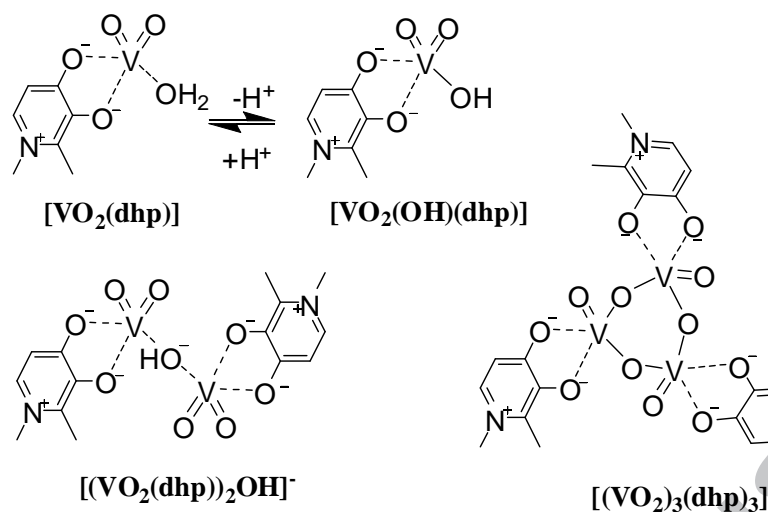
Fig. 3 Representative ^{51}V -NMR spectrum series over the pH range 2-11.4 in the V(V)-dhp system. $\{c_{\text{V(V)}} = 3.3 \times 10^{-3} \text{ mol dm}^{-3}, c_{\text{dhp}} = 6.6 \times 10^{-3} \text{ mol dm}^{-3}, I = 0.2 \text{ mol dm}^{-3} (\text{KCl}), T = 298 \text{ K}\}$.

process of the mono complex takes place parallel with the formation of this species which rather suggests the $[(\text{VO}_2(\text{dhp}))_2\text{OH}]^-$ composition. The dimerization and the composition were proved by a series of ^{51}V -NMR spectra. (see Figs. SI3C and SI4) Formation of this complex is favoured with increasing concentration of vanadate and decreasing excess of ligand. The solution structure of this complex is depicted in Scheme 3. As the chemical shift of this complex does not change with pH probable does not undergo simple protonation or deprotonation processes.

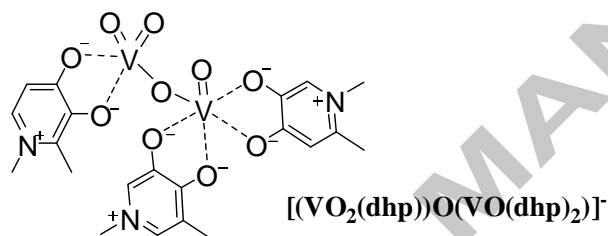
3.2.3 The $[(\text{V(V)}\text{O}_2)_3(\text{dhp})_3]$ species

In the literature [14] an X-ray structure was published for this complex but the species was assigned to $\delta(^{51}\text{V}) = -489 \text{ ppm}$ which is definitely belongs to its monomeric form. (In a later publication it was corrected [15].) We assigned this species to another, earlier also observed but not identified peak (-466 ppm see Table 2). The trinuclear complex was observed (see Fig. 2) in the pH range 4-5.5 at 1 : 1 metal ion-to-ligand ratio, the increase of the total concentration of vanadate favours the formation of it (see Fig. SI 4). The species has no observed protonation equilibria. Based on our measurements formation of a dinuclear complex could not be completely ruled out besides the trinuclear one, only the existing X-ray structure was the reason to identify it as a trinuclear species.

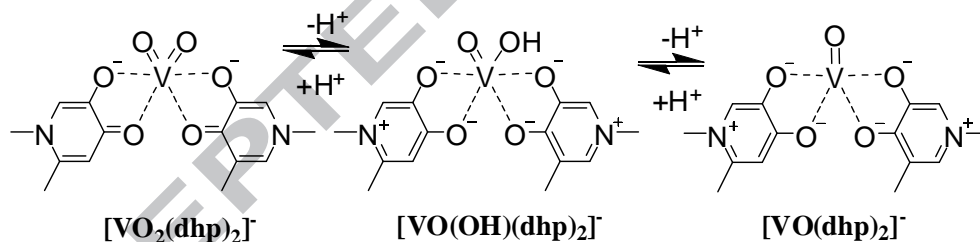
mono-ligandum complexes, V:dhp 1:1 (or 2:2, 3:3)



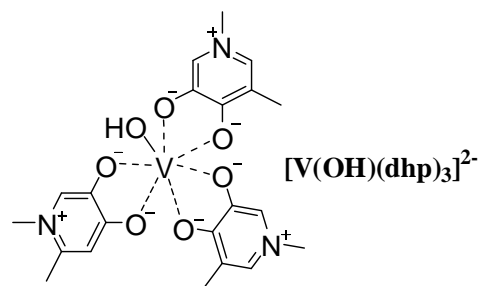
mixed mono/bis-ligandum complex



bis-ligandum complexes, V:dhp 1:2 (or 2:4)



tris-ligandum complex, V:dhp 1:3



Scheme 3 The most likely solution structures of the formed complexes in the V(V)-dhp system.

