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Interaction of oxovanadium(IV) with tricarboxylic ligands in aqueous solution: a thermodynamic and spectroscopic study

Silvia Berto* [a], Pier Giuseppe Daniele[a], Enrico Prenesti[a], Enzo Laurenti[b]

[a]*Dipartimento di Chimica Analitica dell'Università, Via Pietro Giuria 5, 10125 Turin, Italy*

[b]*Dipartimento di Chimica IFM dell'Università, Via Pietro Giuria 7, 10125 Turin, Italy*

*Corresponding author: address: Dipartimento di Chimica Analitica dell'Università

via Pietro Giuria 5, 10125 Turin, Italy

phone: +39 011 6707626;

fax: +39 011 6707615;

e-mail address: silvia.berto@unito.it

Abstract

In this paper we report an investigation on coordination compounds of vanadyl ion with citric, D(+)-*threo*-isocitric, *cis*- and *trans*-aconitic acids in aqueous solution. The different binary systems have been studied by potentiometric and spectroscopic techniques (t = 25 °C, I = 0.1 mol dm^{-3}). Electronic paramagnetic resonance spectroscopy (EPR) at room temperature and molecular absorption spectrophotometry were employed in order to obtain a structural characterization of complexes. A speciation model was proposed for all the metal/ligand systems from potentiometric data. For all the systems studied the chemical model assumes the formation of complexes with stoichiometry MLH_r and binuclear species $M_2L_1H_{-r}$. Citric and D(+)-*threo*-isocitric acids also form stable dimeric species $M_2L_2H_{-r}$. The EPR investigation reveals that the dimeric complexes of hydroxyacids present magnetic interaction supporting the hypothesis of an alcoholate-bridged dinuclear structure. For each system investigated we recorded visible absorption spectra and estimated the individual spectrum of species in solution. A particular spectrophotometric behaviour of citrate system was recognized and the

joint examination of our spectroscopic results and the literature data leads us to suppose that the *trans* position of vanadyl is occupied by the bridged alcoholate group.

1. Introduction

The main scientific interest for both vanadium and vanadium compounds is related to their involvement in metallurgical, fuels and coal (energetic), catalytic, electrochemical (vanadium fuel cells) and pharmaceutical fields. In particular, the biological effects, bio-distribution, toxicology and pharmacological activity of vanadium compounds are areas of increasing research interest. In this regard, the European Food Safety Authority (EFSA) delivered a scientific opinion on the safety and bioavailability of vanadium compounds added to foods and food supplements [1]. The toxicity of vanadium is correlated to its degree of oxidation, vanadyl is less toxic than vanadate ion, and to its chemical form, the organic form is less toxic than inorganic one [2,3]. This is why experiments that involve vanadium compounds with multidentate ligands have been carried out. These studies show that the organic form provides higher absorption of ion from the alimentary duct and reduces the risk of side effects [4-8]. Vanadium citrate, bismaltolato oxovanadium(IV) and bisglycinato oxovanadium(IV), for example, are vanadium organic compounds added to foods for particular nutritional uses [1]. Recent studies report that bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) is a potent cardioprotective agent [9] and improves memory and cognitive deficits following brain ischemia [10]. Moreover, the chemical form of vanadium can influence the biological effects [11] and, in particular, vanadium compounds with organic ligands can increase specificity of biological action [12,13]. In view of these remarks, the investigation on vanadyl ion chemistry and on structural characteristics of vanadium complexes may provide information useful to better understand its biochemical behaviour and to identify vanadium chemical forms suitable for human health.

This study is an enhancement of a previous investigation on the vanadyl chemistry in water solution [14]. Our attention is addressed to the study of coordination compounds between vanadyl oxocation and carboxylic acids and to their structural characterisation. In this paper we report the investigation on the stability of coordination compounds of vanadyl ion with citric, D(+)-*threo*-isocitric, *cis*- and *trans*-aconitic acids in aqueous solution. The four acids were chosen in order to characterize the effect of the functional groups position in the ligand molecule on the vanadyl coordination capability and on the structure of complexes.

The different binary systems have been studied by potentiometric technique at $t = 25$ °C and spectrometric techniques were employed in order to obtain a structural characterization of complexes. In particular, molecular visible absorption spectrophotometry and electronic paramagnetic resonance spectroscopy (EPR), at room temperature, were joined to reach our

goals. The processing of the visible absorption spectra was performed for those vanadyl complexes reaching significant percentage of formation in solution, with the aim of giving a better characterization of single species. EPR spectra were recorded on metal-ligand solutions with chemical conditions favourable to the formation of specific coordination species. This technique is rather utilized for the study of vanadyl chemistry, whereas the visible absorption spectra of these oxocation are seldom considered for its speciation. At this regard, Kiss et al. [15] and Lodyga-Chruscinska et al. [16] reported studies on vanadyl/citric acid interaction in aqueous solution by potentiometry, absorption spectrophotometry and EPR, and provide structural hypothesis. In particular, the ref. 16 is an enhancement of the work of Kiss et al. [15] aimed to characterize the vanadyl complexes in aqueous solution at high ligand to metal molar ratio. In these conditions the mononuclear species formation is favoured, while, at low ligand to metal molar ratio, the dimeric species are prevalent. A spectroscopic study centred on the dimeric complexes vanadyl/citrate was also carried out by Tsaramyrsi et al. [17]. There are no similar studies on vanadyl speciation in solution with D(+)-*threo*-isocitric, *cis*- and *trans*-aconitic acids. Moreover, in the above mentioned papers the spectroscopic investigation was based on the observation of experimental visible absorption spectra, whereas in this work, the experimental spectra were elaborated in order to obtain the spectrum for each complex formed in solution only assuming the additivity of the signal in the investigated concentration range. This may be particularly useful for interpretation of spectra recorded on metal ligand systems in which, with raising pH value, the type of complex species formed in solution significantly varies showing wide overlapping between species laying different structures.

2. Experimental

2.1 Chemicals

Vanadyl sulfate (vanadium(IV) oxide sulphate pentahydrate, purity ~ 96%, Riedel-de Haën or Aldrich), stock solution (~ 0.1 mol dm^{-3}) was weekly prepared without previous purification of the salt; it was standardized by redox titration with permanganate solution [18] (Carlo Erba), followed by photometric detection [14].

