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

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By reaction of salicylaldehyde and GlyGly or GlyGlyGly, followed by reduction with $NaBH_4$, *N*-salicyl-glycylglycine **1** and *N*-salicyl-glycylglycylglycine **2**, the reduced Schiff bases H_2sal-RGG and H_2sal-RGGG, containing the GlyGly or GlyGlyGly moities, are prepared and characterised. Their acid–base properties and complexation with $V^{IV}O^{2+}$ and Cu^{II} 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_2sal-RGG systems with the formation of the (O-phenolate, N-amine, N-amide, COO^-) (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_2 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 $V^{IV}O$–H_2sal-RGG system the N-amide deprotonation/co-ordination is strongly promoted, it is not so favoured in the Cu^{II}–H_2sal-RGG and Cu^{II}–H_2sal-RGGG systems. The increased stability induced by the additional O-phenolate donor is particularly relevant for sal-RGG and hydrolysis of the MLH_{-1} complexes starts several pH units higher than in the GlyGly systems. In contrast to V^{IV}, the V^V 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,[1] in certain mushrooms[2] and in polychaete worms.[3] In addition, two classes of vanadium enzymes – vanadium-nitrogenases[4,5] and vanadate-dependent haloperoxidases,[6] both found in nature – as well as the vanadium's insulin-like action[7,8] and anticancer activity[9] 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.[8] 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_2 group together with the peptide C=O (O_{amide}), or through the terminal COO^- ($O_{carboxylate}$) alone or together with the O_{amide}, 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_{amide}) 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).[10]

The Cu^{2+} 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.[10] It is shown that the deprotonated N_{amide} acts as the strongest donor, but the terminal COO^- and NH_2 groups, as well as the O_{amide}, are also involved in metal binding.[10,11] In contrast, it has been found that GlyGly, GlyAla and AlaAla are not strong enough binders to prevent the hydrolysis of $V^{IV}O^{2+}$.[12] 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 [$VOLH_{-1}$] containing co-ordinated N_{amide} (see Structure **I** in Fig. 1).[5] Interestingly, no clear evidence of N_{amide} co-ordination was found for Gly- and Ala-containing tripeptides. It was concluded that neither the terminal NH_2 nor the terminal COO^- is a strong enough $V^{IV}O^{2+}$ binder to behave as an efficient anchoring donor in the promotion of N_{amide} 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 $V^{IV}O$ and Cu^{II} complexes, visible absorption spectra and EPR spectra of the $V^{IV}O$- and Cu^{II}-ligand systems. See http://www.rsc.org/suppdata/dt/b2/b206835b/

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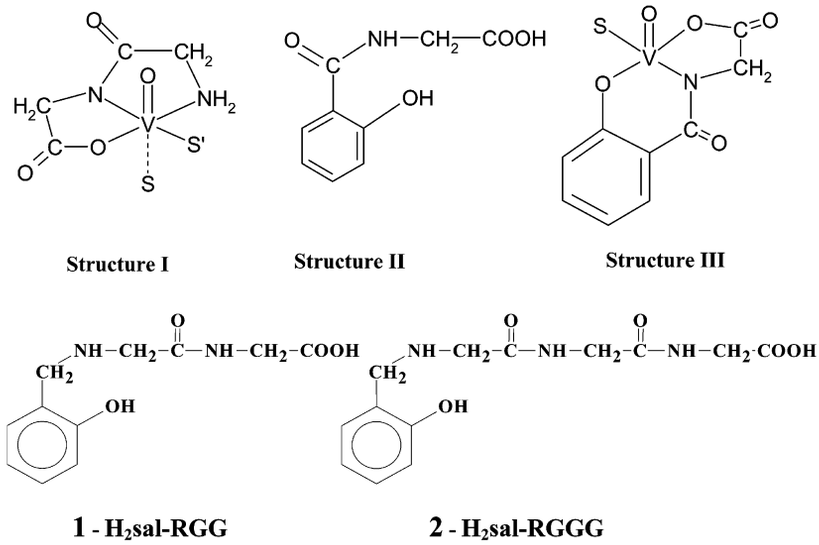

Fig. 1 Structure **I**: binding mode expected for the $VO(GlyGly^{2-})$ complex;[12] structure **II**: formula of H_2salGly; structure **III**: binding mode expected for the $VO(salGly^{3-})$ complex;[13] ligands H_2sal-RGG (**1**) and H_2sal-RGGG (**2**). S and S′ represent solvent molecules.

necessary. Accordingly, in the case of dipeptides, $V^{IV}O$ can induce deprotonation of the NH_{amide}, while with tripeptides, where two peptide groups separate the two terminal anchors, this does not occur. N_{amide} binding has been documented for several vanadium(v)-dipeptide systems, *e.g.* GlyTyr,[14] AlaHis,[15] GlyGly,[16] and a set of dipeptides,[17] and for several vanadium(IV) systems.[18]

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 $O_{phenolate}$, is known to have a high affinity for $V^{IV}O^{2+}$.[19] Accordingly, replacement of the terminal NH_2 group by $O_{phenolate}$ may enhance the metal-binding ability of the molecule. In fact, the dipeptide analogue 2-OH-hippuric acid (salicylglycine – H_2salGly – structure **II**) proved to be a much stronger $V^{IV}O^{2+}$ binder, several $V^{IV}O$:salGly complexes forming in the pH range 2–12, even at a L : M ratio of *ca.* 1.[13] The strong co-ordination to the $V^{IV}O^{2+}$ can now induce deprotonation of the NH_{amide} group, and the compound corresponding to the binding mode with ($O_{phenolate}$, N_{amide}, $O_{carboxylate}$) (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 $O_{phenolate}$ group, derived from the condensation of salicylaldehyde and dipeptides have been prepared and characterized.[20,21] The binding mode involves ($O_{phenolate}$, N_{imine}, O_{amide}, $O_{carboxylate}$) (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^{2+} the compound $Na[Cu(salGlyGly)]\cdot 6H_2O$[22] was prepared and the proposed binding mode was ($O_{phenolate}$, N_{imine}, N_{amide}, $O_{carboxylate}$). For the vanadium Schiff base complexes with this type of systems, racemisations, deaminations,[21–23] β-eliminations[24,25] and aldol-type condensations[21] have been reported. For the Cu^{II} systems, hydrolytic cleavage of tripeptides,[26] racemisations[27] and aldol-type condensations[27,28] 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,[23] 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_2sal-RGG (**1**) – and GlyGlyGly – H_2sal-RGGG (**2**) – (see Fig. 1), the preparation of their $V^{IV}O$ and Cu^{II} 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 ^{51}V and ^{1}H NMR spectrometries.