3.2.4 The $[V(OH)(dhp)_3]$ species

In the presence of ligand excess, a new and never published peak appeared in our spectra at $\delta(^{51}V) = -276$ ppm in the pH range 2.0-5.5. The concentration dependent spectrum series (see Fig. SI6) at pH 3.6 suggests that at least three ligands coordinate to V(V). The quantitative evaluation of three spectrum series provided the best fit with the assumption of a species composition $[V(OH)(dhp)_3]$. The composition and the structure seems to be unique for vanadate, although, “naked”, oxido-oxygen free $[V(dhp)_3]^+$ tris-complexes were identified also in the VO(IV)-dhp system [30], and an X-ray structure of the tantalum(V) complex with similar composition ($Ta(V)O(dhp)_3$) was also published [35]. The most probable structure of this complex is depicted in Scheme 3.

3.2.5 The $[(VO_2(dhp))O(VO(dhp)_2)]^-$ and $[(VO(dhp)_2)_2O]$ complexes

Two other, only qualitatively identified ^{51}V -NMR peaks (dinuclear bis complexes) were described in the literature [14,15]. Sometimes they form an equivalent pair at $\delta(^{51}V) = -399$ and -406 ppm, however, the latter peak was observed also separately. Based on our measurements the ratio of the peak pair is usually 1:1 (in the pH range 5-7), however, at pH ~5, it is difficult to measure them accurately due to the overlap with the peak of the bis dhp-complex (their chemical shifts move with the pH). Furthermore, when the peak areas of the pair may start to change and finally they collapse into a single one, this could not be followed clearly by ^{51}V -NMR. The situation can be followed in Fig. 2. Spectra recorded at various metal-to-ligand ratios may prove (see Fig. SI 4C) that these complexes are not mono complexes. The quantitative evaluation of the measured spectra gives the best fit with the composition of $[(VO_2(dhp))O(VO(dhp)_2)]^-$ and $[(VO(dhp)_2)_2O]$. The most probable structures of these complexes are depicted in Scheme 3. The $[(VO(dhp)_2)_2O]^{2-}$ species can be derived from two $[(VO(OH)(dhp)_2)]$ mononuclear complexes assuming formation of a μ -oxo bridge, the other species can be formed in a reaction between $[(VO(OH)(dhp)_2)]$ and $[(VO_2(OH)(dhp))]^-$ having a similar μ -oxo bridge. A water molecule is liberated in the reaction in both cases. As both complexes contain the same $(VO(dhp)_2)$ -O- moiety, it is not surprising that there is no significant difference in their chemical shift values.

3.3 X-ray structure of the $K[VO_2(dhp)_2] \cdot 2H_2O$

The bis complex was crystallized at pH 8.5 with the aid of slow addition of acetone. The unit cell contains two individual complexes with a pseudo- C_2 symmetry, only one of them is depicted in Fig. 4. The selected distances and angles can be found in Table 4, comparing the data with two related systems, the vanadate complex of maltol [36] and 2-hydroxypyridine-N-oxide (hpno) [32]. The

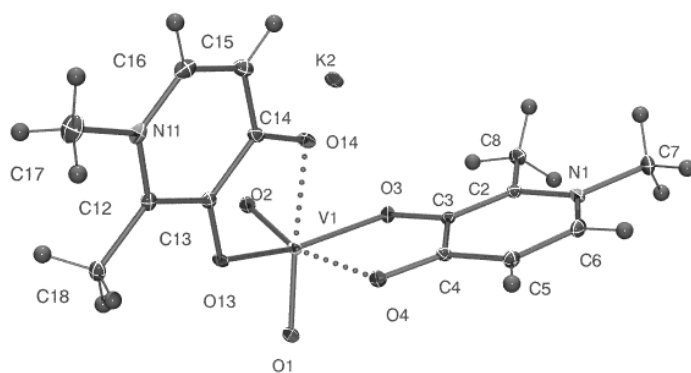


Fig. 4. ORTEP view of $\text{K}[\text{VO}_2(\text{dhp})_2] \cdot 2\text{H}_2\text{O}$ with 50% thermal ellipsoids, water molecules are omitted for clarity.

