

Thermal and long period stability of series of V(V), V(IV) and V(III) complex with Schiff base ligands in solid state

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Abstract

The synthesis and physicochemical properties of three new complexes of vanadium at +5, +4 and +3 oxidation state are described and discussed. The octahedral surrounding of vanadium for V(III) complexes of $[V(L^1)(HL^1)]$ general formula is filled with two ONO tridentate ligand L, for V(IV) one ONO ligand L, oxido ligand and 1,10-phenanthroline (phen) as a co-ligand are presented in complexes of $[VO(L^2)(phen)]$. For V(V) the complexes of $[VO_2(L_1)(sol)]$ type were formed. As ligands, the H_2L Schiff bases were formed in reaction between 5-hydroxysalicylaldehyde and phenylacetic hydrazide (H_2L_1) and 3,5-dichlorosalicylaldehyde and 4-hydroxybenzhydrazide (H_2L^2). The magnetic moment measurements, in 8 year period, show, that V(III) complexes slowly oxidise to V(IV) with preservation of the nonoxido character of the complexes, while V(IV) complexes were found to be stable. The TG and SDTA measurements indicate, that thermal stability depends mainly on the oxidation state of vanadium. The less thermally stable are the V(V) complexes, while V(IV) and V(III) are stable up to ca. 200°C. In solution, at pH 2.0 (similar to that in human digestion system), again the V(IV) are the most stable, only at pH 7.0 V(III) complexes had higher stability. The most stable, thus best for pharmaceutical use, are V(IV) complexes.

Keywords: Vanadium, complexes, Schiff base, salicylaldehyde, hydrazide, thermogravimetry

Introduction

The insulin mimetic properties of vanadium complexes are known for over a hundred years (since 1899) [1, 2]. It was also shown, that in patients suffering from diabetes type 2, lower level of vanadium in body than normal was found [3]. Moreover, up to now, there are no data suggesting, that there is any toxic effect for human both in short, and long term excluding gastrointestinal discomfort [4,5]. Vanadium is a bio element and is used as a diet supplement, for example to enhance weight training in athletes in doses much higher than recommended [6]. In the beginning of XXI century, it was suggested however that the vanadium administration is unnecessary [7,8]. In spite of these publications the investigations on vanadium continued, and entered for phases I and II of clinical treatment [8,9]. In last decade the increase of interest in vanadium starts again [10]. The most study on vanadium insulin mimetic properties comes from *in vitro* studies, in 2017 a review in this subject appeared [11]. Still in study on its influence on humans the simplest V(IV) salts as vanadyl sulphate or vanadate's were used [10].

The main problem with simple inorganic vanadium salts is their bioavailability. In 1990s first complex of vanadium(IV) with maltolato ligand (BMOV) was synthesized [12]. Today

BMOV is used as a standard to access vanadium complexes effectiveness in a Type II diabetes. Still for bidentate organic ligands, as in BMOV, the mechanism shows that unliganded vanadium is the active component [13]. The investigation of more sophisticated vanadium complexes with polydentate ligands, and also with biologically active ligands, is now undertaken, in most cases Schiff base ligands are used [14-16]. The inorganic ligands show better bioavailability of vanadium and show much higher stability towards ligand dissociation, giving a new insight into our knowledge on a role of vanadium in biological systems.

In present paper we describe synthesis of three new vanadium complexes with tridentate ONO Schiff base ligands. The aim of this work was to investigate the stability of these complexes both in solution and in solid state, as a crucial information for practical applications in *in vitro* and *in vivo* studies. Three oxidation states of vanadium were investigated: +3, +4 and +5. The correlation between oxidation state metal center and stability is discussed.

Materials and Methods

$[V(acac)_3]$, $[VO(acac)_2]$, 1,10-phenanthroline (phen), 5-hydroxysalicylaldehyde, 3,5-dichlorosalicylaldehyde, phenylacetic hydrazide and 4-hydroxybenzhydrazide were of analytical grade (Aldrich) and were used as supplied in synthesis of complexes **1-3**. The complexes $[V(L^3)(HL^3)] \cdot EtOH$ (**4**), $[VO(L^4)$

