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Uncovering the Energies and Reactivity of Aminoxyl Radicals by Mass Spectrometry

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Uncovering the Energetics and Reactivity of Aminoxyl Radicals by Mass Spectrometry

A thesis submitted in (partial) fulfilment of the requirements for the award of the Degree

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by

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DECLARATION

I, David L. Marshall, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

D. Marshall

David L. Marshall

April 2014
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ABSTRACT

Aminoxyl radicals (R₁R₂NO•) are a well-known class of stable free radicals and play a major role in polymer chemistry as both reagents for controlled polymerisation processes and polymer stabilisers. Both of these processes involve cycling between aminoxyl radical and alkoxyamine (R¹R²NO–R³) forms. Despite their widespread industrial use, there is surprisingly little known about the intrinsic energetics and reactivity of these species that could inform our understanding of their behaviours in complex polymer matrices or reaction mixtures. In this thesis mass spectrometry is used to uncover these fundamental properties of aminoxyl radicals in the gas phase.

Alkoxyamines were prepared with an ionisable carboxylic acid moiety, remote from the NOR³ functional group. These precursors were subjected to electrospray ionisation to generate the corresponding gas phase [M – H]⁻ anions. The effect of different radical fragments (R³) on the competitive homolysis of O–C and N–O bonds was examined by collision-induced dissociation (Chapter 2). These results demonstrate that cleavage of the O–C bond is dominant for most of the R³-substituents investigated but examples of preferential N–O homolysis were observed where the O–C bond was strengthened by adjacent heteroatom(s) (e.g., R³ = CH₂F). These experimental findings are supported by theoretical calculations, which confirm trends in relative bond dissociation energetics. Importantly, calculations also predict that O–C bond dissociation energies are lowered by the presence of the remote carboxylate anion. The corollary of this finding is that gas phase acidities (GPAs) of the corresponding carboxylic acid moieties are greater in the presence of aminoxyl radicals than structurally related, but closed-shell, alkoxyamines.

To test computational predictions, relative and absolute GPAs were measured experimentally by applying the kinetic method to proton-bound dimers containing
alkoxyamines and aminoxyl radicals bearing carboxylic acid groups. The results confirm the decreased basicity of anions in the presence of aminoxyl radicals, and by extension, the increased stability of aminoxyl radicals in the presence of an ostensibly remote anion (Chapter 3). Further experiments were undertaken to elucidate the relationship between the magnitude of stabilisation and the nature of the charge-tag (e.g., carboxylates, sulfates, alkoxides) and the spatial separation between charge and radical moieties. These studies demonstrate that stabilisation of the radical can be measured at intramolecular separations of almost 8 Å (Chapter 4). The consequences for this discovery in the use of distonic radical anions as models of neutral radicals are evaluated (Chapter 7).

Encouraged by the selective release of carbon-centred radicals upon collisional activation of alkoxyamines, this moiety was incorporated onto peptide $N$-termini with the aim of photodissociative radical-directed structure elucidation (i.e., peptide sequencing). Upon isolation of desired ions in a linear ion trap mass spectrometer, homolysis of the oxygen-carbon was studied as a function of laser wavelength, charge state and peptide structure (Chapter 5). Finally, combined electron spin resonance spectroscopy and mass spectrometry methods were employed to study the degradation of piperidine-based aminoxyl radicals in solution. In the presence of hydroxyl radicals generated by irradiation of photocatalytic TiO$_2$ suspensions, multiple products are identified. The elucidation of these reaction mechanisms by experiment and theory provide a rationale for the well-documented time-dependent decrease in efficacy of piperidine stabilisers in polymer coatings.
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**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APPI</td>
<td>Atmospheric pressure photoionisation</td>
</tr>
<tr>
<td>BDE</td>
<td>Bond dissociation energy</td>
</tr>
<tr>
<td>BDFE</td>
<td>Bond dissociation free energy</td>
</tr>
<tr>
<td>CE</td>
<td>Collision energy</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical ionisation</td>
</tr>
<tr>
<td>CID</td>
<td>Collision-induced dissociation</td>
</tr>
<tr>
<td>CRDS</td>
<td>Cavity ring-down spectroscopy</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECD</td>
<td>Electron capture dissociation</td>
</tr>
<tr>
<td>EI</td>
<td>Electron ionisation</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionisation</td>
</tr>
<tr>
<td>ESR</td>
<td>Electron spin resonance</td>
</tr>
<tr>
<td>ETD</td>
<td>Electron transfer dissociation</td>
</tr>
<tr>
<td>FRIPS</td>
<td>Free-radical initiated peptide sequencing</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas chromatography / mass spectrometry</td>
</tr>
<tr>
<td>GPA</td>
<td>Gas-phase acidity</td>
</tr>
<tr>
<td>HALS</td>
<td>Hindered amine light stabiliser</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>ICR</td>
<td>Ion cyclotron resonance</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption ionisation</td>
</tr>
<tr>
<td>MCSCF</td>
<td>Multi-configurational self-consistent field</td>
</tr>
<tr>
<td>( m/z )</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MS(^n)</td>
<td>Multiple-stage mass spectrometry (( n ) product ion stages)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MS/MS</td>
<td>Tandem mass spectrometry</td>
</tr>
<tr>
<td>NMP</td>
<td>Nitroxide-mediated polymerisation</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>ONIOM</td>
<td>Our N-layered integrated molecular orbital and molecular mechanics</td>
</tr>
<tr>
<td>OPO</td>
<td>Optical parametric oscillator</td>
</tr>
<tr>
<td>PA</td>
<td>Proton affinity</td>
</tr>
<tr>
<td>PBN</td>
<td>Phenyl tert-butyl nitrene</td>
</tr>
<tr>
<td>PD</td>
<td>Photodissociation</td>
</tr>
<tr>
<td>PROXYL</td>
<td>2,2,5,5-tetramethylpyrrolidine-N-oxyl</td>
</tr>
<tr>
<td>PTM</td>
<td>Post-translational modification</td>
</tr>
<tr>
<td>RDD</td>
<td>Radical-directed dissociation</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
</tr>
<tr>
<td>RSE</td>
<td>Radical stabilisation energy</td>
</tr>
<tr>
<td>SOMO</td>
<td>Singly occupied molecular orbital</td>
</tr>
<tr>
<td>TCID</td>
<td>Threshold collision-induced dissociation</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-N-oxyl</td>
</tr>
<tr>
<td>TEMPOL</td>
<td>4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl</td>
</tr>
<tr>
<td>TEMPONE</td>
<td>4-oxo-2,2,6,6-tetramethylpiperidine-N-oxyl</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>YAG</td>
<td>Yttrium aluminium garnet</td>
</tr>
</tbody>
</table>
1. **INTRODUCTION**

---

*M: “Too many free radicals. That’s your problem.”*

*James Bond: “Free radicals, sir?”*

*M: “Yes. They’re toxins that destroy the body and the brain. Caused by eating too much red meat and white bread, and too many dry martinis.”*

*James Bond: “Then I shall cut out the white bread, sir.”*

*Never Say Never Again (1983)*
1.1 Free Radicals

Free radicals (or more simply, radicals) are defined as atomic or molecular entities possessing an odd number of electrons, and hence one of these electrons is unpaired.\(^1\) Free radicals therefore have non-zero spin and multiplicity greater than unity. The atomic centre that possesses the unpaired electron (or highest spin density) typically describes the radical; \(e.g., \) carbon-, oxygen-, or sulfur-centred. Further, radicals can be characterised by the type of molecular orbital in which the lone electron resides. Unpaired electrons in \(s\)-type orbitals are described as \(\sigma\)-radicals, while those in orbitals with more \(p\)-orbital character are termed \(\pi\)-radicals.\(^1\) To the physical organic chemist, the nomenclature of free radicals is typically derived from the name of the corresponding even-electron molecule, with the addition of the \(-\text{yl}\) suffix. For example, the simplest carbon-centred radical is a methyl radical (\(\cdot\text{CH}_3\)), based on methane, whilst removal of an electron from hydroxide anion (\(\text{HO}^-\)) produces an oxygen-centred hydroxyl radical (\(\text{HO}^\cdot\)).

The electronic configuration of an atom or molecule is governed by the Aufbau principle, which states that electrons of opposing spin are added pairwise into orbitals in order of increasing orbital energy, described by a wavefunction \(\psi\) (Figure 1.1). The frontier orbital is titled the highest occupied molecular orbital (HOMO) and the first vacant orbital is the lowest unoccupied molecular orbital (LUMO). The same principle applies to the electronic configuration of electron-deficient free radicals, however the final electron is unpaired and thus occupies a singly occupied molecular orbital (SOMO). The lone electron in the SOMO drives the reactivity of free radicals.
Free radicals are often highly reactive species as a further electron is sought to reach a stable octet. At ambient temperatures, free radicals are therefore susceptible to reaction with air, moisture and solvents, most often making them troublesome to synthesise, isolate, and characterise. In some instances, the radical site can be stabilised by delocalisation over conjugated \( \pi \)-systems, and/or by steric crowding to inhibit interaction of the radical with a reaction partner. These species are termed persistent radicals, unlike their fleeting transient cousins.\(^2\) In 1900 Moses Gomberg – whilst attempting to synthesise hexaphenylethane – prepared the persistent triphenylmethyl radical (\( \text{Ph}_3C\cdot \)), the first organic radical to be discovered.\(^3\) Triphenylmethyl radicals derive their stability from delocalisation of the radical across three phenyl rings, and the steric hindrance that suppresses dimerisation. Gomberg clearly saw the potential of his discovery, concluding his seminal paper:

"This work will be continued and I wish to reserve the field to myself."\(^3\)

and his follow-up paper the following year:

"...and I beg to reserve this field for further work."\(^4\)
1.2 Free Radicals are Everywhere

From proteins to polymers, combustion to cancer, free radical chemistry lies at the heart of many important natural and synthetic processes occurring in solution and the gas phase. Radicals undoubtedly play a crucial role in essential and deleterious processes. Understanding their reactivity, lifetime, and mode of action is critical to the fields of biology, medicine, environment, industry, and atmospheric chemistry.\(^5\)

As a consequence of their reactivity and propensity to degrade organic molecules, it is well-documented that free radicals have adverse effects on human health, by damaging biomolecules such as proteins, lipids, and DNA, hence disrupting their function. Free radicals have been implicated in biomolecular oxidative stress, and linked to numerous diseases including cancer, diabetes and atherosclerosis.\(^{10,11}\) Paradoxically, free radical reactions *in vivo* are essential to life, including cell signalling and anti-bacterial defence.\(^{12}\) Thus, the body has developed enzymatic and antioxidative defences to curtail and repair radical-induced damage.

