Supramolecular Agent–Simulant Correlations for the Luminescence Based Detection of V-Series Chemical Warfare Agents with Trivalent Lanthanide Complexes


Abstract: Solution spectroscopic investigations into the interactions of eight potential bidentate V-series organophosphorus chemical warfare agent (OP CWA) simulants with [Eu(phen)₂(NO₃)₃]·2H₂O demonstrated that the chemical and structural composition of the secondary binding site within the simulant was of paramount importance. Only simulants containing both phosphoryl/phosphonyl and amine moieties generated analogous spectroscopic behaviours to V-series OP CWAs seen in previous studies. The results demonstrated that the bidentate chelation mechanism was driven by the phosphoryl/phosphonyl moieties and that the presence of the amine moieties induced a significant secondary dynamic luminescence quenching mechanism. The binding modes of the simulants VO and TEEP to trivalent lanthanides (Eu and La) were further investigated using ¹H and ³¹P NMR spectroscopic titrations and kinetic IR experiments. VO, with both the phosphoryl and amine binding sites was found to be the most appropriate simulant for V-series OP CWAs in supramolecular studies with trivalent lanthanide ions and we recommend VO for use in supramolecular studies of this type.

Introduction

V-Series Chemical Warfare Agents and Simulants

The V-series chemical warfare agents (CWAs) are a sub-class of the traditional organophosphorus (OP) CWAs and contain characteristic phosphorus–sulfur bonds and tertiary amine moieties. The V-series OP CWAs are comprised of phosphonothioates such as VX, VE and VM and the phosphorothioate, VG (Amiton, Figure 1). V-series OP CWAs are extremely potent and fast acting acetylcholinesterase inhibitors with the lethal dose for VX being approximately 6–10 mg for a 70 kg man[11] making them amongst the most toxic substances ever synthesized on a large scale.

![Chemical structures of selected V-series OP CWAs and the investigated V-series simulants.](http://doc.rero.ch)

Figure 1. Chemical structures of selected V-series OP CWAs and the investigated V-series simulants.

V-series OP CWAs affect the body via the same acetylcholinesterase pathway as the G-series OP CWAs (phosphonofluoridates and phosphoramidocyanidates, Figure 2), however their
differing chemical and physical properties can require modified approaches to hazard mitigation and detection. For example, V-series are viscous liquids with low volatility at room temperature (VX, 75 mg/m³ vs. sarin, GB, 22,000 mg/m³)\(^2\) that pose a persistent (skin) contact hazard rather than a transient inhalation hazard. These differences require specific and appropriate low toxicity simulants for each of the OP CWA series for the safe and accurate testing and evaluation of new and existing hazard mitigation and detection technologies.

Figure 2. Chemical structures of selected G-series OP CWAs and related simulants.

Detection of OP CWAs and Simulants with Complexes of Trivalent Lanthanide Ions

When a trivalent lanthanide ion is coordinated to a suitable ligand and irradiated with light (in the absorption band of the ligand), energy is transferred from the ligand to the lanthanide centre generating a singlet excited state. Upon relaxation this yields an intense, narrow, metal-centred, characteristic luminescent emission.\(^3\) This process is called the antenna effect. These unique photophysical properties, combined with the coordinative affinity of trivalent lanthanide ions for phosphoryl (P=O) bonds, has led to the recent development of numerous trivalent lanthanide ion containing supramolecular OP CWA simulants sensing systems.\(^4\) The sensing events of these systems rely upon modulation of the luminescence emission of the trivalent lanthanide ion in the presence of the target OP CWA. This led to the relative binding affinities of the trivalent lanthanide ion for the phen ligand vs. the titrated OP CWA (or simulant). The V-series OP CWAs were proposed to coordinate via the phosphoryl/phosphonyl and amine coordination sites to form a seven-membered bidentate chelate ring with the lanthanide ion, displacing the phen ligand (Figure 3).\(^9,10,11,12\) This indicated that at low molecular equivalents of the target analyte the system was dominated by a collisional, dynamic quenching mechanism rather than a competitive binding mechanism.

Previously we have demonstrated high selectivity for V-series over G-series OP CWAs and the suitability of low toxicity G-series simulants in lanthanide complexation studies.\(^9,10\) However, no such correlative studies between V-series OP CWAs and simulants exists and thus we address this knowledge gap herein.
This study identifies low toxicity and readily accessible (commercially or synthetically) simulants for V-series OP CWA studies to enable accurate, safe and routine experimentation with lanthanide based detection systems outside of The Organisation for the Prohibition of Chemical Weapons (OPCW) declared facilities. We report V-series OP CWA–simulant correlations that demonstrate that the amine moiety in the V-series OP CWAs results in a significant secondary dynamic quenching component. Relative influence of the dynamic and static components on the luminescence quenching mechanism is presented and binding affinities calculated. This highlights the importance of the secondary binding site on the binding affinities and the specificity of the system for V-series OP CWAs. Finally, selected V-series simulants (VO and TEEP) were used to further elucidate the binding modes of the V-series OP CWAs via $^1$H and $^{31}$P NMR spectroscopic titrations and kinetic IR experiments.