D(+)-*threo*-isocitric ((1R,2S)-1-hydroxy-1,2,3-propanetricarboxylic acid) and *trans*-aconitic (*trans*-propene-1,2,3-tricarboxylic acid) acids are Fluka products with purity of 99%. The citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is a Merck product (purity of 99.5%). The *cis*-aconitic (*cis*-propene-1,2,3-tricarboxylic acid) is a Sigma-Aldrich product with purity

of 98%. The ligand molecule were used without further purification and their purity were checked alkalimetrically. The formulae of the ligands are provided in Fig. 1.

Standard KOH and HCl solutions were prepared by diluting Merck or Fluka concentrate products and standardized against potassium hydrogenphthalate (Fluka, puriss.) and sodium carbonate (Fluka, puriss.), respectively. All solutions were prepared using grade A glassware and ultrapure water (conductivity < 0.1 µS).

2.2 Electromotive force measurements

Potentiometric measurements were performed using a Metrohm mod. 713 potentiometer (resolution of ± 0.1 mV) coupled with a Metrohm 665 Dosimat burette (minimum volume deliverable of ± 0.001 cm^3) and equipped with a Metrohm combined glass electrode (mod. 6.0222.100).

For all the potentiometric measurements the electrode couple was standardized, in terms of pH = $-\log[H^+]$, by titrating HCl 10 mmol·dm^{-3} solution (at the same ionic strength value as the solution under study) with standard KOH in order to determine the standard potential E° before each experiment. The potentiometric titrations were carried out in a stream of purified nitrogen gently bubbled in the titration cell to avoid O_2 and CO_2 contamination. The measurement cells were thermostated at (25± 0.1 °C) by means of a water circulation from a thermocryostat (mod. D1-G Haake).

The experiments were carried out in KCl aqueous solutions with ionic strength 0.1 mol·dm^{-3}. For the investigation of VO^{2+}-carboxylate systems, 25·10^{-3} dm^3 of solution containing VO^{2+}, the carboxylate ligand and KCl was titrated with KOH standard. Each titration was at least twice repeated. The metal concentration ranges from 2 to 12 mmol·dm^{-3} for citrate systems, from 3 to 20 mmol·dm^{-3} for isocitrate systems and from 3 to 7.5 mmol·dm^{-3} for aconitate systems. The ligand concentration ranges from 2 to 15 mmol·dm^{-3} for citrate, from 3 to 20 mmol·dm^{-3} for isocitrate and from 3 to 30 mmol·dm^{-3} for aconitate systems. The metal to ligand ratios are 1:1, 1:2, 1:3 and 3:1 for citrate systems, 1:1, 1:2 and 3:1 for isocitrate systems and 1:1, 1:2, 1:4, 1:5, 1:10 and 2.5:1 for aconitate systems. The considered ranges of pH are 2.1 - 6.7, 2.4 - 6.0, 2.1 - 4.7, 2.2 - 4.1, for citrate, isocitrate, *cis*-aconitate and *trans*-aconitate systems, respectively.

2.3 Spectrophotometric measurements

The visible molecular absorption (500-900 nm) spectra were recorded on VO^{2+}-carboxylate systems, at $I = 0.1$ mol dm^{-3}, with a V-550 Jasco spectrophotometer (optical path 1.000 cm). The solution being examined was transferred from the potentiometric to an optical cell using a peristaltic pump. Due to the low values of molar absorptivity coefficients of vanadyl containing species, the concentration of oxocation was always higher than 5 mmol dm^{-3}, with the suitable metal to ligand ratio.

2.4 EPR measurements

The EPR spectra of VO^{2+}-carboxylate systems were recorded in quartz flat cell at room temperature with an ESP-300E Bruker X-band spectrometer. Experimental parameters were as follows: microwave power 4 mW, microwave frequency 9.68 GHz, modulation amplitude 4 Gauss, modulation frequency 100 KHz, time constant 81.92 ms, time sweep 84 s.

2.5 Data analysis and calculations

To determine all parameters of the acid-base titration, the calculations were performed by using the non linear least squares computer program ESAB2M [19]. This program allow us to refine the analytical concentration of the reagents, the electrode formal potential E°, the coefficient j_a relative to the acidic junction potential (according to the equation: $E_j = j_a [H^+]$) and the ionic product of water K_w; it is also useful to evaluate the purity of the ligand examined.

The refinement of the formation constants was performed by the BSTAC [20] software. It employs an iterative and convergent numerical method, which is based upon the linear combination of the mass balance equations, minimises the error squares sum on electromotive force values and takes into account eventual variations of ionic strength among and/or during titrations. In view of the experimental pH range, the contribution of acidic junction potential was taken into account in the elaboration of data.

Spectrophotometric data were analysed by means of the HYPERQUAD software [21] which calculates the values of molar absorptivity coefficients (ε_λ/dm^3 mol^{-1} cm^{-1}) of the different complexes by using experimental spectra (absorbance vs. wavelength λ/nm), analytical concentrations of the reagents and the proposed chemical model (stoichiometric coefficients

and known stability constant values of all complexes) as input. After the calculation of the species distribution, absorption spectra are estimated for each complex formed in solution, only assuming the additivity of the absorbance in the investigated concentration range. Neither assumptions on the shape of the curves nor on the nature of electronic transitions are taken into account by the program.

The vanadyl complexes spectra are characterized by two absorption bands, whose exact position was identified by a Gaussian deconvolution by means a data analysis software (Origin 6.1).

EPR spectra of VO^{2+} solutions show typical unequal spacing between the lines due to second order effects. Therefore, in order to obtain the correct values of g and A, experimental data were analyzed with the EPR simulation program written by A. Rockenbauer and L. Korecz [22].

3. Results and discussion

3.1 Vanadyl complexes

The potentiometric data were elaborated in order to evaluate a reliable speciation model for each metal–ligand system and to calculate the formation constants of complexes. The formation constants calculated are reported in Table 1 (at I = 0.1 mol dm^{-3}, t = 25 °C), together with protonation constants of carboxylates. Calculations were performed by considering the formation of hydrolytic species of the oxocation and the formation of the ion pair vanadyl – sulphate. The formation constants of these species are reported in ref. 14. The protonation constants were refined from pH-metric data on aqueous solution containing only the acid and KCl. The values obtained for citric acid are in good accordance with that from ref. 23. Fig. 2 and 3 report the species distributions for the four metal-ligand system studied.

