

# Oxovanadium(IV and V) and copper(II) complexes of *N*-salicyl-glycylglycine and *N*-salicyl-glycylglycylglycine †

João Costa Pessoa,<sup>\*a</sup> Isabel Correia,<sup>a</sup> Tamás Kiss,<sup>\*b</sup> Tamás Jakusch,<sup>b</sup>  
 Margarida M. C. A. Castro<sup>c</sup> and Carlos F. G. C. Geraldes<sup>c</sup>

<sup>a</sup> Centro Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 LISBOA, Portugal

<sup>b</sup> Biocoordination Chemistry Research Group of the Hungarian Academy of Sciences, Department of Inorganic and Analytical Chemistry, University of Szeged, PO Box 440, H-6701 Szeged, Hungary

<sup>c</sup> Centro de Neurociências, Departamento de Bioquímica, Universidade de Coimbra, 3000 Coimbra, Portugal

Received 12th July 2002, Accepted 23rd September 2002  
 First published as an Advance Article on the web 31st October 2002

By reaction of salicylaldehyde and GlyGly or GlyGlyGly, followed by reduction with NaBH<sub>4</sub>, *N*-salicyl-glycylglycine **1** and *N*-salicyl-glycylglycylglycine **2**, the reduced Schiff bases H<sub>2</sub>sal-RGG and H<sub>2</sub>sal-RGGG, containing the GlyGly or GlyGlyGly moieties, are prepared and characterised. Their acid–base properties and complexation with V<sup>IV</sup>O<sup>2+</sup> and Cu<sup>II</sup> are studied by pH-potentiometry, visible absorption and EPR spectrometries, and the protonation and complex formation constants are determined. Vanadium and copper complexes are also prepared and characterised. Differences in the metal binding abilities of **1** and **2** are found. Amide deprotonation is proved but its extent depends on the system, being particularly important in the M–H<sub>2</sub>sal-RGG systems with the formation of the (O-phenolate, N-amine, N-amide, COO<sup>–</sup>) (6+5+5)-membered joined chelate system. Depending on the system, at physiological pH this binding mode and/or 2 × (O-phenolate, N-amine) are dominant. The aminophenolate chelates formed with sal-RGG and sal-RGGG behave as anchoring donor sites and the ML and ML<sub>2</sub> complexes, involving O-phenolate, N-amine chelation, form in higher relative concentration than in the corresponding M–GlyGly or M–GlyGlyGly systems. Moreover, while for the V<sup>IV</sup>O–H<sub>2</sub>sal-RGG system the N-amide deprotonation/co-ordination is strongly promoted, it is not so favoured in the Cu<sup>II</sup>–H<sub>2</sub>sal-RGG and Cu<sup>II</sup>–H<sub>2</sub>sal-RGGG systems. The increased stability induced by the additional O-phenolate donor is particularly relevant for sal-RGG and hydrolysis of the MLH<sub>–1</sub> complexes starts several pH units higher than in the GlyGly systems. In contrast to V<sup>IV</sup>, the V<sup>V</sup> complexes formed with sal-RGGG were found to be more stable than those of sal-RGG.

## Introduction

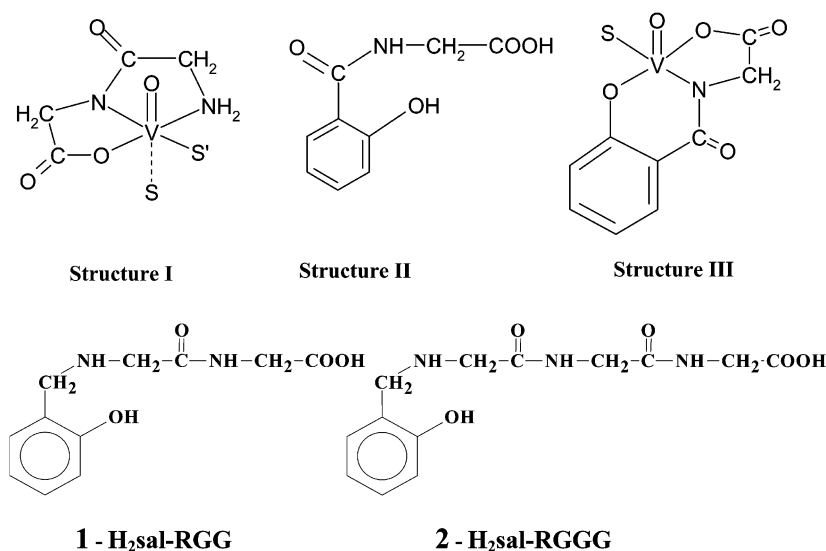
Vanadium is a bioessential element that is found in remarkably high concentrations in marine ascidians,<sup>1</sup> in certain mushrooms<sup>2</sup> and in polychaete worms.<sup>3</sup> In addition, two classes of vanadium enzymes – vanadium-nitrogenases<sup>4,5</sup> and vanadate-dependent haloperoxidases,<sup>6</sup> both found in nature – as well as the vanadium's insulin-like action<sup>7,8</sup> and anticancer activity<sup>9</sup> have spurred a considerable amount of research. Our understanding of the role of vanadium in the insulin-like action is far from complete, but it is known that the originally supplied complexes undergo considerable transformations in the organism, including ligand-exchange processes and redox reactions.<sup>8</sup> A knowledge of the distribution and chemical speciation of vanadium in biological fluids and tissues is therefore of importance.

Proteins may be intricately involved in vanadium binding in organisms. Peptides are the most closely related models of proteins, and investigations on model complexes of vanadium with oligopeptides will certainly contribute to our understanding of the biological roles and actions of vanadium. A simple oligopeptide contains three kinds of potential donors: the

terminal amino and carboxylate groups, and the peptide groups. Chelation through the terminal NH<sub>2</sub> group together with the peptide C=O (O<sub>amide</sub>), or through the terminal COO<sup>–</sup> (O<sub>carboxylate</sub>) alone or together with the O<sub>amide</sub>, is often not a strong enough binding mode to keep metal ions in solution at the physiological pH. In order to achieve this, the amide-N (N<sub>amide</sub>) should also co-ordinate. Such a binding mode may correspond to strong interaction between a metal ion and the oligopeptide. Various metal ions have been found to be able to do this, *e.g.* Pt(II), Pd(II), Cu(II) and Ni(II).<sup>10</sup>

The Cu<sup>2+</sup> ion forms rather stable complexes with di- and tri-peptides and a survey of the main data available for its interaction with simple dipeptides and related ligands has been published by Sóvágó.<sup>10</sup> It is shown that the deprotonated N<sub>amide</sub> acts as the strongest donor, but the terminal COO<sup>–</sup> and NH<sub>2</sub> groups, as well as the O<sub>amide</sub>, are also involved in metal binding.<sup>10,11</sup> In contrast, it has been found that GlyGly, GlyAla and AlaAla are not strong enough binders to prevent the hydrolysis of V<sup>IV</sup>O<sup>2+</sup>.<sup>12</sup> At pH > 7 in the presence of a high excess of ligand [ligand-to-metal (L : M) ratio > 80], spectral measurements indicated the existence of a species [VOLH<sub>–1</sub>] containing co-ordinated N<sub>amide</sub> (see Structure I in Fig. 1).<sup>5</sup> Interestingly, no clear evidence of N<sub>amide</sub> co-ordination was found for Gly- and Ala-containing tripeptides. It was concluded that neither the terminal NH<sub>2</sub> nor the terminal COO<sup>–</sup> is a strong enough V<sup>IV</sup>O<sup>2+</sup> binder to behave as an efficient anchoring donor in the promotion of N<sub>amide</sub> deprotonation. In order to achieve this, the simultaneous coordination of both donors adjacent to the amide group seems

† Electronic supplementary information (ESI) available: additional spectroscopic data, additional comments for the discussion, FTIR data for the ligands and V<sup>IV</sup>O and Cu<sup>II</sup> complexes, visible absorption spectra and EPR spectra of the V<sup>IV</sup>O- and Cu<sup>II</sup>-ligand systems. See <http://www.rsc.org/suppdata/dt/b2/b206835b/>



**Fig. 1** Structure I: binding mode expected for the VO(GlyGly<sup>2-</sup>) complex;<sup>12</sup> structure II: formula of H<sub>2</sub>salGly; structure III: binding mode expected for the VO(salGly<sup>3-</sup>) complex;<sup>13</sup> ligands H<sub>2</sub>sal-RGG (1) and H<sub>2</sub>sal-RGGG (2). S and S' represent solvent molecules.

necessary. Accordingly, in the case of dipeptides, V<sup>VO</sup> can induce deprotonation of the NH<sub>amide</sub>, while with tripeptides, where two peptide groups separate the two terminal anchors, this does not occur. N<sub>amide</sub> binding has been documented for several vanadium(v)-dipeptide systems, *e.g.* GlyTyr,<sup>14</sup> AlaHis,<sup>15</sup> GlyGly,<sup>16</sup> and a set of dipeptides,<sup>17</sup> and for several vanadium(IV) systems.<sup>18</sup>

It has also been found for many metal ions that the presence of a suitable anchoring donor in the molecule, which can bind metal ions strongly enough to be able to promote co-ordination of the amide, plays a crucial role in the metal binding. Phenolate, hereafter designated by O<sub>phenolate</sub>, is known to have a high affinity for V<sup>VO</sup>.<sup>19</sup> Accordingly, replacement of the terminal NH<sub>2</sub> group by O<sub>phenolate</sub> may enhance the metal-binding ability of the molecule. In fact, the dipeptide analogue 2-OH-hippuric acid (salicylglycine – H<sub>2</sub>salGly – structure II) proved to be a much stronger V<sup>VO</sup> binder, several V<sup>VO</sup>:salGly complexes forming in the pH range 2–12, even at a L : M ratio of *ca.* 1.<sup>13</sup> The strong co-ordination to the V<sup>VO</sup> can now induce deprotonation of the NH<sub>amide</sub> group, and the compound corresponding to the binding mode with (O<sub>phenolate</sub>, N<sub>amide</sub>, O<sub>carboxylate</sub>) (Structure III; Fig. 1) becomes the predominant species at pH *ca.* 6.

A range of oxovanadium(IV)–Schiff-base complexes, the Schiff base containing an O<sub>phenolate</sub> group, derived from the condensation of salicylaldehyde and dipeptides have been prepared and characterized.<sup>20,21</sup> The binding mode involves (O<sub>phenolate</sub>, N<sub>imine</sub>, O<sub>amide</sub>, O<sub>carboxylate</sub>) (6+5+7)-membered joined chelate system. The solubility of the complexes in water or methanol is low and increasing the pH the Schiff bases hydrolyse easily. With Cu<sup>2+</sup> the compound Na[Cu(salGlyGly)]·6H<sub>2</sub>O<sup>22</sup> was prepared and the proposed binding mode was (O<sub>phenolate</sub>, N<sub>imine</sub>, N<sub>amide</sub>, O<sub>carboxylate</sub>). For the vanadium Schiff base complexes with this type of systems, racemisations, deaminations,<sup>21–23</sup> β-eliminations<sup>24,25</sup> and aldol-type condensations<sup>21</sup> have been reported. For the Cu<sup>II</sup> systems, hydrolytic cleavage of tripeptides,<sup>26</sup> racemisations<sup>27</sup> and aldol-type condensations<sup>27,28</sup> have also been reported, but these systems are not adequate if one requires metal complexes that preserve their integrity in water or water–alcohol solutions for several hours or days, or at relatively low pH (*e.g.* pH *ca.* 2). As for this type of system it is relatively easy to tune lipophilicity and chemistry of the complexes by changing the aromatic aldehyde and/or the amino acid,<sup>23</sup> as it is desirable to overcome the problems of ligand instability. This can be done by reduction of the Schiff base to give an amine, and presents interesting possibilities as ligands are more flexible and not constrained to remain planar. We

report here the preparation of the reduced Schiff base ligands of salicylaldehyde with GlyGly – H<sub>2</sub>sal-RGG (1) – and GlyGlyGly – H<sub>2</sub>sal-RGGG (2) – (see Fig. 1), the preparation of their V<sup>VO</sup> and Cu<sup>II</sup> complexes, and the characterisation of these systems by pH-potentiometry, visible and EPR spectroscopies. The behaviour of the vanadium complexes in aqueous solution under aerobic conditions is also studied by <sup>51</sup>V and <sup>1</sup>H NMR spectrometries.