**Results and Discussion**

Eight potentially bidentate (or tridentate in some cases) V-series OP CWA simulants were selected in order to vary the secondary binding site, steric bulk or bidentate chelate ring size (number of atoms between the binding sites), Figure 1. Simulants were selected based upon known structure–activity relationships to ensure that compounds with low acetylcholinesterase inhibitory activity were selected. Replacement of P–C bonds (using a phosphorus rather than a phosphonate core), removal of good leaving groups and changing a P–S moiety to a P–O moiety are all known to reduce acetylcholinesterase activity of OP compounds. For example, the LD$_{50}$ of DEPATESE is 500 mg/kg (white rats, topical),$^{[14]}$ and it is known that the thio compounds (VX series OP CWAs bioadducts for the analysis of blood samples to confirm exposure and intoxication with these agents.$^{[16]}$ The synthesis of VO, DEPATESE (phosphoric acid, diethyl 2-ethoxyethyl ester) and DEPATEXE [phosphoric acid, diethyl 2-(ethylthio)ethyl ester] was achieved using the reaction sequence outlined in Scheme 1. Products were characterised by $^1$H, $^{13}$C and $^{31}$P NMR spectroscopy, GC–MS and high resolution mass spectroscopy (see ESI). The simulants Ac-Ser(MPE)-OMe [methyl N-acetyl-O-(ethoxy(methyl)phosphonyl)-L-serinate] and Cbz-Ser(MPE)-OBn [benzyl N-((benzylxoy)carbonyl)-O-ethoxy(methyl)phosphonyl)-L-serinate] were originally prepared as V-series OP CWA analogues) show considerably higher (by a factor of 10$^4$) cholinesterase-inhibiting properties than corresponding oxygen compounds (VO analogues).$^{[15]}$

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![Scheme 1. Synthetic strategy used for the preparation of the V-series OP CWA simulants, a: DEPATESE and DEPATEXE, b: VO.](Image)

It should be noted that the pesticide Demeton-S (O,O-di-methyl S-[2-(ethylsulfanyl)]ethyl phosphorothioate) was used in recent bidentate chelation studies with Eu$^{3+}$. However, in our opinion the very high toxicity and acetylcholinesterase inhibitory behaviour of this compound makes its use as a simulant undesirable and therefore it was not investigated in this study.

**Spectroscopic Luminescence Emission and UV/Vis Absorbance Behaviours**

Solutions of the $[\text{Eu(phen)}_2(\text{NO}_3)_3]_2\text{H}_2\text{O}$ complex (1 × 10$^{-3}$ mol dm$^{-3}$) were prepared in 1:9 anhydrous DMF/MeCN. These solutions were further diluted in MeCN ([complex] = 1 × 10$^{-5}$ mol dm$^{-3}$) and the emission and absorbance spectra recorded. Working with low toxicity simulants allowed us to use a higher resolution instrument than employed in the original OP CWA studies. This later instrument detected finer structures in the $[\text{Eu(phen)}_2(\text{NO}_3)_3]_2\text{H}_2\text{O}$ complex absorbance that were not observed in our previous studies. UV/Vis spectra of free 1,10-phenanthroline and $[\text{Eu(phen)}_2(\text{NO}_3)_3]_2\text{H}_2\text{O}$ standards were obtained on both instruments to demonstrate the resolution differences and to determine that the changes in the complex spectra were not due to degradation. These spectra were comparable to those found in the literature on higher resolution spectrophotometers and are available in the ESI (Figures S37 and S38).

In luminescence studies the characteristic narrow emission band for Eu$^{3+}$-complexes at 617 nm was observed. Solutions of the V-series simulants in anhydrous MeCN were titrated into the prepared solution of the europium complex.

**Amine Containing Simulants: VO, DADSA and TMBD**

Additions of VO to a solution of $[\text{Eu(phen)}_2(\text{NO}_3)_3]_2\text{H}_2\text{O}$ resulted in immediate reduction of the luminescence emission (Figure 4), with complete quenching achieved upon the addition of seven equivalents (94 % quenching achieved with four equivalents). As found with VX and VG$^{[9]}$ a Stern–Volmer (SV) plot of $I_0/I$ (where $I_0$ is the complex emission intensity, and $I$ is the intensity in the presence of the analyte or quencher) vs. the concentration of the analyte did not yield a straight line and thus the static quenching constants for VO ($K_q$) could not be determined via this methodology (Figure 5). Similarly, the plot

![Figure 4. Quenching of the luminescence emission of $[\text{Eu(phen)}_2(\text{NO}_3)_3]_2\text{H}_2\text{O}$ upon the addition of VO; where $\lambda_{em} = 617$ nm and $[\text{complex}]_{final} = 1 \times 10^{-5}$ mol dm$^{-3}$, 293 K. Inset luminescence quenching titration profile.](Image)
for VO displays an upward curvature away from the x-axis at VO concentrations of approximately $1.6 \times 10^{-5}$ mol dm$^{-3}$ (1.6 molar equivalents) and higher. This slight upward curvature is indicative of the presence of multiple quenching mechanisms (static quenching dominant), and becomes increasingly pronounced as the VO concentration increases during the titration.