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(phen)]·2H₂O (**5**) and [VO(L⁵)EtO] (**6**) (where H₂L³, H₂L⁴ and H₂L⁵ denote *N*'-[(*E*)-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)methylidene]-2-phenylacetohydrazide, *N*'-[(*E*)-(5-chloro-2-hydroxyphenyl)methylidene]-4-hydroxybenzohydrazide and *N*'-[(*E*)-(5-chloro-2-hydroxyphenyl)methylidene]-2-hydroxybenzohydrazide respectively) were synthesized as described elsewhere [17]. Ethanol (98%) of pharmaceutical grade was from Polmos and used as supplied. All other solvents were of analytical grade and were used as supplied. Microanalysis of carbon, hydrogen and nitrogen were performed using Elementar Vario MICRO Cube elemental analyzer. The magnetic susceptibility measurements were performed on a SHERWOOD SCIENTIFIC magnetic susceptibility balance. Thermogravimetric measurements were performed on a TGA/SDTA 851e Mettler Toledo Microthermogravimeter in air or under argon atmosphere with scan speed 10 °C/min. The electronic absorption spectra were recorded on Shimadzu UV-3600 UV-VIS-NIR spectrophotometer equipped with a CPS-240 temperature controller. Complex stability measurements in DMSO/H₂O (20 μL/3mL) mixture were performed at a native pH (7.00) and at pH = 2.00 (0.1M KCl, HCl added to adjust the pH). The spectra were measured every 340 s at 37.0 °C on Shimadzu UV-3600 UV-Vis-NIR spectrophotometer in 1 cm UV cuvettes.

Synthesis

The reaction was carried in one step. The Schiff base ligands H₂L¹ and H₂L² were synthesized *in situ* in reaction between aldehydes (5-hydroxysalicylaldehyde for H₂L¹ and 3,5-dichlorosalicylaldehyde for H₂L²) and hydrazides (phenylacetic hydrazide for L₁ and 4-hydroxybenzhydrazide for H₂L²) in 1 : 1 molar ratio and after completing of the reaction [V(acac)₃] (for **1** and **4**) or [VO(acac)₂] (for **2**, **3** and **5** complexes) or VOSO₄·aq (for **6**) was added. The reaction proceeds under anaerobic conditions (Ar) due to fast oxidation of V(IV) to V(V) in condition used as can be seen comparing synthesis of **1** and **3**.

The addition of phen as a co-ligand (for **2**) results in fast precipitation of the complex by filling the coordination sphere of vanadium and in stabilization of V(IV) oxidation state. The products are stable in air, soluble in organic solvents and almost insoluble in water.

[V(L¹)(HL¹)], **1**

5-hydroxysalicylaldehyde (0.278 g, 2.0 mmol), phenylacetic hydrazide (0.301 g, 2.0 mmol) and EtOH (21 ml) were refluxed under Ar for 13 minutes with formation of yellow transparent solution. Then [V(acac)₃] (0.349 g, 1.0 mmol) was added and the reflux was continued for 28 minutes. The solution immediately turns brown. In two minutes after addition of [V(acac)₃] the brown product starts to precipitate. Then it was filtered off, washed with EtOH and dried in air. Yield 0.2665 mg. MW = 589.49. Anal. Calcd. for C₃₀H₂₆N₄O₆V: C, 61.12; H, 4.45; N, 9.50 %. Found: C, 60.52; H, 4.18; N, 9.47 %. FT-IR (KBr, cm⁻¹): 3026

(w), 2355 (w), 1571 (w), 1554 (s), 1518 (s), 1480 (w), 1432 (m), 1438 (m), 1392 (w), 1364 (w), 1329 (w), 1276 (w), 1245 (w), 1227 (m), 1173 (s), 1116 (w), 1006 (w), 951 (w), 827 (s), 791 (w), 726 (m), 694 (w), 652 (w), 566 (w), 519 (w), 490 (w), 450 (w), 427 (w). Complex is paramagnetic, μ = 2.38 μ_B.