Table 4 Selected bond distances (Å) and angles (deg) in $\text{K}[\text{V}(\text{V})\text{O}_2(\text{dhp})_2] \cdot 2\text{H}_2\text{O}$ with estimated standard deviations, compared with related complexes ($\text{K}[\text{VO}_2(\text{maltol})_2] \cdot \text{H}_2\text{O}$ and $\text{NH}_4[\text{VO}_2(\text{hpno})_2] \cdot 3\text{H}_2\text{O}$)

$\text{K}[\text{V}(\text{V})\text{O}_2(\text{dhp})_2] \cdot 2\text{H}_2\text{O}$				a	b
Atom	Atom	Bond	Bond	Bond	Bond
V1	O1	1.634(4)	1.634(4)	1.633	1.641
V1	O2	1.638(4)	1.638(4)	1.643	
V1	O3	1.956(4)	1.956(4)	1.963	1.977
V1	O13	1.966(4)	1.966(4)	1.999	
V1	O4	2.215(4)	2.215(4)	2.189	2.182
V1	O14	2.233(4)	2.233(4)	2.276	
Bond angles		Angle	Angle	Angle	Angle
O1—V1—O2		105.5(2)	104.5(2)	103.7	104.4
O1—V1—O3		104.24(18)	106.98(19)	102.0	
O2—V1—O13		102.47(19)	101.6(2)	102.9	102.1
O1—V1—O13		93.78(17)	92.80(19)	91.2	
O2—V1—O3		92.78(17)	93.10(19)	93.9	90.9
O1—V1—O4		89.18(18)	90.8(2)	89.9	
O2—V1—O14		89.04(19)	88.01(19)	92.0	89.5
O13—V1—O4		82.69(16)	82.89(15)	83.0	
O3—V1—O14		80.87(16)	80.09(15)	85.1	88.6
O3—V1—O4		76.95(14)	76.82(15)	76.7	
O13—V1—O14		76.60(15)	76.32(14)	76.8	75.1
O4—V1—O14		77.26(16)	77.71(16)	75.7	79.4
O2—V1—O4		163.93(19)	163.68(18)	164.9	162.2
O1—V1—O14		164.17(17)	164.96(18)	162.2	
O3—V1—O13		152.41(17)	151.63(17)	155.6	158.9

^a $\text{K}[\text{V}(\text{V})\text{O}_2(\text{maltol})_2] \cdot \text{H}_2\text{O}$ [36]

^b $\text{NH}_4[\text{V}(\text{V})\text{O}_2(\text{hpno})_2] \cdot 3\text{H}_2\text{O}$ [32]

bond distances are similar for the three complexes, the trans effect of the oxido-oxygen of the vanadate makes one of the V-O-dhp distances much longer than the other. In the three cases of the asymmetric ligands the donor atom with the worse coordination properties goes to the trans position. The average difference between the two type bond distances are 0.26 Å in the case of the maltolato-type coordination mode. In the other case the catechol type coordination, which can be observed for example in the tridentate complex [14], when the trans effect of the oxido-oxygens does not appear, the difference between the two V-O-dhp bond distances is only 0.03-0.04 Å.

3.4 Reduction of vanadium(V) complexes by GSH and ASC

The reduction of the $[\text{V(V)O}_2(\text{dhp})_2]^+$ complexes of pic, maltol and dhp by GSH or ASC was followed spectrophotometrically. The redox reaction was found to be moderately fast which allowed us to record the UV-Vis spectra immediately after mixing the samples containing the given $[\text{V(V)O}_2(\text{dhp})_2]^+$ complex and the reducing agent at various concentrations under strictly anaerobic conditions at pH 7.40. Kinetic runs were always made at high ligand excess (at 1:10 metal-to-ligand ratio) to provide the predominant formation of the bis $[\text{V(V)O}_2(\text{L}_2)]$ complexes at this pH (Scheme 1, Fig. 5). Both $[\text{V(IV)O}(\text{dhp})_2]$ and $[\text{V(V)O}_2(\text{dhp})_2]^-$ complexes show characteristic