Additions of the diamines DADSA and TMBD displayed complete luminescence quenching of the [Eu(phen)$_2$(NO$_3$)$_3$]·2H$_2$O ($\lambda_{em} = 617$ nm) with VO with up to seven molar equivalents added, outlier removed at [VO] = $4 \times 10^{-5}$ mol dm$^{-3}$. Inset: selected region of the Stern–Volmer plot (0–3 molar equivalents where [complex]$_{initial} = 1 \times 10^{-5}$ mol dm$^{-3}$, 293 K).

Figure 5. Full Stern–Volmer plot for the quenching of the luminescence emission of [Eu(phen)$_2$(NO$_3$)$_3$]·2H$_2$O ($\lambda_{em} = 617$ nm) with VO with up to seven molar equivalents added, outlier removed at [VO] = $4 \times 10^{-5}$ mol dm$^{-3}$. Inset: selected region of the Stern–Volmer plot (0–3 molar equivalents where [complex]$_{initial} = 1 \times 10^{-5}$ mol dm$^{-3}$, 293 K).

Significant changes were also observed in the absorption spectra immediately upon addition of VO (Figure 6). Specifically, the loss of absorption intensity related to the coordinated phen ligand at 271 nm with the simultaneous absorbance increase and bathochromic shift to the free phen absorbance to 263 nm (isosbestic point at 266 nm) was observed. This indicated competitive binding of VO to the lanthanide ion. Similar absorbance behaviours were observed for TMBD and DADSA (ESI Figures S20 and S21). The presence of the disulfide moiety imparts an additional unknown electronic influence onto the system.

As seen previously with the structural differences between VX and VG, replacement of a P–C bond with an additional O–alkyl moiety resulted in a decrease in binding affinity. Analogously, the replacement of the P–S bond (in VX) with a P–O moiety appears to have a similar effect in reducing the Lewis basicity of the phosphonyl moiety of VO and hence the binding affinity.

Amine-Free Simulants: TEEP, DEPATESE, DEPATEXE, Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn

With the exception of TEEP, the other bi- (and tri-) dentate simulants and bioadducts investigated all generated similar changes to the Eu$^{3+}$ complex luminescence to those observed with the monodentate G-series OP CWA, sarin, and related G-series simulants. After titration of up to one hundred equivalents, neither DEPATESE, DEPTAEXE, Ac-Ser(MPE)-OMe or Cbz-Ser(MPE)-OBn had fully quenched the luminescence of the [Eu(phen)$_2$(NO$_3$)$_3$]·2H$_2$O complex solution (Figure 7). However, TEEP demonstrated luminescence quenching activity between that observed for VO and the amine-free simulant with ca. 75 % quenching with the addition of fifty equivalents (Figure 7, a).

Table 1. log $K_{assoc}$ constants for the selected V-series simulants by [Eu(phen)$_2$(NO$_3$)$_3$]·2H$_2$O, where [complex]$_{initial} = 1 \times 10^{-5}$ mol dm$^{-3}$, 293 K.

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<thead>
<tr>
<th>Analyte</th>
<th>log $K_{assoc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEEP</td>
<td>5.4 ± 0.075</td>
</tr>
<tr>
<td>VO</td>
<td>4.8 ± 0.041</td>
</tr>
<tr>
<td>DADSA</td>
<td>4.0 ± 0.025</td>
</tr>
<tr>
<td>TMBD</td>
<td>3.8 ± 0.060</td>
</tr>
<tr>
<td>VX$^{[a]}$</td>
<td>5.1 ± 0.12$^{[b]}$</td>
</tr>
<tr>
<td>VG$^{[a]}$</td>
<td>4.5 ± 0.03</td>
</tr>
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</table>

(a) Values reproduced with permission from The Royal Society of Chemistry.[9] (b) Standard deviation of the refined parameters from the least-squares fitting of binding isotherms.
Figure 7. A comparison of the luminescence titration profiles of \([\text{Eu(phen)}_2(\text{NO}_3)_3] \cdot 2\text{H}_2\text{O}\) upon additions of the simulants DEPATESE, DEPATEXE, Ac-Ser(MPE)-OMe, Cbz-Ser(MPE)-OBn and TEEP, where \([\text{complex}]_{\text{initial}} = 1 \times 10^{-5} \text{ mol dm}^{-3}, 293 \text{ K}.\) a) Ten molar equivalents added with a comparison to VO included. b) Full one hundred equivalent concentration range.

The full SV plots for DEPATESE and DEPATEXE displayed downward curvature towards the \(x\)-axis (DEPATEXE in Figure 8 inset). This was also observed in our previous studies\(^{[10]}\) with the collisional (dynamic) non-radiative luminescence G-series simulants DCP (at low concentrations) and DMMP and with GB at high concentrations. It was postulated that such curvature may be the result of steric hindrance preventing some quenching processes.\(^{[10]}\) At low equivalents, the SV plots of DEPATESE (ESI Figure S27) and DEPATEXE (Figure 8) yielded straight lines giving quenching constants \((\log K_{sv})\) of 3.45 and 3.49 respectively (Table 2). These quenching constants were similar to those obtained for the G-series OP CWAs and simulants presented previously \((\log K_{sv} 3.56 \text{ for GB})\) and are indicative of a dynamic quenching mechanism.\(^{[10]}\)