Experimental

Preparation of ligands and complexes

Synthesis of the reduced Schiff base 1 containing GlyGly (H_2sal-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%. $C_{11}H_{14}N_2O_4\cdot 0.8H_2O$ requires: C, 52.29; H, 6.22; N, 11.09%. MS: mol. ion at m/z = 238. ^{1}H NMR (500 MHz, $CDCl_3$, standard $SiMe_4$): δ 3.79 (2H, s, CH_2-COOH), 3.87 (2H, s, CH_2-NH), 4.15 (2H, s, CH_2-phenol), 6.83 (2H, m, CHaromatic), 7.84 (2H, m, CHaromatic).

Synthesis of the reduced Schiff base 2 containing GlyGlyGly (H_2sal-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%. $C_{13}H_{17}N_3O_5\cdot 0.2H_2O$ requires: C, 52.24; H, 5.87; N, 14.06%. MS: mol. ion at m/z = 295. ^{1}H NMR (500 MHz, $CDCl_3$, standard $SiMe_4$): δ 3.80 (2H, s, CH_2-COOH), 3.85 (4H, s, CH_2-NH), 4.15 (2H, s, CH_2-phenol), 6.83 (2H, m, CHaromatic), 7.21 (2H, m, CHaromatic).

Vanadium(IV) complex with H_2sal-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 $VOSO_4 \cdot 5H_2O$ (0.256 g, 1.0 mmol) aqueous solution. A greyish-green precipitate formed during the addition of the $V^{IV}O^{2+}$ 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%. $C_{11}H_{12}N_2O_5V \cdot 2.1H_2O$ [formulation: $V^{IV}O(sal\text{-}RGG)(H_2O)_{2.1}$] requires: C, 38.74, H, 4.79; N, 8.22%.

Copper(II) complex with H_2sal-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%. $C_{11}H_{12}N_2O_4Cu \cdot 0.3H_2O$ [formulation: $Cu(sal\text{-}RGG)(H_2O)_{0.3}$] requires: C, 43.29, H, 4.16; N, 9.18%.

Vanadium(IV) complex with H_2sal-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%. $C_{13}H_{15}N_3O_6V$ [formulation: $V^{IV}O(RsalGGG)$] requires: C, 43.35, H, 4.20; N, 11.66%.

Copper(II) complex with H_2sal-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%. $C_{13}H_{15}N_3O_5Cu \cdot 1.8H_2O$ [formulation: $Cu(sal\text{-}RGGG)(H_2O)_{1.8}$] requires: C, 40.11; H, 4.82; N, 10.80%.

Physical and spectroscopic studies

^{51}V and ^1H 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 ± 0.5 °C. ^{51}V NMR chemical shifts were externally referenced to a $VOCl_3$ solution at 0 ppm. The ^{51}V NMR acquisition parameters were: 33 kHz spectral width, 30 μs pulse width, 1 s acquisition time and 10 Hz line broadening. A pre-saturation pulse sequence was used for ^1H 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.[29] 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 $V^{IV}O^{2+}$ 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 ± 0.1 °C, and for VIS spectra 25.0 ± 0.3 °C with circulating water.

TLC

These were performed on Merck TLC plates (Art. 5626, 10 × 20 cm). Usually, 2 μL 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.[32] 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 cm^3 samples. The ligand concentrations were in the range 0.002–0.004 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 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.[33] The ionic product of water was $pK_w = 13.76$.

The concentration stability constants $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$ were calculated with the aid of the PSEQUAD computer program.[34] The formation of the hydroxo complexes of $V^{IV}O$ was taken into account. The following species were assumed: $[V^{IV}O(OH)]^+$ ($\log\beta_{10-1} = -5.94$), $[(V^{IV}O)_2(OH)_2]^{2+}$ ($\log\beta_{20-2} = -6.95$), with stability constants calculated from the data of Henry *et al.*[35] and corrected for the different ionic strengths using the Davies equation, $[(V^{IV}O)_2(OH)_5]^-$ ($\log\beta_{20-5} = -22.0$) and $[V^{IV}O(OH)_3]^-$ ($\log\beta_{10-3} = -18.0$).[30,36,37] The maximum pH values of the data in the input files of PSEQUAD were 8.1, 7.8 and 11.7 for the $V^{IV}O$–H_2sal-RGG, $V^{IV}O$–H_2sal-RGGG and both Cu^{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 $M_pL_qH_r$ notation will be used, where L = sal-RGG^{2-} (the deprotonated form of **1**) or sal-$RGGG^{2-}$ (the deprotonated form of **2**). Some comparisons will be made with the peptides GlyGly and GlyGlyGly. For this purpose the notations $M_pGG_qH_r$ or $M_pGGG_qH_r$ will be used, where GG and GGG correspond to $GlyGly^{2-}$ or $GlyGlyGly^{2-}$, respectively.

Spectroscopic measurements

Unless otherwise stated, by visible (VIS) spectra we mean a representation of ε_m values *vs.* λ [ε_m = 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 $V^{IV}O$ systems some interference due to the co-ordination of ethylene glycol was found for 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 Cu(II) systems.