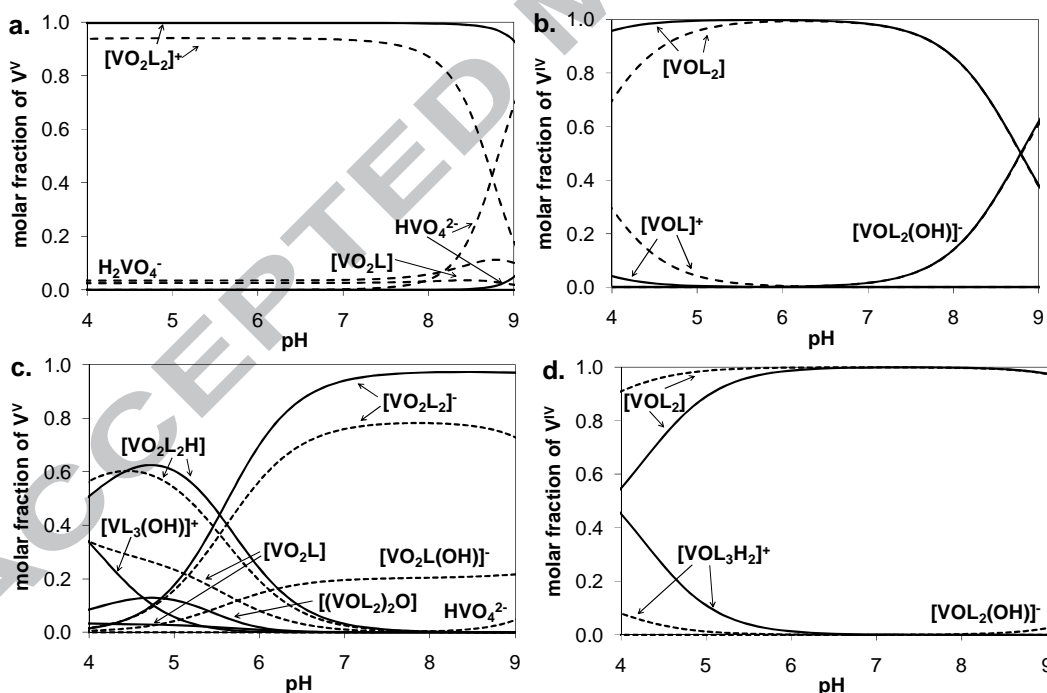


Fig. 5. Concentration distribution curves for vanadium complexes formed in the V(V)-maltol (a); V(IV)O-maltol (b); V(V)-dhp (c); V(IV)O-dhp (d) systems at 1:10 metal-to-ligand ratio, $c_V = 2 \times 10^{-3} \text{ mol dm}^{-3}$ (solid lines) or $0.2 \times 10^{-3} \text{ mol dm}^{-3}$ (dashed lines) based on the stability constants taken from ref. [9,37]. $\{tT = 298 \text{ K}, I = 0.2 \text{ mol dm}^{-3} (\text{KCl})\}$.

Table 5. Molar absorbance values (ϵ , $\text{mol}^{-1}\text{dm}^3\text{cm}^{-1}$) calculated for the V(IV/V)-ligand systems at 1:10 metal-to-ligand ratio at various wavelengths ($T = 298\text{ K}$; $I = 0.20\text{ mol dm}^{-3}$ (KCl); $\text{pH} = 7.40$ (0.1 mol dm^{-3} HEPES)).

ϵ	maltol		dhp		pic	
	V(V)O ₂ ⁺	V(IV)O ²⁺	V(V)O ₂ ⁺	V(IV)O ²⁺	V(V)O ₂ ⁺	V(IV)O ²⁺
364 nm	960	550	8750	550	94	410
410 nm	305	50	5690	230	22	250
450 nm	70	48	4620	110	7	60

absorption in the visible range due to the charge-transfer bands, however, significant differences are seen in the two oxidation states as indicated by the representative molar absorbance values collected in Table 5. *E.g.* the $[\text{V(V)O}_2]^+$ complexes of maltol and dhp absorb light at 410 nm much more intensively than the $[\text{V(IV)O}]^{2+}$ complexes, while the molar absorbance of the bis picolinato- $[\text{V(IV)O}]^{2+}$ complex is much higher at this particular wavelength than in its oxidized form. On the other hand, the oxidation of the reducing agents GSH, ASC by purging oxygen through their solutions results in significant decrease in the absorbance at their λ_{max} values (262 and 265 nm, respectively) as it is shown in Fig. SI8. Oxidation of GSH gives GSSG and dehydro-L-ascorbic acid is the oxidized form of ASC. It is noteworthy that neither the reduced and oxidized form of GSH nor ASC absorbs light in the visible range.

In order to obtain comparable data relating to the oxidizing power of $[\text{V(V)O}_2]^+$ complexes of pic, maltol and dhp against GSH or ASC a systematic kinetic study was performed. In these measurements the concentration of vanadium(V) and the metal-to-ligand ratio (1:10) was kept as constant in one series and the concentration of the reducing agent was in large excess in order to provide pseudo-first order conditions. Spectral changes were followed in the visible range where only the various vanadium complexes absorb light as the time-dependence spectra of V(V)-maltol-GSH system show in Fig. 6.a. In well accordance with the expectations the reduction of this vanadium(V) maltolato complex by the reductant results in diminished absorbance values between 370 and 450 nm (see data in Table 5) followed with progress of time. Similar changes were observed in the case of dhp, although absorbance was increased for the picolinato complex (Fig. SI 9). Most probably not merely the transformation of $[\text{V(V)O}_2(\text{L})_2]$ to $[\text{V(IV)O}(\text{L})_2]$ takes place at the excess of the reducing agents since formation of their binary and ternary complexes with both vanadium ions is also possible leading to complicated speciation [19,38]. Thus, our interpretation of these kinetic runs can be considered only as a semi-quantitative description, but can give information about the different abilities of GSH and ASC to reduce the chosen complexes. The rate