The bioadducts Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn with their additional secondary and tertiary amide and carbonyl binding sites displayed limited luminescence quenching at low concentrations \((< \text{ten equivalents; Figure 7, a). This behaviour diverged at higher concentrations (more than twenty equivalents; Figure 7, b) such that at one hundred equivalents Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn quenched the luminescence of the system by 57 \% and 40 \%, respectively. The more pronounced quenching differences between these two bioadducts at high analyte concentrations is likely to be the result of disparities in steric bulk around the coordination centres. Accordingly, the SV plot for Ac-Ser(MPE)-OMe demonstrated only limited negative curvature suggesting steric hindrance was less of a factor with this compound (ESI, Figure S29) than for Cbz-Ser(MPE)-OBn which displayed negative curvature (ESI Figure S31) over the whole concentration range. At low equivalents the plot was fitted to a straight line that gave a \(\log K_{sv}\) generated for Ac-Ser(MPE)-OMe at low concentrations \((< \text{ten equivalents})\) was 3.30 (Table 2).

The UV/Vis absorbance spectra for the amine-free simulants \([\text{DEPATESE, DEPATEXE, Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn}]\) displayed only minor decreases in the absorbance band attributed to the complexed phen ligand \((271 \text{ nm})\) with addition of less than ten equivalents of simulant. This indicated the presence of a dynamic collisional process and not competitive binding, i.e. there was no change to the ground state of the luminescent complex.

Again, TEEP generated similar changes to the absorbance spectra as seen for the amine containing simulants (ESI Figure S42), indicating competitive binding and displacement of the phen ligand as the mechanism of luminescence quenching. Using HypSpec gave \(\log K_{\text{assoc}}\) for TEEP as 5.4 ± 0.075 (Table 1) which was the highest affinity binding event out of the simulants tested.

### Analysis of Combined Static and Dynamic Quenching Processes

Although TEEP demonstrated the highest affinity competitive complexation event, the exact mode of complexation was un-
clear. By comparison to closely related ligands, a bidentate seven-membered chelate was postulated to form in solution.[20] Conversely, solid-state X-ray crystallographic structures of TEEP complexes with lanthanide ions indicate the formation of dinuclear and/or polymeric complexes in which TEEP plays the role of a bridging ligand,[18] although inference of solution structures from solid-state data can be misleading. Nonetheless, the observed behaviour of TEEP establishes that the phosphonyl/phosphoryl moiety is a suitable ligand for Ln3+ centres. This observed behaviour of TEEP establishes that the phosphonyl/phosphoryl moieties from solid-state data can be misleading. Nonetheless, the trend in luminescence quenching behaviours (Figure 9) demonstrates that the amine containing ligands are more effective quenchers than phosphonyl/phosphoryl groups alone.

Table 3. Log Kassoc and estimated dynamic quenching constants (log KD) for the various V-series OP CWAs and simulants investigated.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>log Kassoc</th>
<th>log KD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO</td>
<td>4.8</td>
<td>2.5</td>
</tr>
<tr>
<td>VX</td>
<td>5.1</td>
<td>2.3</td>
</tr>
<tr>
<td>VG</td>
<td>4.5</td>
<td>3.2</td>
</tr>
<tr>
<td>TMBD</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>DADSA</td>
<td>4.0</td>
<td>=0</td>
</tr>
<tr>
<td>TEEP</td>
<td>5.4</td>
<td>=0</td>
</tr>
</tbody>
</table>

Derivation of the association constants Kassoc from analysis of the quantitative UV/Vis titration data allows us to subsequently use the luminescence quenching data to estimate the extent of dynamic quenching occurring in solution. Extended SV plots of the luminescence data, in which [(I/I0−1)/Q] is plotted against [Q] yields both static, Kassoc and dynamic, KD quenching constants as the slope of the extended SV plot is equal to Kassoc × KD. KD can therefore be estimated using the Kassoc values determined from the UV/Vis titration data.

Using this estimation method, it was found that VO and VX behaved similarly, with log KD values of 2.5 and 2.3, respectively (Table 3). For VG, the dynamic quenching component was found to be somewhat greater at log KD 3.2, and for TMBD a significantly larger value of log KD 4.1 was determined. Both DADSA and TEEP luminescence quenching behaviour was not amenable to extended SV analysis, and the KD values are assumed to tend towards zero (absent) for low quencher concentrations.

With only a relatively small set of quenchers it is challenging to draw conclusions as to the exact nature of the dynamic quenching processes, though the impact of the amine moiety at low equivalents is obvious. Notably, the monodentate simulants DMMP and DCP, both lacking in amine groups were only capable of dynamically quenching the complex luminescence at much higher concentrations.[10] Dynamic quenching by tertiary amines could arise from collisional non-radiative decay (with appropriate oscillators) or intermolecular photoinduced electron transfer (PET). The absence of dynamic quenching in the case of TEEP further supports the conclusion that tertiary amines play a dominant role in the dynamic quenching processes for the other ligands. In contrast to the ambiguity of the dynamic quenching mechanism, static (ground state) quenching is clearly the result of displacement of the phen antenna ligand.