Antioxidants also play a role in the protection of synthetic materials against radical degradation. Polymeric materials – themselves often produced by radical chain-reaction processes – are susceptible to chain scission and peroxidation\(^{13}\) initiated by heat or UV radiation, and propagated by a series of free radical reactions. In fact, polymers such as paints, varnishes, construction materials, homewares and electronics would be rendered nearly useless without such antioxidant additives or protective coatings abating the harmful effects of free radical degradation.\(^{14}\)

Free radicals implicated in these processes are present at low concentration, and are short lived before being consumed by a reaction partner. Thus our understanding of key radical intermediates is often based on indirect end product analysis.
1.3 Formation of Free Radicals

The predominant mechanism for formation of neutral free radicals is through homolytic cleavage of a covalent bond where one bonding electron is retained on each atomic or molecular fragment (Reaction 1.1).

\[
AB \rightarrow A' + B'
\]  
(1.1)

Given that molecules do not spontaneously decompose, the formation of free radicals by Reaction 1.1 is an endothermic process that requires energy, in the form of heat, light, or nuclear radiation. These processes are termed pyrolysis, photolysis and radiolysis, respectively. For example, the molecular halides \((X_2, \ X = \text{Cl, Br or I})\) are photolysed by ultraviolet (UV) or visible light to form two halogen atoms \((X')\), each with an odd number of electrons. The energy required to break a given covalent bond at room temperature is the bond dissociation energy (BDE); that is, the enthalpy of Reaction 1.1:

\[
\text{BDE}(AB) = \Delta_{\text{rxn}} H_{298}^{\circ}(1.1) = \Delta_f H_{298}^{\circ}(A') + \Delta_f H_{298}^{\circ}(B') - \Delta_f H_{298}^{\circ}(AB)
\]  
(1.2)

where \(\Delta_f H_{298}^{\circ}(M)\) represents the enthalpy of formation of molecular species \(M\) under standard conditions. BDEs are readily determined either experimentally by kinetic (equilibrium), or threshold (e.g., photoionisation) measurements,\(^{15,16}\) or from theoretical methods.\(^{17}\) BDEs in organic molecules are strongly influenced by the local molecular structure, and particularly the two atoms between which the bond is broken. BDEs in organic molecules range from \(ca.\ 190 \text{ kJ mol}^{-1}\) for a weak carbon – iodine bond in allyl iodide, to \(ca.\ 560 \text{ kJ mol}^{-1}\) for the \(C_{\text{ring}} - C_{\text{nitile}}\) bond in benzonitrile.\(^{16}\) By corollary, the formation of a closed-shell molecule from two free radicals is highly exothermic.

BDEs are often taken as proxies for comparisons of radical ‘stability.’ A higher BDE implies a less stable radical. In some instances it may be necessary to invoke a more rigorous description of radical stability. It is well known that the order of R-H
BDEs in alkanes decreases in the order of $R = \text{methyl, ethyl, isopropyl, and tert-buty}$.

On this basis, one would (correctly) conclude that the tert-butyl radical is the most stable. Conversely, the R-OH BDEs in alcohols exhibit the opposite trend, because of differing stabilisation effects contributed by the ionic $R^+ \cdot \text{OH}$ configuration in the intact molecule.\(^\text{18}\) Thus the concept of radical stability can only be unambiguously defined in the context of a balanced reaction, not in isolation. Radical stabilisation energy (RSE) is defined by the reaction enthalpy of the isodesmic reaction of the radical of interest ($R'$) with some similar reference species, for example a methyl radical (Reaction 1.3).\(^\text{2,19,20}\)

\[
R' + \text{CH}_4 \rightarrow \cdot \text{CH}_3 + \text{RH}
\] (1.3)

The overall reaction enthalpy is described by Reaction 1.4, which is rearranged by substituting for $\text{BDE(\text{CH}_4)}$ and $\text{BDE(\text{RH})}$ using Reaction 1.2, and cancelling the common term of $\Delta H_{298}(\text{H}^\cdot)$.

\[
\text{RSE} = \Delta_{\text{rxn}}H_{298}(1.3) = \Delta_{\text{f}}H_{298}(\text{RH}) + \Delta_{\text{f}}H_{298}(\cdot \text{CH}_3) - \Delta_{\text{f}}H_{298}(R') - \Delta_{\text{f}}H_{298}(\text{CH}_4) = \text{BDE(\text{CH}_4)} - \text{BDE(\text{RH})}
\] (1.4)

Thus an RSE greater than zero results when $R'$ is more stable than $\cdot \text{CH}_3$, and if the RSE is less than zero, $\cdot \text{CH}_3$ is the more stable species. That is, Reaction 1.3 will proceed favourably if BDE(RH) is greater than BDE(CH\(_4\)).

The second mechanism for neutral free radical formation is through electron detachment from an even-electron anion by photolysis or electrolysis (Reaction 1.5).

\[
A^- \rightarrow A^\cdot + e^-
\] (1.5)

Reaction 1.5 is similarly endothermic. The required energy can be supplied in the laboratory by a UV laser source to calculate electron affinities and electron binding energies (the difference between the energy of the incident photon and the kinetic energy of the liberated electron) by negative ion photoelectron spectroscopy.\(^\text{21-23}\)
1.4 Radical Ions

In the preceding section, only the production, reactions, and characterisation of neutral free radicals were considered. Adding an electron to a neutral molecule (Reaction 1.6) by chemical or electrochemical means results in a chemical species with an odd number of electrons and is thus a radical. However, it also possesses an overall negative charge, and is thus termed a radical anion. Similarly, removal of an electron results in a radical cation (Reaction 1.7). Electrons can also be added or subtracted from an ionic precursor (Reactions 1.8 and 1.9).

\[
\begin{align*}
A + e^- & \rightarrow A^- & \quad (1.6) \\
A & \rightarrow A^{+*} + e^- & \quad (1.7) \\
A^{n-} & \rightarrow A^{(n-1)-*} + e^- & \quad (1.8) \\
A^{n+} + e^- & \rightarrow A^{(n-1)+*} & \quad (1.9)
\end{align*}
\]

Radical ions are commonly encountered in the chemistry laboratory. Perhaps the simplest commonly encountered radical anion is superoxide (\(O_2^-\)), a reactive oxygen species formed from the one-electron reduction of triplet molecular oxygen (\(\text{^3O}_2\)). Superoxide is commonly encountered in nature as a precursor to hydrogen peroxide and singlet oxygen (\(\text{^1O}_2\)).\(^{12}\) In theory, all molecules contain unoccupied molecular orbitals into which an extra electron can be added to form radical anions. However, only those with sufficiently low reduction potentials are amenable to electrolytic or chemical reduction (e.g., by alkali metals). In this regard, persistent radical anions of polycyclic aromatic hydrocarbons are readily prepared by reaction with sodium metal, due to delocalisation of spin density across the \(\pi\)-system, lowering the energy of the LUMO. Such radical anions act as electron donors and strong proton acceptors to ultimately produce partially reduced aromatic hydrocarbons (Birch reduction),\(^{24}\) and initiating further reactions on the substrate to which the electron is transferred.\(^{25,26}\) Due to their
facile reactivity, radical anions can be utilised as probes for the presence of oxygen or water (a proton donor) in organic solvents. Reduction of benzophenone with potassium metal produces the corresponding bright blue ketyl radical anion, a persistent radical in the absence of water or oxygen due to conjugation across both phenyl rings and the carbonyl $\pi$-bond. In the presence of water or oxygen, the blue colour is quenched due to the consumption of the radical anion. Ketyl radical anions also form a key step in the pinacol reaction of ketones and aldehydes into 1,2-diols (Scheme 1.1).$^{27}$ The carbonyl is reduced to a ketyl radical anion by an alkaline earth or lanthanide metal (M). Addition to a second ketyl radical forms the new carbon-carbon bond of the diol product.

Scheme 1.1: Ketyl radical anions as intermediates in the pinacol reaction.