For the $V^{IV}O$–H_2sal-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} ≈ 7 mM), 4 (C_{VO} ≈ 5.0 mM) and 1.0 (C_{VO} ≈ 4.0 mM); and (ii) at pH = 7.5 and C_{VO} ≈ 4 mM, varying L : M ratios in the 10–1 range, by addition of excess of the ligand H_2sal-RGG to the equimolar solution of the metal–ligand system. For the Cu^{II} systems, EPR spectra were recorded varying the pH with L : M = 4 and 1 with C_{Cu} ≈ 6 mM. VIS spectra were run in the pH range 2–12 at L : M = 1, 2, 4 and 8 with C_{Cu} ≈ 3 mM, and L : M = 4 and 1 with C_{Cu} ≈ 1 mM. For the H_2sal-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 $V^{IV}O$–H_2sal-RGG and $V^{IV}O$–H_2sal-RGGG systems, several VIS spectra were also recorded in the low pH range 2–3.2 and C_L ≈ 0.016 M, varying the L : M ratios in the 4–1 range by addition of $V^{IV}O^{2+}$ stock solution. These spectra were used to establish the formation constant of the $VOLH_2$ 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 $VOSO_4$ (0.0020 M) with the pH adjusted to 7 with NaOH was left with stirring under N_2 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 $V^{IV}O$ (and Cu^{II}) complexes with the Schiff base ligands derived from the reaction of salicylaldehyde and GlyGly and other simple dipeptides.[21] Most compounds present broad bands in the range 2400–3500 cm^{-1} corresponding to hydrogen-bonded (symmetrical and asymmetrical) O–H and N–H and overtone bands.[38,40,41] 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.[38,40] The strong amide I (C=O) band at 1672–1676 cm^{-1} is shifted to 1629 and 1607 cm^{-1} in the FTIR of $V^{IV}O$ and Cu^{II} complexes of **1**. In the corresponding complexes with ligand **2** the band at 1670 cm^{-1} 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 $\nu < 3200$ cm^{-1}. For **3** and **4** their IR features are compatible with the co-ordination of the O_{amide} group. In **5** and **6** the presence of the amide I band at *ca.* 1672 cm^{-1} suggests that either one of the O_{amide} groups is not involved in the binding to the metal or that its binding is very weak.

Solution studies

Species H_3Sal-RGG$^+$ and H_3sal-RGGG$^+$ contain three protons that may dissociate in the measurable pH range. The corresponding protonation and complex formation constants with $V^{IV}O$ and Cu^{II} are included in Table 1. The pK_{COOH} of 3.1–3.2 agree well with the characteristic acidity of the terminal carboxylic groups of dipeptides[42] and also with that of H_2SalGly (pK_1 = 3.37, I = 0.2 M).[13] 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_2salGly (pK_2 = 8.16, I = 0.2 M),[13] salicylaldehyde (pK = 8.13, I = 0.15 M) and salicylamide (pK = 8.89, I = 3.0 M),[42] and Schiff bases derived from pyridoxal-5′-phosphate (and derivatives) and phenylglycine (and derivatives) (pK = 7.1–7.3, I = 0.10 M),[43] we suggest that the pK values of *ca.* 7.4–7.6 may be ascribed to the phenolic protons.

In the $V^{IV}O$ systems the metal ion hydrolysis is rather extensive and the nature (and formation constants) of the $(V^{IV}O)_n(OH)_m$ products formed are not accurately known,[37] and in the present systems these hydrolysis products are relevant for pH > 8. Therefore, while for the Cu^{II} systems it was possible to use data obtained in the pH range 2–12 in the calculations, for the $V^{IV}O$ 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[34] 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.

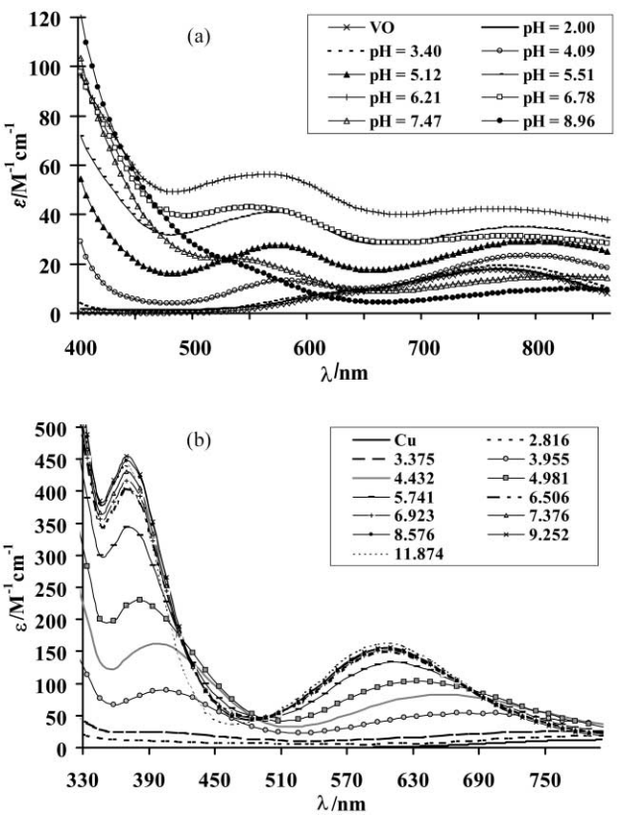

Fig. 2 Visible absorption spectra of solutions containing: (a) $V^{IV}O^{2+}$ and H_2sal-RGG at L : M = 5 and C_{VO} ≈ 6 mM {the spectrum of $[V^{IV}O(H_2O)_5]^{2+}$ (VO) is also included}; and (b) Cu^{2+} and H_2sal-RGG at L : M = 8 and C_{Cu} ≈ 3 mM {the spectrum of $[Cu(H_2O)_n]^{2+}$ (Cu) is also included}. The pH values corresponding to each spectrum are indicated.