## Experimental

### Preparation of ligands and complexes

**Synthesis of the reduced Schiff base 1 containing GlyGly (H<sub>2</sub>sal-RGG).** To a 10 mL solution of GlyGly (2.645 g, 20.0 mmol) in water containing NaOH (0.405 g, 10 mmol), salicylaldehyde (1.05 mL, 10 mmol) in ethanol (10 mL) was added. The resulting yellow solution was stirred at room temperature for 30 min, and then cooled to *ca.* 2 °C. Sodium borohydride (0.460 g, 12 mmol) in ethanol (5 mL), containing a few drops of a 2 M NaOH solution, was then slowly added, a colourless solution being obtained. A few drops of 3 M HCl were added till pH 4–5 was reached and the mixture was left overnight with stirring. A white precipitate formed, was collected by filtration and washed with cold water and diethyl ether. To this white solid water at *ca.* 40 °C was added, the mixture stirred for a few minutes and left at *ca.* 4 °C for several hours. The solid was filtered, washed with water and diethyl ether and dried. Yield: 35%. Analysis of 1: C, 52.2; H, 6.1; N, 11.0; Na < 0.2%. C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>·0.8H<sub>2</sub>O requires: C, 52.29; H, 6.22; N, 11.09%. MS: mol. ion at *m/z* = 238. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, standard SiMe<sub>4</sub>): δ 3.79 (2H, s, CH<sub>2</sub>-COOH), 3.87 (2H, s, CH<sub>2</sub>-NH), 4.15 (2H, s, CH<sub>2</sub>-phenol), 6.83 (2H, m, CH<sub>aromatic</sub>), 7.84 (2H, m, CH<sub>aromatic</sub>).

**Synthesis of the reduced Schiff base 2 containing GlyGlyGly (H<sub>2</sub>sal-RGGG).** The procedure was similar to that for 1. Yield: 66%. Analysis of 2: C, 52.3; H, 6.0; N, 13.8; Na ≈ 0%. C<sub>13</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>·0.2H<sub>2</sub>O requires: C, 52.24; H, 5.87; N, 14.06%. MS: mol. ion at *m/z* = 295. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, standard SiMe<sub>4</sub>): δ 3.80 (2H, s, CH<sub>2</sub>-COOH), 3.85 (4H, s, CH<sub>2</sub>-NH), 4.15 (2H, s, CH<sub>2</sub>-phenol), 6.83 (2H, m, CH<sub>aromatic</sub>), 7.21 (2H, m, CH<sub>aromatic</sub>).

**Vanadium(IV) complex with H<sub>2</sub>sal-RGG (3).** The ligand (0.254 g, 1.00 mmol) was dissolved in 5 mL of water containing sodium acetate (0.274 g, 2.0 mmol), and a few drops of a 4 M

NaOH solution were added to promote dissolution. Ethanol (2.5 mL) was added followed by 2 mL of a  $\text{VOSO}_4 \cdot 5\text{H}_2\text{O}$  (0.256 g, 1.0 mmol) aqueous solution. A greyish-green precipitate formed during the addition of the  $\text{V}^{\text{IV}}\text{O}^{2+}$  solution, which was collected by filtration, washed with ethanol : water (2 : 1), ethanol and diethyl ether, and dried. Yield: 56%. Analysis of **3**: C, 38.5; H, 4.4; N, 8.0; Na < 0.3%.  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_5\text{V} \cdot 2.1\text{H}_2\text{O}$  [formulation:  $\text{V}^{\text{IV}}\text{O}(\text{sal-RGG})(\text{H}_2\text{O})_{2.1}$ ] requires: C, 38.74, H, 4.79; N, 8.22%.

**Copper(II) complex with  $\text{H}_2\text{sal-RGG}$  (**4**).** The ligand (0.29 g, 1.1 mmol) was dissolved in 15 mL of water. A solution of cupric acetate (0.204 g, 1.0 mmol) in 15 mL of ethanol was added and the pH adjusted to 4.7 with 1 M HCl. A blue precipitate formed after about 1 h, which was collected by filtration, washed with water, ethanol and diethyl ether, and dried. Yield: 73%. Analysis of **4**: C, 43.3; H, 4.1; N, 8.9%.  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_4\text{Cu} \cdot 0.3\text{H}_2\text{O}$  [formulation:  $\text{Cu}(\text{sal-RGG})(\text{H}_2\text{O})_{0.3}$ ] requires: C, 43.29, H, 4.16; N, 9.18%.

**Vanadium(IV) complex with  $\text{H}_2\text{sal-RGGG}$  (**5**).** The procedure was similar to that for **3**. Yield: 61%. Analysis of the bluish-grey solid **5**: C, 43.5; H, 4.3; N, 11.5; Na < 0.2%.  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_6\text{V}$  [formulation:  $\text{V}^{\text{IV}}\text{O}(\text{RsalGGG})$ ] requires: C, 43.35, H, 4.20; N, 11.66%.

**Copper(II) complex with  $\text{H}_2\text{sal-RGGG}$  (**6**).** The procedure was similar to that for **4**, the pH being adjusted to *ca.* 5. Yield: 80%. Analysis of the green solid **6**: C, 40.3; H, 4.5; N, 10.5%.  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_5\text{Cu} \cdot 1.8\text{H}_2\text{O}$  [formulation:  $\text{Cu}(\text{sal-RGGG})(\text{H}_2\text{O})_{1.8}$ ] requires: C, 40.11; H, 4.82; N, 10.80%.

#### Physical and spectroscopic studies

$^{51}\text{V}$  and  $^1\text{H}$  NMR spectra were obtained on a Varian Unity-500 Spectrometer operating at 131.404 MHz and 499.824 MHz, respectively, using a 5 mm broad band probe, at  $25.0 \pm 0.5$  °C.  $^{51}\text{V}$  NMR chemical shifts were externally referenced to a  $\text{VOCl}_3$  solution at 0 ppm. The  $^{51}\text{V}$  NMR acquisition parameters were: 33 kHz spectral width, 30  $\mu\text{s}$  pulse width, 1 s acquisition time and 10 Hz line broadening. A pre-saturation pulse sequence was used for  $^1\text{H}$  NMR spectra to eliminate the residual water signal. IR spectra were recorded with a BioRad FTS 3000 MX FTIR spectrometer. Visible spectra were recorded either with a Hitachi U-2000 or with an HP 8452A diode array spectrophotometer. The EPR spectra were usually recorded at 77 K (on glasses made by freezing solutions in liquid nitrogen) with a Bruker ESP 300E X-band spectrometer.

#### Solution studies

The purity of the ligands was checked pH-potentiometry and the exact concentrations of solutions were determined by the Gran method.<sup>29</sup> The experimental difficulties in the present systems were discussed in refs. 30 and 31. All solutions were manipulated in an inert atmosphere (high purity dinitrogen or purified argon). The stock solutions of  $\text{V}^{\text{IV}}\text{O}^{2+}$  were prepared and standardised as described in ref. 30. The ionic strength was adjusted to 0.2 M KCl. For pH-potentiometric measurements the temperature was  $25.0 \pm 0.1$  °C, and for VIS spectra  $25.0 \pm 0.3$  °C with circulating water.

#### TLC

These were performed on Merck TLC plates (Art. 5626,  $10 \times 20$  cm). Usually, 2  $\mu\text{L}$  samples were applied to the plates 20 mm from the bottom. Elution was carried out in Camag twin chambers with walls covered with filter-paper impregnated with the eluent. This was either (A) butanol–ethanol–propionic acid–water (10 : 10 : 2 : 5) or (B) ethanol–water (70–30). When it reached 120 mm from the bottom the plates were removed and

dried. The chromatogram was developed with a ninhydrin–collidine (2,4,6-trimethylpyridine)–copper solution prepared according to Moffat and Lytle.<sup>32</sup> In some cases, after such development, the plate was placed in an enclosed chamber for development with iodine vapours.

#### pH measurements

**Spectroscopic measurements.** For preparation of the solutions and pH calibrations we used a special glass vessel with a double wall, with entries for the combined electrode (Radiometer 'Red Rod' pHC2015-8), thermometer, nitrogen and reagents (*e.g.* base). A computerized system developed locally was used to control the titration conditions for pH calibrations. The e.m.f. measurements were made with a Denver Model 15 pH meter.

**pH-potentiometric titrations.** Stability constants were determined by pH-metric titration of 25.0  $\text{cm}^3$  samples. The ligand concentrations were in the range 0.002–0.004  $\text{mol dm}^{-3}$  and the metal ion : ligand molar ratio was ranged from 1 to 6. Titrations were normally carried out from pH 2.0 to 12 with KOH solution of known concentration (*ca.* 0.2  $\text{mol dm}^{-3}$ ) under a purified argon atmosphere, unless extensive hydrolysis or very slow equilibration was detected. The pH was measured with an Orion 710A precision digital pH meter equipped with an Orion Ross 8103BN type combined glass electrode, calibrated for hydrogen ion concentration as described earlier.<sup>33</sup> The ionic product of water was  $\text{p}K_w = 13.76$ .

The concentration stability constants  $\beta_{pqr} = [\text{M}_p\text{L}_q\text{H}_r]/[\text{M}]^p[\text{L}]^q[\text{H}]^r$  were calculated with the aid of the PSEQUAD computer program.<sup>34</sup> The formation of the hydroxo complexes of  $\text{V}^{\text{IV}}\text{O}$  was taken into account. The following species were assumed:  $[\text{V}^{\text{IV}}\text{O}(\text{OH})]^+$  ( $\log \beta_{10-1} = -5.94$ ),  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_2]^{2+}$  ( $\log \beta_{20-2} = -6.95$ ), with stability constants calculated from the data of Henry *et al.*<sup>35</sup> and corrected for the different ionic strengths using the Davies equation,  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_2]^-$  ( $\log \beta_{20-5} = -22.0$ ) and  $[\text{V}^{\text{IV}}\text{O}(\text{OH})_3]^-$  ( $\log \beta_{10-3} = -18.0$ ).<sup>30,36,37</sup> The maximum pH values of the data in the input files of PSEQUAD were 8.1, 7.8 and 11.7 for the  $\text{V}^{\text{IV}}\text{O}-\text{H}_2\text{sal-RGG}$ ,  $\text{V}^{\text{IV}}\text{O}-\text{H}_2\text{sal-RGGG}$  and both  $\text{Cu}^{\text{II}}$  systems, respectively. The reproducibility of titration points included in the evaluation was within 0.005 pH units in the whole pH range.

When referring to stoichiometries of complexes present in solution the normal  $\text{M}_p\text{L}_q\text{H}_r$  notation will be used, where L =  $\text{sal-RGG}^{2-}$  (the deprotonated form of **1**) or  $\text{sal-RGGG}^{2-}$  (the deprotonated form of **2**). Some comparisons will be made with the peptides GlyGly and GlyGlyGly. For this purpose the notations  $\text{M}_p\text{GG}_q\text{H}_r$  or  $\text{M}_p\text{GGG}_q\text{H}_r$  will be used, where GG and GGG correspond to  $\text{GlyGly}^{2-}$  or  $\text{GlyGlyGly}^{2-}$ , respectively.