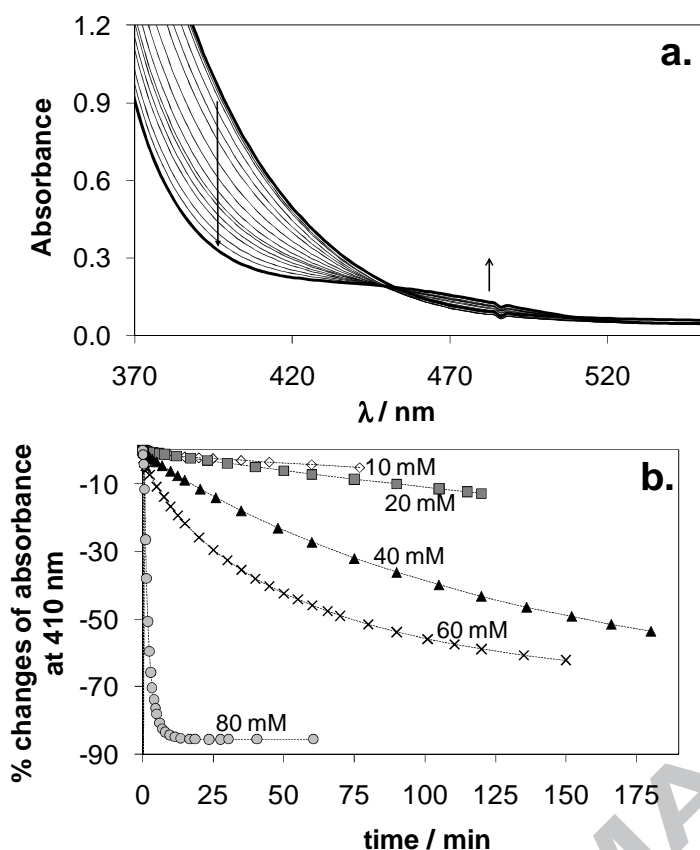


Fig. 6. Time-dependence UV-Vis spectra of V(V)-maltol-GSH system at 1:10:60 ratio (a) and absorbance changes plotted against the time at $\lambda = 410 \text{ nm}$ at various GSH concentrations ($10\text{--}80 \times 10^{-3} \text{ mol dm}^{-3}$) (b). $\{c_V = 2 \text{ mM}; T = 298 \text{ K}, I = 0.2 \text{ mol dm}^{-3} (\text{KCl}); \text{pH} = 7.40 (\text{HEPES})\}$.

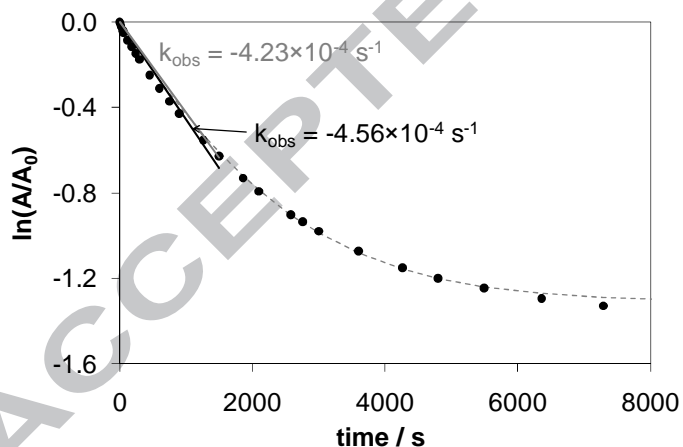


Fig. 7. $\ln(A/A_0)$ values at $\lambda = 410 \text{ nm}$ plotted against the time for the V(V)-dhp-GSH system at 1:10:40 ratio obtained from the experimental (\bullet) and the simulated (dashed grey line) absorbance-time curves with the calculated observed rate constants $\{c_V = 0.2 \times 10^{-3} \text{ mol dm}^{-3}; T = 298 \text{ K}, I = 0.2 \text{ mol dm}^{-3} (\text{KCl}); \text{pH} = 7.40 (\text{HEPES})\}$.

Table 6. Calculated observed rate constants (k_{obs}) and half-lives ($t_{1/2}$) in the V(V)-ligand-reducing agent (GSH or ASC) systems from the spectral changes at 410 nm {V-to-ligand ratio: 1:10; $c_V = 2 \times 10^{-4}$, 5×10^{-4} or 2.0×10^{-3} mol dm $^{-3}$; $I = 0.2$ mol dm $^{-3}$ (KCl), $tT = 298$ K; pH = 7.40 (HEPES)}.