Radical ions form the cornerstone of this thesis, with a particular emphasis on their formation, isolation, reactivity, and stability in the gas phase. Applications of mass spectrometry to radical ion chemistry will be considered in further detail below.
1.5 Aminoxyl Radicals

Aminoxyl radicals (variously described as ‘nitroxides’ or ‘nitroxy radicals’) are persistent \(\text{N}_2\text{N}\)-disubstituted \(\text{N}-\text{O}\) radicals (\(\text{R}_1\text{R}_2\text{NO}^\cdot\)). Noteworthy alkynitroxides are illustrated in Figure 1.2, but a diverse array of structures have been studied.\(^{28}\) Unlike most radicals, aminoxyl radicals are easily stored, manipulated and characterised. The first reported aminoxyl radical was the inorganic salt potassium nitrosodisulfonate, discovered in the 19\(^{\text{th}}\) century by Frémy.\(^ {29}\) The first fully organic nitroxide \textit{porphyrexide} was prepared by Piloty and Schwerin in 1901.\(^ {30}\)

\[\text{KO}_2\text{S}\text{N}^\cdot\text{SO}_3\text{K} \quad \text{Frémy’s salt} \quad \text{DTBN} \quad \text{TEMPO} \quad \text{TMIO} \quad \text{Porphyrexide} \quad \text{TEDIO} \quad \text{TIPNO} \quad \text{SG1} \]

Figure 1.2: Commonly used and historically significant aminoxyl radicals.

<table>
<thead>
<tr>
<th>Radical</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTBN</td>
<td>di-\textit{tert}-butylnitroxide</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-(\text{N})-oxyl</td>
</tr>
<tr>
<td>TMIO</td>
<td>2,2,10,10-tetramethyl-isoinodin-(\text{N})-oxyl</td>
</tr>
<tr>
<td>Porphyrexide</td>
<td>2,4-diimino-5,5-dimethyl-imidazolidine- (\text{N})-oxyl</td>
</tr>
<tr>
<td>TEDIO</td>
<td>2,2,10,10-tetraethyl-isoinodin-(\text{N})-oxyl</td>
</tr>
<tr>
<td>TIPNO</td>
<td>2,2,5,5-tetramethyl-4-phenyl-3-azaheksane-(\text{N})-oxyl</td>
</tr>
<tr>
<td>SG1</td>
<td>(\text{N})-(2-methylpropyl)-(\text{N})-(1-diethyl-phosphono-2,2-dimethylpropyl)-(\text{N})-oxyl</td>
</tr>
</tbody>
</table>

Aminoxyl radicals are commonly prepared by oxidation of the corresponding secondary amine. The first reported synthesis of 4-oxo-TEMPO was achieved by oxidising the precursor piperidine with hydrogen peroxide over tungsten, molybdenum
or vanadium salts. Hydroperoxides, silver oxide, and peracids are also suitable oxidising agents for this purpose. Aminoxyl radicals are also formed from reduction of nitro or nitroso compounds, or the addition of free radicals to nitrones (the basis of ‘spin trapping’). Aminoxyl radical synthesis is the subject of a review by Rozantsev.

The unique thermal and chemical stability of aminoxyl radicals arises from delocalisation of the unpaired electron over the N-O bond (Scheme 1.2). Dimerisation is unlikely, due to the ca. 120 kJ mol\(^{-1}\) required to overcome delocalisation. With two electrons in an N-O \(\pi\)-orbital, one in the corresponding \(\pi^*\), and two in an N-O \(\sigma\)-orbital, the bond order of 1.5 is confirmed by the N-O bond length (ca. 1.28 Å) and vibrational frequency falling between that of an N-O single and double bond. Moreover, typical radical reactions are suppressed by steric hindrance provided by neighbouring alkyl groups. Disproportionation to hydroxylamines and nitrones is also inhibited, as the required \(\beta\)-hydrogen is absent.

![Scheme 1.2: Resonance stabilisation across the nitrogen-oxygen bond in TEMPO.](image)

Aminoxyl radicals undergo reversible one-electron oxidation to oxoammonium cations, and one-electron reduction to hydroxylamines, shown in Scheme 1.3. From cyclic voltammetry, the oxidation potential for aminoxyl radicals lies between 0.6 – 1.1 V (versus standard hydrogen electrode), depending on the aminoxyl radical substituents and solvent. Chemical oxidants including potassium ferricyanide are also applicable. Oxoammonium cations are postulated to be the key intermediate responsible for the oxidation of alcohols by aminoxyl radicals. Reduction to hydroxylamines occurs at a higher potential of 1.4 – 2.2 V, or by employing reductants such as ascorbate.
1.5 Aminoxyl Radicals

Scheme 1.3: Reversible oxidation and reduction of TEMPO.

Aminoxyl radicals are unreactive toward most common solvents and reagents, allowing synthetic derivatisation to proceed without involvement of the radical site.\textsuperscript{51-55} Numerous substituted aminoxyl radicals are now commercially available as crystalline solids. Products starting from 4-oxo-TEMPO are illustrated in Scheme 1.4,\textsuperscript{31,56-59} each of which are suitable for further manipulation. A diverse array of molecular scaffolds can be prepared with chemical and physical properties tailored to specific applications, an attribute that will be exploited in the following chapters of this work.

Scheme 1.4: Substituted TEMPO derivatives from 4-oxo-TEMPO.
1.5 Aminoxyl Radicals

Perhaps the most important reactions of aminoxyl radicals are the scavenging of carbon-centred radicals to form alkoxyamines. Efficient radical scavenging forms the basis of aminoxyl radicals as antioxidants and polymerisation mediators. From the works of Beckwith and Ingold,\textsuperscript{60-63} rate constants for such radical-radical coupling reactions are known to be orders of magnitude lower than the diffusion-controlled limit. Reaction rates depend on the structure of the alkyl and aminoxyl radical; from \textit{ca.} $10^9$ M$^{-1}$ s$^{-1}$, decreasing to $10^5$ M$^{-1}$ s$^{-1}$ with increasing radical stabilisation and steric bulk.

1.5.1 Applications

The ability of aminoxyl radicals to reversibly cycle between multiple oxidation states makes them attractive alternatives to iodide as a redox mediator in dye-sensitised solar cells.\textsuperscript{64-66} Aminoxyl radicals mimic the enzymatic antioxidant action of superoxide dismutase,\textsuperscript{67,68} by scavenging and nullifying reactive oxygen species and protein-derived radicals.\textsuperscript{69,70} Consequently, aminoxyl radicals show promise in preventing cellular cytotoxicity, oxidative stress, hypertension and tissue damage.\textsuperscript{71-74}

1.5.2 Polymer Stabilisers

Free radicals are responsible for degradation of polymeric materials. Once generated, free radicals propagate along the polymer backbone in a self-sustaining autoxidation reaction,\textsuperscript{13} causing chain scission. These molecular level changes manifest as changes to the aesthetic and mechanical properties of the material, and ultimately lead to failure. Two core strategies exist for combating this problem: (i) inhibiting radical initiation by blocking the source of radical generation (\textit{e.g.}, an exterior UV-absorbing layer to prevent photolysis); or (ii) quenching radical propagation by employing radical-trapping anti-oxidants, such as hindered phenols and hindered amines. Hindered phenols are widely employed in polymers and lubricants (as well as naturally occurring Vitamin E), and act by trapping two equivalents of peroxyl radicals.
by every hindered phenol, as shown in Scheme 1.5.\textsuperscript{75} The hindered phenols are sacrificial antioxidants, as the cyclohexadienone product can no longer contribute to antioxidant activity.

\textbf{Scheme 1.5}: Sacrificial peroxy radical trapping by 2,6-di-\textit{tert}-butyl-4-methylphenol.

By contrast, hindered amine light stabilisers (HALS) are regenerative antioxidants, trapping many deleterious alkyl and peroxy radicals per stabiliser molecule. For this reason, HALS are the most effective polymer antioxidants commercially available, even at low concentrations. Despite the clear benefits of their use on inhibiting polymer degradation and improving polymer performance and lifetime,\textsuperscript{76} for many years the mechanism of their action remained ambiguous.\textsuperscript{77-81} Understanding the mechanisms of HALS action is further complicated by the intricate polymer matrix, which contains other additives to aid processing and performance. For this reason, model studies\textsuperscript{82} have been employed on representative systems to understand the chemistry of HALS; a strategy employed in this research by studying reactions of HALS in the gas-phase. From earlier model studies, a general (but oversimplified) model for the mechanism of HALS action was proposed, commonly referred to as the Denisov cycle.\textsuperscript{83} According to this cycle (Scheme 1.6), the parent amine initially added to the polymer formulation during processing is oxidised to an aminoxyl radical – which is the active radical scavenging anti-oxidant. Unlike hindered phenols, the alkoxyamine product is recycled back to the nitroxide, with even-electron by-products.
The precise mechanisms for activation\textsuperscript{84-88} and regeneration\textsuperscript{89-91} of aminoxyl radicals remain contentious. Coote \textit{et al.}\textsuperscript{92-94} have recently employed high level quantum chemical calculations to conduct critical evaluations of the myriad mechanistic proposals, and discounted the majority as energetically unfeasible. Instead they propose the involvement of a nitrogen-centred aminyl radical as a key intermediate in aminoxyl radical regeneration from alkoxyamines,\textsuperscript{92} illustrated in Scheme 1.7. Accessing aminyl radicals \textit{via} homolysis of the alkoxyamine N–O bond has been considered computationally,\textsuperscript{95} but as yet has proved difficult to directly examine experimentally.

\textbf{Scheme 1.6}: Catalytic action of TEMPO-based HALS in the Denisov cycle.