The speciation models reported in Table 1 show that all the ligands studied form complexes with stoichiometry MLH_r and binuclear species $M_2L_1H_{-r}$. Citric and D(+)-*threo*-isocitric acids form also stable dimeric species.

The species with stoichiometry MLH_2 was proposed in the speciation models of citrate and aconitate systems. For the systems with citrate the species MLH_2 is required in order to better explain the trend of experimental data in the pH range 2.1 – 3.0. The processing, with the BSTAC software [20], of experimental data of 19 titrations (104 experimental points), in this

pH range, with and without the species MLH_2 provides fit standard deviations of 1.42 and 3.09 respectively. In view of this results and of the correspondence between experimental and calculated data (proton concentration and potential values), we chose to insert this species in the speciation model. In the same way, for aconitate systems, the processing of experimental data without the species MLH_2 provides fit standard deviations of 3.05 and 3.29, for *cis-* and *trans-*aconitic acid respectively. These values of standard deviation are noticeably higher than those reported in Table 1 for the two systems.

The species with stoichiometry MLH can involve a seven membered chelate ring with a protonated carboxylic function. This is reliable for *cis-* and *trans-*aconitic acids: the MLH species have a $logK_{M(HL)}$ of 3.13 and 2.86 respectively, values a little lower than that of succinic acid: $logK_{ML}$ = 3.25 [14] ($logK$ is referred to the reaction VO^{2+} + H_rL^{r-z} $\leftrightarrows$ VOH_rL^{2+r-z}, with z = charge of the fully deprotonated ligand). The $logK_{M(HL)}$ values of citric and D(+)-*threo*-isocitric acid are fairly higher than that of succinic acid (see Table 1). Kiss et al. [15] asserted the formation of a five-membered chelate ring through the central donor pair for citric acid with (COO⁻, O⁻) coordination mode. While, Lodyga-Chruscinska et al. [16] proposed a (COO⁻, COO⁻) coordination mode. Both binding modes seem to be not much probable in view of the particularly high value of the formation constant that indicates the reasonable presence of a third donator. Moreover, the hydroxyl group involvement seems to be probable, in fact the presence of a third carboxylic group in the aconitic acids does not provide particularly high value of the formation constant for the same species.

It is interesting to observe the different stability of complexes of citric and D(+)-*threo*-isocitric acids. The potentiometric experimental data obtained with these ligands can be explained by a similar speciation model, but the stability of the complex species are quite different. The species formed by citric acid are always more stable than the corresponding of D(+)-*threo*-isocitric and this is marked, in particular, for the dimeric species. The formation constants of complexes of citric acid with stoichiometry $M_2L_2H_{-2}$ and $M_2L_2H_{-1}$ are about five order of magnitude higher than those of D(+)-*threo*-isocitric acid. The distribution diagrams of species, significantly different for the two acids, reported in Fig. 2, reflect this stability difference. A big difference between stability of citric and D(+)-*threo*-isocitric acid complexes was observed for copper(II) ion as well [23,24]. These stability comparisons show the importance of hydroxyl group position in the ligand molecule in the formation of coordination compounds. The speciation model and the formation constants of citrate system are in quite accordance with the results obtained by Kiss et al. [15]. They hypothesized a

speciation model including MLH, ML, $M_2L_2H_{-1}$, $M_2L_2H_{-2}$, but without binuclear species, M_2LH_{-r}.

Binuclear species are hypothesized for all the metal-ligand systems, but, excluding the isocitrate systems (see Fig. 2b), they were formed with a relevant percentage only in that solution with metal in excess. For a solution with citrate and metal to ligand ratio of 3:1 the species M_2LH_{-1} has a maximum formation percentage of 25% at pH 3.5, the M_2LH_{-2} has a maximum formation percentage of 38% at pH 4.2 and the M_2LH_{-3} has a formation percentage of 66% at pH 6.7. For a solution with isocitrate, in the same experimental conditions, the species M_2LH_{-1} reaches a maximum formation percentage of 31% at pH 3.9 and the species M_2LH_{-3} reaches a formation percentage of 67% at pH 6.0. In the solutions containing *cis*-aconitate with a metal to ligand ratio of 2.5:1 the species M_2LH_{-1} reaches a formation percentage of 18% at pH 4.1, maximum pH value before precipitation. Analogues systems with *trans*-aconitate presents a formation percentage of 11% at the same pH.

The *cis-trans* isomerism provides a little difference on the stability of the aconitic acids complexes. The speciation model hypothesized is the same for the two ligands, but the *cis* isomer shows higher formation constants, as expected. The position of carboxylates in the *cis*-isomer is more favourable to a simultaneous metal coordination by the donors. The distribution diagrams of vanadyl-*cis*-aconitic and vanadyl-*trans*-aconitic systems were reported in Fig. 3.

3.2 Visible spectrophotometric spectra

The visible spectrophotometric measurements were performed at different pH values, in the same experimental conditions as potentiometric ones. As an example, the experimental spectra of vanadyl with *cis*-aconitate, D(+)-*threo*-isocitrate and citrate are reported in Fig. 4. In agreement with the scheme proposed by Ballhausen and Gray [25], in all the binary systems here investigated, two bands can be evidenced: the former (I) in the range 820-760 nm and the latter (II) in the range 630-550 nm, which can be assigned to $b_2(d_{xy}) \rightarrow e(d_{xz}, d_{yz})$ and $b_2(d_{xy}) \rightarrow b_1(d_{x^2-y^2})$ transitions, respectively. Furthermore, it must be remarked the most evident bathochromic effect of the band I for isocitrate complexes and, above all, the particular behaviour (see Fig. 4) of the vanadyl-citrate containing solutions: the increasing of pH provides a clear hypsochromic effect of the band I and an intense increasing of absorption values of the band II. For solutions with pH higher than 3, the relative intensity of the vanadyl bands I and II is inverted with respect to spectra of the most of vanadyl solution containing

ligands and the solution exhibits a violet hue. The singular spectrophotometric behaviour of citrate systems was also recognized by other researchers [15,17,26].

For each system investigated, from the whole of the spectra recorded on solutions at different concentrations and pH values, we have also estimated the individual visible spectrum of the relevant species in solution. The principal spectral features of these complexes are listed in Table 2. For each complex, the values of λ_{max} of the two bands were identified by the Gaussian deconvolution of spectra. This procedure is useful to better locate the band position when the spectrum presents a shoulder instead of a well defined maximum.