For the $V^{IV}O$ systems, pH-metric calculations could not give acceptable values for the formation constants of MLH. The logβ_{111} values were always rather low and this species does not appear in the speciation diagrams. For the Cu^{II} systems, the formation constants of MLH could be consistently refined and this stoichiometry appears in significant concentrations in the speciation diagrams. The MLH_2 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_{112} can be varied within ±0.5 without any significant change in the fitting. However, the formation of such a species is unquestionable for the $V^{IV}O$ systems (not so evident in the Cu^{II} 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,[12,30] is clearly seen. In Table 1 all constants except logβ_{112} were calculated from the pH-potentiometric data.

Table 1 Protonation and formation constants[a] 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[34]

	H_2sal-RGG		H_2sal-RGGG	
	pK_a	logβ	pK_a	logβ
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)

	logβ (VO)	logβ (Cu)	logβ (VO)	logβ (Cu)
MLH_2[b]	19.2 ± 0.5	19.3 ± 0.5	19.1 ± 0.5	18.9 ± 0.5
MLH	—	15.41 ± 0.05	—	14.9 ± 0.1
ML	11.60 ± 0.03	11.51 ± 0.03	11.18 ± 0.05	10.65 ± 0.06
MLH_{-1}	6.17 ± 0.03	6.19 ± 0.05	—	4.94 ± 0.06
MLH_{-2}	−2.09 ± 0.06	—	Yes (EPR)[c]	−4.4 ± 0.1
ML_2	18.47 ± 0.06	17.11 ± 0.08	18.29 ± 0.06	17.0 ± 0.3
ML_2H	—	24.8 ± 0.1	—	—
ML_2H_{-1}	—	7.72 ± 0.1	—	—
MLH_{-3}	—	—	—	−15.7 ± 0.1

[a] 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[34] 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[34] computer program, including those based on the visible absorption spectra. [b] The logβ_{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.[34] [c] 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,[12] whereas H_2salGly [13] 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 GlyGlyGly, 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(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,[11] and the pK_2(amide) is 9.3 for ligand **2**, being 6.9 for GlyGlyGly.[11] 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.[11]

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[11,44] and may help in confirming the differences found in the systems studied. For the $V^{IV}O$-systems, Chasteen[44] introduced an additivity rule

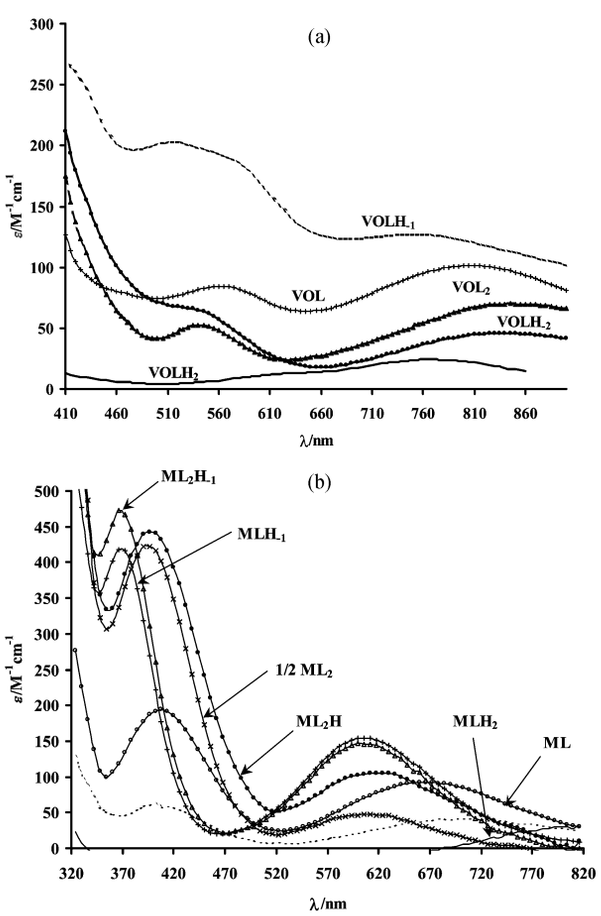

Fig. 3 Visible absorption spectra (calculated by means of the program PSEQUAD[34] – see the text for details) for the individual complexes formed in the (a) $V^{IV}O$–H_2sal-RGG, and (b) Cu^{II}–H_2sal-RGG systems.

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 $\pm 3 \times 10^{-4}$ 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[34]

Stoichiometry	$V^{IV}O$–H_2sal-RGG λ/nm, ε/M^{-1} cm^{-1}	$V^{IV}O$–H_2sal-RGGG λ/nm, ε/M^{-1} cm^{-1}	Cu^{II}–H_2sal-RGG λ/nm, ε/M^{-1} cm^{-1}	Cu^{II}–H_2sal-RGGG λ/nm, ε/M^{-1} cm^{-1}
MLH_2	*ca.* 760, 25	*ca.* 760, 33	*ca.* 800, *ca.* 32	*ca.* 710, *ca.* 65
MLH	—	—	402, 62 696, 42	$\lambda < 410$, $\varepsilon > 170$ 680[a], 69
ML	565, 84 808, 102	565, 41 808, 35	408, 195 666, 92	413, 493 715, 103
MLH_{-1}	*ca.* 415, 267 515, 203 569, 190 753, 126	—	370, 417 606, 155	$\lambda < 400$, $\varepsilon > 300$ 613, 158
ML_2	$\lambda < 400$, $\varepsilon > 180$ 542, 53 852, 70	$\lambda < 400$, $\varepsilon > 40$ 540, 35 856, 36	396, 874 620, 96	433, 1070 660, 122
ML_2H	—	—	396, 453 630, 107	—
ML_2H_{-1}	—	—	366, 473 600, 148	—
MLH_{-2}	$\lambda < 400$, $\varepsilon > 220$ 532, 66 838, 45	—	[b]	$\lambda < 410$, $\varepsilon > 160$ 554, 189
MLH_{-3}	—	—	—	$\lambda < 410$, $\varepsilon > 130$ 551, 205

[a] Very broad band. [b] No formation constant is available for the calculation of the spectra.