#### Spectroscopic measurements

Unless otherwise stated, by visible (VIS) spectra we mean a representation of  $\epsilon_m$  values *vs.*  $\lambda$  [ $\epsilon_m = \text{absorption}/(bC_M)$  where  $b$  = optical path (either 0.5, 1 or 3 cm cells were used) and  $C_M$  = total concentration of the metal ion]. The spectral range covered was normally 400–900 nm. Most measurements and operations were computer controlled. In the absence of ethylene glycol a relatively broad background was present in most of the frozen solution EPR spectra, therefore most spectra were run with solutions containing 5% ethylene glycol. In the studies involving the  $\text{V}^{\text{IV}}\text{O}$  systems some interference due to the co-ordination of ethylene glycol was found for  $\text{pH} > 10$ , and a few sets of EPR spectra were also recorded by adding 5% DMSO to the solutions. No such interference was found in the  $\text{Cu}(\text{II})$  systems.

For the  $\text{V}^{\text{IV}}\text{O}-\text{H}_2\text{sal-RGG}$  system, EPR and VIS spectra were recorded: (i) changing the pH with approximately fixed total vanadium and ligand concentrations, at L : M ratios of

5 ( $C_{VO} \approx 7$  mM), 4 ( $C_{VO} \approx 5.0$  mM) and 1.0 ( $C_{VO} \approx 4.0$  mM); and (ii) at pH = 7.5 and  $C_{VO} \approx 4$  mM, varying L : M ratios in the 10–1 range, by addition of excess of the ligand H<sub>2</sub>sal-RGG to the equimolar solution of the metal–ligand system. For the Cu<sup>II</sup> systems, EPR spectra were recorded varying the pH with L : M = 4 and 1 with  $C_{Cu} \approx 6$  mM. VIS spectra were run in the pH range 2–12 at L : M = 1, 2, 4 and 8 with  $C_{Cu} \approx 3$  mM, and L : M = 4 and 1 with  $C_{Cu} \approx 1$  mM. For the H<sub>2</sub>sal-RGGG systems, similar conditions were used in the measurements (except L : M = 8) and additional EPR spectra were recorded for pH > 6 at L : M of 10 and 1: (i) without addition of ethylene glycol or DMSO; and (ii) adding DMSO.

For the V<sup>IV</sup>O–H<sub>2</sub>sal-RGG and V<sup>IV</sup>O–H<sub>2</sub>sal-RGGG systems, several VIS spectra were also recorded in the low pH range 2–3.2 and  $C_L \approx 0.016$  M, varying the L : M ratios in the 4–1 range by addition of V<sup>IV</sup>O<sup>2+</sup> stock solution. These spectra were used to establish the formation constant of the VOLH<sub>2</sub> species (see below).

**Stability of ligands in solution at pH = 7 in the presence of vanadium.** A solution containing the ligand (1 or 2) (0.020 M) and VOSO<sub>4</sub> (0.0020 M) with the pH adjusted to 7 with NaOH was left with stirring under N<sub>2</sub> for ca. 74 h. 2 μL samples were periodically applied on a TLC plate. This was eluted with eluent A. No decomposition of any of the ligands was found.

## Results and discussion

### Solid state studies

**Infrared spectra.** FTIR data for 1–6 are summarised as ESI † in Table SM-1. The assignments were made based on refs. 38–41, and by comparison with the V<sup>IV</sup>O (and Cu<sup>II</sup>) complexes with the Schiff base ligands derived from the reaction of salicylaldehyde and GlyGly and other simple dipeptides.<sup>21</sup> Most compounds present broad bands in the range 2400–3500 cm<sup>-1</sup> corresponding to hydrogen-bonded (symmetrical and asymmetrical) O–H and N–H and overtone bands.<sup>38,40,41</sup> In the case of 1 and 2 sharp ν(N–H) bands emerge from this broad band as well as several other weak or very weak bands (Table SM-1). This is characteristic of compounds containing secondary amide groups.<sup>38,40</sup> The strong amide I (C=O) band at 1672–1676 cm<sup>-1</sup> is shifted to 1629 and 1607 cm<sup>-1</sup> in the FTIR of V<sup>IV</sup>O and Cu<sup>II</sup> complexes of 1. In the corresponding complexes with ligand 2 the band at 1670 cm<sup>-1</sup> is still present.

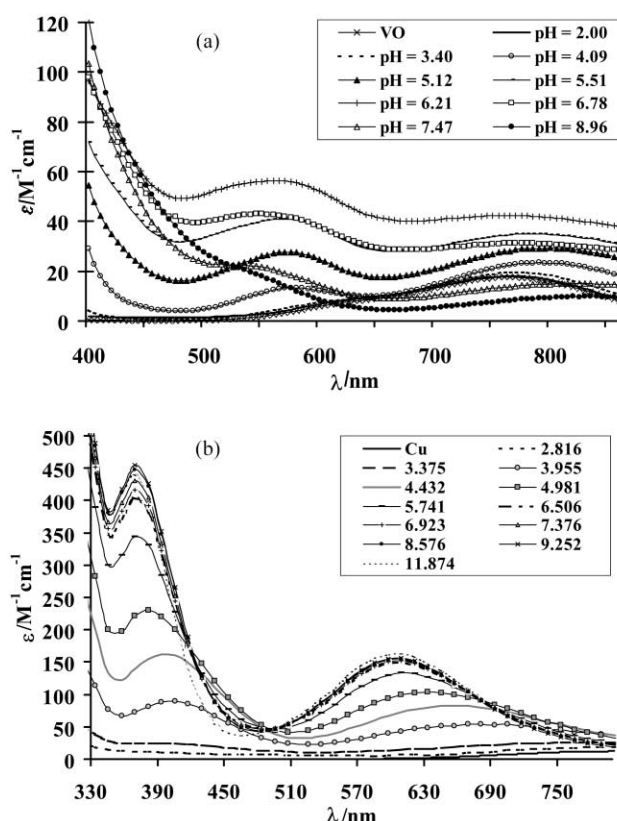
The IR of 3 and 4 show a broad band in the ν(O–H) and ν(N–H) range (not so broad in 3), but this differs from the corresponding free ligand, particularly for ν < 3200 cm<sup>-1</sup>. For 3 and 4 their IR features are compatible with the co-ordination of the O<sub>amide</sub> group. In 5 and 6 the presence of the amide I band at ca. 1672 cm<sup>-1</sup> suggests that either one of the O<sub>amide</sub> groups is not involved in the binding to the metal or that its binding is very weak.

### Solution studies

Species H<sub>3</sub>Sal-RGG<sup>+</sup> and H<sub>3</sub>sal-RGGG<sup>+</sup> contain three protons that may dissociate in the measurable pH range. The corresponding protonation and complex formation constants with V<sup>IV</sup>O and Cu<sup>II</sup> are included in Table 1. The pK<sub>COOH</sub> of 3.1–3.2 agree well with the characteristic acidity of the terminal carboxylic groups of dipeptides<sup>42</sup> and also with that of H<sub>2</sub>SalGly (pK<sub>1</sub> = 3.37, I = 0.2 M).<sup>13</sup> In 1 and 2 intramolecular H-bonding is possible between the phenolic O atom and the amine H atoms. By comparison with the corresponding acidity constants of H<sub>2</sub>salGly (pK<sub>2</sub> = 8.16, I = 0.2 M),<sup>13</sup> salicylaldehyde (pK = 8.13, I = 0.15 M) and salicylamide (pK = 8.89, I = 3.0 M),<sup>42</sup> and Schiff bases derived from pyridoxal-5'-phosphate (and derivatives) and phenylglycine (and derivatives) (pK = 7.1–7.3, I = 0.10 M),<sup>43</sup> we suggest that the pK values of ca. 7.4–7.6 may be ascribed to the phenolic protons.

In the V<sup>IV</sup>O systems the metal ion hydrolysis is rather extensive and the nature (and formation constants) of the (V<sup>IV</sup>O)<sub>n</sub>(OH)<sub>m</sub> products formed are not accurately known,<sup>37</sup> and in the present systems these hydrolysis products are relevant for pH > 8. Therefore, while for the Cu<sup>II</sup> systems it was possible to use data obtained in the pH range 2–12 in the calculations, for the V<sup>IV</sup>O systems only data obtained in the pH range 2–8 was used.

Fig. 2 (see also Fig. SM-1 in ESI †) depicts the VIS spectra of the vanadium(IV) and copper(II) systems. The pH-metric titration curves and the VIS spectra could be evaluated using the PSEQUAD computer program<sup>34</sup> and the calculated protonation and formation constants are given in Table 1. No other chemically relevant and reasonable models gave better fits with the experimental data.



**Fig. 2** Visible absorption spectra of solutions containing: (a) V<sup>IV</sup>O<sup>2+</sup> and H<sub>2</sub>sal-RGG at L : M = 5 and  $C_{VO} \approx 6$  mM {the spectrum of [V<sup>IV</sup>O(H<sub>2</sub>O)<sub>5</sub>]<sup>2+</sup> (VO) is also included}; and (b) Cu<sup>2+</sup> and H<sub>2</sub>sal-RGG at L : M = 8 and  $C_{Cu} \approx 3$  mM {the spectrum of [Cu(H<sub>2</sub>O)<sub>6</sub>]<sup>2+</sup> (Cu) is also included}. The pH values corresponding to each spectrum are indicated.

For the V<sup>IV</sup>O systems, pH-metric calculations could not give acceptable values for the formation constants of MLH. The logβ<sub>111</sub> values were always rather low and this species does not appear in the speciation diagrams. For the Cu<sup>II</sup> systems, the formation constants of MLH could be consistently refined and this stoichiometry appears in significant concentrations in the speciation diagrams. The MLH<sub>2</sub> species forms in a relatively low concentration and is not easy to detect by pH-potentiometric measurements because its formation is hardly accompanied by base-consumption. The values of logβ<sub>112</sub> can be varied within ±0.5 without any significant change in the fitting. However, the formation of such a species is unquestionable for the V<sup>IV</sup>O systems (not so evident in the Cu<sup>II</sup> systems), because the increase of absorbance values without change of the pattern of the spectra (see Fig. 2 and also Fig. SM-1 in ESI †), typical of monodentate carboxylate co-ordination,<sup>12,30</sup> is clearly seen. In Table 1 all constants except logβ<sub>112</sub> were calculated from the pH-potentiometric data.