All the species are characterized in the visible range by two bands with higher intensity with respect to the vanadyl oxocation. According to Ballhausen and Gray [25], the energy associated to the band II ($b_2 \rightarrow b_1$) corresponds to $10Dq$ value, while the energy associated to $b_2 \rightarrow e$ transition (band I) corresponds to $-3Ds + 5Dt$ value. The term $-3Ds + 5Dt$ is independent of Dq and it is related to the tetragonal distortion. In Table 2 the values of $10Dq$ and $-3Ds + 5Dt$ (expressed in kK = 10^{-3} cm^{-1} units) are collected for all the complexes for which a reliable spectrum was calculated. Spectrophotometric data coming from a previous study [14] on vanadyl coordination chemistry were also collected. The light bathochromic (for the I band) and hypsochromic (for the II band) effects, outlined above for the experimental spectra at different pH, were kept in the spectra of the single complex species, as one would expect. The Dq values collected in Table 2 show a general trend as a function of the number of donor groups co-ordinated to oxovanadium. If neglecting the ML, $M_2L_2H_{-1}$ and $M_2L_2H_{-2}$ complexes of citrate and $M_2L_2H_{-2}$ complex of isocitrate, the value of $-3Ds + 5Dt$ calculated for all the species here concerned ranges from 12.4 to 13.1, suggesting that the substitution of co-ordinated water molecules by a variable number of oxygen donor groups, in the most of complexes reported in Table 2, does not affect to a great extent the tetragonal distortion. If looking at literature reports, also the values of the parameter $-3Ds + 5Dt$ proposed for VO(edta)$^{2-}$ and VO(oxalate)$_2^{2-}$ (12.8 and 12.6, respectively [25]) fall within the range 12.4-13.1. The ML, $M_2L_2H_{-1}$ and $M_2L_2H_{-2}$ complexes of citrate are marked by a high value of $-3Ds + 5Dt$ parameter indicating an increase in tetragonal distortion. On the other hand the species $M_2L_2H_{-2}$ of isocitrate shows an opposite behaviour: the value of $-3Ds + 5Dt$ parameter is particularly low. Previous investigations on vanadyl-citrate system [17,26] indicate, for the dimeric species, the presence of alcoholate bridges between two metal centre with the alcoholate groups in *trans* position respect the vanadyl oxygen. Bartecki and Kurzak [27], pointed out that the vanadyl complexes could change their hue from greenish-blue to purple or red for higher Dq values and Sacconi et al. [28] showed that, when the strong donor

nitrogen of pyridine coordinated the vanadium in *trans* position with respect to the oxygen linked through a double bond to the central atom, the bands shift toward the violet. Likewise, for the citrate complexes, we can suppose that the presence of an alcoholic group in axial position explains the particular effect on tetragonality. In addition, as mentioned above, we obtained high value of $-3Ds + 5Dt$ parameter also for the species ML (not for MLH_2^+ and $MLH°$). Since for ML complex a penta-coordination by the ligand is unlikely, one of the four equatorial position might be occupied by a water molecule. As we have found for ML complex of isocitrate a quite different behaviour (Table 2), it is evident that the position of alcoholic group in the citrate molecule is critical. Lodyga-Chruscinska et al. [16] suppose, for the species ML of citrate, a coordination mode (COO⁻, COO⁻, COO⁻) where a carboxylate is in *trans* position with respect to the vanadyl oxygen.

3.3 EPR spectra

EPR spectroscopy has been extensively used in the study of ligand environment in oxovanadium(IV) complexes since the separation in the typical eight lines EPR pattern, due to the coupling of the unpaired electron with ^{51}V nucleus ($I = 7/2$), strictly depends from the nature of the ligands donor atoms [29,30].

EPR spectra of VO^{2+}-polycarboxylate systems were recorded at room temperature in experimental conditions similar to those previously described for the other experiments and data obtained were then simulated with appropriate software [22] in order to obtain the correct experimental values of A_0 shown in Table 3.

For all ligands, the value of the isotropic hyperfine coupling constant A_0 recorded for the complexes decreases with increasing pH, indicating the replacement of one or more molecules of water in the aquoion $[VO(H_2O)_5]^{2+}$ with donor groups belonging to the ligands. Moreover, the intensity of the spectrum decreases due to the contemporary formation of magnetically coupled species [15] $M_2L_2H_{-1}$, $M_2L_2H_{-2}$ and M_2LH_{-1}, so that only the complex M_2LH_{-3} of isocitric acid can be observed at pH 6.0. The magnetic coupling of dimeric species of vanadyl oxocation is widely described in previous papers dealing with vanadyl complexation [15,17,26]. We suppose magnetic coupling also for the dinuclear species M_2LH_{-1} because the EPR spectra of aconitic acids show an intensity decrease for an increasing pH, and the M_2LH_{-1} is the only species with two metal units proposed in the speciation model (see Fig. 3).

In order to assign each EPR spectrum to a particular VO^{2+}-complex, we tried to apply the classic additivity criterion to calculate the theoretical values of A_0 for each species [29,30].

This method assumes that the value of the isotropic hyperfine coupling constant is the result of the average of the individual contributions of the ligands and thus can be generally calculated by the following equation (1) where $A_{0,i}$ is the experimental value for a complex that has all the same donor groups (data taken from the literature [30]), and n_i is the number of donor groups (water included) of each type present in the complex. This equation takes into account only the interactions on the equatorial plane and therefore the value of the sum of i is divided by four.

$$A_{0,calc} = \frac{\sum_i n_i A_{0,i}}{4} \qquad (1)$$

However, the values of the isotropic constants, calculated by equation (1) on the basis of the most abundant species resulting from the speciation profiles, were all lower than the experimental ones and show a mean error equal to 2.65×10^{-4} cm^{-1}, which is considered significant of a difference in coordinating functional groups or in the complex geometry [30]. These results seemed to highlight the presence of a systematic error in the determination of $A_{0,calc}$ due to the inadequacy of equation (1) applied to these systems, consisting of a mixture of paramagnetic species with similar A_0 values. Moreover, the $A_{0,i}$ values taken from the literature probably are not suitable to describe these species because, as previously reported by Chasteen [30], different VO^{2+}-complexes with equal coordination groups can have experimental A_0 values significantly different due to the diversity in the size of their chelate rings. On the other hand, also bi or tridentate ligand chelation can impose structural constraints to the complexes that change their coordination geometry, thus altering their EPR parameters.