Most of the $A_{||,i}$ were presented by Chasteen, and others by Cornman *et al.* {$A_{||}(N_{amide})$ = 34–35 × 10^{-4} cm^{-1}},[45] Tasiopoulos *et al.* {$A_{||}(N_{amide})$ = 34–43 × 10^{-4} cm^{-1}},[46] Hamstra *et al.* {$A_{||}(O_{amide})$ = 43–44 × 10^{-4} cm^{-1}}[47] and Costa Pessoa *et al.* {$A_{||}(N_{amide})_{dipeptides}$ = 34–36 × 10^{-4} cm^{-1}}.[48] In this work we used 36 × 10^{-4} and 43.7 × 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,[49] or charge of the ligand.[46] The influence of the axial donor groups (if any) is not taken into account. Chasteen[44] estimated the contribution of the COO^- group in eqn. (1) as 42.7 × 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 × $O_{carboxylate}$, $H_2O)_{eq}$,[50] so this contribution should be 42.1 × 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.[51]

The speciation curves (Figs. 6 and 7) indicate that complex formation starts at relatively low pH with the protonated species MLH_2. 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_2 species. The binding mode of MLH_2 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):

$$H_3L + M^{2+} \rightleftarrows MLH_2 + H^+ \qquad (2)$$

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),[12] Ala (−1.33)[30] or H_2salGly (−1.29).[13] 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 $VOLH_2$. The same comment applies to the $CuLH_2$ complexes. In all systems the VIS spectra of MLH_2 complexes were calculated with relatively high standard deviations (SD of *ca.* 10%). As with simple amino acids and peptides[12,30] with the same binding mode, the VIS spectra calculated for $VOLH_2$ complexes are similar to that of $[V^{IV}O(H_2O)_5]^{2+}$, but with higher ε values.

The **MLH stoichiometry** is only relevant for the Cu^{II} 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).[52] 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 × 10^{-4} cm^{-1} is obtained, which compares well with those in Table 3. The EPR parameters for the Cu^{II} systems are also compatible with a similar binding mode.[52]

In agreement with the involvement of $O_{phenolate}$ in the M–L bonding, the ε 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.[19,53,54]

The formation of complexes with the **MLH_{-1}** stoichiometry is relevant for both Cu^{II} 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

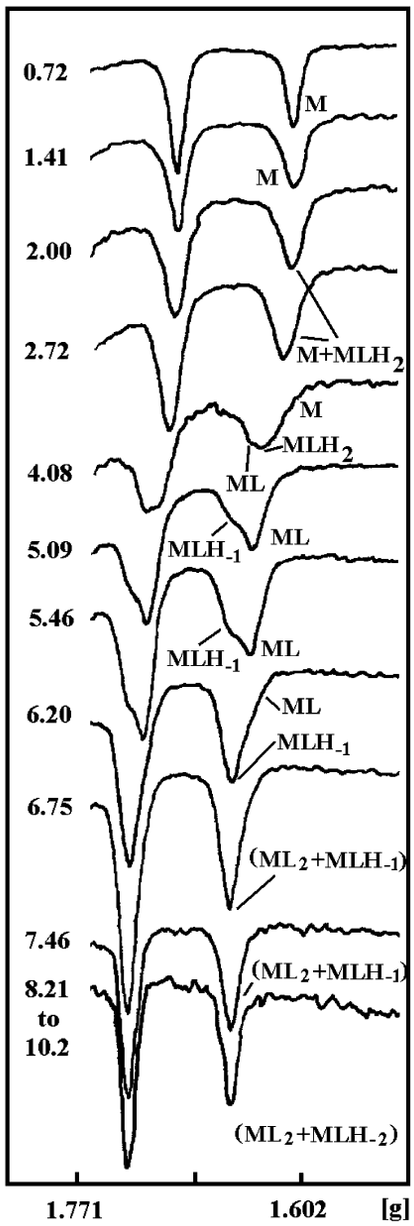

Fig. 4 High-field range (*g* values of *ca.* 1.6–1.8) of the first-derivative EPR spectra at 77 K of frozen 'solutions' containing $V^{IV}O^{2+}$ and H_2sal-RGG with $C_{VO} \approx 7$ mM and L : M = 5. The pH values are indicated as well as the species that originate the peaks (see text).

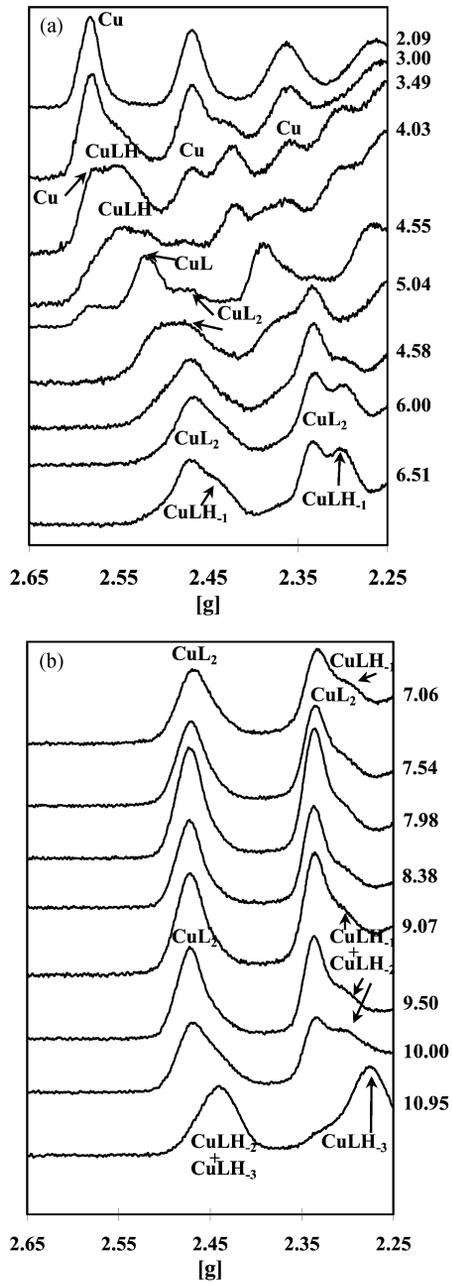

Fig. 5 First-derivative EPR spectra (*g* values of *ca.* 2.2–2.6) at 77 K of frozen 'solutions' containing Cu^{2+} and H_2sal-RGGG, with $C_{Cu} \approx 6$ mM and L : M = 4. The pH values are indicated as well as the species that originate the peaks (see text).