**Table 1** Protonation and formation constants<sup>a</sup> of species  $M_pL_qH_r$  formed in the  $V^{IV}O-$  and  $Cu^{II}-H_2sal-RGG$  and  $-H_2sal-RGGG$  systems calculated from the pH-potentiometric data with the PSEQUAD computer program<sup>34</sup>

	$H_2sal-RGG$		$H_2sal-RGGG$	
	$pK_a$	$\log \beta$	$pK_a$	$\log \beta$
HL	10.06(1)	10.06(1)	9.99(1)	9.99(1)
$H_2L$	7.57(2)	17.63(1)	7.38(3)	17.38(2)
$H_3L$	3.13(3)	20.76(2)	3.23(4)	20.61(2)

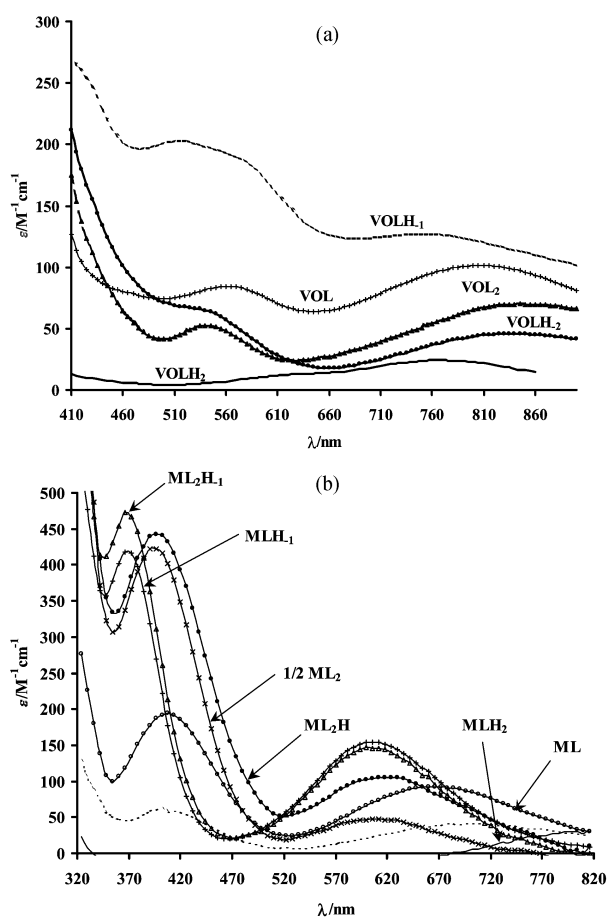
	$\log \beta$ (VO)	$\log \beta$ (Cu)	$\log \beta$ (VO)	$\log \beta$ (Cu)
	$MLH_2^b$	$19.2 \pm 0.5$	$19.3 \pm 0.5$	$19.1 \pm 0.5$
MLH	—	$15.41 \pm 0.05$	—	$14.9 \pm 0.1$
ML	$11.60 \pm 0.03$	$11.51 \pm 0.03$	$11.18 \pm 0.05$	$10.65 \pm 0.06$
$MLH_{-1}$	$6.17 \pm 0.03$	$6.19 \pm 0.05$	—	$4.94 \pm 0.06$
$MLH_{-2}$	$-2.09 \pm 0.06$	—	Yes (EPR) <sup>c</sup>	$-4.4 \pm 0.1$
$ML_2$	$18.47 \pm 0.06$	$17.11 \pm 0.08$	$18.29 \pm 0.06$	$17.0 \pm 0.3$
$ML_2H$	—	$24.8 \pm 0.1$	—	—
$ML_2H_{-1}$	—	$7.72 \pm 0.1$	—	—
$MLH_{-3}$	—	—	—	$-15.7 \pm 0.1$

<sup>a</sup> For each protonation constant listed, a range of uncertainty is given equal to 3 times the standard deviations obtained in the corresponding calculations with the PSEQUAD<sup>34</sup> computer program. For each of the metal–ligand formation constants listed, a plausible range of uncertainty is given, established by a critical examination of all calculations with the PSEQUAD<sup>34</sup> computer program, including those based on the visible absorption spectra. <sup>b</sup> The  $\log \beta_{112}$  values were calculated from the VIS spectra at low pH (2.0–2.8) at variable L : M ratios with the PSEQUAD computer program.<sup>34</sup> <sup>c</sup> The  $MLH_{-2}$  species forms for pH > 10, but no formation constant could be calculated (see text).

Light-blue solutions are obtained when ligands **1** or **2** are mixed with  $V^{IV}O^{2+}$  at L : M ratios of 1 to 10 in the pH range 2–4. As the pH is increased the solutions become bluish grey, grey and finally brown (the pH values for these changes depend on the L : M ratio and  $C_{VO}$ ). In  $V^{IV}O$ -dipeptide systems a hydroxide precipitate starts to form at *ca.* pH 5 even at a 180-fold excess of ligand,<sup>12</sup> whereas  $H_2salGly$ <sup>13</sup> is able to keep  $V^{IV}O$  in solution even at a L : M of 1. The present ligands do not bind  $V^{IV}O$  as strongly as  $H_2salGly$ , but their binding ability towards this metal ion is much higher than that of GlyGly or GlyGly-Gly, in agreement with  $O_{phenolate}$  (or the aminophenolate) being a good anchoring function. Overall, from our pH-metric and spectroscopic data it is also clear that ligand **1** has a stronger  $V^{IV}O$ -binding capability than ligand **2** (see below). In the  $Cu^{II}$  systems, the relative importance of the  $CuL$  complex is higher than in the case of GlyGly or GlyGlyGly. Besides, the deprotonation and co-ordination of the  $N_{amide}$  starts at higher pH values as compared with GlyGly and GlyGlyGly. In fact, the  $pK_1(\text{amide})$  values are 5.3 and 5.7 for ligands **1** and **2**, respectively, while these are 4.2 and 5.4 for GlyGly and GlyGlyGly, respectively,<sup>11</sup> and the  $pK_2(\text{amide})$  is 9.3 for ligand **2**, being 6.9 for GlyGlyGly.<sup>11</sup> The increased stability of the ML species induced by the additional  $O_{phenolate}$  group is particularly relevant in **1**, where hydrolysis of the  $CuLH_{-1}$  species starts at *ca.* 2 pH units higher than for GlyGly. Moreover,  $CuL_2$  species may form in solution while in the  $Cu^{II}$ -GlyGly and  $Cu^{II}$ -GlyGlyGly systems complexes with this stoichiometry do not form.<sup>11</sup>

The VIS spectral data and the PSEQUAD program were used to calculate the visible spectrum of each individual species included in the equilibrium models of Table 1. These are shown in Fig. 3 (see also Fig. SM-2 in ESI†), the main spectrophotometric features being collected in Table 2. The fact that all spectra could be calculated satisfactorily indicates that the proposed models and the values of the formation constants are correct.

Different equilibrium models were obtained for the  $V^{IV}O-H_2sal-RGG$  and  $-H_2sal-RGGG$  systems based on data in the pH range 2–8. Hydrolysis and the corresponding significant increase in the absorbance values of the solutions precluded the use of data to accurately determine formation constants of species that form only at pH > 8. The EPR spectra may help to elucidate which groups co-ordinate in solution<sup>11,44</sup> and may help in confirming the differences found in the systems studied. For the  $V^{IV}O$ -systems, Chasteen<sup>44</sup> introduced an additivity rule



**Fig. 3** Visible absorption spectra (calculated by means of the program PSEQUAD<sup>34</sup> – see the text for details) for the individual complexes formed in the (a)  $V^{IV}O-H_2sal-RGG$ , and (b)  $Cu^{II}-H_2sal-RGG$  systems.

to estimate the hyperfine coupling constant  $A_{\parallel}^{est}$  [eqn. (1)], based on the contributions  $A_{\parallel,i}$  of each of the four equatorial donor groups. The estimated accuracy of  $A_{\parallel}^{est}$  is  $\pm 3 \times 10^{-4} \text{ cm}^{-1}$ .

$$A_{\parallel}^{est} = \sum_{i=1}^4 A_{\parallel,i} \quad (1)$$

**Table 2** Some spectroscopic properties of species  $M_pL_qH_r$  formed in the  $V^{IV}O$ - and  $Cu-H_2sal-RGG$  and  $-H_2sal-RGGG$  systems. Visible absorption spectra calculated with the PSEQUAD computer program<sup>34</sup>

Stoichiometry	$V^{IV}O-H_2sal-RGG$ $\lambda/nm, \epsilon/M^{-1} cm^{-1}$	$V^{IV}O-H_2sal-RGGG$ $\lambda/nm, \epsilon/M^{-1} cm^{-1}$	$Cu^{II}-H_2sal-RGG$ $\lambda/nm, \epsilon/M^{-1} cm^{-1}$	$Cu^{II}-H_2sal-RGGG$ $\lambda/nm, \epsilon/M^{-1} cm^{-1}$
MLH <sub>2</sub>	ca. 760, 25	ca. 760, 33	ca. 800, ca. 32	ca. 710, ca. 65
MLH	—	—	402, 62 696, 42	$\lambda < 410, \epsilon > 170$ 680 <sup>a</sup> , 69
ML	565, 84 808, 102	565, 41 808, 35	408, 195 666, 92	413, 493 715, 103
MLH <sub>-1</sub>	ca. 415, 267 515, 203 569, 190 753, 126	—	370, 417 606, 155	$\lambda < 400, \epsilon > 300$ 613, 158
ML <sub>2</sub>	$\lambda < 400, \epsilon > 180$ 542, 53 852, 70	$\lambda < 400, \epsilon > 40$ 540, 35 856, 36	396, 874 620, 96	433, 1070 660, 122
ML <sub>2</sub> H	—	—	396, 453 630, 107	—
ML <sub>2</sub> H <sub>-1</sub>	—	—	366, 473 600, 148	—
MLH <sub>-2</sub>	$\lambda < 400, \epsilon > 220$ 532, 66 838, 45	—	<sup>b</sup>	$\lambda < 410, \epsilon > 160$ 554, 189
MLH <sub>-3</sub>	—	—	—	$\lambda < 410, \epsilon > 130$ 551, 205

<sup>a</sup> Very broad band. <sup>b</sup> No formation constant is available for the calculation of the spectra.

Most of the  $A_{||}$  were presented by Chasteen, and others by Cornman *et al.*  $\{A_{||}(N_{amide}) = 34-35 \times 10^{-4} cm^{-1}\}$ ,<sup>45</sup> Tasiopoulos *et al.*  $\{A_{||}(N_{amide}) = 34-43 \times 10^{-4} cm^{-1}\}$ ,<sup>46</sup> Hamstra *et al.*  $\{A_{||}(O_{amide}) = 43-44 \times 10^{-4} cm^{-1}\}$ <sup>47</sup> and Costa Pessoa *et al.*  $\{A_{||}(N_{amide})_{dipeptides} = 34-36 \times 10^{-4} cm^{-1}\}$ .<sup>48</sup> In this work we used  $36 \times 10^{-4}$  and  $43.7 \times 10^{-4} cm^{-1}$  for the  $A_{||}(N_{amide})$  and  $A_{||}(O_{amide})$  contributions, respectively. These data can be used to establish the most probable binding mode of the complexes formed, but care must be taken as the contributions of the donor groups to the hyperfine coupling may depend on their orientation,<sup>49</sup> or charge of the ligand.<sup>46</sup> The influence of the axial donor groups (if any) is not taken into account. Chasteen<sup>44</sup> estimated the contribution of the  $COO^-$  group in eqn. (1) as  $42.7 \times 10^{-4} cm^{-1}$ , based on the  $A_{||}$  of the  $VO(oxalato)_2^{2-}$  complex, assuming that the four  $COO^-$  groups co-ordinate equatorially. The solution structure presumably involves the set  $(3 \times O_{carboxylate}, H_2O)_{eq}$ ,<sup>50</sup> so this contribution should be  $42.1 \times 10^{-4} cm^{-1}$ , the value used in the present work.