[VO(L²)(phen)]·H₂O, **2**

3,5-dichlorosalicylaldehyde (0.287 g, 1.5 mmol), 4-hydroxybenzhydrazide (0.226 g, 1.5 mmol) and EtOH (50 ml) were refluxed under Ar for 10 minutes. The light yellow transparent solution was formed and then solid [VO(acac)₂] (0.393 g, 1.5 mmol) was added (the mixture turns to dark brown) and the mixture was refluxed for additional 20 minutes. Then phen (0.272 g, 1.5 mmol in 10 ml of EtOH) was added and the mixture was heated for additional 8 minutes. Next day the formed crystals were filtered off, washed with small amount of cold EtOH and dried in air. Yield 0.649 g. MW = 588.29. Anal. Calcd. for C₂₆H₁₈Cl₂N₄O₅V: C, 53.08; H, 3.08; N, 9.52 %. Found: C, 52.89; H, 3.15; N, 9.33 %. FT-IR (KBr, cm⁻¹): 3436 (m), 1607 (vs), 1520 (w), 1497 (vs), 1436 (w), 1423 (vs), 1375 (m), 1352 (m), 1301 (w), 1274 (w), 1211 (m), 1183 (m), 1166 (m), 1141 (w), 1101 (w), 1034 (w), 954 (s), 907 (w), 848 (m), 760 (m), 724 (m), 644 (w), 618 (w), 562 (w), 511 (w), 448 (w), 431 (w). Complex is paramagnetic, μ = 1.32 μ_B.

[VO₂(L¹)]·H₂O, **3**

5-hydroxysalicylaldehyde (0.208 g, 1.5 mmol) and phenylacetic hydrazide (0.227 g, 1.5 mmol) in 50 ml of EtOH were refluxed for 15 minutes under Ar yielding a transparent light yellow solution. Then [VO(acac)₂] (0.399 g, 1.5 mmol) was added and the mixture was refluxed for 40 minutes giving yellow-black transparent solution. Then it was evaporated in part (in air) yielding a dark-green oil liquid. After three days black muddy-like product was filtered off, washed with EtOH and dried in air. Yield 0.200 g. MW = 370.23. Anal. Calcd. for C₁₅H₁₅N₂O₆V: C, 48.66; H, 4.08; N, 7.57 %. Found: C, 48.14; H, 3.83; N, 6.31. FT-IR (KBr, cm⁻¹): 3254 (w,br), 1657 (w), 1521 (s), 1445 (w), 1370 (w), 1351 (w), 1281 (w), 1191 (w), 1119 (w), 1079 (w), 1033 (w), 977 (m), 835 (w), 802 (w), 726 (w), 696 (w), 653 (w), 596 (w). Complex is diamagnetic.

Results and Discussion

The reaction of tridentate ONO Schiff base and vanadium source in anaerobic conditions yield the formation of respective V(III) and V(IV) complexes. For this latter phen ligand was used as a co-ligand to fill the octahedral sphere. When phen was not added, the products were contaminated with by-products. When, after synthesis, reaction mixture was exposed to air, rapid oxidation to V(V) was observed with precipitation of **3**. For synthesized complexes **1-3** their formula was proposed basing on analytical data, IR spectra and magnetic moment measurements. The magnetic moment shows, that complex **1** contains

mainly V(III), but is slightly contaminated with V(IV) - all presented here data are for freshly prepared complex. After 8 years (in period 2010-2018) of keeping **1** in a solid state in air, the magnetic susceptibility was found to be equal to $1.63 \mu_B$ indicating oxidation of V(III) to V(IV), but the IR spectrum remains almost unaltered, indicating no presence of intense bands which could be attributed to V=O group. All this shows that oxidation does not change the nonoxido character of the complex. Complexes **2** and **3** were found to be stable in a solid state.

Thermal stability measurements

The thermogravimetric analysis of complexes **1-6** was performed in ca 25-1000 °C at scan speed 10 °C. The SDTA spectra were measured in the same temperature range. For two complexes (**3** and **6**) the measurements were repeated under anaerobic conditions (Ar). The obtained results are presented separately for V(III), V(IV) and V(V) complexes in Figs 1, 2 and 3 respectively for measurements performed in air, while at Fig. 4 results under Ar are presented.

