Ternary complex formation between vanadium(III)–salicylic acid and histidine, cysteine, aspartic and glutamic acids as a basis to obtain new antidiabetic agents

Formación de complejos ternarios de vanadio(III)-ácido salicílico con los aminoácidos histidina, cisteina, ácido aspártico y ácido glutámico como base para la obtención de nuevos agentes antidiabéticos

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RESUMEN

En el presente trabajo se estudió los equilibrios en solución de los complejos ternarios de Vanadio(III)-Ácido Salicílico (H_2Sal) con los aminoácidos Cisteína (H_2Cys), Histeína (HHis), Ácido Aspártico (H_2Asp) y Ácido Glutámico (H_2Glu) vía potenciométrica. Las constantes de formación reportadas fueron obtenidas a 25 °C empleando en el medio una fuerza iónica constante igual a 3,0 mol.L^{-1}, de manera de mantener los coeficientes de actividad constantes. Todas las contantes de formación reportadas fueron obtenidas empleando LETAGROP como Software de cálculo, usando el nivel (HL) como referencia; mientras que los diagramas de distribución de especies fueron obtenidos empleando HySS como Software de Modelaje. Para el sistema Vanadio(III)-H_2Sal-H_2Asp se observó la formación de las siguientes especies: $\{V(HSal)(H_2Asp)\}^{2+}$, $\{V(Sal)(H_2Asp)\}^{+}$, V(Sal)(HAsp), $\{V(Sal)(Asp)\}^{-}$ y $\{V(Sal)(Asp)(OH)_2\}^{3-}$, para el sistema Vanadio(III)-H_2Sal-H_2Glu se observó las especies: $\{V(Sal)(H_2Glu)\}^{+}$, V(Sal)(HGlu), $\{V(Sal)(Glu)\}^{-}$ y $\{V(Sal)(Glu)(OH)\}^{2-}$, en el sistema Vanadio(III)-H_2Sal-H_2Cys, se observó la formación de cuatro complejos: $\{V(Sal)(H_2Cys)\}^{2+}$, $\{V(Sal)(HCys)\}^{+}$, V(Sal)(Cys) y $\{V(Sal)(Cys)(OH)\}^{-}$; mientras que para el Sistema Vanadio(III)-H_2Sal-HHis, se observó los siguientes complejos en solución: $\{V(HSal)(HHis)\}^{2+}$, $\{V(Sal)(HHis)\}^{+}$, V(Sal)(His), $\{V(Sal)(His)(OH)\}^{-}$ y $\{V(Sal)(His)(OH)_2\}^{2-}$.

Palabras clave: Constantes de formación de complejos, estudios potenciométricos, especiación química, complejos ternarios.

ABSTRACT

Solution equilibria of the ternary complexes Vanadium(III)-Salicylic Acid (H_2Sal) and the amino acids Cysteine (H_2Cys), Histidine (HHis), Aspartic acid (H_2Asp) and Glutamic acid (H_2Glu), were studied pH-metrically. The formation constants of the resulting mixed ligand complexes have been determined at 25 °C using an ionic strength 3,0 mol.L^{-1} KCl to maintain constant the activity coefficients. All the formation complex constant reporters were obtained using LETAGROP as calculation Software using (HL) level of reference, while the species distribution diagrams were obtained using Hyss as modeling software. In the Vanadium(III)-H_2Sal-H_2Asp system was observed the formations of the

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following species: $\{V(HSal)(H_2Asp)\}^{2+}$, $\{V(Sal)(H_2Asp)\}^{+}$, V(Sal)(HAsp), $\{V(Sal)(Asp)\}^{-}$ and $\{V(Sal)(Asp)(OH)_2\}^{3-}$, for the Vanadium(III)-$H_2$Sal-$H_2$Glu is observed the species: $\{V(Sal)(H_2Glu)\}^{+}$, V(Sal)(HGlu), $\{V(Sal)(Glu)\}^{-}$ and $\{V(Sal)(Glu)(OH)\}^{2-}$, in Vanadium(III)-H_2Sal-H_2Cys system four complexes were detected: $\{V(Sal)(H_2Cys)\}^{2+}$, $\{V(Sal)(HCys)\}^{+}$, V(Sal)(Cys) and $\{V(Sal)(Cys)(OH)\}^{-}$; while the Vanadium(III)-H_2Sal-HHis system, were detected the following complexes: $\{V(HSal)(HHis)\}^{2+}$, $\{V(Sal)(HHis)\}^{+}$, V(Sal)(His), $\{V(Sal)(His)(OH)\}^{-}$ and $\{V(Sal)(His)(OH)_2\}^{2-}$.