Modifying the equation (1) in a way that takes into account the percentage of each species present at a given pH value, and using different $A_{0,i}$ values (except for that related to the water oxygen, in agreement with the aquoion species), a good agreement between experimental and calculated values could be obtained (data not shown). However this approach seems to be suitable only for an homogeneous group of ligands, in fact the calculated parameters are also applicable to the VO^{2+}- succinic acid complexes [14,31], but not to other systems, such as those with malonic acid. Therefore, only a more elaborate model that takes into account all the factors involved (steric, geometric and electronic) may provide a broader applicability.

4. Conclusions

The joint elaboration of potentiometric and spectroscopic data obtained on the vanadyl-ligand containing solutions allowed us to strengthen the speciation model proposed and to achieve a deeper knowledge of the structure of complexes in solution.

The interpretation of EPR data by the application of solution speciation model is a novelty and, although it was applied only to systems rather similar in donor groups and molecular size, could be a new experimental procedure aimed to better characterize coordination compounds in solution, without perturbing the chemical equilibrium. Furthermore this approach, if supported by a suitable mathematical model and if applied to systems that differ in molecular size and rigidity of their ligands, could help to clarify the role played by these factors in determining the geometry and stability of vanadyl complexes in solution.

The results acquired highlight the weight of the position of alcoholic group in citric and D(+)-*threo*-isocitric acids on their coordination capability and on the complexes structure. Both the ligands form complexes with the same stoichiometry and their dimeric complexes have an alcoholate-bridged structure. On the other hand, the species $M_2L_2H_{-2}$ of isocitrate and citrate, in relation to the tetragonality of the complex, shows an opposite behaviour and the citrate dimeric complexes have higher stability. These observations lead us to hypothesize two different structure for the dimeric complexes of the two ligands (see Fig.5): it is reasonable suppose an axial coordination of alcoholate group in the case of citrate dimers. Taking as reference the structures proposed by Plass [32] for the dimeric complexes of vanadyl with magnetic interaction, we can suppose for the dimeric complexes of isocitrate an *anti-* or *syn-*orthogonal structure. In the case of citrate our spectroscopic results are compatible with an *anti-* or a *syn-*coplanar conformation where the alcoholate groups are in *trans* position with respect to the oxygen of oxocation. This hypotheses is in accordance with the complex structures proposed by Tsaramyrsi et al. [17] and Velayutham et al. [26] for the species $M_2L_2H_{-2}$.

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Table 1
Protonation constants of carboxylic acids and formation constants for VO^{2+}-carboxylate systems, at $I = 0.1$ mol dm^{-3} (KCl) and $t = 25$ °C.

Ligand	Species	$\log\beta_{pqr}$ [a]	$\log K$ [b]
citric	HL	5.68 ± 0.01 [c]	
	H_2L	10.04 ± 0.01	4.34
	H_3L	12.96 ± 0.01	2.92
	MLH_2 [d]	13.26 ± 0.06	3.23
	MLH	11.20 ± 0.02	5.51
	ML	8.62 ± 0.03	
	$M_2L_2H_{-1}$	16.37 ± 0.05	
	$M_2L_2H_{-2}$	11.39 ± 0.06	
	M_2LH_{-1}	7.95 ± 0.07	
	M_2LH_{-2}	4.27 ± 0.05	
	M_2LH_{-3}	-0.4 ± 0.1	
	weighted standard deviation of the fit [e]	1.46	
	12 titration curves	371 experimental points	
D(+)-*threo*-isocitric	HL	5.70 ± 0.01	
	H_2L	9.99 ± 0.01	4.29
	H_3L	13.06 ± 0.02	3.07
	MLH	10.46 ± 0.01	4.73
	ML	7.12 ± 0.01	
	$M_2L_2H_{-1}$	11.69 ± 0.03	
	$M_2L_2H_{-2}$	6.92 ± 0.04	
	M_2LH_{-1}	6.02 ± 0.03	
	M_2LH_{-3}	-2.23 ± 0.03	
	weighted standard deviation of the fit	2.03	
	7 titration curves	282 experimental points	
cis-aconitic	HL	6.17 ± 0.01	
	H_2L	10.28 ± 0.01	4.12
	H_3L	12.13 ± 0.01	1.85
	MLH_2	11.88 ± 0.08	1.60
	MLH	9.28 ± 0.05	3.11
	ML	5.03 ± 0.07	
	M_2LH_{-1}	3.31 ± 0.06	
	weighted standard deviation of the fit	1.01	
	7 titration curves	178 experimental points	
trans-aconitic	HL	5.56 ± 0.01	
	H_2L	9.50 ± 0.01	3.94
	H_3L	12.24 ± 0.01	2.74
	MLH_2	11.47 ± 0.03	1.98
	MLH	8.42 ± 0.02	2.86
	ML	4.32 ± 0.03	
	M_2LH_{-1}	2.57 ± 0.02	
	weighted standard deviation of the fit	0.60	
	13 titration curves	236 experimental points	

[a] refer to the general reaction: $pVO^{2+} + qL^{z-} + rH^+ \leftrightarrows (VO)_pL_qH_r^{2p+r-qz}$.

[b] $\log K$ values are calculated from $\log\beta$ values and refer to the reaction: $pVO^{2+}+H_rL^{r-z} \leftrightarrows (VO)_pH_rL^{2p+r-z}$, with z = charge of the fully deprotonated ligand.

[c] $\pm$ standard deviation.

[d] $M = VO^{2+}$.

[e] weight for each experimental point is given as $w = 1/s^2$ (see ref. 20).

Table 2

Values of λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}, $-3Ds+5Dt$ (kK) and $10Dq$ (kK) for relevant species in solution.