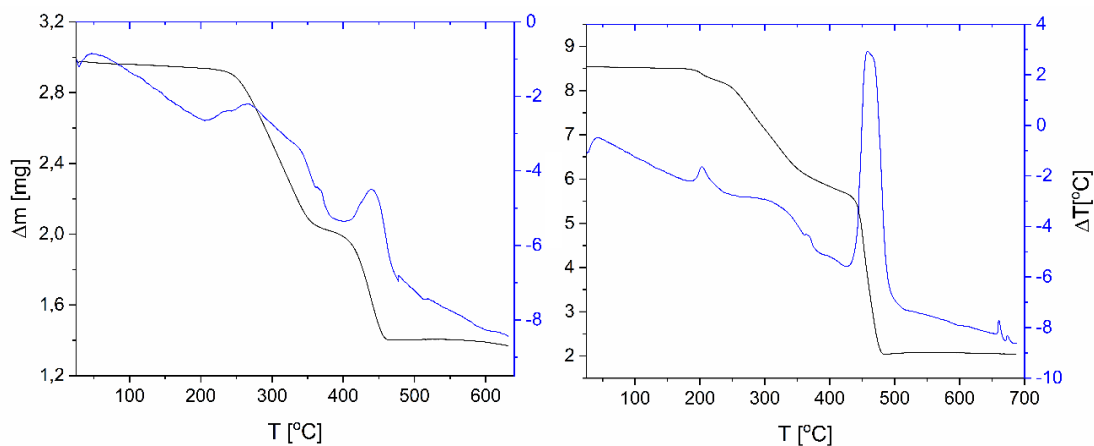


Figure 1. The TG (black, solid line) and SDTA (blue, dotted line) curves for complexes **1** (left) and **4** (right) in air, scan speed 10°C/min.

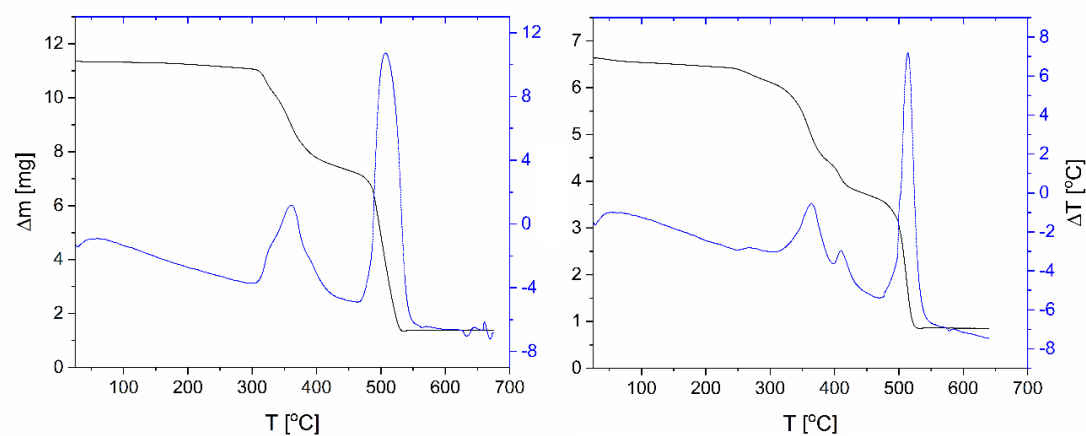


Figure 2. The TG (black, solid line) and SDTA (blue, dotted line) curves for complexes **5** (left) and **2** (right) in air, scan speed 10°C/min.

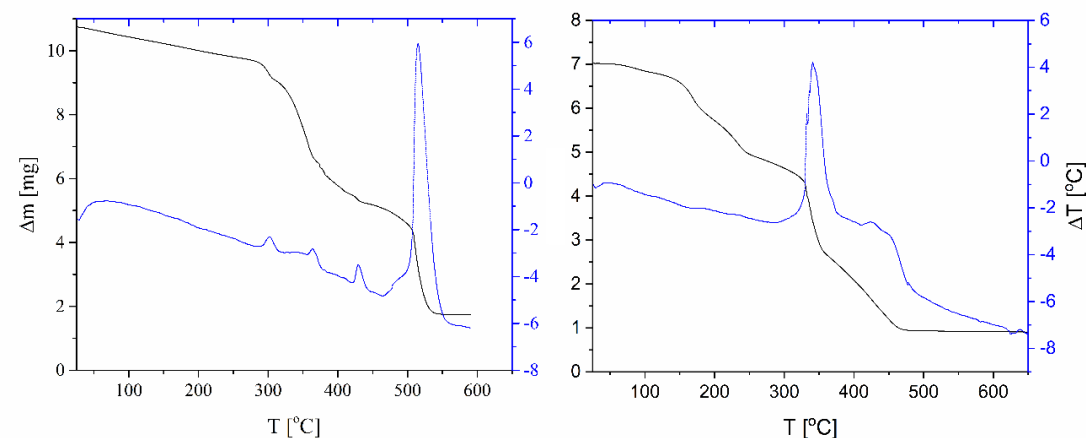


Figure 3. The TG (black, solid line) and SDTA (blue, dotted line) curves for complexes **3** (left) and **6** (right) in air, scan speed 10°C/min.