Key words: Complexation constants formation, potentiometric studies, chemical speciation, ternary complexes.

Introduction

Diabetes mellitus (DM) is one of the biggest problems in global public health (American Diabetes Association, 2015; World Health Organization, 2015), about 387 million people suffer this disease worldwide and estimates an additional increase of 205 million patients by 2035 (Federación Internacional de la Diabetes, 2013). In Venezuela, it is the fourth leading cause of death and there are 1.252 million people with diabetes (Ministerio del Poder Popular para la Salud, 2014; Federación Internacional de la Diabetes, 2013).

Hyperglycemia is the main clinical sign of DM and reflects the altered metabolism of glucose characteristic of this disease. Many factors related to lifestyle have been linked to diabetes, most notably the high consumption of carbohydrates and lipids in the diet (American Diabetes Association, 2015). Deficiency of trace elements also have been associated with this cardiometabolic disease, such is the case of zinc, chromium, selenium and vanadium, therefore the supplementation of these elements in diet has been widely studied (Yeh et al., 2003; Thompson et al., 2004; Wiernsperger and Rapin, 2010; Lin and Huang, 2015). Interestingly, salts and coordination complex of Vanadium have shown insulin-mimetic and antidiabetic activity, making this trace element in a promissory therapeutic agent for the treatment of DM (Brichard and Henquin, 1995; Sakurai, 1999; Melchior et al., 2001; Thompson and Orvig, 2006; Mehdi et al., 2006; Nankar and Doble, 2013; Clark et al., 2014; Mohammadi et al., 2014; Rehder, 2015).

The treatment with salts of vanadium decreases hyperglycemia in experimental models of diabetes, such as rats with streptozotocin-induced diabetes, diabetic Zucker rats, and diabetic obese Zucker rats, ob/ob mice and db/db mice (Meyerovitch et al., 1991; Wang et al., 2001; Sakurai, 2008; Mohammadi et al., 2014). Also, vanadium sulfate has been shown to be effective in reducing fasting glucose and glycosylated hemoglobin (HbA1C) in patients with type 1 and type 2 DM (Goldwaser et al., 2000; Smith et al., 2008; Thompson et al., 2009; Souveid et al., 2013.). This antidiabetic activity has been linked to a competitive inhibition of the protein tyrosine phosphatase (PTP) (Huyer et al., 1997; Poucheret et al., 1998; Semiz and McNeill, 2002; McLauchlan et al., 2010; Lu et al., 2011) and the increase of the activity of some cytosolic protein kinases (Shisheva and Shechter, 1993); resulting increases of several key proteins activity in the insulin signaling, such as the IRS-1 (substrate insulin receptor 1), Akt/PKB (protein kinase B), GSK3 (glycogen synthase kinase 3), transcription factor FOXO-1 (Forkhead box protein O1) and some MAPK (mitogen activated kinase), leading to increased glycolysis and gluconeogenesis and a decreased in gluneogenesis and stimulation of cell proliferation (Mehdi et al., 2006; Vardatsikos et al., 2009; Wiernsperger and Rapin, 2010). The evidence supports that insulin-mimetic effects induced by vanadium are independent of the insulin receptor (Mehdi et al., 2006; García-Vicente et al., 2007).

From the pharmacokinetic point of view, the inorganic vanadium is poorly absorbed by the oral route and binds significantivly to plasma proteins (Srivastava 2000; Correia et al., 2012; Willsky et al., 2013). Additionally, treatment with vanadium salts have been associated with certain toxic effects, such as decreased body weight and gastrointestinal discomfort (Srivastava, 2000). The use of coordination complex of vanadium have not only shown insulin-mimetics and anti-diabetic effects, but they also have shown improvement in the kinetic profile and decreased toxicity in vanadium inorganic compounds (Srivastava, 2000; Domingo, 2002; Zhang et al., 2008). These findings have prompted us to propose new vanadium compounds that are promising as anti-diabetic and with greater safety index than common inorganic salts.