The similarities between the VO and VX quenching constants (Kassoc and KD) reinforce the importance of structural similarities when selecting appropriate supramolecular simulants. Further, a decrease in steric bulk around the amine nitrogen atom could account for the greater dynamic quenching component in the case of VG. Although the phosphorus centre is also modified with respect to VO and VX, this is more likely a factor in the differences in observed Kassoc values. The low steric bulk around the two nitrogen centres in TMBD (vs. one nitrogen centre in VX, VG and VO) may account for its high dynamic quenching component, although in this ligand there are also a large number of C–H oscillators available for collisional deactivation of the complex excited states. Surprisingly, DADSA showed little evidence of any dynamic quenching process with only slight upward curvature in the SV plot not sufficient to allow for further analysis. This anomalous result could be the result of multiple steric, structural and electronic differences between DADSA and the other simulants.

Ultimately, these results demonstrate that the competitive bidentate chelate complexation event is sensitive to changes to the composition of the secondary binding site. Specifically, the presence of an amine group is required, as species incorporating ethers, thioethers, carbonyls and amides demonstrated spectroscopic behaviours analogous to monodentate G-series OP CWA and their simulants.

The luminescence quenching behaviour of diamine containing simulants was shown to be analogous to V-series OP CWAs, whilst their binding behaviours were not. The reverse was true for the diphosphonate, TEEP (Figure 9). This allowed for the identification of a significant secondary dynamic quenching mechanism from the presence of the amine moieties in VO, VX and VG and thus complete luminescence quenching of the system is not necessarily the end point of the competitive binding process as multiple quenching processes are at play (i.e. static and dynamic).
In an effort to gain further insight into the interaction between the V-series OP CWAs and simulants with trivalent lanthanide ions, $^1$H and $^{31}$P NMR titrations were undertaken using the diamagnetic La$^{3+}$ ion with VO and TEEP. VO was chosen as it was the only simulant shown to generate analogous UV/Vis and luminescence behaviours to the V-series OP CWAs, while TEEP was investigated as a low toxicity, commercially available example of a static quencher, despite the absence of a secondary dynamic quenching mode.

Titrations of La(NO$_3$)$_3$(H$_2$O)$_6$ in anhydrous MeCN into solutions of VO (4 × 10$^{-5}$ mol dm$^{-3}$) and TEEP (2 × 10$^{-5}$ mol dm$^{-3}$) were performed with a phosphoric acid in D$_2$O external standard (internal NMR tube). $^1$H and $^{31}$P NMR spectra were obtained after each addition of lanthanum salt. Blank additions of MeCN were also performed (ESI Figures S47 and S50) to demonstrate that chemical shift and peak shape changes observed were the result of lanthanum nitrate additions and not minor concentration changes. Full titration data (chemical shifts) and spectra are available in the ESI (Table S1, Figures S47 and S48).

Analysis of the $^{31}$P signal of VO upon 0.05 equiv. additions of La(NO$_3$)$_3$(H$_2$O)$_6$ demonstrates signal broadening with additions to up to 0.8 equiv. of La$^{3+}$ which appears to indicate exchange between several VO:La species in solution (Figure 10). This downfield progression ($\Delta \delta \approx 4$ ppm) is complete after the addition of 0.8 equiv. with further additions leading to small upfield perturbations and deformation of the $^{31}$P signal. These changes to peak shape and chemical shift of the $^{31}$P signal are consistent with deshielding of the phosphorus nucleus due to the electronic effects of the oxygen–lanthanum interaction (P=O···La$^{3+}$).

Of particular interest are the downfield shifts of the resonances arising from the disopropylamino moiety, at $\delta = 1.69$ ppm (doublet) and 3.70 ppm (septet). The doublet is shifted downfield from 1.69 to 1.97 ppm ($\Delta \delta = +0.28$ ppm), whereas the septet is shifted from 3.70 to 4.48 ppm ($\Delta \delta = +0.78$ ppm). There is a small (2 %) impurity in the VO sample resulting from its synthesis (1.75, 3.33, 3.80, 4.12 ppm). This material interacts with the lanthanum ion in a similar manner to VO and its resonances are shifted during the titration making the $^1$H NMR spectra appear more complicated than anticipated.

Given the isopropyl position within VO, if coordination to the lanthanum ion was occurring only through the phosphonyl moiety, such a substantial downfield perturbation would not be expected. Thus changes in these signals are indicative of the amine moiety interacting with the lanthanum center adding further evidence towards the bidentate chelation mechanism. Significant downfield shifts were also observed for the bridging ethylene proton resonances (Figure 11, a), with the O-methylene peaks shifted from 4.50 to 5.08 ppm ($\Delta \delta = +0.58$ ppm) and the N-methylene resonances shifted from 3.39 to 4.07 ppm ($\Delta \delta = +0.68$ ppm). The changes in the bridging ethylene group proton resonances upon the addition of La$^{3+}$ appear consistent with hindered conformations due to the formation of a chelate ring involving the VO side chain. Such significant changes in the $^1$H NMR signals of the amine substituents and ethylene bridge are indicative of the amine moiety interacting with the lanthanum centre and support a bidentate chelation mechanism.

The $^1$H NMR downfield shift of the P-CH$_3$ doublet at approximately 2.08 to 2.31 ppm ($\Delta \delta = +0.23$ ppm, Figure 11, b) is addi...
tional evidence of a significant change in the chemical environment of the phosphorus nucleus upon complexation to La$^{3+}$. The chemical shifts from the P-CH$_3$ signal and the $^{31}$P signal upon titration of La(NO$_3$)$_3$(H$_2$O)$_6$ (Figure 10) were analysed using the software package HypNMR (ESI Figure S57 and Figure S58). Whilst convergence of the data (fitting) was not able to be achieved, we have derived approximate log $K_{assoc}$ values for each of the potential species in solution based on a close visual fit of the data to a model containing the potential VO:La species shown in Table 4 (visual fit of the $^{31}$P and $^1$H NMR spectroscopic data is available in ESI Figures S57 and S58). If any of the species specified in Table 4 are removed from the model, the fit of the data significantly deteriorates.