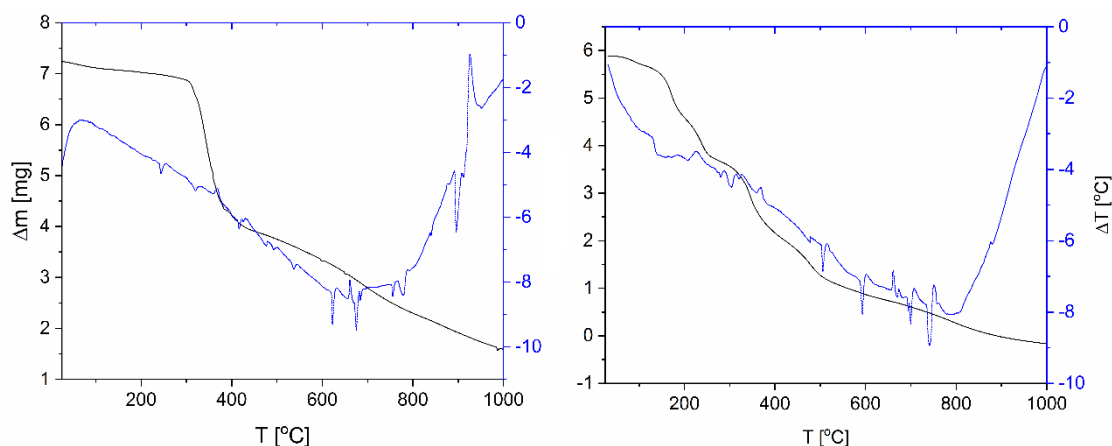


Figure 4. The TG (black, solid line) and SDTA (blue, dotted line) curves for complexes **3** (left) and **6** (right) under Ar, scan speed 10°C/min.

The numerical data of TG and SDTA measurements are collected in Table 1. The T_{max} denote the SDTA maximum or minimum, the type of process is given (exothermic or endothermic). The mass of the sample at every step of decomposition were calculated when SDTA curve reaches the background level, thus at the plateau or shoulder on TG curve.

Table 1. The numerical data for TG and SDTA measurements for complexes **1-6**

Complex	m [mg]	SDTA T_{max} [°C]	Process	Δm_{exp} [%]
1	2.97628			
	2.96016	205	egzo	0.54
	2.02328	240	egzo	32.0
	1.40161	401	egzo	52.9
	1.37041			54.0
2	6.64212	364	endo	
	6.52974	410	endo	1.69
	6.26106	514	endo	5.74
	4.37507			34.1
	3.64895			45.1
0.85567			87.1	
3	10.75178			
	9.03846	302	endo	15.9
	5.07847	365	endo	52.8
	1.73502	429	endo	83.9
		515	endo	
3 (argon)	7.22262	a	a	
	7.08502			1.91
	3.80825			47.3
	1.59059			78.0
4	8.54098	201	endo	
	8.52179	425	egzo	0.22
	8.23637	460	endo	3.57
	5.85304			31.5
	2.04012			76.1
5	11.3519	363	endo	
	11.3115	507	endo	0.36
	11.0885			2.32
	7.29449			35.7
	1.39075			87.7

6	7.02301	340	endo	
	6.88612	425	endo	1.95
	5.90808			15.9
	4.771			32.1
	2.56346			63.5
0.9114			87.0	

^a The SDTA T_{max} was not listed due to the problems with T_{max} determination, as shown in Fig. 4.

Presented TG and SDTA curves indicate that the decomposition processes are very complicated. The solvent release is probably possible after structure damage, thus decomposition temperature overlaps with ligand L decomposition. Similar situation is for coordinated EtOH molecules. The presented TG curves show, that ligand L is decomposed in two steps, in the first one hydrazide part is released, in second, overlapped with the first one, salicylaldehyde part is decomposed. As all processes overlaps it is difficult to assign the observed mass loss with calculated one. For complexes with phen ligand, it is released first, and then ligand L is decomposed. For example for complex **2**, calculated for phen and H_2O release $\Delta m_{calc} = 33.7\%$, while observed 34.1%, total mass loss calculated for phen, H_2L^2 and $2H_2O$ release $\Delta m_{calc} = 88.6\%$, observed $\Delta m = 87.1$. For complex **5**, for phen and H_2O release $\Delta m_{calc} = 34.6\%$, observed $\Delta m = 35.7\%$, for H_2L^4 , phen and H_2O release $\Delta m_{calc} = 88.8\%$, observed $\Delta m = 87.7\%$. In most cases decompositions are the endothermic processes, the only exclusions are complexes of V(III) (**1** and **4**) where exothermic processes dominate. This is probably connected with the oxidation energy of V(III) to finally V(V). The decompositions under Ar are much more complicated, as shown in Fig. 4. At the beginning of the process, TG curves in air and under Ar are relatively similar, but later processes complicates, and even at 1000 °C plateau is not reached on TG curve. This is probably connected with formation of carbides of vanadium in anaerobic conditions.