Figs. 4 and 5 (and Figs. SM-3 and SM-4 in ESI†) include the high-field region of some of the frozen solution EPR spectra recorded. Clearly the type of spectrum changes as the pH is increased and, at least until pH 10, these spectra indicate that the ligand field around the metal ions increases. Table 3 summarises the EPR parameters obtained, mostly from simulation of the spectra using a computer program from Rockenbauer and Korecz.<sup>51</sup>

The speciation curves (Figs. 6 and 7) indicate that complex formation starts at relatively low pH with the protonated species MLH<sub>2</sub>. In all systems the EPR parameters and VIS spectra at low pH (ca. 2–3) indicate the presence of a mixture of  $M(H_2O)_n$  and MLH<sub>2</sub> species. The binding mode of MLH<sub>2</sub> either involves the carboxylate group alone or also an  $O_{amide}$  donor. To evaluate the basicity-adjusted formation constants (proton displacement constants), we may consider the equilibrium depicted in eqn. (2):



For the  $V^{IV}O$  systems this is  $-1.56$  for **1** and  $-1.51$  for **2**, which may be compared with the values obtained for GlyGly ( $-1.43$ ), GlyGlyGly ( $-1.59$ ),<sup>12</sup> Ala ( $-1.33$ )<sup>30</sup> or  $H_2salGly$  ( $-1.29$ ).<sup>13</sup> As no significant increase in the basicity-adjusted formation constants was found for ligands **1** and **2**, there is no definite

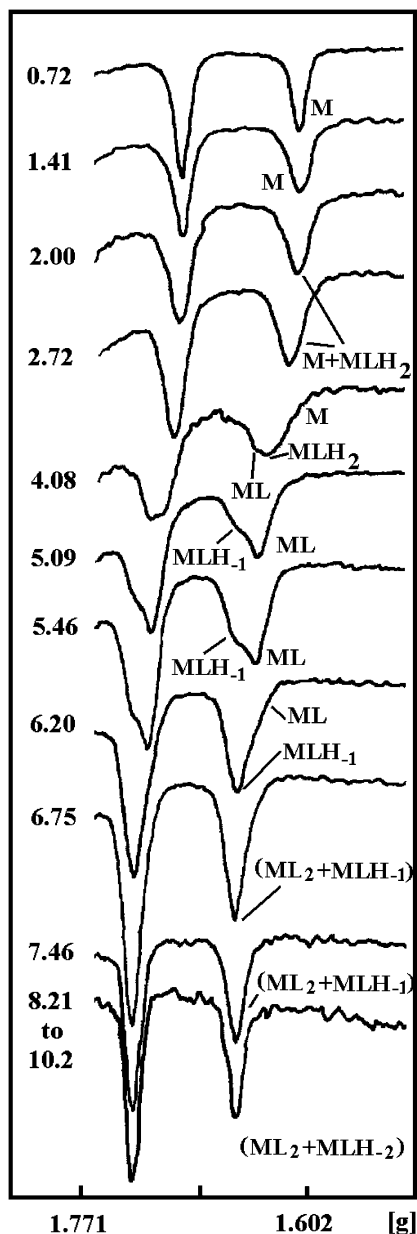
argument to support the involvement of  $O_{amide}$  groups in the binding mode of VOLH<sub>2</sub>. The same comment applies to the CuLH<sub>2</sub> complexes. In all systems the VIS spectra of MLH<sub>2</sub> complexes were calculated with relatively high standard deviations (SD of ca. 10%). As with simple amino acids and peptides<sup>12,30</sup> with the same binding mode, the VIS spectra calculated for VOLH<sub>2</sub> complexes are similar to that of  $[V^{IV}O(H_2O)_5]^{2+}$ , but with higher  $\epsilon$  values.

The **MLH stoichiometry** is only relevant for the Cu<sup>II</sup> systems but their concentration is never high. The EPR parameters (Table 3) are consistent with a binding mode including  $(N_{amine}, O_{amide}, O_{carboxylate}, H_2O)$ , but do not rule out  $(O_{phenolate}, O_{amide}, O_{carboxylate}, H_2O)$ .<sup>52</sup> In the calculated VIS spectra (Fig. 3 and Fig. SM-2 in ESI†) the intensity of the band at ca. 400 nm is not high, which indicates that the  $O_{phenolate}$  donor atom of the ligand is not involved in the co-ordination. Accordingly, the former binding mode seems to be more probable for this species.

The **ML stoichiometry** is important in all systems. It should correspond to complexes where the  $N_{amide}$  group is not deprotonated. The compounds **3–6** (plausible structures are in Fig. 8) correspond to this stoichiometry and were obtained from solutions in the pH range 4–5.5. They do not contain any counter-ion, which should be present if the  $N_{amide}$  group was deprotonated. The binding mode in solution thus corresponds to a  $(O_{phenolate}, N_{amine}, O_{amide}, H_2O)_{equatorial}$  set, and could also include the  $(O_{carboxylate})_{axial}$ . For the  $V^{IV}O$  systems, by using the additivity relation [eqn. (1)] a value of  $A_{||}^{est} = 168 \times 10^{-4} cm^{-1}$  is obtained, which compares well with those in Table 3. The EPR parameters for the Cu<sup>II</sup> systems are also compatible with a similar binding mode.<sup>52</sup>

In agreement with the involvement of  $O_{phenolate}$  in the M–L bonding, the  $\epsilon$  values at ca. 380–420 nm in the calculated VIS spectra of VOL and CuL complexes are relatively high (Fig. 3 and Fig. SM-2 in ESI†). These bands should have an important contribution from charge transfer transitions.<sup>19,53,54</sup>

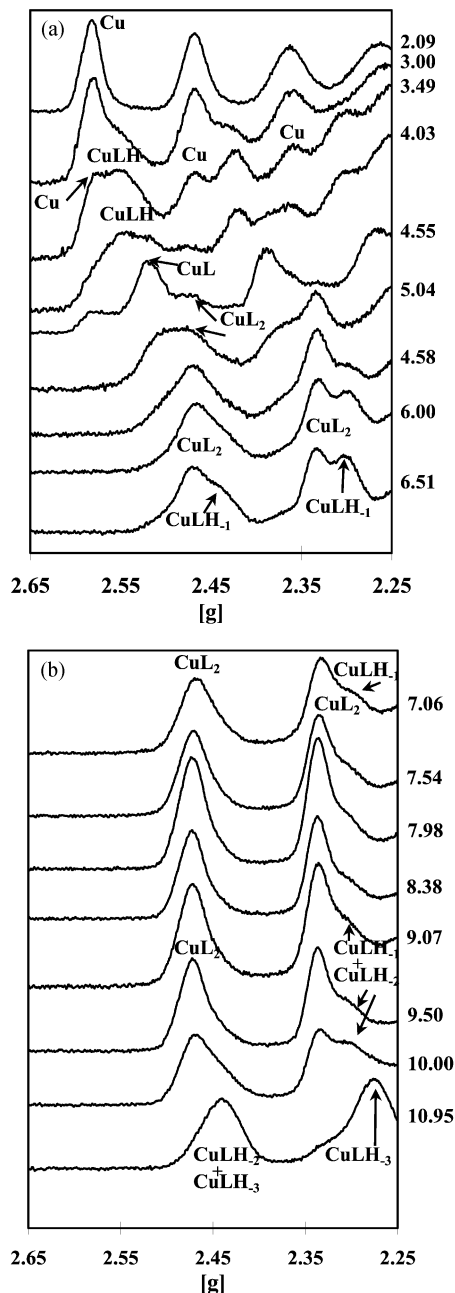
The formation of complexes with the **MLH<sub>-1</sub>** stoichiometry is relevant for both Cu<sup>II</sup> systems, and for the  $V^{IV}O-H_2sal-RGG$  system. We could not find evidence for its formation in the  $V^{IV}O-H_2sal-RGGG$  system. For the  $V^{IV}O-H_2sal-RGG$  system the binding mode corresponds to an  $(O_{phenolate}, N_{amide}, O_{carboxylate}, H_2O)_{equatorial}$  set, possibly also including the  $(N_{amine})_{axial}$ . By using the additivity relation [eqn. (1)] a value of



**Fig. 4** High-field range ( $g$  values of *ca.* 1.6–1.8) of the first-derivative EPR spectra at 77 K of frozen ‘solutions’ containing  $V^{IV}O^{2+}$  and  $H_2sal$ -RGG with  $C_{VO} \approx 7$  mM and  $L : M = 5$ . The pH values are indicated as well as the species that originate the peaks (see text).

$A_{\parallel}^{est} = 163 \times 10^{-4} \text{ cm}^{-1}$  is obtained, which compares well with that in Table 3. The VIS spectrum calculated for  $VOLH_{-1}$  shows three d–d bands (Table 2), two of them resulting from the non-degeneracy of the  $d_{xz}$  and  $d_{yz}$  energy levels in a low symmetry environment, this being consistent with the equatorial donor set proposed. The absorption with  $\lambda_{max}$  at *ca.* 400 nm is assigned to a charge transfer band. In agreement with the aminophenolate acting as a good anchoring moiety, the  $pK(\text{amide})$  is 5.4, while in the  $V^{IV}O$ -GlyGly system the amide deprotonation/co-ordination is only detected at  $pH > 7$ , at very high  $L : M$  ratios.<sup>12</sup>

For the  $Cu$ - $H_2sal$ -RGG system species  $CuLH_{-1}$  is the dominating species in the pH range 6–12 [Fig. 7(a)] and the donor set involves the ( $O_{phenolate}$ ,  $N_{amine}$ ,  $N_{amide}$ ,  $O_{carboxylate}$ ). For the  $Cu$ - $H_2sal$ -RGGG system  $CuLH_{-1}$  is also a relevant stoichiometry and the binding mode probably includes one  $O_{amide}$  instead of  $O_{carboxylate}$ , its lower stability accounting for the more significant formation of  $ML_2$  in this system. The EPR parameters are quite similar in both systems (see Table 3) and agree well with an  $N_2O_2$  donor set.<sup>52</sup> Moreover, very small features may



**Fig. 5** First-derivative EPR spectra ( $g$  values of *ca.* 2.2–2.6) at 77 K of frozen ‘solutions’ containing  $Cu^{2+}$  and  $H_2sal$ -RGGG, with  $C_{Cu} \approx 6$  mM and  $L : M = 4$ . The pH values are indicated as well as the species that originate the peaks (see text).

be seen in the EPR spectra due to  $^{14}N$  superhyperfine (shf) couplings.

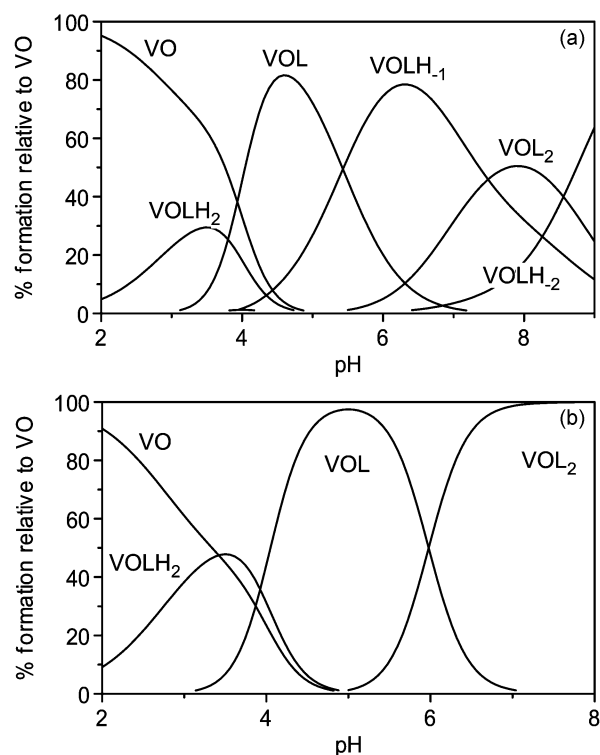
The formation of complexes with the  $ML_2$  stoichiometry is relevant in all systems studied here. This is in contrast with what was found for the  $V^{IV}O$  and  $Cu^{II}$  complexes with simple dipeptides, where the  $ML_2$  stoichiometry does not form even in a very high excess of peptide.<sup>11,12</sup> For the  $V^{IV}O$  systems the binding mode corresponds to ( $2 \times O_{phenolate}$ ,  $2 \times N_{amine}$ ) (one axial), and an equatorial  $H_2O$ . By using the additivity relation [eqn. (1)] a value of  $A_{\parallel}^{est} = 163 \times 10^{-4} \text{ cm}^{-1}$  is obtained, which compares well with those in Table 3. The relatively high intensity of the absorption band in the range 360–420 nm indicates that  $O_{phenolate}$  belongs to the co-ordination sphere.