Ligand	Species	λ_{max}/nm		ε_{max}/dm^3 mol^{-1} cm^{-1} [c]		Transition energy [d]/kK[e]	
		I [a]	II [a]	I [a]	II [a]	$-3Ds+5Dt$	$10Dq$
water [14]		765	635 sh [b]	16	7	13.0	15.9
citric acid	MLH$_2$	763	610 sh	14	3	13.1	16.4
	MLH	785	608 sh	25	3	12.7	16.4
	ML	692 sh	566	14	16	14.5	17.5
	M$_2$L$_2$H$_{-1}$	647 sh	553	44	30	15.5	18.1
	M$_2$L$_2$H$_{-2}$	666 sh	558	37	31	15.0	17.9
	M$_2$LH$_{-1}$	767	592 sh	48	18	13.0	16.9
	M$_2$LH$_{-2}$	760	593 sh	29	20	13.2	16.9
D(+)-*threo*-isocitric acid	MLH	766	588 sh	21	8	13.1	17.0
	ML	782	597 sh	15	7	12.8	16.8
	M$_2$L$_2$H$_{-1}$	807	582	56	41	12.4	17.2
	M$_2$L$_2$H$_{-2}$	835	601	47	17	12.0	16.6
	M$_2$LH$_{-1}$	788	591	49	14	12.7	16.9
	M$_2$LH$_{-3}$	809	578	29	14	12.4	17.3
cis-aconitic acid	MLH$_2$	770	623 sh	21	9	13.0	16.1
	MLH	783	599 sh	25	4	12.8	16.7
	ML	789	605 sh	40	15	12.7	16.5
trans-aconitic acid	MLH$_2$	768	618 sh	21	8	13.0	16.2
	MLH	773	626 sh	15	3	13.0	16.5
	ML	793	604 sh	46	23	12.6	16.7
acetic acid [14]	ML	778	626 sh	18	5	12.9	16.0
succinic acid [14]	MLH	774	612 sh	17	6	12.9	16.3
	ML	790	600 sh	40	21	12.6	16.7
malonic acid [14]	MLH	770	603 sh	18	6	13.0	16.6
	ML	783	585 sh	27	7	12.8	17.1
	ML$_2$H	801	573	44	22	12.8	17.2
	ML$_2$	802	576	39	8	12.5	17.4
1,2,3-propantricarboxylic acid [14]	MLH	793	604 sh	32	12	12.6	16.6
	ML	791	600 sh	21	8	12.6	16.7
1,2,3,4-butantetracarboxylic acid [14]	MLH$_2$	788	605 sh	22	8	12.7	16.5
	MLH	786	604 sh	27	11	12.7	16.6
	ML	792	597 sh	29	12	12.6	16.7

[a] transition band.

[b] sh = shoulder.

[c] The uncertainty on ε_{max} values ranges between 1 and 10%.

[d] The transition energy uncertainty is of 0.1 kK units considering that the λ_{max} values are obtained elaborating the experimental absorbance values sampled each 5 nm.

[e] kK = 10^{-3} cm^{-1}.

Table 3

Relative distribution and EPR data for relevant species in solution at critical pH values

Acid	pH	Relative abundance (%)					A_0 (10^{-4} cm^{-1})
		VOaq.	MLH_2	MLH	ML	M_2LH_{-3}	
citric	2,5	24,0	11,5	31,4	26,4	0,0	104,58
	3,2	3,2	0,7	9,9	41,6	0,1	102,89
	3,7	0,5	0,1	2,2	29,3	0,2	102,43
D(+)-*threo*-isocitric	3,2	19,1	0,0	43,0	31,4	0,1	104,04
	4,2	1,6	0,0	10,2	59,1	6,4	102,27
	6,0	0,0	0,0	0,0	3,0	44,8	97,90
cis-aconitic	3,4	23,0	9,3	58,6	8,4	0,0	104,51
	4,5	3,3	0,4	31,4	56,9	0,0	103,05
trans-aconitic	3,2	65,6	12,5	19,0	2,5	0,0	105,70
	4,0	31,7	3,4	30,2	24,4	0,0	104,67

FIGURES
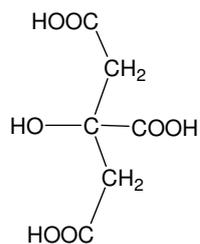
citric acid

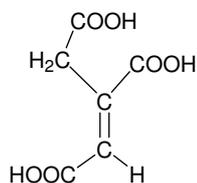(2-hydroxy-1,2,3-propanetricarboxylic acid)
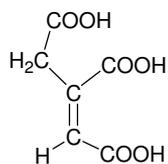
***cis*-aconitic acid**

(*cis*-propene-1,2,3-tricarboxylic acid)
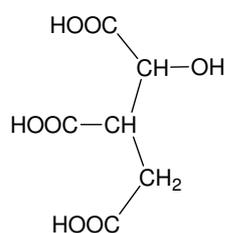
D(+)-*treo*-isocitric acid

((1R,2S)-1-hydroxy-1,2,3-propanetricarboxylic acid)

***trans*-aconitic acid**

(*trans*-propene-1,2,3-tricarboxylic acid)