The most important is the comparison of complex thermal stability. The V(V) complexes (**3** and **6**) decomposition processes start almost at room temperature as shown in Fig. 3. It

indicates, that solvent molecule (EtOH) is weakly coordinated to the vanadium center and, after it release at crystal faces, the structure starts to collapse and constant decrease of mass, without defined plateau on TG curve, is observed. The most stable seems the V(IV) complexes, with decomposition processes starting over 250 °C (for **2** only water of hydration is released). For V(III) complexes (**1** and **4**) complexes are thermally stable up to ca. 200 °C. Similar results were obtained also for other vanadium complexes of that type of ligands. For example for [VO(L)(EtO)(EtOH)] (where H_2L = Schiff base ligand derived from 5-methoxysalicylaldehyde and phenylacetic hydrazide) [18] the V(V) complex is stable up to ca 50 °C, then solvent molecules are released but still after this process the complex is stable up to 133 °C.

Spectroscopic measurements

The stability of the solutions of the complexes were studied in DMSO- H_2O (20 μ L + 3mL) mixture. This volume ratio was the same, as in later biological studies both *in vivo* and *in vitro*. As all investigated complexes were almost insoluble in water, DMSO was used as it had the best solubility of the complexes studied and is biologically neutral. To lower the DMSO con-

centration and to prevent complex precipitation, the mixtures of DMSO- H_2O were used. The stability was studied by measuring the UV-Vis spectra versus time at $T = 37$ °C to mimic behavior in human body. As vanadium complexes were later used in biological studies and as pharmaceuticals should be admitted orally, the complexes should be stable enough to pass through human digestion system (the most challenging is the pH of the stomach). We decided to study the solution stability both at neutral pH (7.0) as reference (pH resembling the *in vitro* conditions), and at pH = 2.0 (close to that of stomach; in range 1.5–2.5). The spectra were recorded every 340s and were measured in period of 2 hours (time much longer than required for passage through digestion system). The results are presented in Figs. 5-7.

At the first look the spectra at pH = 2.0 seem to change slower than that at pH 7.0. But looking in detail, at Figs 5 - 7, it can be seen, that at pH = 2.0 the band above 400 nm, attributed to coordinated ligand L, is or not present (Figs 5, 7) or its intensity is very low (Fig. 6). This indicates, that before the first spectrum measurement the complexes **1-3** decomposed. Still some remaining absorption in this range for complex **2** indicates, that this is the most stable complex at pH = 2.0. At pH = 7.0 both complexes **1** and **2** are relatively stable, while complex **3** is not.

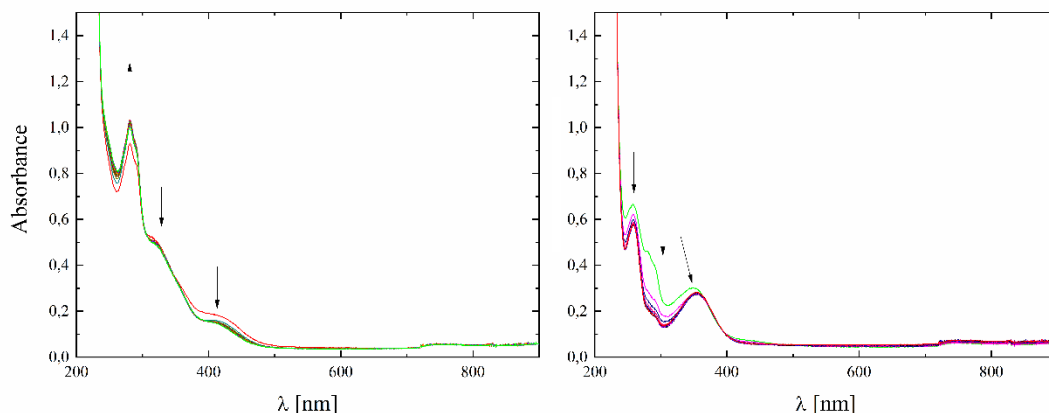


Figure 5. The electronic spectra of **1** in DMSO- H_2O mixture (20 μ l + 3 ml) at pH = 7.00 (left) and 2.00 (right). $T = 37^\circ\text{C}$, $d = 1\text{cm}$, $c = \text{mol}/\text{dm}^3$, 15 spectra measured every 340s. The arrows indicate the direction of the changes