Even in solutions containing  $Cu^{II}$  and 1 with  $L : M = 8$  the maximum relative concentration of  $CuL_2$  is only about 14% at  $pH \approx 7.5$ . Its EPR parameters could not be obtained but the calculated VIS spectrum indicates a binding mode ( $2 \times O_{phenolate}$ ,  $2 \times N_{amine}$ ). In contrast with the  $Cu^{II}$ - $H_2sal$ -RGG

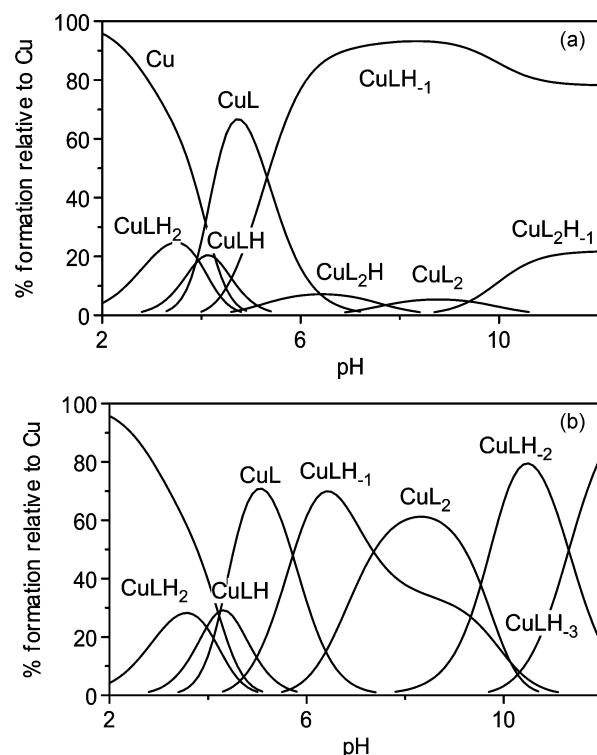
**Table 3** EPR parameters obtained from spectra where the individual species are present in significant relative concentration. The  $A_{\parallel}$  and  $A_{\perp}$  values are in  $\text{cm}^{-1}$  and multiplied by  $10^4$

	$\text{H}_2\text{sal-RGG}$				$\text{H}_2\text{sal-RGGG}$			
	$\text{V}^{\text{VO}}$		$\text{Cu}^{\text{II}}$		$\text{V}^{\text{VO}}$		$\text{Cu}^{\text{II}}$	
	$g_{\parallel} (g_{\perp})$	$A_{\parallel} (A_{\perp})$	$g_{\parallel} (g_{\perp})$	$A_{\parallel} (A_{\perp})$	$g_{\parallel} (g_{\perp})$	$A_{\parallel} (A_{\perp})$	$g_{\parallel} (g_{\perp})$	$A_{\parallel} (A_{\perp})$
M	1.938	182.5	2.418 (2.080)	131 (12)	1.945	182.0	2.416 (2.081)	133 (12)
MLH <sub>2</sub>	1.940 <sup>a</sup> (1.982)	179 <sup>a</sup> (66.1)	2.382 (2.070)	140 (13)	<sup>b</sup>	<sup>b</sup>	2.377 (2.072)	139 (13)
MLH	—	—	ca. 2.323	ca. 157	—	—	ca. 2.325 <sup>b</sup>	ca. 154 <sup>b</sup>
ML	1.943 (ca. 1.98)	169 (58.8)	ca. 2.282	ca. 168	1.943 (1.978)	172.2 (61)	ca. 2.305 <sup>b</sup>	ca. 166 <sup>b</sup>
MLH <sub>-1</sub>	1.953 (1.982)	164 (55.4)	2.231 <sup>e</sup> (2.051)	170 <sup>e</sup> (26)	—	—	2.240 <sup>f</sup> (2.053)	162 <sup>f</sup> (27)
MLH <sub>-2</sub>	1.955 (1.978)	158 (49)	—	—	1.960 (1.984)	157 ± 1 (ca. 52)	2.200 <sup>e</sup> (2.040)	205 <sup>e</sup> (26)
ML <sub>2</sub>	1.951 (1.976)	163 (53.9)	ca. 2.265	ca. 163	1.952 (1.978)	163.3 (54.2)	2.274 <sup>f</sup> (2.057)	166 <sup>f</sup> (15)
ML <sub>2</sub> H	—	—	<sup>b</sup>	<sup>b</sup>	—	—	—	—
ML <sub>2</sub> H <sub>-1</sub>	—	—	<sup>b</sup>	<sup>b</sup>	—	—	—	—
MLH <sub>-3</sub>	—	—	—	—	—	—	ca. 2.206 <sup>c</sup>	ca. 201 <sup>c</sup>
VO(OH <sub>3</sub> ) <sup>-b</sup>	—	—	—	—	1.949 (1.975)	163.3 (53.1)	—	—

<sup>a</sup> This species is never present in high relative concentration. The parameters shown are those obtained by simulation for a solution with L : M = 8 and  $C_{\text{VO}} = 0.005$  M at pH = 2.7. <sup>b</sup> This species is always present in low relative concentration and cannot easily be distinguished from other major species. It is not possible to give reliable estimates of its  $A_{\parallel}$  and  $g_{\parallel}$ . <sup>c</sup> Obtained from solutions also containing CuLH<sub>-2</sub>. <sup>d</sup> There is no indication for the formation of this species. <sup>e</sup> Shf (superhyperfine) coupling is clearly seen for MLH<sub>-2</sub> (see text). <sup>f</sup> From small perturbations of the lineshape, shf couplings are expected, but it is only possible to give approximate estimates for the corresponding parameters (see text and Supplementary Material).



**Fig. 6** Concentration distribution curves of complexes formed in solutions containing (a)  $\text{V}^{\text{VO}2+}$  and  $\text{H}_2\text{sal-RGG}$ , with  $C_{\text{VO}} = 5$  mM and L : M = 4, and (b)  $\text{V}^{\text{VO}2+}$  and  $\text{H}_2\text{sal-RGGG}$  with  $C_{\text{VO}} = 7$  mM and L : M = 5, calculated using the stability constants listed in Table 1.



**Fig. 7** Concentration distribution curves of complexes formed in solutions containing (a)  $\text{Cu}^{2+}$  and  $\text{H}_2\text{sal-RGG}$ , with  $C_{\text{Cu}} = 3$  mM and L : M = 4, and (b)  $\text{Cu}^{2+}$  and  $\text{H}_2\text{sal-RGGG}$  with  $C_{\text{Cu}} = 6$  mM and L : M = 4, calculated using the stability constants listed in Table 1

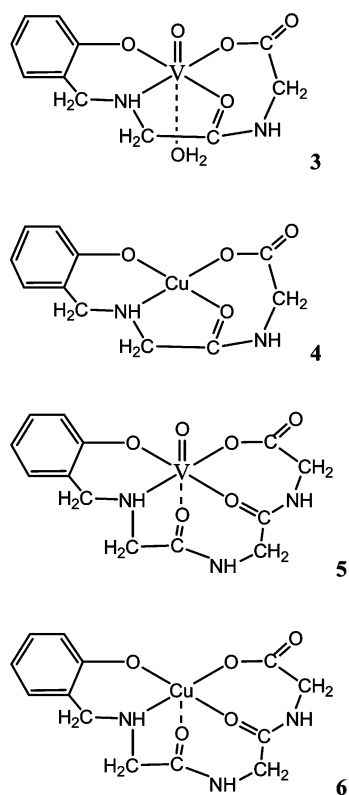
system where for solutions with L : M = 1–4 the VIS spectra do not change significantly with the pH; for the  $\text{Cu}^{\text{II}}-\text{H}_2\text{sal-RGGG}$  system the  $\text{CuL}_2$  stoichiometry is relevant for L : M > 1 [e.g. Fig. 7(b)]. As for CuL, the presence of  $\text{O}_{\text{phenolate}}$ , which forms relatively strong bonds with  $\text{Cu}^{\text{II}}$ , favours  $\text{CuL}_2$  formation as compared with species  $\text{CuGGGH}_{-1}$  and  $\text{CuGGGH}_{-2}$  in the  $\text{Cu}^{\text{II}}-\text{GGG}$  system. The intensity of the charge transfer band in the range 360–450 nm, more than twice than that of CuL (see

ESI† Fig. SM-2B and Table 2), indicates the presence of two  $\text{O}_{\text{phenolate}}$  donors. The EPR parameters are also compatible with an  $\text{N}_2\text{O}_2$  donor set.<sup>52</sup>

The formation of complexes with the  $\text{MLH}_{-2}$  stoichiometry is relevant in most systems studied here.

Only for the  $\text{Cu}-\text{H}_2\text{sal-RGG}$  system there is no pH-metric or spectroscopic indication of its formation. With L =  $\text{sal-RGGG}^{2-}$  this stoichiometry starts being relevant at pH > 8





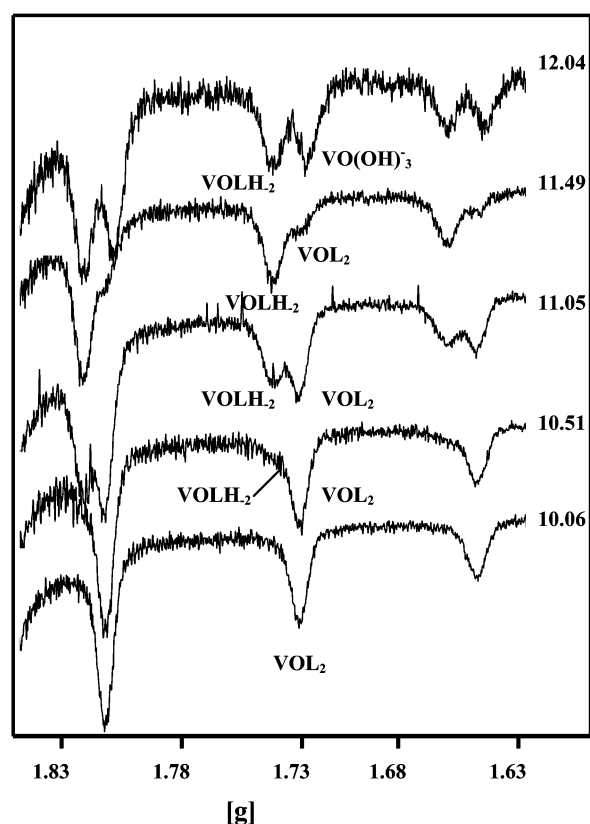
**Fig. 8** Plausible binding modes for compounds 3–6, all corresponding to ML stoichiometries (see text).

(for  $V^{VO}$  complexes) and at pH > 10 (for  $Cu^{II}$  complexes), in agreement with the difference in the relative stability of the  $MLH_{-1}$  species in both systems and with the greater tendency of the  $V^{VO}$  complexes to hydrolyse.