Fig. 1 Formulae of the ligands. In parenthesis are reported the IUPAC names.
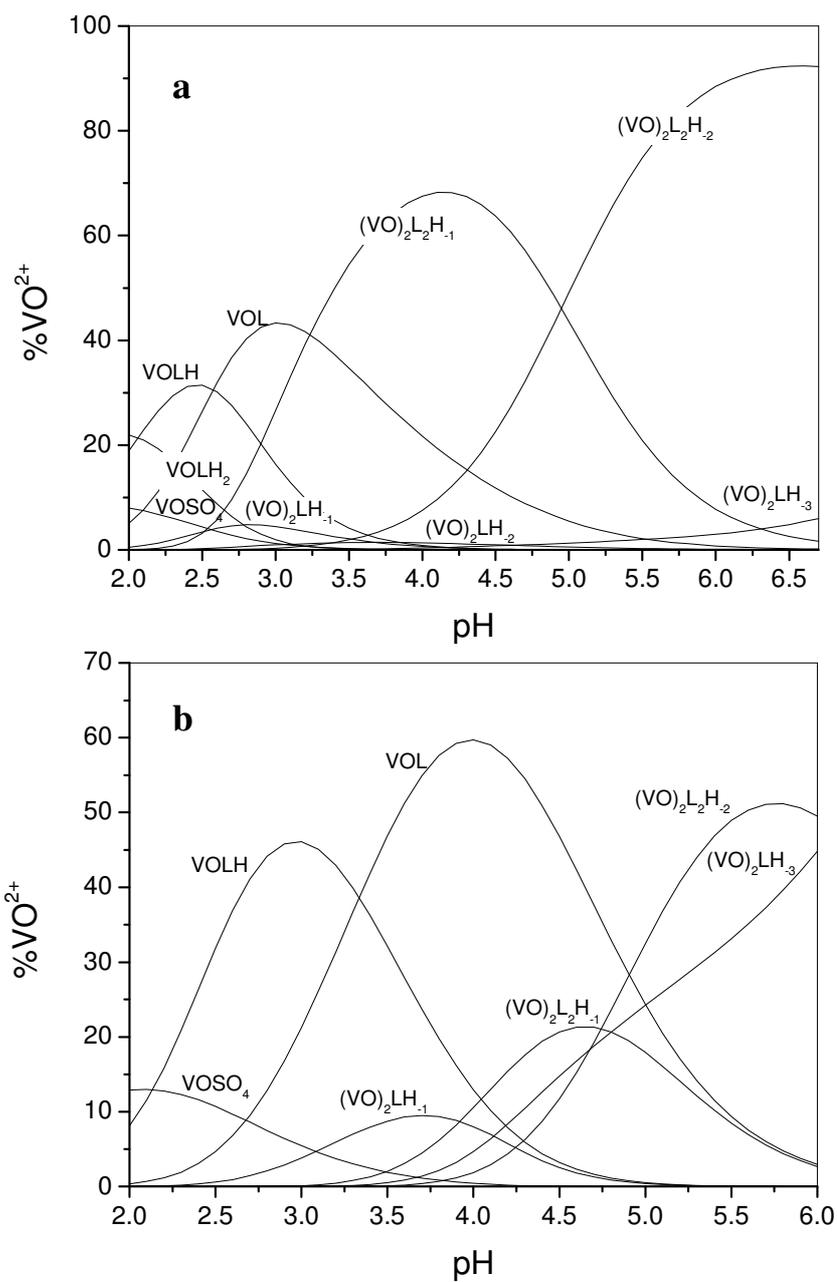
Fig. 2. Distribution diagram of: a) vanadyl-citric system for a solution with $C_M = 5$, $C_L = 5$ mmol L^{-1}, b) vanadyl- D(+)-*threo*-isocitric system for a solution with $C_M = 5$, $C_L = 5$ mmol L^{-1}; $I = 0.1$ mol L^{-1} (KCl) and at t = 25 °C.
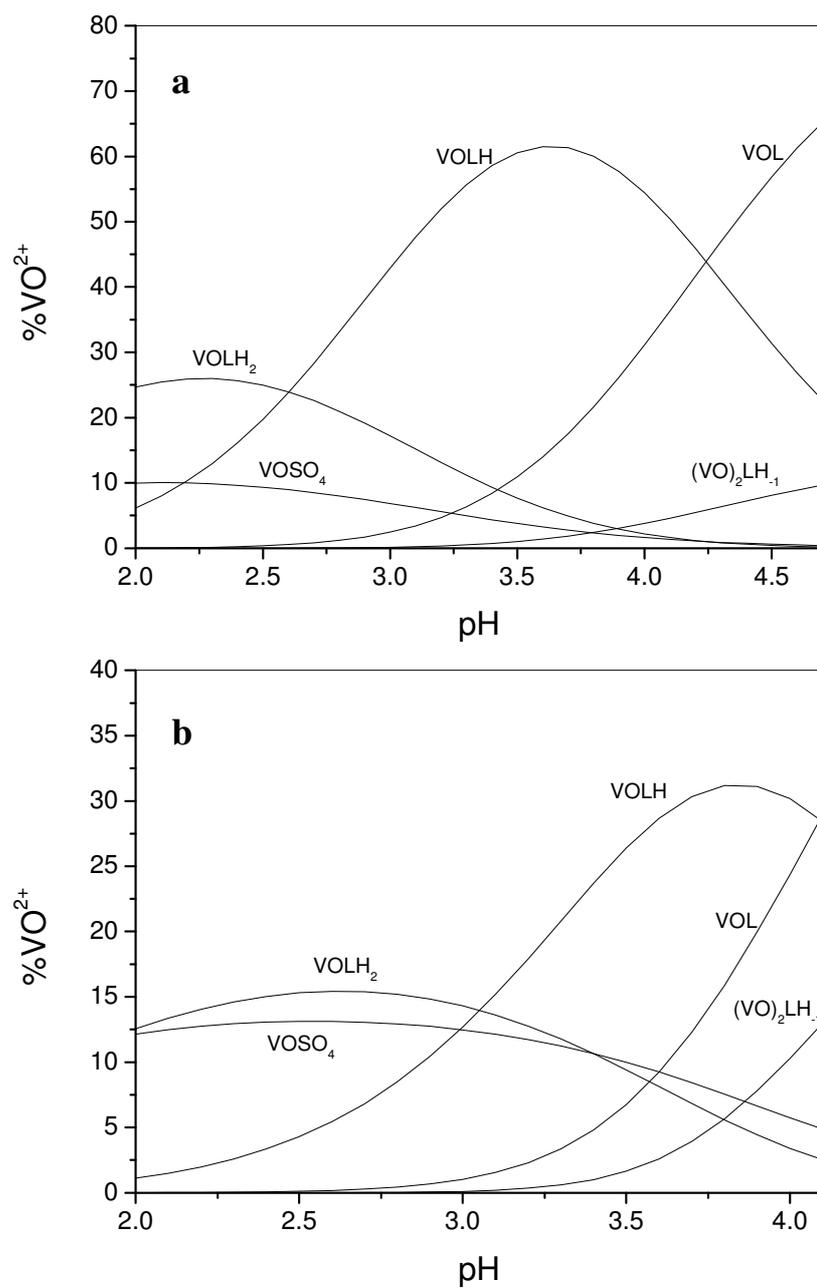
Fig. 3. Distribution diagram of: a) vanadyl-*cis*-aconitic system for a solution with $C_M = 5$, $C_L = 20$ mmol L^{-1}, b) vanadyl-*trans*-aconitic system for a solution with $C_M = 5$, $C_L = 20$ mmol L^{-1}, $I = 0.1$ mol L^{-1} (KCl) and at t = 25 °C.