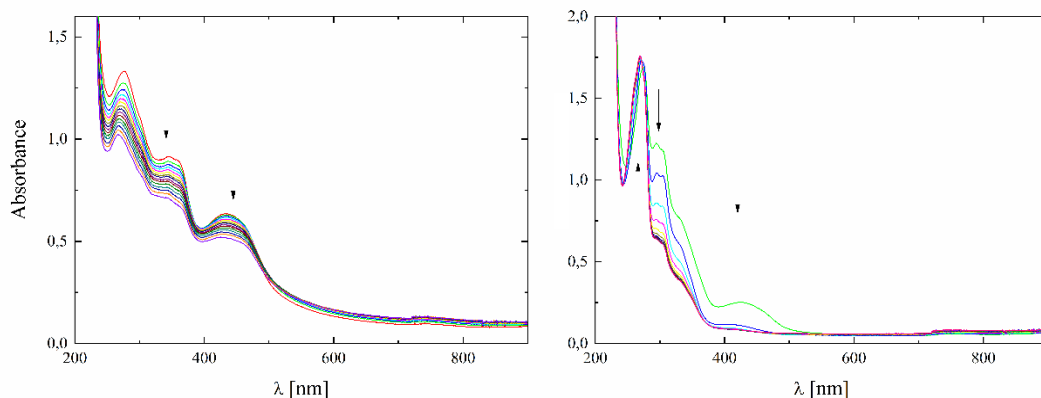


Figure 6. The electronic spectra of **2** in DMSO- H_2O mixture (20 μ l + 3 ml) at pH = 7.00 (left) and 2.00 (right). $T = 37^\circ\text{C}$, $d = 1\text{cm}$, $c = \text{mol}/\text{dm}^3$, 15 spectra measured every 340s. The arrows indicate the direction of the changes

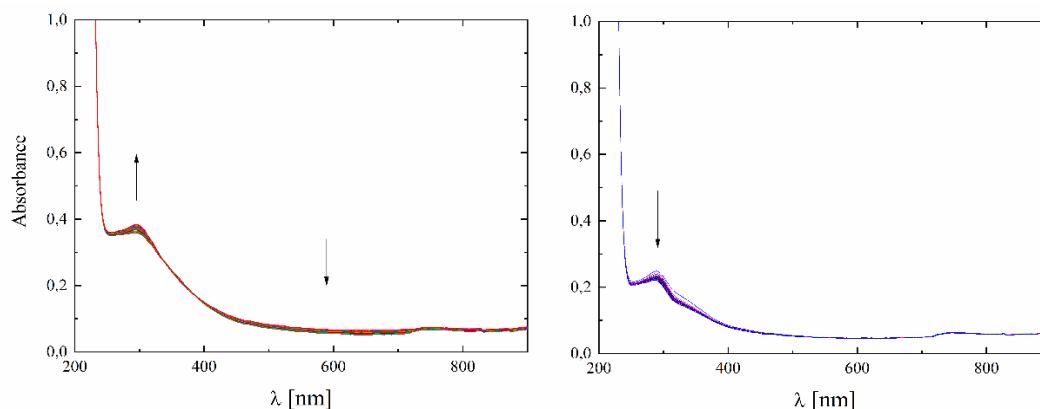


Figure 7. The electronic spectra of **3** in DMSO-H₂O mixture (20 μl + 3 ml) at pH = 7.00 (left) and 2.00 (right). T = 37°C, d = 1 cm, c = mol/dm³, 15 spectra measured every 340s. The arrows indicate the direction of the changes

Conclusions

The thermal stability of vanadium at different oxidation state was described and compared, both for solid state and in solution. It was found, that V(IV) complexes are the most stable in all conditions studied. The V(III) complexes are relatively stable in a solid state and in solution at pH 7.0 but slow process of V(III) oxidation to V(IV) (observed in 8 year period), with preserving the nonoxido character of vanadium center, exclude them for future applications as metalopharmaceutics. The V(V) complexes, possessing solvent molecules as ligands, are the least stable both in solution and in a solid state. In a solid state this is probably connected with solvent release on the crystal border, resulting in structure collapse and stepwise decomposition of whole crystals. In solution, it seems that **2** is the most stable among V(V) complexes and at least part of the complex can pass through stomach without decomposition. In conclusion we suggest, that V(IV) complexes are most suitable for applications in biological systems.