\textbf{Scheme 1.7}: Regenerative cycling of aminoxyl radicals \textit{via} aminyl radicals.
The rate of polymer chain scission is undoubtedly decreased by HALS, and degradation is inhibited compared to non-stabilised samples. However, the benefits afforded by HALS are finite. Over time, the concentration of available HALS decreases and polymer performance is compromised, implying that the stabilisation cycle is disrupted. The full scope of chemical and physical transformations to impede the ability of HALS to act as efficient stabilisers is still not fully understood.

1.5.3 Nitroxide-Mediated Polymerisation

A subset of the broader living free radical polymerisation family, nitroxide-mediated polymerisation (NMP) produces high molecular weight polymers with low polydispersity and controlled molecular weight distributions. In 1993, Georges and co-workers used TEMPO as a mediator to produce polystyrene with a narrower molecular weight distribution (Figure 1.3, solid trace) compared to conventional free radical polymerisation (dotted trace). NMP has since diversified into a robust technique suitable for a wide range of monomers, solvents, and reaction conditions.

![Figure 1.3: Gel-permeation chromatograms of polystyrene.](image)

(a) NMP with TEMPO; (b) Conventional radical polymerisation. From Reference 101.

In a typical NMP experiment illustrated in Scheme 1.8, free radical polymerisation is carried out in the presence of an aminoxyl radical. At low reaction temperatures, growing polystyrene chains with a terminal alkyl radical are trapped by...
TEMPO, forming a new oxygen-carbon bond in an alkoxyamine, which is no longer available for polymerisation. Raising the reaction temperature cleaves the O–C bond, and the carbon-centred radical adds to excess monomer, continuing polymer growth. This process can be repeated many times to achieve the desired molecular weight.

Scheme 1.8: Nitrooxide-mediated polymerisation of styrene with TEMPO.

Initial experiments used TEMPO as the mediating nitroxide, but now a diverse array of aminoxyl radicals specifically designed for compatibility with NMP are available. Moreover, the molecular structure in the vicinity of the nitroxide affects the reversibility and rates of alkoxyamine formation and dissociation, and thus controls the molecular weight distribution of the product. The success of NMP requires suppressing the significance of side-reactions at high temperatures, such as disproportionation or cleavage of the N–O bond, thus terminating polymerisation and introducing an alkoxy radical onto the polymer chain terminus. Furthermore, due to the thermal sensitivity of some monomers, there has been an increasing focus on designing aminoxyl radicals that would reduce the required reaction temperature, or indeed respond to different stimuli such as light or pH to control combination and dissociation.
1.6 Characterisation of Free Radicals

The kinetic and thermodynamic stability of aminoxyl radicals is the exception rather than the rule when it comes to free radicals. The inherent reactivity of transient radicals is both central to their role in chemical systems, and a significant drawback to their isolation and characterisation in the chemistry laboratory. The initially formed radical will react rapidly (the rate can approach the diffusion-controlled limit depending on the specific reaction) by transfer of hydrogen atoms or by radical-radical coupling (e.g., addition of molecular oxygen). Radicals can react with either a suitable co-reagent, another radical, or with components of adventitious moisture or air. The secondary radicals formed in these reactions are then subsequently available for further reaction. Thus, insight into radical reaction mechanisms may be limited to analysis of downstream end-products, and therefore susceptible to ambiguity.

Isolating a pure population of the primary radical to investigate its intrinsic reactivity can prove challenging. At any one time, the radical of interest is likely present as part of a complex mixture, and only in low concentration before being consumed. Manipulating experimental variables to increase the number density of radical species to facilitate detection regrettably also increases the likelihood of undesirable radical-radical recombination reactions, thus decreasing sensitivity. Specialised apparatus are often required to produce, characterise and quantify free radicals.

1.6.1 Solid Phase

Matrix isolation was developed by Pimentel and co-workers\textsuperscript{105} to provide an inert environment in which to study reactive intermediates, extending their lifetime by removing all potential reaction partners. Reactive species (e.g., radicals, carbenes, carbanions, nitrenes or aryynes)\textsuperscript{106} are confined in an inert matrix of solid argon or xenon. By using a large excess of matrix relative to analyte (ca. 100:1)\textsuperscript{107} and
temperatures below 20 K, trapped species are unable to diffuse through the matrix to react with each other. In a typical matrix isolation experiment, a suitable precursor and matrix are sprayed together through a nozzle onto a cold glass plate. The active species can be formed by pyrolysis, microwave discharge, UV irradiation of photolabile precursors, or co-condensation of multiple gas streams that each contain one reaction component. Active species are formed immediately prior to, or – in the case of photolysis – following condensation, and hence the time between synthesis and isolation is minimised. After generation and confinement, an uncontaminated population of radicals is available for characterisation. Advantageously, typical matrices are transparent in the visible and IR regions, so non-invasive spectroscopic analyses may be undertaken to interrogate the electronic structure of the trapped species. Spectra acquired from matrix isolation experiments have been shown to be comparable to the gas-phase structure.

1.6.2 Solution Phase

Matrix isolation experiments require specialised apparatus and cryogenic temperatures, making it unfeasible for many laboratories. Much of our contemporary knowledge of free radical behaviour stems from solution-phase characterisation. Electron spin resonance spectroscopy (ESR) detects odd-electron radicals with a signal characteristic of the local environment around the unpaired electron. Energetic splitting of nuclear spin states in the presence of an external magnetic field forms the basis of nuclear magnetic resonance (NMR) spectroscopy. In the presence of an applied magnetic field, the electrons’ magnetic moment is similarly aligned parallel or antiparallel to the field, the latter being higher in energy by \( \Delta E \):

\[
\Delta E = h\nu = g_e\mu_B B_0
\]  

(1.10)
where $h$ is Planck’s constant, $g_e$ is the electron $g$-value, $\mu_B$ the Bohr magneton, and $B_0$ the applied field. ESR spectra are typically generated by exposing the sample to fixed frequency microwave radiation ($9 \text{–} 10$ GHz), while varying the field (ca. 0.3 – 0.4 T) until Equation 1.10 is satisfied. At this point, the unpaired electron can alternate between the two energy levels by absorption or emission of electromagnetic radiation. As the lower state is more heavily populated, there is a net energy absorption, which is converted to a spectrum (typically presented as the first derivative of absorption) as a function of applied field (Figure 1.4). Integration of the resulting spectrum provides kinetic data of radical formation or consumption.

![Figure 1.4](image_url)

Figure 1.4: ESR spectrum of the persistent aminoxyl radical 4-carboxy-TEMPO in water at 298 K. Equation 1.10 is used to derive $g_e$.

Spectral features depend on many factors, including temperature, concentration, and solvent polarity. If these influences are held constant, spectral variations can be attributed to the local chemical environment of the unpaired electron. The electron $g$-value ($g_e$) is analogous to an NMR chemical shift. In a vacuum, the electron $g$-value is 2.0023. However, the unpaired electron also experiences a local field from neighbouring atoms such that the net field is not equal to $B_0$, which is reflected in the value of $g_e$ for a particular molecule. Although $g$-values can differ significantly for metal complexes, for organic radicals the variation is rarely sufficient to be used as a diagnostic tool.\(^{110}\)
The second spectral feature of note is the hyperfine coupling (or hyperfine splitting, denoted A). Comparable to the coupling constant in an NMR spectrum, perturbations in the effective field strength by neighbouring nuclei with non-zero spin result in hyperfine splitting into \((I + 1)\) lines. In the case of 4-carboxy-TEMPO (Figure 1.4), the unpaired electron on oxygen is split into 3 lines of equal height by the adjacent nitrogen (for \(^{14}\text{N}\), nuclear spin \(I = \pm 1\)). Each line can be further split by distant nuclei, however in Figure 1.4 this fine splitting is masked by the broad peak width. Hyperfine splitting patterns are central to deciphering the local environment of the unpaired electron, however, information about the broader molecular structure is limited. For example, the nitrogen hyperfine splitting \((A_N)\) of 4-carboxy-TEMPO (Figure 1.4) is ca. 0.01 mT larger in high pH solutions,\(^{111}\) due to a long-range polar effect increasing the spin-density on oxygen. Other pH-sensitive aminoxyl radicals have been designed with larger \(\Delta A_N\) values to serve as pH probes, particularly across lipid membranes.\(^{112-114}\)

ESR is ideal for selective detection of radical species, as common solvents and other even-electron components will not display a signal. In some circumstances it is desirable to deliberately incorporate a persistent ‘spin label’ into otherwise diamagnetic molecules such as proteins, to derive kinetic or structural information by ESR.\(^{115-117}\) The radical under investigation must be sufficiently persistent to enable detection (cooling the apparatus and excluding oxygen to extend the radical lifetime may be necessary). Transient radicals may be trapped by a non-radical precursor, or ‘spin trap’, the product of which is a stable radical that exhibits a spectrum characteristic of the primary trapped radical.\(^{118,119}\) Particularly relevant are the works of Gigmes et al.\(^{120}\) and Paine et al.;\(^{121}\) who used the spin trapping technique to distinguish between the formation of alkyl and alkoxy radicals during the thermolysis of alkoxyamines \((R_1^1 R_2^2 \text{NOR}_3)\) in the presence of phenyl tert-butyl nitron (PBN), outlined in Scheme 1.9.
1.6 Characterisation of Free Radicals

Scheme 1.9: Spin trapping transient radicals with PBN produces aminoxyl radicals, the characteristic spectra of which distinguish alkyl and alkoxy radicals.