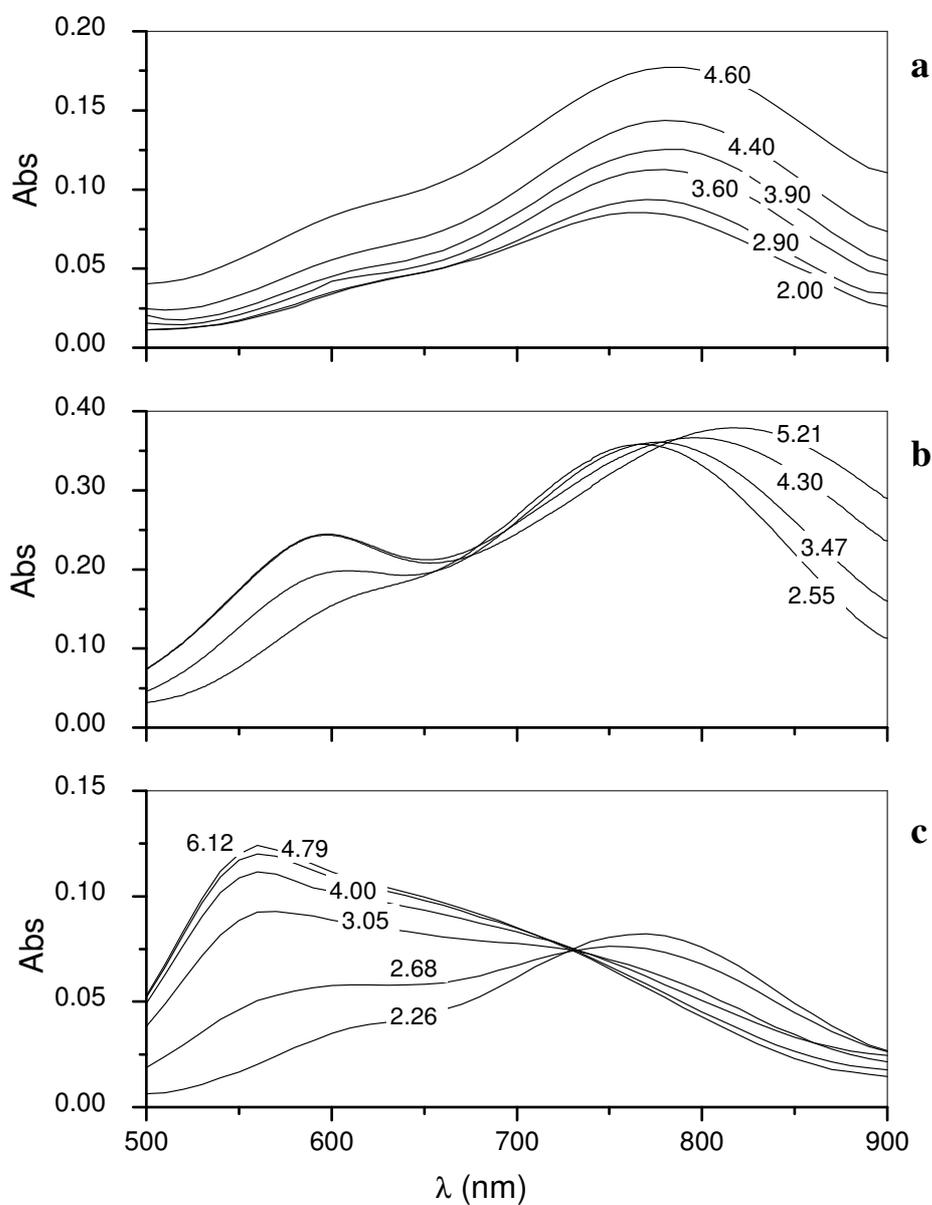
Fig. 4. Experimental visible spectra in function of pH condition of : a) VOSO$_4$ 5.0 mmol dm^{-3} and *cis*-aconitic acid 20.2 mmol dm^{-3} solution; b) VOSO$_4$ 20.0 mmol dm^{-3} and D(+)-*threo*-isocitric acid 20.1 mmol dm^{-3} solution; c) VOSO$_4$ 5.0 mmol dm^{-3} and citric acid 5.1 mmol dm^{-3} solution. I = 0.1 mol dm^{-3} (KCl) and t = 25 °C. The pH values of solutions are reported on each spectrum.
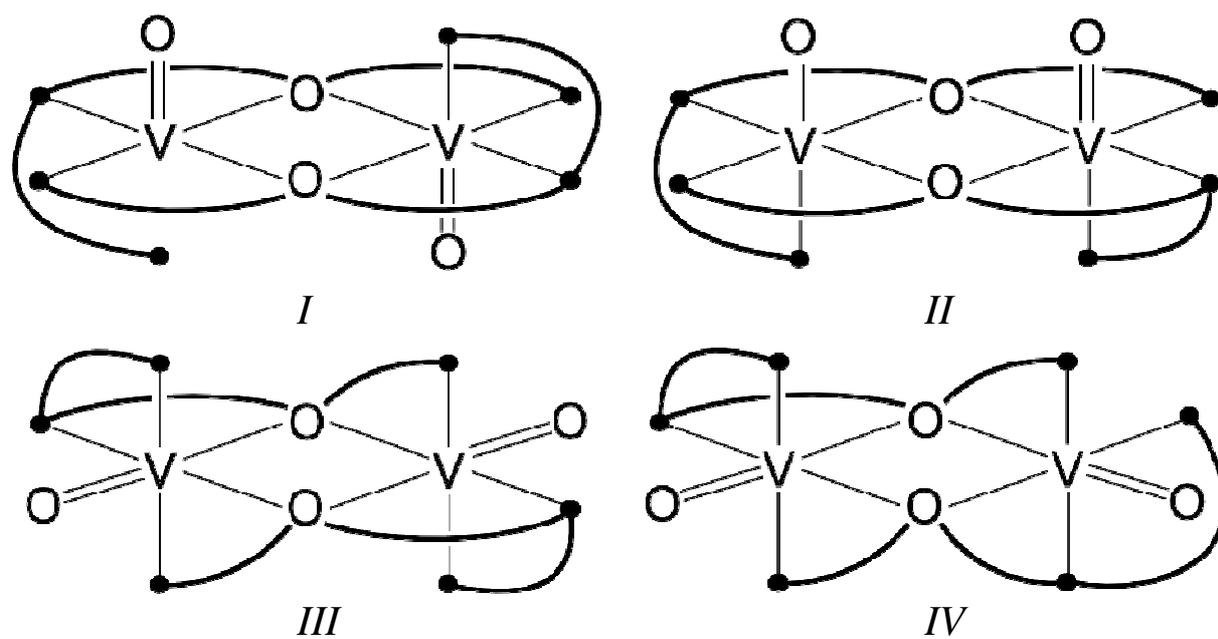
Fig.5. Schemes with the proposed binding modes for the dimeric complexes ($M_2L_2H_{-2}$) of D(+)-*threo*-isocitric (*I* and *II*) and citric (*III* and *IV*) acids.