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References

1. Brannick B, Kocak Solomon S. Vanadium in Glucose Metabolism: Past, Present and Future. *Journal of Toxicology and Pharmacology*. 2017; 1:1-011.
2. Lyonnet B, Martz, Martin E. L'emploi du vanadium. *La Presse Medicale*. 1989; 1:191-192.
3. Hamel FG, Solomon SS, Jespersen AS, Blotcky A, Rack E, Duckworth WC. Alteration of tissue vanadium content in diabetes. *Metabolism*. 1993; 42:1503–1505.
4. Smith DM, Pickering RM, Lewith GT. A systematic review of vanadium oral supplements for glycaemic control in type 2 diabetes mellitus. *Q.J.Med.* 2008; 101:351-358.
5. Wang J, Yuen VG, McNeill JH. Effects of vanadium on insulin sensitivity and appetite. *Metabolism*. 2001; 50:667-673.
6. Barceloux DG. Vanadium. *Journal of Toxicology: Clinical Toxicology*. 1999; 37:265-278.
7. Domingo JL, Gomez M. Vanadium compounds for the treatment of human diabetes mellitus: A scientific curiosity? A review of thirty years of research. *Food and Chemical Toxicology*. 2016; 95:137-141.
8. Thompson KH, Orvig Ch. Vanadium in diabetes: 100 years from Phase 0 to Phase I. *Journal of Inorganic Biochemistry*. 2006; 100:1925-1935.
9. Cusi K, Cukier S, DeFronzo RA, Torres M, Puchulu FM, Redondo JC. Vanadyl Sulfate Improves Hepatic and Muscle Insulin Sensitivity in Type 2 Diabetes. *The Journal of Clinical Endocrinology and Metabolism*. 2001; 86:1410–1417.
10. Thompson KH, Lichter J, Lebel C, Scaife MC, McNeill JH, Orvig Ch. Vanadium treatment of type 2 diabetes: A view to the future. *Journal of Inorganic Biochemistry*. 2009; 103:554-558.
11. Jakusch, T, Kiss, T. In vitro study of the antidiabetic behavior of vanadium compounds. *Coordination Chemistry Reviews*. 2017; 351: p. 118-126.
12. McNeill JH, Yuen VG, Hoveyda HR, Orvig C. Bis(maltolato) oxovanadium(IV) is a potent insulin mimic. *Journal of Medicinal Chemistry*. 1992; 35:1489–1491.
13. Peters KG, Davis MG, Howard BW, Pokross M, Rastogi V, Diven C, Greis KD, Eby-Wilkens E, Maier M, Evdokimov A, Soper S, Genbauffe F. Mechanism of insulin sensitization by BMOV (bis maltolato oxo vanadium); unliganded vanadium (VO₄) as the active component. *Journal of Inorganic Biochemistry*. 2003; 96:321-330.
14. Ki J, Mukherjee A, Rangasamy S, Purushothaman B, Song JM. Insulin-mimetic and anti-inflammatory potential of a

- vanadyl-Schiff base complex for its application against diabetes. RSC Advances. 2016;6:57530- 57539.
15. Lofti N, Sheikhshoei I, Ebrahimipour SY, Krautscheid H. Synthesis, characterization, crystal structure and DFT studies of a *cis* dioxo-vanadium(V) complex containing a tridentate (NNO) Schiff base ligand. Journal of Molecular Structure. 2017; 1149:432-438.
 16. Gryboś R, Szklarzewicz J, Jurowska A, Hodorowicz M. Properties, structure and stability of V(IV) hydrazide Schiff base ligand complex. Journal of Molecular Structure. 2018;1171:880-887.
 17. Szklarzewicz, J, Jurowska, A, Olszewska, A, Hodorowicz, M, Synthesis, structure and properties of V(III,IV and V) complexes with ONO Schiff bases. Science, Technology and Innovation. 2019; 4(1):37-46.
 18. Jurowska A, Szklarzewicz J, Hodorowicz M, Gryboś R. Synthesis, structure and properties of V(V) monooxido complex with ONO tridentate Schiff base. Science, Technology and Innovation. 2019; 4(1):21-29.