Different types of Vanadium complexes have been studied, varying the metal oxidation state, these studies include species containing V(V), V(IV) and V(III), being V(III) complexes the least studied (Serio, 1986; Sakurai, et al., 1999; Crans, et al., 2004; Thompson and Orvig, 2001; Osin ska-Królicka, et al., 2004; Papaioannou, et al., 2004; Shiozawa, 2009). The first synthesis and characterization of complexes

of V(III) with Maltol (Mal) as anti-diabetic agents candidate was accomplished in 2001 (Thompson and Orvig, 2001; Zhang et al., 2008). As with other metal chelates of these and related ligands, reasonable hydrolytic and thermodynamic stabilities were anticipated; the air stability of $V(Mal)_3$ was an unexpected advantage. Treatment with either $V(Mal)_3$ or $VO(Mal)_2$ resulted in glucose-lowering in streptozotocin-induced diabetic rats of comparable and significant magnitudes, both when administered intraperitoneally or orally, with no overt toxicity other than gastrointestinal distress, and no fatalities (Melchior et al., 2001; Thompson and Orvig, 2001). Other complexes of V(III) have shown antidiabetic activity in experimental models of diabetes, such as the following systems: Dipicolinates and cloropicolinates (Buglyó et al., 2005; Li et al., 2009; Xie et al., 2014). Furthermore, this class of compounds has shown inhibitory activity of enzymes: Acid phosphatase, alkaline phosphatase and PTP (McLauchlan et al., 2010)

Taking into account the possible importance in medicine of the vanadium(III) complexes, it is known that after oral administration of these complexes, they may encounter many other potential vanadium(III) binding molecules present in extracellular or intracellular biological fluids. These latter ligands may partially or completely displace the original vanadium(III) carrier molecules from the metal coordination sphere. Accordingly, ternary complex formation should be taken into account in a speciation description of these complexes in biological fluids. Such ternary complexes might be of great importance in the absorption and transport process of vanadium(III) complexes and even in their physiological activity (Sakurai et al., 1999). There are no reports on the speciation of the ternary vanadium(III)-H_2Sal (H_2Sal = Salicylic acid) complexes with Aspartic acid (H_2Asp), Glutamic acid (H_2Glu), Cysteine (H_2Cys) and Histidine (HHis) (Martell et al., 1993; Powell and Pettit, 1997).

In the present work we study the ternary complex formation between vanadium(III)-H_2Sal and the amino acids H_2Asp, H_2Glu, H_2Cys and HHis as a contribution to the knowledge of the speciation of these complexes in biofluids, laying the groundwork for future applications in diabetes.

Materials and Methods

REAGENTS

The VCl_3 (Merck p.a) and the Salicylic Acid (H_2Sal) (Merck p.a.) and all the amino acids, Aspartic acid (H_2Asp), Glutamic acid (H_2Glu), Cysteine (H_2Cys) and Histidine (HHis) (Merck p.a) were used without more purification. The HCl and KOH solutions were prepared using 100,0 mmol.L^{-1} Titrisol Merck ampoules. The KOH solution was standardized against potassium hydrogen phthalate. The solutions were prepared using triple glass-distilled water, boiled before the preparation of the solutions in order to remove dissolved CO_2. To prevent the hydrolysis of the VCl_3 stock solution, it contained 200 mmol.L^{-1} HCl and was maintained under a H_2 atmosphere in the presence of a Pt platinized net in order to avoid oxidation of the V(III) solution to V(IV) (Brito and Goncalvez, 1982). Under these conditions, the vanadium(III) solution can be maintained and the stability of the vanadium(III) solution was checked periodically by spectrophotometric measurements and it was shown to be stable for several weeks. The emf (H) measurements were carried out in aqueous solution at an ionic strength of 3.0 mol.L^{-1} in KCl. Nitrogen free O_2 and CO_2 was used.