Table 4. Approximate log $K_{assoc}$ values for the binding of VO to La(NO$_3$)$_3$(H$_2$O)$_6$ from the $^{31}$P and $^1$H NMR titrations based on a visual fit of the data in HypNMR spectroscopy.

<table>
<thead>
<tr>
<th>Species</th>
<th>log $K_{assoc}$</th>
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<tbody>
<tr>
<td>La(VO)</td>
<td>4.2</td>
</tr>
<tr>
<td>La(VO)$_2$</td>
<td>5.3</td>
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<tr>
<td>La(VO)$_3$</td>
<td>6.9</td>
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<td>La(VO)$_4$</td>
<td>8.3</td>
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<tr>
<td>La$_2$(VO)</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Although, the log $K_{assoc}$ values obtained from the NMR titrations are not directly comparable to the log $K_{assoc}$ values obtained from the luminescence titrations, they provide further qualitative evidence a bidentate chelate mechanism. In the spectroscopic investigations the association of the VO to Eu$^{3+}$ was determined relative to the stability of the [Eu(phen)$_2$(NO$_3$)$_3$]·2H$_2$O complex. The NMR investigations provide the association constant of VO with La$^{3+}$.

The high association constants estimated for La:VO speciation of 1:2, 1:3 and 1:4 (Table 4) is associated with bidentate chelate behaviour in our previous work, rather than monodentate interactions$^{9,10}$ and supports the conclusions based on the $^1$H NMR shifts noted previously.

**TEEP**

An analogous $^{31}$P NMR titration to that carried out with VO was performed with TEEP (ESI Figure S49). In this case only minimal changes to the $^{31}$P resonance chemical shift were observed ($\Delta \delta \approx 0.2$ ppm) with the peak first shifting upfield (0–0.4 equiv.) then downfield (0.4 + equivalents). In addition, the $^{31}$P signal is broadened after the addition of 0.4 equiv. of La$^{3+}$. These small changes are likely the result of equilibrium between free TEEP and various complexes involving TEEP and were unexpected given the substantial changes observed with VO. This may reflect steric requirements around the bis-phosphonyl ligand, reduced Lewis basicity or the lack of the second nitrogen coordination site in TEEP. This observation may also correlate to the solid state coordination behaviour of TEEP (as a bridging ligand rather than a bidentate chelate ligand). Due to these observations $^1$H NMR titrations were not performed using TEEP.

**In-Situ FTIR Kinetic Analysis**

In order to investigate the reaction kinetics of the lanthanide-VO binding event and to provide information on the potential detection (binding) rates for V-series OP CWAs and simulants with lanthanides, in-situ FTIR experiments were undertaken. In these experiments one equivalent of Eu(NO$_3$)$_3$(H$_2$O)$_5$ in MeCN was added all at once to a solution of TEEP in MeCN or VO in MeCN/DMF and IR spectra were collected over a period of 10 min. Concentration calibration curves for TEEP and VO were also obtained (ESI Figure S59 and Figure S60).

**TEEP**

Upon addition of one equivalent of Eu(NO$_3$)$_3$(H$_2$O)$_5$ to the solution of TEEP, significant changes to the fingerprint region of the IR spectra were observed over a 25 second timeframe. In the region of interest shown in Figure 12 (a), development of a nitrate stretch at 1296 cm$^{-1}$ was observed, whilst the signal intensity of the P–O–C stretches at 1030 cm$^{-1}$ and 1054 cm$^{-1}$ were noted to increase and broaden. Of particular interest were the characteristic phosphonyl stretches of free TEEP at 1247 cm$^{-1}$ (major band), 1197 cm$^{-1}$ and 1157 cm$^{-1}$ (minor bands). Upon addition of the lanthanide the major TEEP phosphonyl band was observed to shift 27 cm$^{-1}$ to 1220 cm$^{-1}$. The shifting of this band over time can be clearly seen in the 3D waterfall image in Figure 12 (b) and is consistent with previous solid state (non-kinetic) studies of diphosphonate complexation.
to lanthanide ions, where low frequency shifts of the P=O band of around 30–80 cm$^{-1}$ were noted.\cite{20} The changes in concentration with time of the initial phosphonyl stretch at 1247 cm$^{-1}$ and the final coordinated phosphonyl stretch at 1220 cm$^{-1}$ can been seen in Figure 13.

Using this data a rate constant of 0.0019 mol L$^{-1}$ s$^{-1}$ was calculated for the binding event. The concentrations of the product phosphonyl signal at 1220 cm$^{-1}$ were calculated using the concentration curve generated for TEEP. Despite the instrument obtaining spectra at maximum rate, insufficient data points were obtained to generate the required plots for quantitative reaction order analysis. However, the data collected confirmed that the binding event and any associated luminescence quenching via a competitive binding mechanism is likely to be rapid.