	GSH or ASC (mM)	GSH or ASC/ V ratio	k_{obs} (s $^{-1}$)	$t_{1/2}$ (s)
maltol GSH	10	5.0	1.00×10^{-5}	69315
	20	10.0	2.28×10^{-5}	30441
	40	20.0	8.87×10^{-5}	7815
	60	30.0	2.30×10^{-4}	3011
	80	40.0	7.61×10^{-3}	91
	100	50.0	1.27×10^{-2}	55
	120	60.0	1.86×10^{-2}	37
	140	70.0	1.92×10^{-2}	36
pic GSH	160	80.0	2.07×10^{-2}	34
	60	30.0	2.74×10^{-5}	25260
	80	40.0	4.96×10^{-5}	13966
	100	50.0	1.69×10^{-4}	4101
	120	60.0	8.07×10^{-5}	8589
	140	70.0	2.17×10^{-4}	3200
dhp GSH	160	80.0	8.23×10^{-4}	842
	4	20.0	3.32×10^{-4}	2088
	8	40.0	4.82×10^{-4}	1439
maltol ASC	60	30.0	1.38×10^{-2}	50
	1.4	2.9	2.10×10^{-3}	330
	2.9	5.7	5.29×10^{-3}	131
	4.3	8.6	1.75×10^{-2}	40
	5.7	11.4	2.63×10^{-2}	26
	8.6	17.1	9.60×10^{-2}	7.2
	40	20.0	6.93×10^{-2}	10
pic ASC	80	40.0	1.44×10^{-1}	4.8
	0.7	1.4	5.06×10^{-3}	137
	1.4	2.9	1.73×10^{-2}	40
	2.9	5.7	2.73×10^{-2}	25
	3.6	7.1	4.36×10^{-2}	16
	4.3	8.6	4.54×10^{-2}	15
	5.7	11.4	6.33×10^{-2}	11
	7.1	14.3	7.75×10^{-2}	8.9
	10.0	20.0	9.85×10^{-2}	7.0
dhp ASC	0.07	0.29	8.05×10^{-2}	8.6
	0.14	0.57	1.14×10^{-1}	6.1
	0.18	0.71	1.34×10^{-1}	5.2
	0.21	0.86	1.55×10^{-1}	4.5
	0.29	1.14	1.68×10^{-1}	4.1

of the reduction was very sensitive to the concentration of GSH or ASC (see Fig. 6.b as an example) and the absorbance (A)-time (t) curves were fitted and analysed at various wavelengths (Fig. 7.). Observed rate constants (k_{obs}) were obtained as the average of at least two kinetic runs and as the slope of the $\ln(A/A_0)$ vs. t plots. The k_{obs} values and half-lives calculated at various metal complex-to-reducing agent ratios at 410 nm are collected in Table 6. On the basis of these data the k_{obs} values were increased by increasing GSH or ASC concentrations. The rate constants are appreciably different in the case of the different ligands. The reduction of the complex $[\text{V(V)O}_2(\text{dhp})_2]$ by GSH was found to be the fastest, while complex of maltol and especially that of pic could be reduced much slower by this antioxidant. At the same time the k_{obs} values are much higher in the case of ASC showing that these vanadium(V) complexes can be reduced much faster by the ascorbate compared with GSH. This trend was also observed by Orvig *et al.* [17]. The spectral changes are so fast in the presence of ASC that the vanadium concentration has to be decreased down to $5 \times 10^{-4} \text{ mol dm}^{-3}$ (maltol, pic) and $2.5 \times 10^{-4} \text{ mol dm}^{-3}$ (dhp) for the measurements. The reduction of $[\text{V(V)O}_2(\text{dhp})_2]$ by ASC was also the fastest among the studied vanadate complexes. However, the order of the reaction rate is somewhat different namely, $\text{dhp} > \text{pic} > \text{maltol}$ compared with the case of GSH. (It should be noted that a large excess of ASC could not be applied in the V(V)-dhp-ASC system owing to the extremely fast redox reaction, stopped-flow technique would be needed for a better description.)

The outstandingly fast reduction of the complex $[\text{V(V)O}_2(\text{dhp})_2]$ by the studied naturally occurring reducing agents can be explained by the relatively high stability of the complex $[\text{V(IV)O}(\text{dhp})_2]$ formed in the reaction (*vide supra*). The reducibility of the vanadium(V) complexes strongly depends on the formal potential of the corresponding V(V)/V(IV) redox couples, which depend on the ratio of the conditional stability constants of the complexes $[\text{V(V)O}_2(\text{L}_2)]$ and $[\text{V(IV)O}(\text{L}_2)]$. The pM values ($\text{pM} = -\log[\text{M}]$), thus the unbound metal fractions, under the conditions employed: $\text{pH} = 7.4$ $c_{\text{V}} = 2 \text{ mM}$; $\text{V}:\text{ligand} = 1:10$) can provide a comparable basis of the relative chelating ability of the ligands at physiological pH; thus higher pM value represents the stronger binding. In order to compare the stabilities of the vanadium(IV/V) complexes of maltol, pic and dhp pM values were computed (Table 7). These data undoubtedly reveal the higher stabilities of the vanadium(IV) complexes in all cases, but the difference between the values of the two metal ions is the greatest with dhp. Additionally, the formal potentials of the $[\text{V(V)O}_2(\text{L}_2)] / [\text{V(IV)O}(\text{L}_2)]$ redox couples were measured at pH 7.40 by cyclic voltammetry. Quasi reversible redox processes were observed (see ΔE values in Table 7) in all cases, most probably as a result of the dioxo / monooxo rearrangement in the coordination sphere of the vanadium ion during reduction-oxidation, which