METHODS

The emf (H) measurements were done using the following instruments: Thermo Orion model 520A pH meter, Metrohm EA 876–20 titration vessel, Lauda Brikmann RM6 thermostat bath, Shimadzu UV-1601 PC spectrophotometer, and a quartz cell with a 10.0 mm path length. The sealed 100 mL thermostatted double-walled glass titration vessel was fitted with a combined Orion Ross 8102BN pH electrode with a titrant inlet, magnetic stirrer, and an inert nitrogen atmosphere inlet with outlet tubes. The temperature was maintained at (25.0 ± 0.1) °C by constant circulation of water from the thermostat bath. Figure 1 show the experimental arrangement employed to carry out potentiometric measurements.

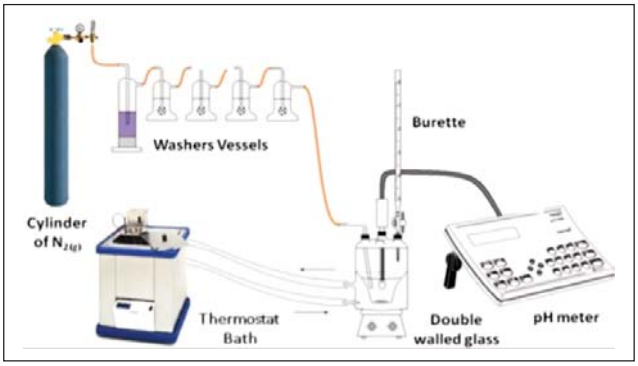

Figure 1. Experimental arrangement employed to carry out potentiometric measurements.

The emf (H) measurements were carried out by means of the REF//S/GE cell, where REF = Ag/AgCl/3,0 M KCl; S = equilibrium solution and GE = glass electrode. At 25 °C the emf (mV) of this cell follows the Nernst equation, $E = E^0 + jh + 59.16 \log h$, where h represents the free hydrogen ion concentration, E^0

is the standard potential and *j* is a constant which takes into account the liquid junction potential (Biedermann and Sillén, 1952). The experiments were carried out as follows: a fixed volume of 0.100 mol.L^{-1} HCl was titrated with successive additions of 0.100 mol.L^{-1} KOH until near neutrality in order to get the parameters E^0 and *j*. Then, volume of a stock solution of the ligands and an aliquot of the Vanadium(III) stock solution were added sequentially. Finally, the titration was continued with 0.100 mol.L^{-1} KOH. The measurements were done using a total metal concentration, M_T = 2–3 mmol.L^{-1} and Vanadium(III)-H_2Sal-Amino acid under study molar ratios R = 1:1:1, 1:2:1 and 1:1:2, respectively. First, the dissociation constants of H_2Sal and the amino acids: Aspartic acid (H_2Asp), Glutamic acid (H_2Glu), Cysteine (H_2Cys) and Histidine (HHis) were determined (Table I). The values of pK_i obtained in a 3.0 mol.L^{-1} KCl ionic medium are also presented in Table I. The values obtained are in good agreement with those previously reported in the literature (Martell et al., 1993; Powell and Pettit, 1997).

The V^{3+}-H_2Sal-Amino acids (H_iL) systems were studied according to the reaction scheme:

$$pH_2O + qV^{3+} + rH_2Sal + sH_iL \rightleftharpoons \{V_q(OH)_p(H_2Sal)_r(H_iL)_s\} + pH^+ \beta_{pqrs} \quad (1)$$

Where (H_iL) represents the amino acids: H_2Asp, H_2Glu, H_2Cys and HHis, being i = 1 for HHis and 2 for H_2Asp, H_2Glu, H_2Cys and $\{V_q(OH)_p(H_2Sal)_r(HiL)_s\}$ is the ternary (p, q, r, s) complex and $\beta_{p,q,r,s}$ is the respective stability constant.

The potentiometric data were analysed using the program LETAGROP (Sillén, 1964; Brito et al., 2005), in order to minimize the function $Z_B = (h - H)/M_T$, being Z_B the number of proton dissociate per mole of V(III), where *H* is the total analytical concentration of H^+, *h* represents the equilibrium concentration of H^+, and *C* represents the total analytical concentration of H_2Sal.