The reverse experiment is also possible, whereby kinetic data is obtained by monitoring decay of a persistent aminoxyl radical in the presence of a transient radical, the product of which is a diamagnetic alkoxyamine \( \textit{via} \) radical-radical coupling.\(^{62}\) Clearly, little structural information concerning the transient radical can be obtained from ESR alone and in this spin-trapping experiment, chromatographic or mass spectrometric methods are required for structural elucidation.\(^{122}\)

### 1.6.3 Gas Phase

Although many radical processes take place in the condensed phases, there are several benefits to studying radical reactions in the gas phase. Primarily, the complicating effects of competing radical-solvent reactions are eliminated, thus simplifying the potential reaction mechanisms. Free radicals and other such reactive intermediates may be formed in the gas-phase by irradiation, microwave discharge or combustion of suitable precursors, and further reactions can be quenched by rapid cooling (\textit{e.g.}, supersonic expansion). Conventional diagnostic techniques for gas-phase free radicals include non-intrusive spectroscopic techniques (fluorescence, UV, Raman,
etc.), which are able to detect and identify simple intermediates. However, these techniques lack the selectivity and sensitivity to detect more complex intermediates that exhibit weak or featureless spectra. Cavity ring-down spectroscopy\textsuperscript{123,124} (CRDS) is a sensitive technique for gas-phase kinetics and spectroscopic measurements of either trace species or weak absorptions. The cavity itself consists of highly reflective mirrors, increasing the effective pathlength to the order of kilometres, improving sensitivity in accordance with the Beer-Lambert law. Unlike conventional absorption spectroscopy, CRDS measures the rate of absorption of a laser pulse by a molecule in the optical cavity, rather than the absolute magnitude of absorption. As the absorption rate – or ring-down time – is independent of laser intensity, CRDS is largely immune to errors arising from fluctuations in light source intensity that plague most absorption measurements.\textsuperscript{124} However, analysis may be complicated by cavity modes or gas components overlapping with the spectral features of interest.

Alternatively, extractive sampling methods such as GC/MS exhibit greater sensitivity and selectivity compared to spectroscopic methods, but are disadvantaged by indirect detection of radical species and the disruption caused by the sampling process. An alternative strategy is therefore sought, in which radical synthesis and characterisation are accomplished \textit{in vacuo} in a mass spectrometer.
1.7 Radical Ions and Mass Spectrometry

As great a role as radical ions play in the condensed phases in synthetic and biological processes, the same difficulties that plague the preparation and characterisation of neutral radicals are also relevant to radical ions. Inert atmospheres and specialised apparatus are required to characterise these often transient species. Many of these shortcomings are overcome by the formation and subsequent analysis of radical ions in the gas phase. Radical ions are studied in isolation, in the absence of solvent, reagents, and container surfaces. Coulombic repulsion between radical ions of the same polarity excludes the possibility of radical-radical recombination. Mass spectrometry is a versatile technique suitable for achieving the preparation, isolation, and subsequent characterisation of radical ion species, even at low concentrations and in the presence of other ions or neutral molecules. Widely considered to be the father of mass spectrometry, in 1913 Sir J. J. Thompson wrote of its benefits:

“The method is surprisingly sensitive... requires an infinitesimal amount of material, and does not require this to be specially purified: the technique is not difficult if appliances for producing high vacua are available.”

Thomson’s words remain pertinent to this day. Mass spectrometry in all its diverse forms offers sensitivity, selectivity, and speed unequalled in the analytical laboratory. Moreover, analytes can be sequestered from complex mixtures (either by coupling to chromatography or by using the discriminatory powers of the mass analyser) and studied in isolation in a controlled atmosphere, free from the complications of solvents, catalysts, reagents and other unknowns.

At its simplest, a mass spectrometer consists of three components: an ion source for volatilising and ionising the analyte, one or more mass analysers to discriminate between ions on the basis of their mass-to-charge ratio ($m/z$), and a detector. Myriad
arrangements of ion sources and mass analysers exist, (and additionally, hybrid combinations of mass analysers) each offering particular advantages and drawbacks, depending on the experimental requirements. In the following sections, the theory and technologies underpinning various mass spectrometer configurations will be described in the context of producing, isolating, and analysing radical ions in the gas phase. Production of radical ions can occur directly in the ion source, or by subsequent interrogation of a closed-shell ion in the mass analysis stage.

1.7.1 Ion Sources

The role of the ion source is to produce gas-phase ions from a solid, liquid or gas sample, including the eluent from a gas- or liquid-chromatograph. Ionisation is achieved by electron capture or ejection, protonation, deprotonation, formation of adducts (metal or organic ions), or by transferring a condensed-phase ion into the gas phase.

1.7.1.1 Electron Ionisation

The first electron ionisation (EI) source was described by Dempster as early as 1918, in which metal salts were bombarded with electrons accelerated through a potential difference of 128 volts.\textsuperscript{126} EI is now widely used for the analysis of organic compounds, particularly due to its compatibility with gas chromatography.\textsuperscript{127}

A typical EI source is illustrated in Figure 1.5. Electrons produced in a heated filament are accelerated across a potential difference – typically a standardised 70 V – toward the anode. Electrons interact with gaseous sample molecules that are introduced orthogonally though the sample inlet. Gases and volatile liquids can be directly introduced, whilst samples with low vapour pressure require a heated source for volatilisation, which occurs prior to – and distinct from – ionisation. EI is therefore suitable only for the analysis of volatile, thermally stable compounds.
The interaction between the gaseous sample molecule and a 70 eV electron expels an additional electron, forming the molecular radical cation $M^{++}$ (Reaction 1.11). Ionisation potentials of organic compounds lie in the range of 7-10 eV,\(^\text{128}\) and thus the molecular ion is formed with excess internal energy. This additional energy is dissipated by unimolecular dissociation, either generating a radical ion product through rearrangement and elimination of a neutral molecule (Reaction 1.12); or by homolytic bond cleavage to form a closed-shell fragment ion and a neutral radical (Reaction 1.13).

$$AB + e^- \rightarrow AB^{++} + 2e^- \quad (1.11)$$

$$AB^{++} \rightarrow A^{++} + B \quad (1.12)$$

$$AB^{++} \rightarrow A^+ + B^\cdot \quad (1.13)$$

Although this highly energetic ionisation process may result in a minor or absent molecular ion, advantageously the fragmentation pattern is highly reproducible and dependent on molecular structure. Vast spectral databases have therefore been compiled and the fragmentation can act as a fingerprint to identify unknown compounds.
1.7.1.2 Chemical Ionisation

Electron ionisation is a highly energetic process that produces ions with excess internal energy, resulting in rich fragmentation. Although this reproducible fragmentation increases specificity, the molecular ion is often very weak or absent, leading to ambiguity over the analyte molecular mass. Chemical ionisation (CI) is a complementary method that produces ions with less excess energy and therefore results in a spectrum with fewer fragment ions and a greater abundance of molecular ions. For example, the EI and CI mass spectra of tributylamine are compared in Figure 1.6.

![Figure 1.6](image)

**Figure 1.6:** EI and CI mass spectra of tributylamine, adapted from Reference 129.

In a CI experiment,\textsuperscript{129,130} a reagent gas – typically methane – is introduced into the ionisation source at a pressure of \textit{ca.} 1 Torr. As the analyte sample pressure is several orders of magnitude lower, the electron beam will preferentially ionise methane by Reaction 1.14. The methane radical cation will predominantly react with another methane molecule to produce protonated methane and a methyl radical (Reaction 1.15).

\[
\text{CH}_4 + e^- \rightarrow \text{CH}_4^{++} + 2e^- \quad (1.14)
\]

\[
\text{CH}_4^{++} + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3^+ \quad (1.15)
\]
Further unimolecular and bimolecular reactions of methane-derived radicals and ions may occur, the extent of which depend on the pressure of methane in the source. The desired reaction is proton transfer from CH$_5^+$ to the analyte molecule M (Reaction 1.16).

$$M + CH_5^+ \rightarrow MH^+ + CH_4 \quad (1.16)$$

Methane is a weak Brønsted base (proton affinity = 543 kJ mol$^{-1}$) and therefore its conjugate acid CH$_5^+$ is a strong proton donor. Reaction 1.16 will proceed favourably so long as the sample molecule has a greater proton affinity than methane. By changing the reagent gas, different Brønsted acids are produced, and the energetics of the proton transfer reaction can be tuned.

Reagent gases with a high ionisation potential (e.g., N$_2$, CO, noble gases) interact with analyte molecules by charge exchange, rather than proton transfer. The resulting spectra resemble those obtained by EI, with the degree of fragmentation dependent on the exothermicity of electron transfer (Reaction 1.18).

$$Xe + e^- \rightarrow Xe^{++} + 2e^- \quad (1.17)$$

$$Xe^{++} + M \rightarrow M^{++} + Xe \quad (1.18)$$

Data obtained by negative ion mass spectrometry is often confirmatory and/or complementary to that obtained in the positive ion mode. Negative ions can be studied using most mass spectrometers, by switching the appropriate potentials and fields used for ion manipulation. Negative ions can be produced in CI either by electron capture or an ion-molecule reaction. Interaction of near-thermal electrons with a neutral molecule may result in: (i) associative resonance electron capture, forming a molecular radical anion (Reaction 1.19); (ii) dissociative resonance electron capture, forming a neutral radical and closed-shell anion (Reaction 1.20); or (iii) ion pair production, which also results in fragmentation of the molecule (Reaction 1.21).
1.7 Radical Ions and Mass Spectrometry

\[ AB + e^- (\text{slow}) \rightarrow AB^- \quad (1.19) \]

\[ AB + e^- (\text{slow}) \rightarrow A^- + B^+ \quad (1.20) \]

\[ AB + e^- (\text{slow}) \rightarrow A^- + B^+ + e^- \quad (1.21) \]

An ion-molecule reaction of a strong Brønsted base with sample molecules results in deprotonated analyte ions by proton transfer, analogous to Reaction 1.16. A mixture of methane and nitrous oxide is often used for this purpose, forming O\(^-\) and subsequently hydroxide anion, which acts as the proton acceptor.