**VO**

Analogous kinetic IR investigations were attempted with VO, however, issues with sensitivity of the VO signal at the concentrations previously investigated for TEEP were encountered. Increasing the concentrations of VO in MeCN resulted in insolubility of the europium complexes formed in the solution. Changing the solvent system to DMF/MeCN (1:1) and utilising 800 mg of VO in 20 mL (0.15 mol dm$^{-3}$) gave adequate signal intensity and solubility. The difference in signal intensity was attributed to the molecular asymmetry of VO relative to TEEP.

Addition of 1 equiv. of Eu(NO$_3$)$_3$(H$_2$O)$_5$ to the solution of VO (in DMF/MeCN) resulted in rapid and significant increases in signal intensity throughout the spectrum, which was not observed with TEEP. Again, the fingerprint region demonstrated numerous spectral changes (ESI, Figure S61a). As seen with TEEP, an increase in intensity of the P–O–C bands between 980–1070 cm$^{-1}$ was observed. However, the P–CH$_3$ band was masked by the nitrate stretching signal at 1308 cm$^{-1}$. The phosphonyl band (P=O) at 1239 cm$^{-1}$ in VO, maintained intensity but subtly shifted and broadened, whilst the neighbouring (minor) phosphonyl signals at 1208 cm$^{-1}$ and 1193 cm$^{-1}$ broadened and merged into one signal (ESI, Figure S61b).

The spectral changes from the VO reaction with Eu$^{3+}$, though rapid on the timescale of the instrument, were not amenable to kinetic analysis. Comparison of the VO and TEEP reactions was further complicated by the different solvent systems necessary enforced by solubility limitations of the Eu-VO complex(es). The high concentration of DMF was undesirable given the potential for lanthanides to act as catalysts for a range of organic reactions\cite{21} including the degradation of G- and V-series CWA simulants.\cite{22} Nevertheless, the observed changes were consistent with complexation of VO to the europium centre.

The rapid response rates (chelation times) observed with VO and TEEP in these kinetic investigations, demonstrate that the complexion of V-series OP CWAs and the simulates VO and TEEP to trivalent lanthanides ions is rapid and is thus an extremely desirable starting point for the development of V-series OP CWA sensors.

**Conclusions**

This investigation describes the identification of a high fidelity simulant for luminescence sensing of V-series CWA such as VX with lanthanides. Additionally, the structural and chemical interactions contributing to the lanthanide quenching mechanism of V-series CWA and their simulants were probed using a variety of spectroscopic means including luminescence, UV/Vis, $^1$H and $^{13}$P NMR and in situ IR. The results suggest that a bidentate chelate mechanism in phosphonyl/phosphoryl-amine systems is responsible for the rapid and selective quenching of lanthanide luminescence through a competitive binding mechanism. This bidentate chelation is extremely sensitive to the composition of the secondary binding site on the phosphoryl/phosphoryl ligand. Of those investigated, only simulants containing secondary binding sites comprised of amines or phosphoryl groups resulted in competitive binding of the analyte (displacement of phen) to the europium center; amides, carbonyls, ethers and thioethers did not result in competitive binding.

Comparison of the spectroscopic behaviour of the simulants with OP CWAs\cite{9,10} demonstrated that the most appropriate simulant for supramolecular studies with V-series OP CWAs and trivalent lanthanide ions was VO. VO closely mimicked structural, spectroscopic and binding behaviours of the V-series OP CWAs. VO displays low toxicity and can be readily synthesised. We recommend the use of this material to researchers in the field.

These findings demonstrate that detection systems based upon competitive binding of V-series OP CWAs to trivalent lanthanide ions is an extremely favourable pathway to rapid, sensitive and selective detection of these compounds. Determination of the analogous behaviour of the low toxicity simulant VO to V-series OP CWAs (VX and VG) enables safe and routine experimentation outside of declared defence facilities which will facilitate the safe and accurate development and testing of V-series OP CWA detection technologies in the future.

**Experimental Section**

**Materials and Methods:** Unless otherwise indicated, all reagents and solvents were obtained from commercial suppliers (Sigma–Aldrich, Alfa Aesar, Merck) and were used without further purification.
acetonitrile with a secondary internal tube containing a phosphoric acid internal standard in D2O at 25 °C. Coupling constants (J) are reported in Hz. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), sept (septet) and m (multiplet).

GC–MS (EI) analyses were performed using an Agilent Technologies 7890A instrument equipped with an Agilent Technologies 5975C inert MSD. The measurements were made with the HP-1701 (30 m × 0.25 mm × 0.25 μm, 14 % cyanopropyl-phenyl/86 % PDMS) high resolution gas chromatography column using a temperature program (40 °C for 3 min, 13 °C/min until 280 °C and 280 °C for 3.54 min). The injector and the detector temperatures were 220 and 250 °C, respectively. Volumes of 1 μL (c = 0.5 mg/mL in hexane) were injected in the splitless injection mode. The carrier gas was helium (1 mL/min).

Luminescence experiments were performed on an Ocean Optics portable USB 4000 fluorimeter with 1000 nm fibre optic cables and a PX-2 pulsed xenon light source [Integration time (t/s) 100000, Spectra Averaged: 10, Boxcar smoothing: 0].