By using the additivity relation [eqn. (1)], the donor set ( $O_{phenolate}, N_{amine}, N_{amide}, OH^-$ )<sub>equatorial</sub>( $O_{amide}$  or  $O_{carbox}$ )<sub>axial</sub> gives  $A_{||}^{est} = 154 \times 10^{-4} \text{ cm}^{-1}$ , which compares reasonably well with the value of  $(158 \pm 2) \times 10^{-4} \text{ cm}^{-1}$  obtained for  $VOLH_{-2}$  ( $L = \text{sal-RGGG}^{2-}$ ) from spectra also containing other species. The VIS spectrum calculated for  $VOLH_{-2}$  [Fig. 3(a)], particularly the red shift of band I as compared with  $VOLH_{-1}$ ,<sup>37</sup> agrees with the equatorial co-ordination of an  $OH^-$  group. For the  $V^{VO}$ - $H_2\text{sal-RGGG}$  system no formation constant could be obtained for  $VOLH_{-2}$  as it is formed in solution only at pH > 10, where no stable pH-meter reading could be obtained. However, in Fig. 9 the formation of  $VOLH_{-2}$  is emphasised: *e.g.* at pH  $\approx$  12 it is present at about the same concentration as  $VO(OH)_3^-$ . As far as the EPR parameters indicate, the binding mode for  $VOLH_{-2}$  ( $L = \text{sal-RGGG}^{2-}$ ) appears to be similar to that for  $L = \text{sal-RGG}^{2-}$ .

The  $CuLH_{-2}$  ( $L = \text{sal-RGGG}^{2-}$ ) stoichiometry starts being important for pH > 10 and corresponds to a donor set involving at least the ( $N_{amine}, N_{amide}, N_{amide}, O_{carboxylate}$ ). The EPR parameters (Table 3) agree well with an  $N_3O$  donor set in a  $Cu^{II}$  complex with a charge of  $-1$  or  $-2$ .<sup>52</sup> Confirming the equatorial co-ordination of three N atoms, the EPR spectra, in conditions where  $CuLH_{-2}$  ( $L = 2$ ) is dominant, clearly show features due to  $^{14}N$  shf couplings, and the EPR spectra could be simulated<sup>51</sup> taking two  $N_{amide}$  ( $N_{\perp} = 15 \text{ G}, N_{||} \approx 11 \text{ G}$ ) and one  $N_{amine}$  ( $N_{\perp} = 13 \text{ G}, N_{||} \approx 9 \text{ G}$ ).

Under the conditions used in this work, the formation of complexes with the  $ML_2H$  and  $ML_2H_{-1}$  stoichiometries was only found to be relevant in the  $Cu^{II}$ - $H_2\text{sal-RGG}$  system for  $L : M > 4$ . For the  $Cu$ -GlyGly system these stoichiometries were also found (MGG<sub>2</sub>H only for very high  $L : M$  ratios, no VIS spectral data being available).<sup>11</sup> No reliable EPR parameters can be obtained from our spectra for these stoichiometries, and only the calculated VIS spectra are available (*e.g.* Table 2). The



**Fig. 9** High-field range ( $g$  values of *ca.* 1.6–1.8) of the first-derivative EPR spectra at 77 K of frozen ‘solutions’ containing  $V^{VO}O^{2+}$  and  $H_2\text{sal-RGGG}$  with  $C_{VO} = 8 \text{ mM}$ ,  $L : M = 4$  and 5% DMSO in the pH range 10–12. The pH values are indicated as well as the species that originate the peaks (see text).

VIS spectrum calculated for  $CuL_2H$  ( $L = 1$ ) [Fig. 3(b)] is similar to that of  $CuL_2$ , the  $\epsilon$  values of the bands with  $\lambda_{max}$  at 396 nm being about half of those for  $CuL_2$ . This suggests that one of the ligands is bound through the  $O_{phenolate}$  and  $N_{amine}$ , while the binding of the second ligand presumably does not involve the  $O_{phenolate}$  group.

The VIS spectrum of  $CuL_2H_{-1}$  ( $L = 1$ ) [Fig. 5(a)], is very similar to that of  $CuLH_{-1}$ , the intensity of the band at *ca.* 370 nm being significantly higher. This suggests that in the process  $CuLH_{-1} \rightarrow CuL_2H_{-1}$  the second  $\text{sal-RGGG}^{2-}$  binds through the  $O_{phenolate}$  and  $N_{amine}$  groups, the binding mode of  $CuL_2H_{-1}$  being ( $O_{phenolate}, N_{amine}, N_{amide}, O_{carboxylate}$ ), ( $O_{phenolate}, N_{amine}$ )<sub>axial</sub>.

As far as we may conclude, the formation of complexes with the  $MLH_{-3}$  stoichiometry is only relevant for the  $Cu$ - $H_2\text{sal-RGGG}$  system, its formation being only significant at pH > 10. The corresponding  $\log \beta_{11-3}$  could be refined consistently, but it was not possible to obtain the correct EPR parameters as until pH 12 the solutions also contain  $CuLH_{-2}$ . The binding mode appears to be similar for  $CuLH_{-2}$ ,  $CuGGH_{-3}$  and  $CuGGH_{-3}$ ,<sup>11</sup> *i.e.* it includes ( $N_{amine}, N_{amide}, N_{amide}, HO^-$ ).

#### Stability of the ligands and vanadium(IV) complexes.

**Formation of vanadium(V) complexes.** In contrast with the corresponding Schiff base ligands, which in solutions and in the presence of vanadium hydrolyse or are involved in reactions which may be activated by the metal ion,<sup>21,23–28</sup> ligands **1** and **2** are stable for several days in aqueous solutions in the presence or absence of metal ion at physiological pH, no decomposition being detected by TLC or  $^1H$  NMR. However, in the case of the  $V^{VO}$  complexes, oxidation of the metal ion takes place easily (see ESI<sup>†</sup>).

The  $^{51}V$  NMR spectra of solutions containing vanadate (3 mM) and ligand **1** (15, 25 or 30 mM) (*i.e.*  $L : M \geq 5$ ) at pH

ca. 7 and ca. 8 in aerobic conditions, after ca. 24 h of the preparation showed a low intensity signal at  $-490$  ppm, which is indicative of the formation of a small amount of a complex. This complex could not be detected by  $^1\text{H}$  NMR spectroscopy. The  $^1\text{H}$  NMR spectra of these solutions agree with those of the free ligand at identical pH values. For freshly prepared solutions containing  $\text{V}^{\text{IV}}\text{O}$  (3 mM) and **1** (15 mM), the room temperature EPR spectra could be measured. After around 12 h the EPR signal disappeared, while the  $^{51}\text{V}$  NMR and  $^1\text{H}$  NMR were identical to those obtained when starting with  $\text{V}^{\text{V}}$  instead of  $\text{V}^{\text{IV}}$  salts.

In the  $^{51}\text{V}$  NMR spectrum of freshly prepared aqueous solutions containing  $\text{V}^{\text{V}}$  (3 mM) and **2** (ca. 20 mM) at pH 8, besides the  $\text{V}_1$ ,  $\text{V}_2$ ,  $\text{V}_4$  and  $\text{V}_5$  peaks, a signal was observed at  $-528$  ppm, corresponding to ca. 14.2% of the total  $\text{V}^{\text{V}}$  signal. After about 24 h the intensity of the signal of this  $\text{V}^{\text{V}}$ -sal-RGGG complex increased up to ca. 22% of the total  $\text{V}^{\text{V}}$  (Fig. 10). These results indicate that ligand **2** forms more stable

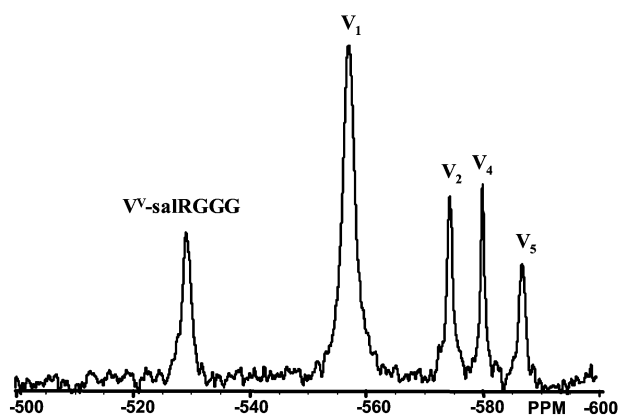


Fig. 10  $^{51}\text{V}$  NMR spectra of a solution containing 3 mM vanadate and 20 mM  $\text{H}_2\text{sal-RGGG}$  at pH = 8 after 24 h of mixing.

$\text{V}^{\text{V}}$  complexes than ligand **1**, the chemical shifts differing from those of GlyGly- and GlyGlyGly- $\text{V}(\text{v})$  complexes (at  $-505$  ppm).<sup>16</sup> The chemical shift of the  $\text{V}^{\text{V}}$ -sal-RGGG complex formed is different from that of the  $\text{V}^{\text{V}}$ -sal-RGG complex, but is close to that for  $\text{V}^{\text{V}}\text{O}(\text{BSsal-Gly})(\text{H}_3\text{pt})$  ( $\text{BSsal-Gly}^{2-} = \text{N-salicylidene glycinato}^{2-}$ ;  $\text{H}_3\text{pt} = \text{glycerol}$ ).<sup>55</sup> In the latter complex the binding mode involves ( $\text{O}_{\text{phenolate}}$ ,  $\text{N}_{\text{imine}}$ ,  $\text{O}_{\text{carboxylate}}$ ,  $\text{O}^-_{\text{glycerol}}$ ) and  $\text{OH}_{\text{glycerol}}$  (weak). This suggests that the complexes that correspond to the  $-528$  ppm peak involve at least ( $\text{O}_{\text{phenolate}}$ ,  $\text{N}_{\text{amine}}$ ,  $\text{O}_{\text{carboxylate}}$ ,  $\text{HO}^-$ ). No clear indication of  $\text{N}_{\text{amide}}$  co-ordination is available but this group presumably also binds to  $\text{V}(\text{v})$ .

## Conclusions

The reduced Schiff bases  $\text{H}_2\text{sal-RGG}$  and  $\text{H}_2\text{sal-RGGG}$  containing the GlyGly or GlyGlyGly moieties form stable complexes with  $\text{V}^{\text{IV}}\text{O}$  and  $\text{Cu}^{\text{II}}$ , which are much less susceptible to ligand hydrolysis or metal-activated reactions than the parent Schiff base complexes. This allowed the study of their acid–base properties and complexation capacity with the above metal ions by pH-potentiometry, visible absorption and EPR spectroscopy, and the determination of the respective protonation and complex formation constants. The aminophenolate chelates formed with  $\text{H}_2\text{sal-RGG}$  and  $\text{H}_2\text{sal-RGGG}$  behave as good anchoring donor sites and  $\text{ML}$  and  $\text{ML}_2$  complexes form in higher concentrations than in the corresponding  $\text{M-GlyGly}$  or  $\text{M-GlyGlyGly}$  systems. The increased stability induced by the additional phenolate-O donor was found to be particularly relevant for  $\text{H}_2\text{sal-RGG}$ : hydrolysis of the  $\text{MLH}_{-1}$  complexes starts several pH units higher than for the GlyGly system and the  $\text{ML}_2$  complexes also form in

significant concentrations. While for the  $\text{V}^{\text{IV}}\text{O-H}_2\text{sal-RGG}$  system the N-amide deprotonation/co-ordination is promoted significantly, it was not so favoured in the  $\text{V}^{\text{IV}}\text{O-H}_2\text{sal-RGGG}$  system, and the  $\text{ML}_2$  species is much more important, namely in the physiological pH range. A somewhat similar situation was found in the corresponding  $\text{Cu}^{\text{II}}$  systems.

Comparing the metal ion binding abilities of the ligands studied, it is interesting to note that in the case of simple oligopeptides the binding to  $\text{Cu}^{\text{II}}$  is significantly more favoured than to  $\text{V}^{\text{IV}}\text{O}$ . This can be explained by the difference in the affinity of the two metal ions to the anchoring  $\text{NH}_2$  group. However, the presence of an additional phenolate at the N terminus makes the  $\text{V}^{\text{IV}}\text{O}$  binding ability of the ligands more favoured, and very similar to that of  $\text{Cu}^{\text{II}}$ .