$A_{\parallel}^{est} = 163 \times 10^{-4}$ 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 λ_{max} at *ca.* 400 nm is assigned to a charge transfer band. In agreement with the aminophenolate acting as a good anchoring moiety, the p*K*(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.[12]

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.[52] Moreover, very small features may be seen in the EPR spectra due to ^{14}N superhyperfine (shf) couplings.

The formation of complexes with the $\mathbf{ML_2}$ 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.[11,12] For the $V^{IV}O$ systems the binding mode corresponds to (2 × $O_{phenolate}$, 2 × N_{amine}) (one axial), and an equatorial H_2O. By using the additivity relation [eqn. (1)] a value of $A_{\parallel}^{est} = 163 \times 10^{-4}$ 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 ≈ 7.5. Its EPR parameters could not be obtained but the calculated VIS spectrum indicates a binding mode (2 × $O_{phenolate}$, 2 × 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 cm^{-1} and multiplied by 10^4

	H_2sal-RGG				H_2sal-RGGG			
	$V^{IV}O$		Cu^{II}		$V^{IV}O$		Cu^{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	131	1.945	182.0	2.416	133
			(2.080)	(12)			(2.081)	(12)
MLH_2	1.940[a]	179[a]	2.382	140	[b]	[b]	2.377	139
	(1.982)	(66.1)	(2.070)	(13)			(2.072)	(13)
MLH	—	—	*ca.* 2.323	*ca.* 157	—	—	*ca.* 2.325[b]	*ca.* 154[b]
ML	1.943	169	*ca.* 2.282	*ca.* 168	1.943	172.2	*ca.* 2.305[b]	*ca.* 166[b]
	(*ca.* 1.98)	(58.8)			(1.978)	(61)		
MLH_{-1}	1.953	164	2.231[e]	170[e]	[d]	[d]	2.240[f]	162[f]
	(1.982)	(55.4)	(2.051)	(26)			(2.053)	(27)
MLH_{-2}	1.955	158	[d]	[d]	1.960	157 ± 1	2.200[e]	205[e]
	(1.978)	(49)			(1.984)	(*ca.* 52)	(2.040)	(26)
ML_2	1.951	163	*ca.* 2.265	*ca.* 163	1.952	163.3	2.274[f]	166[f]
	(1.976)	(53.9)			(1.978)	(54.2)	(2.057)	(15)
ML_2H	—	—	[b]	[b]	—	—	—	—
ML_2H_{-1}	—	—	[b]	[b]	—	—	—	—
MLH_{-3}	—	—			—	—	*ca.* 2.206[c]	*ca.* 201[c]
$VO(OH_3)^{-}$ [b]			—	—	1.949	163.3	—	—
					(1.975)	(53.1)		

[a] 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_{VO} = 0.005 M at pH = 2.7. [b] 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 $g_{\parallel}$. [c] Obtained from solutions also containing $CuLH_{-2}$. [d] There is no indication for the formation of this species. [e] Shf (superhyperfine) coupling is clearly seen for MLH_{-2} (see text). [f] 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).

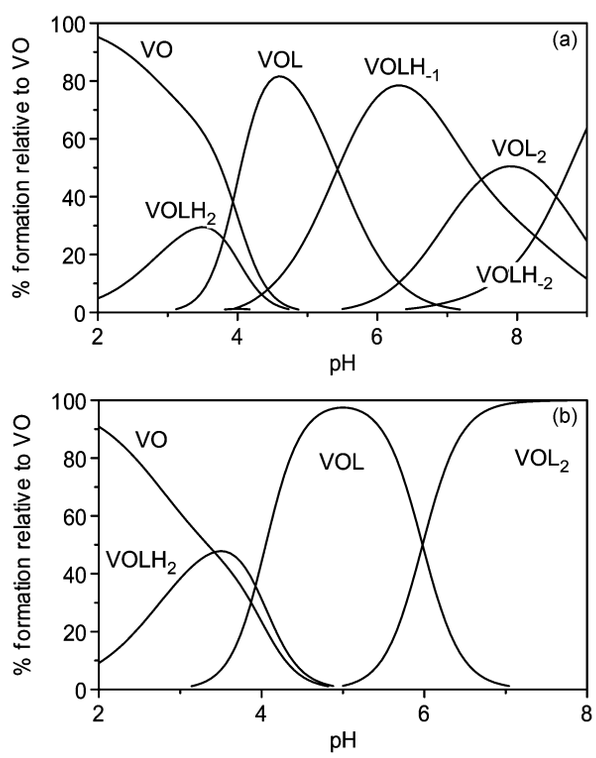

Fig. 6 Concentration distribution curves of complexes formed in solutions containing (a) $V^{IV}O^{2+}$ and H_2sal-RGG, with C_{VO} = 5 mM and L : M = 4, and (b) $V^{IV}O^{2+}$ and H_2sal-RGGG with C_{VO} = 7 mM and L : M = 5, calculated using the stability constants listed in Table 1.