UV/Vis experiments were performed on a Varian Cary 50-Bio spectrophotometer. Absorbance, dual beam, band width 1.5 nm, data interval 1.00 nm, scan speed 600 nm/min, integration time 0.1 s.

IR experiments were performed on a Mettler Toledo React IR 4000 with a K6 conduit and a DiComp diamond probe. The conduit was purged continuously with dry and carbon dioxide free compressed air (via a purge gas generator). Reaction/complexation spectra were obtained using the rapid collect function.

2-Diisopropylaminoethanol Ethyl Methylphosphonate (VO): To ethyl methylphosphonochloridate (3.84 g, 27.0 mmol, 1 equiv.) in MeCN (25 mL) was added dropwise under ice bath cooling and inert atmosphere (N2) a solution of 2-diisopropylaminoethanol (4.3 g, 29.7 mmol, 1.1 equiv.) and 4-(dimethylamino)pyridine (3.6 g, 29.7 mmol, 1.1 equiv.) in MeCN (35 mL). The reaction mixture was further stirred within 0 and 10 °C for 4 h and then at room temperature overnight. After filtration (removal of the white precipitate), the crude product was purified by kugelrohr (bulb-to-bulb) vacuum distillation; b.p. 100 °C/0.1 mbar, yield 49 %, purity > 98 %, 31P-NMR spectroscopy.

2-Diisopropylaminoethanol is available from Sigma Aldrich but is a press or implied endorsement of the results or conclusions of the project by either DST Group or CTTSO or the Department of Defence of either nation.

2-(Hydrazinophosphoryl)acetonitrile was prepared as described previously.[9,10,14] 1H (400 MHz), 13C(1H) (100 MHz) and 31P(1H) (162 MHz) NMR spectra were recorded in CDC13 on a Bruker Avance III Ultrashield Plus 600 MHz NMR spectrometer and 13C NMR in d3-benzene on a Bruker Avance III HD 400 MHz NMR spectrometer in d3-benzene.

Acknowledgments

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[Eu(phen)2(NO3)3·2H2O and the bioadducts, Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn, were prepared as described previously.[9,10,14] 1H (400 MHz), 13C(1H) (100 MHz) and 31P(1H) (162 MHz) NMR spectra were recorded in CDC13 on a Bruker Avance III Ultrashield Plus 600 MHz NMR spectrometer and 13C NMR in d3-benzene on a Bruker Avance III HD 400 MHz NMR spectrometer in d3-benzene.

0.1 mL of this solution was further diluted in anhydrous MeCN (10 mL) to a 1 × 10–5 mol dm–3 solution. 0.02 mol dm–3 solutions of the quenching simulants (TEEP, VO, DADSA, TMBD) and 0.02 m of the non-quenching simulants [DEPATESE, DEPATEXE, Ac-Ser(MPE)-OMe and Cbz-Ser(MPE)-OBn] were prepared in anhydrous MeCN (a small amount of neutralised chloroform was used to help dissolve DADSA). 2 mL of the 1 × 10–5 mol complex solution was placed into a screw cap fluorescence cuvette with a PTFE septum and a small stir bar and reference fluorescence and UV/Vis spectra were obtained for the complex. Additions of the appropriate equivalents of the other solutions were made via a 10 μL syringe through the septum and the solution was allowed to stir at room temperature for one minute in-between additions before spectra were obtained.

NMR Titration Procedure: Solutions of TEEP (2 × 10–5 mol dm–3), VO (4 × 10–6 mol dm–3) and La(NO3)3(H2O)6 (0.4 mol dm–3) were prepared in d3-MeCN. 0.50 μL of the solution was placed into an NMR tube and 31P and 1H spectra of the solution were obtained. 0.05 or 0.1 molar equivalent additions of the La(NO3)3(H2O)6 solution were added to the NMR tube using a 10 μL syringe and solution mixed for approximately 1 min before 31P and 1H NMR spectra were obtained. This was repeated until 2.0 equiv. of La(NO3)3(H2O)6 had been added.

IR Kinetic Experiment Procedure: The solvent for the experiment (20 mL, MeCN or DMF/MeCN, 1:1) was placed in a 2-neck round-bottomed flask with a small oval stir bar. The probe was placed midway into the solution ensuring that the probe tip was completely covered and slow stirring commenced. Care was taken to ensure no bubbles were present on the diamond tip and that a vortex was not present in the solution. A solvent background was obtained and the appropriate simulated solution added to the solution (TEEP 200 mg, VO 800 mg).

The rapid collect function was selected and spectra of the solution in the solvent allowed to collect for 1 min. At the one minute mark, 1 equiv. of Eu(NO3)3(H2O)4 in the solvent (1 mL) was added all at once and the data collected allowed to continue for 10 min.

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Organophosphorus Agents


Supramolecular Agent–Simulant Correlations for the Luminescence Based Detection of V-Series Chemical Warfare Agents with Trivalent Lanthanide Complexes

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Solution spectroscopic investigations with potential bidentate V-series OP CWA simulants with [Eu(phen)2(NO3)3]·2H2O demonstrated the interaction and luminescence quenching mechanism to be specific and selective for bidentate systems containing both amine and phosphoryl/phosphonyl moieties. Only the low toxicity V-series simulant VO generated analogous spectroscopic behaviours to the OP CWAs VX and VG.