### 1.7.1.3 Photoionisation

The basis of photoionisation mass spectrometry is removal of an electron from a molecule by vacuum ultraviolet photons (Reaction 1.22). Photon energies marginally greater than the analyte ionisation potential (ca. 7-10 eV) are typically employed to minimise excess internal energy and fragmentation.

\[ M + h\nu \rightarrow M^{++} + e^- \quad (1.22) \]

Photoionisation mass spectrometry has been extensively applied to the study of reactive intermediates in fuel combustion chemistry, recently reviewed by both Li\(^+\) and Hansen.\(^{135}\) In a typical experiment, fuel combustion products are extracted from the flame region into an ionisation chamber as a controlled molecular beam consisting of a mixture of neutral analytes, including neutral radicals. In this region, the molecular beam perpendicularly intersects a beam of synchrotron UV radiation from a synchrotron, and the ionic products are extracted into the mass analyser. Synchrotron radiation offers the advantages of extensive tunability and high energy resolution of the incident UV photons. Photoproduction ion abundances plotted as a function of photon energy yield photoionisation efficiency spectra. In Figure 1.7,\(^{136}\) species resulting from butanol combustion are characterised according to their \(m/z\) ratio and their ionisation threshold, thus isomers are identified by comparing to calculated or literature ionisation
energies. Photoionisation mass spectrometry offers a greater degree of selectivity than electron ionisation or optical spectroscopic methods for characterising molecular beams.

**Figure 1.7:** Photoionisation efficiency spectra for $m/z$ 72 photoproduction ions of butanol combustion. From Reference 136.

At the turn of the last century, photoionisation mass spectrometry joined the family of ambient mass spectrometric methods, in the form of atmospheric pressure photoionisation (APPI). A solution-phase sample, such as the eluent from a liquid chromatograph, is vaporised by a heated nebuliser. Gaseous analyte molecules are subsequently ionised by photons from a UV discharge lamp, commonly krypton or argon. These lamps are chosen such that the photon energy is greater than the ionisation potential of the analyte, but less than those of solvents and gases, increasing sensitivity. Ionisation efficiency in APPI is improved by the addition of dopant molecules, which act as intermediates to the ionisation of analytes that absorb photons without ionisation (through radiative decay or collisional cooling). If the dopant has a higher ionisation energy than the analyte, charge transfer will produce the analyte molecular ion.

In negative-ion APPI, ionisation occurs via electron capture (Reaction 1.19), dissociative electron capture (Reaction 1.20), proton transfer, or anion attachment.
1.7 Radical Ions and Mass Spectrometry

Molecular radical ions and/or even-electron ions may be observed, depending on the gas-phase acidity and electron affinity of the analyte. Molecular radical anions $M^-$ are produced by electron capture or charge-exchange with $O_2^-$, which is formed from electron capture by molecular oxygen. Analytes with high gas-phase acidity transfer a proton to $O_2^-$ or basic solvent-derived species to form $[M - H]^-$ ions.

1.7.1.4 Electrospray Ionisation

Along with matrix-assisted laser desorption ionisation (MALDI), electrospray ionisation (ESI) revolutionised mass spectrometry in the late 1980s. These ‘soft-ionisation’ methods brought MS into the realms of proteomics, polymer chemistry, and analyses of other large, non-volatile, thermally labile molecules that were inaccessible by existing technologies. So outlandish was the premise that such hefty molecules should “fly” in a mass spectrometer, inventor John Fenn described ESI in his Nobel lecture as “the wings for molecular elephants.” Due to its high sensitivity and straightforward coupling to liquid chromatography, ESI has been applied to an innumerable diverse array of analytes across chemistry and biology, from small polar molecules up to DNA, lipids, oligosaccharides, non-covalent protein complexes and intact viruses. ESI is the primary ionisation method employed in this thesis.

A schematic diagram of an ESI source is shown in Figure 1.8. A solution passes through a thin capillary, held at several kV relative to a counter electrode, producing a mist of charged droplets. Heated gas evaporates the encapsulating solvent until Coulombic repulsion inevitably prevails over the surface tension holding the droplet together, and intact gas-phase ions are produced. Mass spectra produced from ESI typically exhibit solely even-electron ions: namely from protonation, deprotonation, the addition of metal ions or small organic ions.
Figure 1.8: Schematic diagram of an ESI source operating in the positive ion mode.

As Figure 1.8 demonstrates, an ESI source is ostensibly an electrolytic cell. Analytes are oxidised or reduced at the metal spray capillary tip (working electrode) depending on the direction of electron flow. If the analyte exhibits an oxidation or reduction potential within ±1.0 V (relative to a standard calomel electrode), radical ions are formed by acceptance or donation of an electron to the circuit. Examples of species which form radical ions under ESI conditions include reduction of quinones and fullerenes in negative ion mode, as well as oxidation of porphyrins, ferrocenes, and retinoids (e.g., vitamin A) in positive ion mode. When 2,2-diphenyl-1-picrylhydrazyl – a stable radical in solution – is subjected to positive ion ESI, the resulting spectrum predominantly exhibits $M^+$ rather than $[M + H]^+$ ions, indicating oxidation precedes protonation and paramagnetism is not retained into the gas phase. Under positive ion ESI conditions, aminoxyl radicals are detected as both $M^+$ and $[M + H]^+$ ions. Although desorption electrospray ionisation (DESI) is an ambient technique considered analogous to ESI, Cooks and co-workers surprisingly found that electrochemical reactions in an ESI source are not replicated in DESI.
1.7 Radical Ions and Mass Spectrometry

1.7.2 Mass Analysers

The role of the mass analyser is to discriminate between ions based on their mass-to-charge ratios (m/z); the extent to which this is possible is governed by the resolving power of the mass analyser. According to IUPAC definitions, mass resolution is: “the observed m/z value divided by the smallest difference $\Delta(m/z)$ for two ions that can be separated: $(m/z)/\Delta(m/z)$.” In instruments with the greatest resolving power, two peaks with masses differing by less than the electron mass (0.00055 Da) are still baseline resolved.

All mass analysers utilise some combination of static or dynamic electric and/or magnetic fields to facilitate ion separation. Mass analysers can be broadly divided into scanning and trapping analysers. Scanning analysers such as magnetic sectors and quadrupoles separate ions in time and space under the influence of external fields, and allow only the ions of a distinct m/z to pass through to the detector at any one time. Trapping analysers use external fields to simultaneously store all ions, where they are discriminated by temporal ejection from the field or their oscillations within the field. An ideal mass analyser would have a high scan speed, high mass limit, high resolution and highly accurate mass measurement. Multiple mass analysers of the same or different types (hybrids) can be combined sequentially to exploit the advantages of each analyser. The properties of common mass analysers are summarised in Table 1.1.
### 1.7 Radical Ions and Mass Spectrometry

**Table 1.1:** Comparison of common mass analyser properties.

<table>
<thead>
<tr>
<th></th>
<th>Time of Flight</th>
<th>Sector</th>
<th>Quadrupole</th>
<th>Ion Trap</th>
<th>FT-ICR</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
<td>1948</td>
<td>1918</td>
<td>1953</td>
<td>1953</td>
<td>1974</td>
<td>1999</td>
</tr>
<tr>
<td><strong>Basis of Separation</strong></td>
<td>Velocity (flight time)</td>
<td>Kinetic energy or momentum</td>
<td>Stability of ion trajectory</td>
<td>Resonance frequency</td>
<td>Resonance frequency</td>
<td>Resonance frequency</td>
</tr>
<tr>
<td><strong>Mass Limit (Th)</strong></td>
<td>&gt; 1 000 000</td>
<td>20 000</td>
<td>4000</td>
<td>6000</td>
<td>30 000</td>
<td>50 000</td>
</tr>
<tr>
<td><strong>Resolution</strong>*</td>
<td>5000</td>
<td>100 000</td>
<td>2000</td>
<td>4000</td>
<td>500 000</td>
<td>100 000</td>
</tr>
<tr>
<td><strong>Mass Accuracy (ppm)</strong></td>
<td>200</td>
<td>&lt; 10</td>
<td>100</td>
<td>100</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td><strong>Sampling</strong></td>
<td>Pulsed</td>
<td>Continuous</td>
<td>Continuous</td>
<td>Pulsed</td>
<td>Pulsed</td>
<td>Pulsed</td>
</tr>
<tr>
<td><strong>Pressure (Torr)</strong></td>
<td>$10^{-6}$</td>
<td>$10^{-6}$</td>
<td>$10^{-5}$</td>
<td>$10^{-3}$</td>
<td>$10^{-10}$</td>
<td>$10^{-10}$</td>
</tr>
<tr>
<td><strong>Tandem Mass Spectrometry Capabilities</strong></td>
<td>Only in combination</td>
<td>Consecutive sectors</td>
<td>Triple Quadrupole</td>
<td>MS$^n$ fragmentation</td>
<td>MS$^{n+}$ fragmentation</td>
<td>None</td>
</tr>
</tbody>
</table>