When the  $\text{V}^{\text{IV}}\text{O-H}_2\text{sal-RGG}$  and  $\text{V}^{\text{IV}}\text{O-H}_2\text{sal-RGGG}$  complexes are dissolved in water in the presence of air, oxidation of  $\text{V}^{\text{IV}}$  to  $\text{V}^{\text{V}}$  occurs, as demonstrated by  $^{51}\text{V}$  NMR spectroscopy. In contrast to  $\text{V}^{\text{IV}}$ , the  $\text{V}^{\text{V}}$  complexes formed with  $\text{H}_2\text{sal-RGGG}$  were found to be more stable than those of  $\text{H}_2\text{sal-RGG}$ .

## Acknowledgements

This work was carried out in the frame of a COST D21 project. The authors are grateful to the National Research Fund (OTKA T31896/2000), to the Hungarian Academy of Sciences, the Fundo Europeu para o Desenvolvimento Regional, Fundação para a Ciência e Tecnologia, the POCTI Programme (project POCTI/35368/QUI/2000), and the Hungarian–Portuguese Intergovernmental S & T Co-operation Programme for 2000–2001.

## References

- 1 S. W. Taylor, B. Kammerer and E. Bayer, *Chem. Rev.*, 1997, **97**, 333.
- 2 R. E. Berry, E. M. Armstrong, R. L. Beddoes, D. Collison, S. N. Ertok, M. Helliwell and C. D. Garner, *Angew. Chem., Int. Ed.*, 1999, **38**, 795.
- 3 T. Ishii, I. Nakai, C. Numako, K. Ohoshi and K. Otake, *Naturwissenschaften*, 1993, **80**, 268.
- 4 I. Harvey, J. M. Arber, R. R. Eady, B. E. Smith, C. D. Garner and S. S. Hasnain, *Biochem. J.*, 1990, **266**, 929.
- 5 J. Chen, J. Christiansen, R. C. Tittsworth, B. J. Hales, S. J. George, D. Coucouvanis and S. P. Cramer, *J. Am. Chem. Soc.*, 1993, **115**, 5509.
- 6 S. Macedo-Ribeiro, W. Hemrika, R. Revirie, R. Wever and A. Messerschmidt, *J. Biol. Inorg. Chem.*, 1999, **4**, 209 and refs. cited therein.
- 7 K. H. Thompson, J. H. McNeill and C. Orvig, *Chem. Rev.*, 1999, **99**, 2561; K. H. Thompson and C. Orvig, *J. Chem. Soc., Dalton Trans.*, 2000, 2885.
- 8 D. Rehder, J. Costa Pessoa, C. F. G. C. Geraldes, M. M. C. A. Castro, T. Kabanos, T. Kiss, B. Meier, G. Micera, L. Pettersson, M. Rangel, A. Salifoglou, I. Turel and D. Wang, *J. Biol. Inorg. Chem.*, 2002, **7**, 384.
- 9 M. M. Nagar, A. M. Wasef, K. M. Halafawi and I. H. Sayed, *Cancer Lett.*, 1998, **133**, 71; R. Liasko, T. A. Kabanos, S. Karkabounas, M. Malamas, A. J. Tasiopoulos, D. Stefanou, P. Collery and A. Evangelou, *Anticancer Res.*, 1998, **18**, 3609; A. E. Evangelou, *Crit. Rev. Oncol. Hematol.*, 2002, **42**, 249.
- 10 I. Sóvágó, in *Biocoordination Chemistry*, K. Burger, ed., Ellis Horwood, New York, 1990, ch. 4.
- 11 I. Sóvágó, D. Sanna, A. Dessi, K. Varnagy and G. Micera, *J. Inorg. Biochem.*, 1996, **63**, 99.
- 12 J. Costa Pessoa, S. M. Luz, R. Duarte, J. J. G. Moura and R. D. Gillard, *Polyhedron*, 1993, **12**, 2857; J. Costa Pessoa, S. M. Luz and R. D. Gillard, *J. Chem. Soc., Dalton Trans.*, 1997, 569.
- 13 T. Kiss, K. Petrohan, P. Buglyó, D. Sanna, G. Micera, J. Costa Pessoa and C. Madeira, *Inorg. Chem.*, 1998, **37**, 6389.
- 14 D. C. Crans, H. Holst, A. D. Keramidas and D. Rehder, *Inorg. Chem.*, 1995, **34**, 2524.
- 15 K. Elvingsson, M. Fritzsche, D. Rehder and L. Pettersson, *Acta Chem. Scand.*, 1994, **48**, 878; H. Schmidt, I. Andersson, D. Rehder and L. Pettersson, *Chem. Eur. J.*, 2001, **7**, 251.
- 16 J. S. Jaswal and A. S. Tracey, *Can. J. Chem.*, 1991, **69**, 1600.
- 17 A. S. Tracey, J. S. Jaswal, F. Nxumalo and S. J. Angus-Dunne, *Can. J. Chem.*, 1995, **73**, 489.

- 18 A. J. Tasiopoulos, E. J. Tolis, J. M. Tsangaris, A. Evangelou, J. D. Woollins, A. M. Z. Slawin, J. Costa Pessoa, I. Correia and T. A. Kabanos, *J. Biol. Inorg. Chem.*, 2002, **7**, 363 and refs. cited therein.
- 19 J. A. Bonadies and C. J. Carrano, *J. Am. Chem. Soc.*, 1986, **108**, 4088.
- 20 I. Cavaco, J. Costa Pessoa, S. Luz, M. T. L. Duarte, P. M. Matias, R. T. Henriques and R. D. Gillard, *Polyhedron*, 1995, **14**, 429.
- 21 J. Costa Pessoa, I. Cavaco, I. Correia, D. Costa, R. T. Henriques and R. D. Gillard, *Inorg. Chim. Acta*, 2000, **305**, 7 and refs. cited therein.
- 22 A. Nakahara, *Bull. Chem. Soc. Jpn.*, 1959, **32**, 1195.
- 23 J. Costa Pessoa, I. Cavaco, I. Correia, M. T. Duarte, R. D. Gillard, R. T. Henriques, F. J. Higes, C. Madeira and I. Tomaz, *Inorg. Chim. Acta*, 1999, **293**, 1.
- 24 F. Bergel, K. R. Harrap and A. M. Scott, *J. Chem. Soc.*, 1962, 1101.
- 25 Y. Murakami, H. Kondo and A. E. Martell, *J. Am. Chem. Soc.*, 1973, **95**, 7138.
- 26 A. Nakahara, K. Hamada, Y. Nakao and T. Higashima, *Coord. Chem. Rev.*, 1968, **3**, 207.
- 27 K. Harada, S. Suzuki, H. Narita and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 2203.
- 28 D. A. Phipps, *J. Mol. Catal.*, 1979, **5**, 81 and refs. cited therein; Yu. N. Belokon, I. E. Zeltzer, N. M. Loim, V. A. Tsiryapkin, G. G. Sleksandrov, D. N. Kursanov, Z. N. Parnes, Yu. T. Struchkov and V. M. Belikov, *Tetrahedron*, 1980, **36**, 1089.
- 29 G. Gran, *Acta Chem. Scand.*, 1950, **4**, 559.
- 30 J. Costa Pessoa, L. F. Vilas Boas, R. D. Gillard and R. Lancashire, *Polyhedron*, 1988, **7**, 1245.
- 31 I. Nagypál and I. Fábrián, *Talanta*, 1982, **29**, 71; I. Nagypál and I. Fábrián, *Inorg. Chim. Acta*, 1982, **62**, 193.
- 32 E. D. Moffat and R. I. Lytle, *Anal. Chem.*, 1959, **31**, 926.
- 33 J. Costa Pessoa, T. Gajda, R. D. Gillard, T. Kiss, S. M. Luz, J. J. G. Moura, I. Tomaz, J. P. Telo and I. Török, *J. Chem. Soc., Dalton Trans.*, 1998, 3587.
- 34 L. Zekany and I. Nagypál, in *Computational Methods for the Determination of Stability Constants*, D. Leggett, ed., Plenum, New York, 1985, pp. 291–353.
- 35 R. P. Henry, P. C. H. Mitchell and J. E. Prue, *J. Chem. Soc., Dalton Trans.*, 1973, 1156.
- 36 A. Komura, M. Hayashi and H. Imanaga, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 2927.
- 37 L. F. Vilas Boas and J. Costa Pessoa, in *Comprehensive Coordination Chemistry*, G. Wilkinson, R. D. Gillard and J. A. McCleverty, ed., Pergamon Press, Oxford, 1987, vol. 3, pp. 453–583 and refs. cited therein.
- 38 D. H. Williams and I. Fleming, *Spectroscopic Methods in Organic Chemistry*, McGraw-Hill, London, 5th edn., 1995, pp. 42 and 49.
- 39 K. Nakamoto, *Infrared and Raman Spectra of Inorganic Compounds, Part B*, 5th edn., 1997, pp. 73 and 271.
- 40 R. M. Silverstein, G. C. Bassler and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, New York, 5th edn., 1991, pp. 122–125.
- 41 G. Socrates, *Infrared and Raman Characteristic Group Frequencies. Tables and Charts*, John Wiley & Sons, Chichester, 3rd edn., 2001, pp. 108–113.
- 42 L. D. Pettit and K. J. Powell, *Stability Constant Database*, Academic Software-IUPAC, London, 1993.
- 43 V. M. Shanbhag and A. E. Martell, *Inorg. Chem.*, 1990, **29**, 1023.
- 44 N. D. Chasteen, in *Biological Magnetic Resonance*, J. Lawrence, L. J. Berliner and J. Reuben, eds., Plenum, New York, 1981, vol. 3, pp. 53–119.
- 45 C. R. Cornman, E. P. Zovinka, Y. D. Boyajian, K. M. Geiser-Bush, P. D. Boyle and P. Singh, *Inorg. Chem.*, 1995, **34**, 4213.
- 46 A. J. Tasiopoulos, A. N. Troganis, A. Evangelou, C. P. Raptopoulou, A. Terzis, Y. G. Deligiannakis and T. A. Kabanos, *Chem. Eur. J.*, 1999, **5**, 910.
- 47 B. J. Hamstra, A. L. P. Houseman, G. J. Colpas, J. W. Kampf, R. LoBrutto, W. D. Frasch and V. L. Pecoraro, *Inorg. Chem.*, 1997, **36**, 4866.
- 48 J. Costa Pessoa, J. L. Antunes, L. F. Vilas Boas and R. D. Gillard, *Polyhedron*, 1992, **6**, 1449.
- 49 T. S. Smith, C. H. Root, J. W. Kampf, P. G. Rasmussen and V. L. Pecoraro, *J. Am. Chem. Soc.*, 2000, **122**, 767; I. Cavaco, J. Costa Pessoa, M. T. Duarte, R. T. Henriques, P. M. Matias and R. D. Gillard, *J. Chem. Soc., Dalton Trans.*, 1996, 1989.
- 50 P. Buglyó, E. Kiss, I. Fábrián, T. Kiss, D. Sanna, E. Garriba and G. Micera, *Inorg. Chim. Acta*, 2000, **306**, 174.
- 51 A. Rockenbauer and L. Korecz, *Appl. Magn. Reson.*, 1996, **10**, 29.
- 52 J. Peisach and W. E. Blumberg, *Arch. Biochem. Biophys.*, 1974, **165**, 691.
- 53 R. Klement, F. Stock, H. Elias, H. Paulus, P. Pelikán, M. Valko and M. Mazúr, *Polyhedron*, 1999, **18**, 3817.
- 54 A. R. Amundsen, J. Whelan and B. Bosnich, *J. Am. Chem. Soc.*, 1977, **99**, 6730.
- 55 S. Mondal, S. P. Rath, K. J. Rajak and A. Chakravorty, *Inorg. Chem.*, 1998, **37**, 1713.