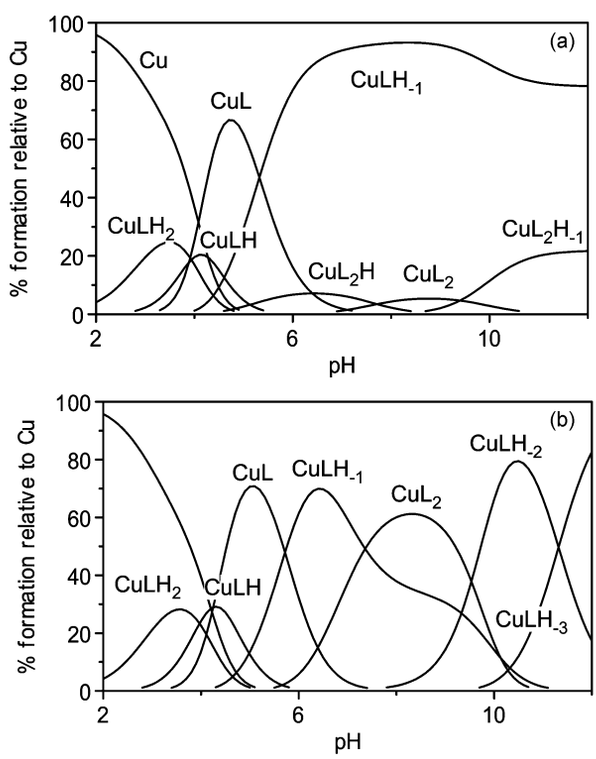

Fig. 7 Concentration distribution curves of complexes formed in solutions containing (a) Cu^{2+} and H_2sal-RGG, with C_{Cu} = 3 mM and L : M = 4, and (b) Cu^{2+} and H_2sal-RGGG with C_{Cu} = 6 mM and L : M = 4, calculated using the stability constants listed in Table 1

system where for solutions with L : M = 1–4 the VIS spectra do not change significantly with the pH; for the Cu^{II}–H_2sal-RGGG system the CuL_2 stoichiometry is relevant for L : M > 1 [*e.g.* Fig. 7(b)]. As for CuL, the presence of $O_{phenolate}$, which forms relatively strong bonds with Cu^{II}, favours CuL_2 formation as compared with species $CuGGGH_{-1}$ and $CuGGGH_{-2}$ in the Cu^{II}-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 $O_{phenolate}$ donors. The EPR parameters are also compatible with an N_2O_2 donor set.[52]

The formation of complexes with the **MLH_{-2}** stoichiometry is relevant in most systems studied here.

Only for the Cu–H_2sal-RGG system there is no pH-metric or spectroscopic indication of its formation. With L = sal-$RGGG^{2-}$ this stoichiometry starts being relevant at pH > 8

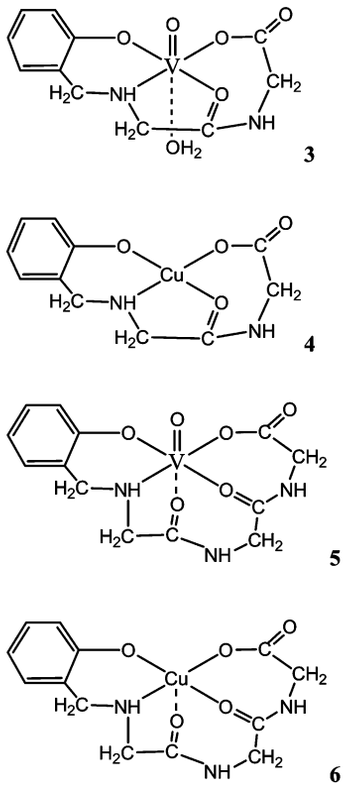

Fig. 8 Plausible binding modes for compounds **3**–**6**, all corresponding to ML stoichiometries (see text).

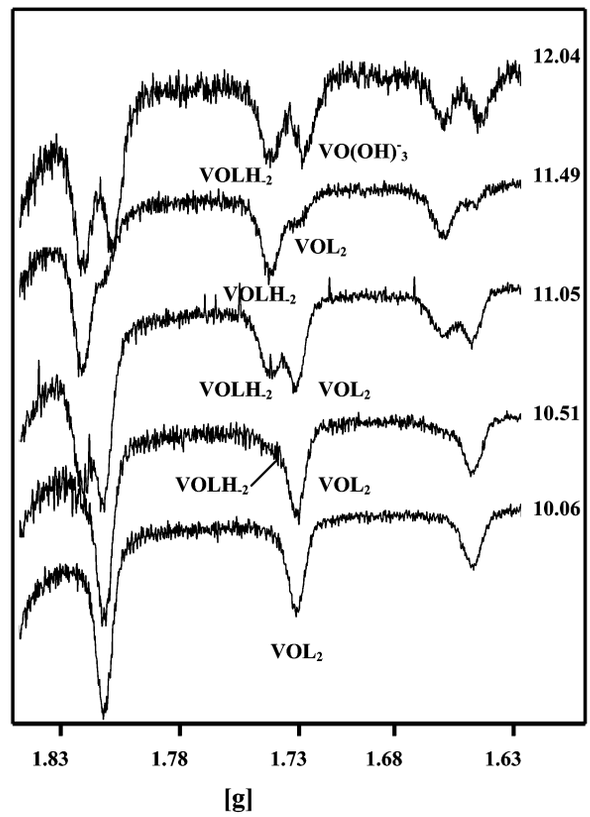

Fig. 9 High-field range (g values of *ca.* 1.6–1.8) of the first-derivative EPR spectra at 77 K of frozen 'solutions' containing $V^{IV}O^{2+}$ and H_2sal-RGGG with C_{VO} = 8 mM, L : M = 4 and 5% DMSO in the pH range 10–12. The pH values are indicated as well as the species that originate the peaks (see text).

(for $V^{IV}O$ 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 $V^{IV}O$ complexes to hydrolyse.

By using the additivity relation [eqn. (1)], the donor set $(O_{phenolate}, N_{amine}, N_{amide}, OH^-)_{equatorial}(O_{amide}$ or $O_{carbox})_{axial}$ gives $A_{\parallel}^{est} = 154 \times 10^{-4}$ cm^{-1}, which compares reasonably well with the value of $(158 \pm 2) \times 10^{-4}$ cm^{-1} obtained for $VOLH_{-2}$ (L = sal-RGG^{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 $VOLH_{-1}$,[37] agrees with the equatorial co-ordination of an OH^- group. For the $V^{IV}O$–H_2sal-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 ≈ 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 = sal-RGGG^{2-}) appears to be similar to that for L = sal-RGG^{2-}.