*Full Width Half Maximum at m/z 1000

Adapted from References 163,164
1.7.2.1 Time-of-Flight

A time-of-flight (TOF) analyser is conceptually simple: ions of different \( m/z \) are distinguished by the time taken to pass through a flight tube to the detector (Figure 1.9a).\(^{165} \) All ions must be initially stored together, and thus have the same start time along the flight tube. This requirement makes TOF analysers ideally suited to pulsed ion sources. Ion packets are accelerated along a flight tube by a potential difference \( V \), whereby electrostatic potential energy is converted to kinetic energy (Equation 1.23). The ion travels at constant velocity \( (v) \) through the flight tube, given by tube length \( L \) divided by flight time \( t \) (Equation 1.24). Combining these equations gives the relationship between flight time and \( m/z \) (Equation 1.25).

\[
E_k = \frac{mv^2}{2} = zeV = E_{el} \tag{1.23}
\]

\[
v = \frac{L}{t} \tag{1.24}
\]

\[
t^2 = \frac{m}{z} \left( \frac{L^2}{2eV} \right) \tag{1.25}
\]

From Equation 1.25, ions arrive at the detector in order of mass, so the mass range of a TOF analyser is theoretically infinite, if one has sufficient patience to wait for the heaviest ions. Mass resolution in a TOF is proportional to the flight time, and thus could be increased by lengthening the flight tube or decreasing the accelerating potential, however, both of these changes adversely affect sensitivity.
1.7 Radical Ions and Mass Spectrometry

Figure 1.9: Principles of separation based on m/z in scanning mass analysers.
(a) TOF analyser, ions separated by flight time; (b) Double sector analyser, only green ions have the correct trajectory to reach the detector; (c) Quadrupole mass filter, only green ions have a stable trajectory through the quadrupole in the presence of an oscillating field. Figure from Reference 165.
1.7 Radical Ions and Mass Spectrometry

1.7.2.2 Electric and Magnetic Sectors

Ions in a magnetic field experience a Lorentz force \( F_L \) perpendicular to both the direction of travel and the magnetic flux \( B \). The ion follows a circular path of radius \( r \), as the Lorentz force is balanced by a centrifugal force \( F_c \) (Equation 1.26).

\[
F_L = zevB = \frac{mv^2}{r} = F_c
\]

\[
r = \frac{mv}{zeB}
\]

That is, in a homogeneous external magnetic field, ions with the same charge and momentum exhibit a characteristic trajectory with a unique radius (Figure 1.9b). Taking into account the potential with which ions are accelerated into the magnetic sector, solving for \( v \) in Equation 1.27 and substituting into Equation 1.23 gives:

\[
\frac{m}{z} = \frac{eB^2r^2}{2V}
\]

Given that the radius is fixed by the instrument geometry, for a constant value of \( V \), scanning the magnetic field \( B \) allows for successive transmission of ions with different \( m/z \) values through to the detector in order to obtain a mass spectrum.\(^{166}\)

Resolution and sensitivity of a magnetic sector is compromised by the assumption that all ions leave the source with the same kinetic energy. Isobaric ions with different velocities will not follow the same trajectory. To improve resolution, an electric sector is added to the ion path to focus ions according to their kinetic energy. The trajectory of an ion in an electric sector is independent of mass, and therefore an electric sector alone is not a mass analyser. All ions with the same kinetic energy pass through the electric sector at a defined field strength (Figure 1.9b). Electric and magnetic sectors in combination can achieve much higher mass resolution than a magnetic sector alone.
1.7.2.3 Quadrupoles

Quadrupole mass analysers separate ions of different \( m/z \) based on the stability of their trajectory in an oscillating electrodynamic field, according to the Mathieu equations of motion. A quadrupole consists of four parallel hyperbolic (more practically, cylindrical) rods (Figure 1.9c). A cross-section of a quadrupole is shown in Figure 1.10. Equal and opposite potentials are applied to each pair of rods, comprising an alternating radio frequency (RF) field \( V \cos(\omega t) \), where \( V \) is the amplitude, and \( \omega \) the angular frequency) superimposed on a fixed DC voltage \( U \).

![Cross-section of a quadrupole mass analyser](image)

**Figure 1.10:** Cross-section of a quadrupole mass analyser: ions travel from source to detector along the \( z \)-axis.

The motion of an ion through the oscillating field depends on its mass. Consider firstly the positively charged rods (along the \( y \)-axis of Figure 1.10). In the absence of an RF field, positively charged ions are focussed along the central axis and continue through to the detector. When the RF field is applied, heavy ions remain predominantly influenced by the average potential applied to the electrodes, that is, the DC potential. The periods when the electrodes are at a net negative potential have a negligible effect on the ion trajectory through to the detector. Conversely, lighter ions are accelerated more rapidly by the oscillating field. During periods of negative potential, the ions are accelerated toward the rods and discharge. In this way, the positive electrodes act as a
high-mass filter. Depending on the magnitude and frequency of the RF waveform, there exists a critical \( m/z \) ratio below which ions will not be transmitted to the detector.\(^{167}\)

The equal and opposite argument stands for the negatively charged rods. Solely in the presence of a DC potential, positive ions are attracted to the rods. When the RF waveform is applied and the overall potential is positive, ions are focussed toward the centre of the quadrupolar field. Lighter ions are deflected more strongly, while the heavier ions once again experience predominantly the DC potential that leads to discharge on the rods. Thus, these rods act as low-mass filters, only allowing ions less than a certain critical \( m/z \) to pass. With careful selection of the potential waveforms, in combination the pairs of rods allow only ions of a single \( m/z \) value through to the detector. Quadrupole mass analysers exhibit modest mass resolving power and have a relatively low mass limit (Table 1.1).

### 1.7.2.4 Ion Traps

The preceding sections have discussed beam-type mass analysers that separate ions in space and time. Like a quadrupole, ion traps use oscillating fields to store ions, prior to sequential ejection on demand by resonant excitation. Ion traps can be classified as linear or 3-dimensional (Figure 1.11).\(^{168,169}\) A 3D (Paul) trap can be envisaged as a quadrupole looped over on itself; the inner rod is reduced to a central point, the outer rod is the ring electrode, and the top and bottom rods make up the endcaps.\(^{163}\) A linear ion trap is essentially a quadrupole with two additional endcap electrodes trapping ions in the axial dimension, and are advantageous insofar as greater ion storage capacity and sensitivity compared to 3D traps.\(^{169}\)
1.7 Radical Ions and Mass Spectrometry

**Figure 1.11:** Central ring and ellipsoid cap electrodes comprise a 3D ion trap (left). In a linear ion trap (right), the ring electrode is replaced by a quadrupole. Figures from References 168 and 169.

As with the quadrupole mass analyser, starting with the Mathieu equations of ion motion in a quadrupolar field, it is possible to calculate ion trajectories as a function of mass to charge (here represented by the elementary charge $e$, to avoid confusion with the $z$-coordinate). One can determine which ions are stable in a trap of radius $r_0$ as a function of the zero-peak amplitude ($V$) and drive frequency ($\Omega$) of the AC field, by the dimensionless trapping parameter $q_z$ (Equation 1.29). A second stability parameter $a_z$ is proportional to the DC potential ($U$), which is zero for a linear trap.\footnote{170}

\[
q_z = \frac{4eV}{mr_0^2 \Omega^2} \tag{1.29}
\]

Ions with a $q$-parameter value between 0 and 0.908 are stable within the trap, as shown by the limit at which the stability region (shaded area) crosses the $q$-axis in Figure 1.12 ($a_z = 0$). By adjusting the applied RF field, the $q$-parameters of different mass ions exceed the stability limit, and are thus ejected from the ion trap, while desired ions of a single $m/z$ value (or range) remain in the trap for further analysis. Isolated ion ensembles tightly focussed in space provide an excellent opportunity to study the interaction of such ions with volatile neutral reagents or laser radiation.
1.7 Radical Ions and Mass Spectrometry

Figure 1.12: Stability diagram (in $r,z$ space) in a quadrupole ion trap parameterised by the voltages and frequencies applied to the trap, from Reference 170.

1.7.3 Tandem Mass Spectrometry

A single $m/z$ value is rarely sufficient to fully elucidate molecular structure. Using the highest resolution mass analysers, an accurate mass will at best identify molecular formulae, but is powerless to discriminate isomers. Tandem mass spectrometry (MS/MS) is a general term for any method that involves two stages of mass analysis, in conjunction with a mass change of the ion under examination. The mass change may be a result of a chemical reaction, or dissociation prompted by an external stimulus. The resulting product ions are studied to aid identification of the precursor ion. Moreover, the products of such reactions may be inaccessible by conventional synthesis and ionisation, and thus MS/MS represents an entirely new field of synthesising novel species – particularly unstable radicals. MS/MS can be accomplished by combining multiple scanning mass analysers of the same or different types (MS/MS in space), or in any of the trapping instruments (MS/MS in time). In this thesis, the nomenclature proposed by Cooks et al.,$^{171}$ is utilised (Figure 1.13) to distinguish different tandem mass spectrometry scan modes.
1.7 Radical Ions and Mass Spectrometry

Figure 1.13: Symbols used to distinguish different tandem mass spectrometry modes.