Table 7. pM values calculated for the V(IV/V)-ligand systems based on the stability constants in Refs. [9,37] ($c_V = 2 \text{ mM}$; V:L = 1:10; $T = 298 \text{ K}$; $I = 0.20 \text{ mol dm}^{-3}$ (KCl); pH = 7.40). Formal potential values (E') and peak separations (ΔE) for the V(IV/V)-ligand systems. ($c_{V(V)} = 0.01 \text{ mol dm}^{-3}$; V:L = 1:2; $T = 298 \text{ K}$; $I = 0.06 \text{ mol dm}^{-3}$ (KCl); pH = 7.40).

	pic	maltol	dhp
pM / V(V)	3.56	5.98	6.77
pM / V(IV)O	7.54	9.15	13.01
E' vs. NHE (V)	+0.33	+0.54	+0.67
ΔE (mV)	277	68	234

is generally considered as a relatively slow process. At the same time, the highest formal potential value was measured for the complex $[V(V)O_2(dhp)_2]$ representing its strongest oxidizing power among these vanadate compounds.

Results demonstrate that reduction of $[V(V)O_2(dhp)_2]$ by ASC is much faster than by GSH at physiological pH and is faster by both reductants compared with the complexes of maltol and pic. In the case of the maltolato complex Orvig *et al.* estimated the chance of the reduction by ASC and GSH *in vivo* considering the actual concentrations in the human body tissues and they considered ASC as relevant biological reductant [17]. As the reaction rate is much higher for the complex of dhp, the reduction by the GSH may be also possible even in the blood plasma where its concentration is lower as compared to the intracellular milieu.

Conclusions

A joint evaluation of the pH-metric and ^{51}V -NMR spectral measurements let us to assign all the NMR peaks, to identify the composition and determine the stability of the complexes formed in the V(V)-dhp system. The results are either in agreement with the earlier findings or solve the published contradictions and finally give a complete speciation picture about this unique system.

At pH 7.4 or above, the speciation of the V(V)-dhp system is basically similar to the V(V)-hpno or V(V)-maltol systems: only one type of bis: $[V(V)O_2(L)_2]^-$ and one type of mono complex: $[V(V)O_2(OH)L]^-$ is formed. It means that in human blood serum, the other forms of the V(V)-dhp complexes practically could not play any role.

The protonation of the mono-dhp-complex occurs at one unit higher pH than for the other usually considered ligands ($pK(\text{hpno}) = 4.50$, $pK(\text{maltol}) = 4.85$, $pK(\text{dhp}) = 5.88$). More significant difference was realized between the acidity of the protonated bis-dhp-complex and the other ligands ($pK(\text{hpno}) = 2.14$, $pK(\text{maltol}) = 2.3$, $pK_2(\text{dhp}) = 5.62$), especially, that in this case it could be

protonated two times ($pK_1(\text{dhp}) = 2.90$). Why is this difference from the other (O,O) donor ligands? The tautomeric structures of the dhp let it to coordinate in two different ways: i) as the other two ligands (maltolato like, with one $-\text{O}^-$ and one $=\text{O}$) and ii) like catecholates (with two $-\text{O}^-$). The maltolato type coordination is caused by the trans effect of the vanadate oxido-oxygens. The catecholate type coordination results in charge increase on the central vanadium ion, which is able to shorten the V–O, vanadium-oxido oxygen bonds, and can increase the negative charge on these oxygens. This is the explanation why the bis dhp-complex protonates more easily.

The crystal structure of the bis complex, $[\text{VO}_2(\text{dhp})_2]^-$, reported here, and the trinuclear form of the mono complex, $[(\text{VO}_2)_3(\text{dhp})_3]$ [14] verifies the above mentioned findings.

In the pH range 2 – 7 several other minor species are also formed. Three of them are dinuclear species and one is trinuclear; and in all cases μ -oxo bridge or bridges are supposed to link the vanadate centers.

When the $\text{pH} < 5$ and the concentrations and the ligand excess are high a totally new and very interesting tris-dhp-complex is also formed.

The comparison of the redox properties of different bis-ligandum complexes are in agreement with the known stability differences. While due to the catecholate type coordination possibility of the ligand the bis-dhp-VO(IV) species has outstanding stability among the VO(IV) complexes, the stability difference is not as significant in the highest oxidation state V(V) where the trans effect of the oxido-oxygen atoms allow only the maltolato type coordination mode for the dhp.

Results also demonstrate that reduction of $[\text{V(V)O}_2(\text{dhp})_2]$ by ASC is much faster than by GSH at physiological pH and is faster by both reductants as compared with the complexes of maltol and pic.

It is worth to mention that the highest stability of the bis-dhp complex of V(IV) among the similar neutral bis-ligand complexes does not bring outstanding antidiabetic effect, the complex shows ordinary insulin-mimetic efficacy in in vitro cell tests [3,11,12]. Immobilisation of vanadium in a given chemical environment is certainly does not enhance its biological efficacy. Although the complex was not as exhaustively studied as BMOV, the results published about its activity [11,12] is somewhat controversial also.