Equilibria corresponding to the formation of the hydroxo complexes of Vanadium(III) were considered in the calculation of the stability constants of ternary complexes. The following species were assumed: $\{V(OH)\}^{2+}$, $\log \beta_{1,-1} = -3.13(8)$; $\{V_2O\}^{4+}$, $\log \beta_{2,-2} = -3.76(6)$; $\{V(OH)_2\}^{+}$, $\log \beta_{1,-2} = -6.86(2)$; and $\{V_3(OH)_8\}^{+}$, $\log \beta_{3,-8} = -27.47(4)$ (Lubes et al., 2010). The stability constants of the binary system were taken into account for the calculation of the stability constant of the ternary complexes, for example in the vanadium(III)–H_2Sal system their stability constants are given in (Goncalves et al., 2011), the vanadium(III)-H_2Asp stability constants are presented (Zambrano, 2004), and the stability constants of systems Vanadium(III)–H_2Glu are given in (Mendoza, 2004) for the Vanadium(III)–H_2Cys and Vanadium(III)-HHis the stability constants are given in (Lubes et al., 2008; Rosas, 2008) the stability constants of the vanadium(III) hydroxo complexes, the stability constants of the ligands and the stability constants of the binary complexes were kept fixed during the analysis. The aim was to find a complex or complexes giving the lowest sum of the errors squared, $U = \sum(Z_B^{exp} - Z_B^{calc})^2$, in the analysis of Z_B function, where the fittings were done by testing different (*p*, *q*, *r*, *s*) combinations. The species distribution diagrams were done with the computer program HYSS (Alderighi et al., 1999), yielding the β_{pqrs} values, which are summarized in Table II.

Table I

Acidity constants ($\log_{10}$) and pK_a values of the ligands studied (3.0 mol.L^{-1} KCl at 25 °C)

	$\log_{10} \beta_{pr}$				
Equilibrium	**H_2Sal**	**H_2Asp**	**H_2Glu**	**H_2Cys**	**HHis**
$H_2L + H^+ \rightleftharpoons H_3L^+$		2.26(4)	2.39(3)	1.94(2)	
$H_2L \rightleftharpoons HL^- + H^+$	-2.89(2)	-3.83(2)	-4.27(2)	-7.81(2)	
$H_2L \rightleftharpoons L^{2-} + 2H^+$	-13.23(3)	-12.92(3)	-13.21(3)	-17.31(2)	
$HL \rightleftharpoons L^- + H^+$					-8.65(3)
$HL + H^+ \rightleftharpoons H_2L^+$					6.15(2)
$HL + 2H^+ \rightleftharpoons H_3L^+$					8.24(4)
Dispersion(σ)	0.029	0.036	0.029	0.022	0.031
pK_i					
pKa_1	2.89	2.26	2.39	1.94	2.09
pKa_2	10.34	3.83	4.27	7.81	6.15
pKa_3		9.09	8.94	9.50	8.65

Values in parentheses are standard deviations ($3(\log_{10})$) on the last significant figure

Table II

Stability constant ($\log_{10\ pqrs}$) of the Vanadium(III)-H_2Sal-H_iL system (3.0 mol.L^{-1} KCl at 25 °C). According to the following reaction scheme:

$$pH_2O + qV^{3+} + rH_2Sal + sH_iL \rightleftharpoons \{V_q(OH)_p(H_2Sal)_r(H_iL)_s\} + pH^+ \beta_{pqrs} \quad (2)$$

Complexes	$\log_{10} \beta_{pqrs}$			
	H_2Asp	**H_2Glu**	**H_2Cys**	**HHis**
$[V(HSal)(H_2L)]^{2+}$	19.78(6)			
$[V(Sal)(H_2L)]^{+}$	16.95(6)	10.85(6)	16.4(1)	
V(Sal)(HL)	13.31(5)	6.87(2)	12.66(8)	
$[V(Sal)(L)]^{-}$	7.2(2)	3.18(8)	7.0(4)	
$[V(Sal)(L)(OH)]^{2-}$		-1.7(3)	2.1(1)	
$[V(Sal)(L)(OH)_2]^{3-}$	-7.1(2)			
$[V(HSal)(HL)]^{2+}$				18.18(9)
$[V(Sal)(HL)]^{+}$				13.5(4)
V(Sal)(L)				9.7(1)
$[V(Sal)(L)(OH)]^{-}$				4.5(1)
$[V(Sal)(L)(OH)_2]^{2-}$				-1.8(1)
Dispersion(σ)	0.088	0.084	0.109	0.105