The $CuLH_{-2}$ (L = 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.[52] 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[51] taking two N_{amide} ($N_{\perp}$ = 15 G, $N_{\parallel}$ ≈ 11 G) and one N_{amine} ($N_{\perp}$ = 13 G, $N_{\parallel}$ ≈ 9 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_2sal-RGG system for L : M > 4. For the Cu-GlyGly system these stoichiometries were also found (MGG_2H only for very high L : M ratios, no VIS spectral data being available).[11] 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 VIS spectrum calculated for CuL_2H (L = **1**) [Fig. 3(b)] is similar to that of CuL_2, the ε values of the bands with λ_{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 $CuLH_{-1} \longrightarrow CuL_2H_{-1}$ the second sal-RGG^{2-} binds through the $O_{phenolate}$ and N_{amine} groups, the binding mode of CuL_2H_{-1} being ($O_{phenolate}$, N_{amine}, N_{amide}, $O_{carboxylate}$), ($O_{phenolate}$, $N_{amine})_{axial}$.

As far as we may conclude, the formation of complexes with the **MLH_{-3}** stoichiometry is only relevant for the Cu–H_2sal-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 $CuGGGH_{-3}$,[11] *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,[21,23–28] 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 ^{1}H NMR. However, in the case of the $V^{IV}O$ complexes, oxidation of the metal ion takes place easily (see ESI †).

The ^{51}V NMR spectra of solutions containing vanadate (3 mM) and ligand **1** (15, 25 or 30 mM) (*i.e.* L : M ≥ 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}H NMR spectroscopy. The ^{1}H NMR spectra of these solutions agree with those of the free ligand at identical pH values. For freshly prepared solutions containing $V^{IV}O$ (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}V NMR and ^{1}H NMR were identical to those obtained when starting with V^{V} instead of V^{IV} salts.

In the ^{51}V NMR spectrum of freshly prepared aqueous solutions containing V^{V} (3 mM) and **2** (*ca.* 20 mM) at pH 8, besides the V_1, V_2, V_4 and V_5 peaks, a signal was observed at −528 ppm, corresponding to *ca.* 14.2% of the total V^{V} signal. After about 24 h the intensity of the signal of this V^{V}-sal-RGGG complex increased up to *ca.* 22% of the total V^{V} (Fig. 10). These results indicate that ligand **2** forms more stable

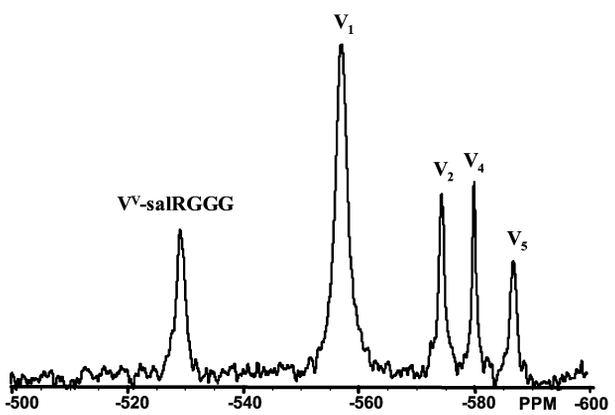

Fig. 10 ^{51}V NMR spectra of a solution containing 3 mM vanadate and 20 mM H_2sal-RGGG at pH = 8 after 24 h of mixing.

V^{V} complexes than ligand **1**, the chemical shifts differing from those of GlyGly- and GlyGlyGly-V(v) complexes (at −505 ppm).[16] The chemical shift of the V^{V}–sal-RGGG complex formed is different from that of the V^{V}–sal-RGG complex, but is close to that for $V^{V}O$(BSsal-Gly)(H_2pt) (BSsal-Gly^{2-} = *N*-salicylidene glycinato^{2-}; H_3pt = glycerol).[55] In the latter complex the binding mode involves ($O_{phenolate}$, N_{imine}, $O_{carboxylate}$, $O^{-}_{glycerol}$) and $OH_{glycerol}$ (weak). This suggests that the complexes that correspond to the −528 ppm peak involve at least ($O_{phenolate}$, N_{amine}, $O_{carboxylate}$, HO^{-}). No clear indication of N_{amide} co-ordination is available but this group presumably also binds to V(v).

Conclusions

The reduced Schiff bases H_2sal-RGG and H_2sal-RGGG containing the GlyGly or GlyGlyGly moieties form stable complexes with $V^{IV}O$ and Cu^{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 H_2sal-RGG and H_2sal-RGGG behave as good anchoring donor sites and ML and ML_2 complexes form in higher concentrations than in the corresponding M-GlyGly or M-GlyGlyGly systems. The increased stability induced by the additional phenolate-O donor was found to be particularly relevant for H_2sal-RGG: hydrolysis of the MLH_{-1} complexes starts several pH units higher than for the GlyGly system and the ML_2 complexes also form in significant concentrations. While for the $V^{IV}O$–H_2sal-RGG system the N-amide deprotonation/co-ordination is promoted significantly, it was not so favoured in the $V^{IV}O$–H_2sal-RGGG system, and the ML_2 species is much more important, namely in the physiological pH range. A somewhat similar situation was found in the corresponding Cu^{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 Cu^{II} is significantly more favoured than to $V^{IV}O$. This can be explained by the difference in the affinity of the two metal ions to the anchoring NH_2 group. However, the presence of an additional phenolate at the N terminus makes the $V^{IV}O$ binding ability of the ligands more favoured, and very similar to that of Cu^{II}.

When the $V^{IV}O$–H_2sal-RGG and $V^{IV}O$–H_2sal-RGGG complexes are dissolved in water in the presence of air, oxidation of V^{IV} to V^{V} occurs, as demonstrated by ^{51}V NMR spectroscopy. In contrast to V^{IV}, the V^{V} complexes formed with H_2sal-RGGG were found to be more stable than those of H_2sal-RGG.

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