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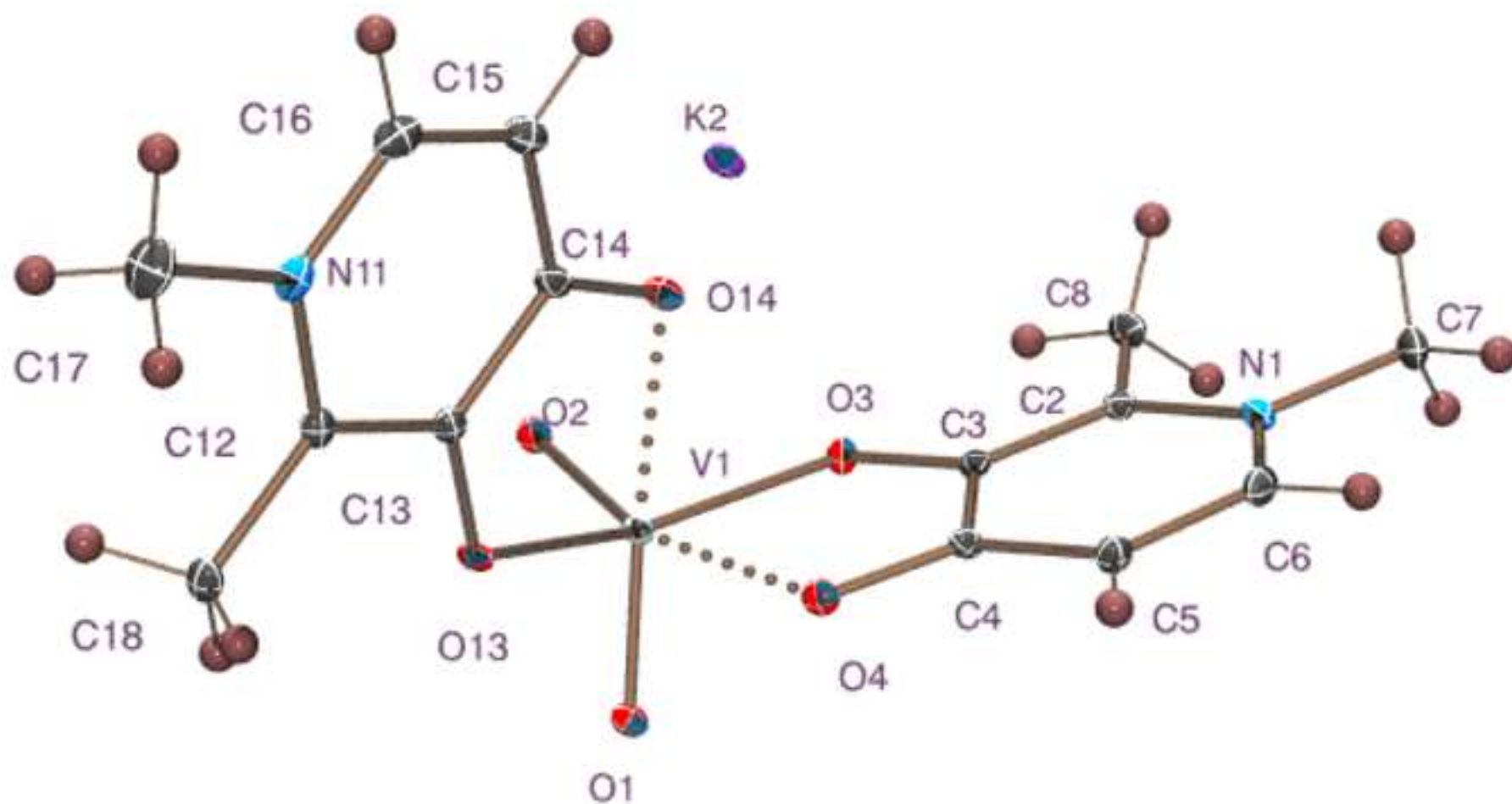
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- Speciation were completed for V(V)-3-hydroxy-1,2-dimethyl-pyridinone (dhp) system
- Four new species, for example a tris-ligand complex was identified
- X-ray structure of $K[V(V)O_2(dhp)_2]$ was determined
- The dhp is able to coordinate both as catecholate and maltolate
- The reduction of the complex $[V(V)O_2(dhp)_2]$ by ASC was found to be the fastest



Speciation in the vanadate - 3-hydroxy-1,2-dimethyl-pyridinone system was clarified by pH-potentiometry and ^{51}V -NMR spectroscopy. X-ray structure of the potassium salt of the bis complex, $[\text{V}(\text{V})\text{O}_2(\text{dhp})_2]^-$ was also determined. The trans effect of the oxido-oxygens results in maltolato-type coordination of the ligand instead of the catecholate-like chelation.

The redox properties of $[\text{V}(\text{V})\text{O}_2(\text{dhp})_2]^-$ and some other prodrug vanadium(V) bis complexes were also investigated. The reduction was found to be much faster by ASC in all cases as compared with GSH. The reduction of the of $[\text{V}(\text{V})\text{O}_2(\text{dhp})_2]^-$ was prominently fast due to significantly high stability of the vanadium(IV)-dhp complex.