Values in parentheses are standard deviations ($3(\log_{10})$) on the last significant figure

Results

IONIZATION CONSTANTS OF STUDIED LIGANDS

The ionization constants (Table I) in the ionic medium 3.0 mol.L^{-1} KCl are in good agreement with the literature values, considering the differences in ionic strength and ionic medium (Martell et al., 1993; Powell and Petit, 1997).

TERNARY VANADIUM(III) COMPLEXES

Vanadium(III)-H_2Sal-H_2Asp system

In Figure 2 the species distribution diagrams is shown, considering the stability constants summarized in Table II. And considering the conditions M_T = 3 mmol.L^{-1} and the molar ratio 1:1:1.

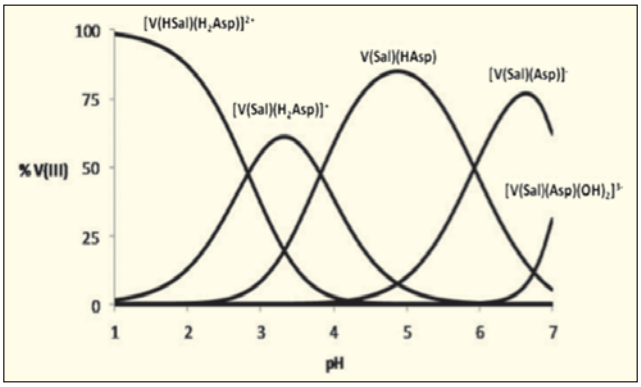

Figure 2. Species distribution diagrams for the V(III)-H_2Sal–H_2 Asp system. M_T = 3 mmol.L^{-1} R = 1:1:1.

higher toxicity profiles, including inhibition of mitochondrial respiration and oxidative phosphorylation (Hosseini et al., 2013), doing very interesting the procurement and evaluation of new Vanadium(III) complexes, especially with common components in blood plasma such as amino acids.

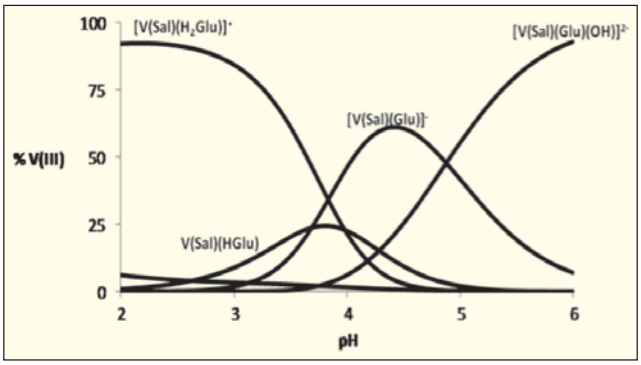

Figure 3. Species distribution diagrams for the V(III)- H_2Sal –H_2Glu system. M_T = 3 mmol.L^{-1} R = 1:1:1.

In the Vanadium(III)-H_2Sal-H_2Asp system the best fit of the experimental data was obtained considering the formations of the species: $\{V(HSal)(H_2Asp)\}^{2+}$,

Vanadium(III)-H_2Sal-H_2Glu system

In Figure 3 are given the results of the species distribution diagrams for this system, considering the stability constants summarized in Table II, and the conditions M_T = 3 mmol.L^{-1} and the molar ratio 1:1:1.

Vanadium(III)-H_2Sal–H_2Cys

In Figure 4 is given the species distribution diagrams, considering the stability constants summarized in Table II. Taking into account the conditions M_T = 3 mmol.L^{-1} and the molar ratio 1:1:1.

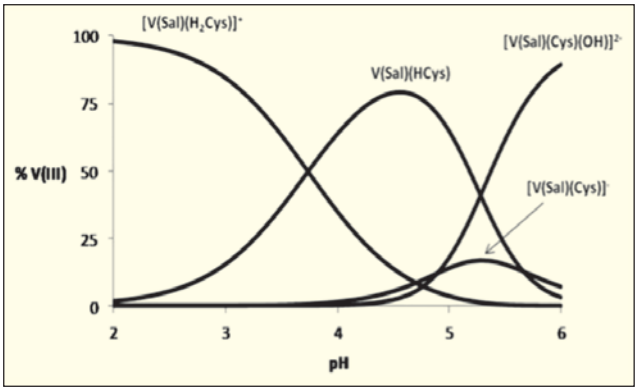

Figure 4. Species distribution diagrams for the V(III)-H_2Sal–H_2 Cys system. M_T = 3 mmol.L^{-1} R = 1:1:1.

Vanadium(III)-H_2Sal-HHis system

Figure 5 show the species distribution diagrams, taking into account the stability constants summarized in Table II, and considering the conditions M_T = 3 mmol.L^{-1} and the molar ratio 1:1:1.

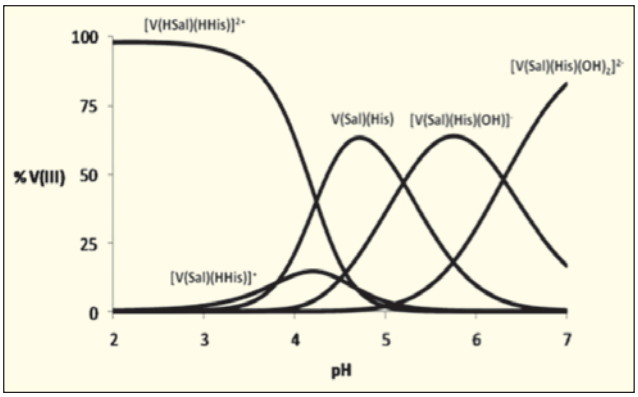

Figure 5. Species distribution diagrams for the V(III)- H_2Sal –HHis system. M_T = 3 mmol.L^{-1} R = 1:1:1

Discussion

Similar as the vanadium(IV) and vanadium(V) complexes, the vanadium(III) complexes have shown antihyperglycemic and insulin-mimetic effects (Buglyó et al., 2005; Li et al., 2009). Although complex in oxidation state V (V^{+5}) have shown better insulin-mimetic potency, these complexes have exhibited

$\{V(Sal)(H_2Asp)\}^+$, V(Sal)(HAsp), $\{V(Sal)(Asp)\}^-$ and $\{V(Sal)(Asp)(OH)_2\}^{3-}$. In Figure 2 the species distribution diagrams are given, in this case is presented the data of the molar ratio (1:1:1), it is observed the abundance of the $\{V(HSal)(H_2Asp)\}^{2+}$, at pH < 2.5, between 2.5 < pH < 3.5 range predominate the species $\{V(Sal)(H_2Asp)\}^+$, the ternary V(Sal)(HAsp) complex is observed between pH 3.5 and 6, the complex $\{V(Sal)(Asp)\}^-$ is important at pH > 6 and the complex $\{V(Sal)(Asp)(OH)_2\}^{3-}$ is formed in low proportion at pH > 6.

For Vanadium(III)-H_2Sal-H_2Glu system the model that best fit to the experimental data contained the species: $\{V(Sal)(H_2Glu)\}^+$, V(Sal)(HGlu), $\{V(Sal)(Glu)\}^-$ and $\{V(Sal)(Glu)(OH)\}^{2-}$. In Figure 3 the species distribution diagrams are shown, we can see that the complex $\{V(Sal)(H_2Glu)\}^+$ is very important at pH < 4 and their relative abundance is about 90 %, so this complex species $\{V(Sal)\ (H_2Glu)\}^+$ could be the easiest complex to isolate in futures works. Between 2 < pH < 5 is formed in a 25 % the species V(Sal)(HGlu), the complex $\{V(Sal)(Glu)\}^-$ predominates in the region 4 < pH < 5, and at pH > 5 the most important species is $\{V(Sal)(Glu)(OH)\}^{2-}$ which increases their relative abundance as the medium becomes more alkaline.

In the case of the Vanadium(III)-H_2Sal-H_2Cys system four complexes were detected (Table II), $\{V(Sal)\ (H_2Cys)\}^{2+}$, $\{V(Sal)(HCys)\}^+$, V(Sal)(Cys) and $\{V(Sal)(Cys)(OH)\}^-$. In Figure 4 the species distribution diagrams is presented, where can be seen that the species $\{V(Sal)(H_2Cys)\}^{2+}$ is very important at pH < 3,5 between 3,5 and 5 pH is observed the complex $\{V(Sal)(HCys)\}^+$, the complex V(Sal)(Cys) is present between the pH values 4-6 but it is formed with a 20 % and the complex $\{V(Sal)(Cys)(OH)\}^-$ is very abundant at pH > 5. Some vanadium complexes with methyl cysteine have shown antidiabetic activity in rats with streptozotocin-induced DM (Sakurai, 2008). However, the species detected in this system have not been evaluated from the biological standpoint.

Finally, the results of the analysis done in the Vanadium(III)-H_2Sal-HHis system, it was detected five complexes in solution: $\{V(HSal)(HHis)\}^{2+}$, $\{V(Sal)(HHis)\}^+$, V(Sal)(His), $\{V(Sal)(His)(OH)\}^-$ and $\{V(Sal)(His)(OH)_2\}^{2-}$. The corresponding species distribution diagrams are given in Figure 5, we see that the $\{V(HSal)(HHis)\}^{2+}$ complex is the most important species at pH < 4, the ternary complex $\{V(Sal)(HHis)\}^+$ is formed in a low proportion between 3 < pH < 5, the species V(Sal)(His) predominates in the range 4 < pH < 5, the complex $\{V(Sal)(His)(OH)\}^-$ is formed between the pH 5-6 and the species $\{V(Sal)(His)(OH)_2\}^{2-}$ predominates at pH > 6. In all systems studied, it was shown a preference to form ternary complexes than binaries complexes.

Importantly, complexes V(III)-salicylates-aminoacids, have not been studied as insulin-mimetic, being the complex of vanadium(III)-dipicolinate and vanadium(III)-maltonate, which have been reported best hypoglycemic effect in rats with streptozotocin-induced DM and insulin-mimetic activity *in vitro* (Buglyó et al., 2005; Li et al., 2009; Islam et al., 2010; Xie et al., 2014).

Moreover, other metal complexes with salicylates have been established as anti-diabetic and antioxidants, that is the case of binary complexes of copper-salicylate and zinc-salicylates (Yoshikawa et al., 2011; Qazzaz et al., 2013). The systems studied provide the basis for the synthesis, characterization and evaluation as new promising antihyperglycemic and insulin-mimetic complexes of vanadium(III), thus contributing to the search for a new anti-diabetic drugs.

Conclusión

In this work we studied the speciation of the ternary complexes formed between the Vanadium(III)-H_2Sal, and the amino acids H_2Asp, H_2Glu, H_2Cys and HHis. The data analysis performed with the program LETAGROP indicate the formation of the complexes $\{V(HSal)(H_2Asp)\}^{2+}$, $\{V(Sal)(H_2Asp)\}^+$, V(Sal)(HAsp), $\{V(Sal)(Asp)\}^-$ and $\{V(Sal)(Asp)(OH)_2\}^{3-}$ in the Vanadium(III)–H_2Sal–H_2Asp system, in the Vanadium(III)–H_2Sal-H_2Glu system, were detected the species $\{V(Sal)(H_2Glu)\}^+$, V(Sal)(HGlu), $\{V(Sal)(Glu)\}^-$ and $\{V(Sal)(Glu)(OH)\}^{2-}$. In the case of the Vanadium(III)–H_2Sal-H_2Cys system were detected the species $\{V(Sal)(H_2Cys)\}^{2+}$, $\{V(Sal)(HCys)\}^+$, V(Sal)(Cys) and $\{V(Sal)(Cys)(OH)\}^-$. And finally, in the Vanadium(III)–H_2Sal–HHis system were observed the complexes $\{V(HSal)(HHis)\}^{2+}$, $\{V(Sal)(HHis)\}^+$, V(Sal)(His), $\{V(Sal)(His)(OH)\}^-$ and $\{V(Sal)(His)(OH)_2\}^{2-}$.

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