In a beam-type analyser such as a triple quadrupole, a product ion scan involves selectively isolating a precursor ion in the first mass analyser, and using the second mass analyser to acquire a spectrum of all product ions resulting from interaction with a target gas, electrons, photons or a surface. In trapping analysers, product ions of interest can be re-isolated and the process repeated (denoted MS$^n$ for $n \geq 2$ mass analysis stages). Precursor ion scanning is the ‘opposite’ configuration, in that the first mass analyser acts in a surveying mode, whilst the second mass analyser is set to isolate a specific $m/z$ ratio. As such, all precursors of a common product ion are identified. Similarly, neutral loss scanning identifies all product ions resulting from common loss of a neutral fragment. Selected reaction monitoring is even more specific, as only a single combination of precursor and product ions are detected, and is useful for product validation. Aside from product ion scanning, these MS/MS modes are only possible on multi-sector or multi-quadrupole geometries. Dissociation techniques for product ion scans are discussed below, with a particular focus on the formation of radical ions.

1.7.3.1 Collision-Induced Dissociation

Collision-induced dissociation (CID) as a structural elucidation tool has its origins in the early works of Haddon and McLafferty in determining the structure of the $\text{C}_3\text{H}_7^+$ ion.$^{172}$ CID presents an unparalleled opportunity to use mass spectrometry for elucidation of molecular structure, which is more powerful than molecular mass
confirmation. Moreover, CID is popular due to the diversity of mass spectrometer configurations on which it can be employed, and its universal applicability to almost any molecular system, including large biomolecules. In a CID experiment, isolated ion(s) of interest are allowed to interact with an inert gas, typically argon or helium. In beam analysers, the collision cell is located between successive quadrupoles or sectors, whereas in ion traps, the inert gas is always present and an additional excitation waveform is applied to stimulate dissociation. The title “collision-induced” is somewhat misleading, as unimolecular dissociation is not a direct result of a single collision in a hard-sphere sense. CID is a form of “slow-heating” method, in which translational energy is converted into internal energy through multiple collisions. The maximum amount of energy transferred under inelastic conditions is given by the energy in the centre of mass frame of reference (\(E_{\text{cm}}\)):

\[
E_{\text{cm}} = E_{\text{lab}} \frac{M_t}{M_i + M_t}
\]

(1.30)

where \(E_{\text{lab}}\) is the ion kinetic energy in the laboratory reference frame, \(M_i\) is the ion mass, and \(M_t\) the mass of the target gas. Each collision occurs in a very short timescale (10\(^{-14}\) to 10\(^{-12}\) s), however the total activation time is in the order of milliseconds. After multiple collisions, the internal energy of the vibrationally excited ion is sufficient to induce unimolecular dissociation. The resulting spectrum is characteristic of the precursor ion structure (Figure 1.14). Similar to photoionisation onsets, ions may also be characterised by their dissociation threshold the amount of energy required to initiate fragmentation (as long as the initial energy distribution of the ion packet is controlled).
Because of the relatively long timescales involved, activated ions have sufficient time to redistribute internal energy among all vibrational modes. Therefore, observed product ions derive from the lowest energy fragmentation exit channel on the precursor ion potential energy surface. Even-electron ions typically do not form radical ions through covalent bond cleavage under CID conditions, an empirical observation known as the ‘even-electron rule.’ Some violations to this phenomenon have been observed in organic ions with labile covalent bonds, however low-energy CID generally remains the realm of even-electron ions.

As mass spectrometry is increasingly used to analyse large and complex biomolecules, greater demands are placed on the ability of tandem mass spectrometry to fragment such ions and produce spectra with sufficient information for unambiguous identification. CID and related slow-heating methods do not always satisfy this requirement. For example, CID induces repeatable fragmentation of peptides and proteins at backbone amide bonds. However, if labile post-translational modifications such as phosphorylation or glycosylation are present, the predominant product ion corresponds to a neutral loss of the PTM (e.g., $\text{H}_3\text{PO}_4$ from phosphates). Such cleavage unambiguously identifies the PTM, but comes at the expense of structurally informative sequence ions. Derivatisation strategies have been developed to deliberately incorporate a labile bond that is susceptible to homolysis by CID, hence generating site-specific
radical ions. In a recent example, Leeming and co-workers demonstrated that protonated nitro-guanidine moieties are susceptible to N–N bond homolysis upon CID, thus releasing NO₂⁻.¹⁸¹,¹⁸²

1.7.3.2  Electron Capture Dissociation and Electron Transfer Dissociation

Alternative tandem mass spectrometry techniques have been developed to complement CID, and circumvent its limitations.¹⁸³,¹⁸⁴ Particular emphasis has been placed on methods to produce radical ions, hence producing product ion spectra exhibiting rich fragmentation. Electron capture dissociation (ECD) involves interacting multiply charged cations with a beam of low-energy (< 0.2 eV) electrons in an ICR cell.¹⁸⁵ Activation occurs rapidly (< 10⁻¹⁴ s), producing an excited charge-reduced radical ion which dissociates prior to energy redistribution.

ECD is limited by being compatible only with costly FT-ICR instruments. The electric fields applied to ion traps perturb electron motion and minimise interactions with trapped ions. Electron transfer dissociation (ETD)¹⁸⁶ is an alternative method to reduce multiply charged cations to radical cations in ion traps, by an ion/ion reaction with a stable radical anion, such as anthracene:

\[ M^{n+} + A^- \rightarrow M^{(n-1)+} + A \]  \hspace{1cm} (1.31)

Fragmentation induced by ETD is similar to that of ECD. As dissociation is not limited to the weakest bonds, extensive fragmentation is observed even for large ions. ECD and ETD provide complementary information to CID, and may be advantageous for protein analysis due to extensive sequence coverage and PTM localisation.¹⁸⁷,¹⁸⁸ For example, Figure 1.15 compares the CID and ETD spectra of a triply charged peptide with a sulfonate PTM. Even when magnified by 50-fold, the CID spectrum contains only one major product ion, corresponding to neutral loss of SO₃, and no sequence ions. By contrast, the ETD spectrum exhibits rich fragmentation along the peptide backbone.
1.7 Radical Ions and Mass Spectrometry

![CID and ETD spectra](image)

**Figure 1.15**: CID (top) and ETD spectra of a sulfonated peptide, from Reference 188.

1.7.3.3 Ultraviolet Photodissociation

Like the electron-based tandem mass spectrometric methods, ultraviolet photodissociation (UVPD) can access higher energy product ion pathways (e.g., bond homolysis) than slow-heating methods, due to the short timescales involved in excitation and dissociation. This is illustrated schematically in Figure 1.16.\(^{189}\)

![Schematic of UVPD](image)

**Figure 1.16**: UVPD of a precursor ion \(M^+\) leads to a wider array of fragment ions \((F_n^+)\) than CID. Figure from Reference 189.

In a typical UVPD experiment, ions of interest isolated in a mass analyser, (usually an ion trap) are irradiated with a pre-determined number of laser pulses at a given wavelength. Precursor ions and photoproduct ions are scanned out to compile the photodissociation mass spectrum, and the trap is re-filled prior to the next laser pulse.
Successful implementation of UVPD requires: (i) modification of the mass spectrometer to gain optical access into the ion trap;\(^{190,191}\) (ii) adequate overlap between the trapped ion ensemble and incident photons; and (iii) the ions of interest having sufficient absorption cross-section at the studied wavelength. The availability of fixed-frequency and tunable laser sources has greatly expanded the scope of UVPD, which can be categorised into selective and indiscriminate dissociation. In the latter, vacuum ultraviolet photons from an excimer laser (e.g., 193 nm from an ArF laser) are used to irradiate trapped ions, with the aim of producing as many fragment ions as possible to elucidate primary structure. UVPD in this wavelength range is particularly useful for the analysis of proteins, which absorb strongly below 200 nm,\(^{192,193}\) due to the large number of amide chromophores and aromatic side chains. Performed in parallel with CID, 193 nm UVPD spectra of proteins,\(^{194}\) lipids,\(^{195}\) and sugars\(^{196}\) provide complementary data and increase the likelihood of complete structural elucidation, demonstrated in Figure 1.17 for the 7\(^+\) – 13\(^+\) charge states of ubiquitin.\(^{194}\)

\[\text{Figure 1.17:} \quad 193 \text{ nm UVPD of ubiquitin results in greater sequence coverage than collisional or electron based methods. Figure from Reference 194.}\]

An alternate UVPD strategy is to target a specific photo-labile bond to selectively produce a clean population of ions with site-specific radicals. A recent example of this strategy is the photolysis of weak carbon – halogen bonds in charge-
remote aryl iodides using 266 nm photons.\textsuperscript{197} Thus formed, the nascent charge-tagged phenyl radical is further characterised by its addition to molecular oxygen.\textsuperscript{198,199}

As noted previously, CID and related slow-heating technologies are inadequate for full structural elucidation of large, complex biomolecules. Selective homolysis of C–I bonds whilst leaving the rest of the molecular structure unperturbed presents an attractive strategy for introducing radicals into specific sites of such molecules, which then induces rich, structurally informative fragmentation. Derivatising the target biomolecular ion to incorporate an aryl iodide motif is one example of radical-directed dissociation (RDD).

Structural modification is typically carried out synthetically prior to mass spectrometric analysis, for example iodination of tyrosine residues in a peptide/protein,\textsuperscript{200} or fatty acid derivatisation as iodobenzyl esters (Figure 1.18).\textsuperscript{201} However, as evidence for the selectivity of bond homolysis, the aryl-iodide motif can also be incorporated as part of a non-covalent complex with the target lipid or protein.\textsuperscript{202,203} Remarkably upon UVPD, the complex remains intact other than the eliminated iodide radical. In either covalent or non-covalent attachment, the charge-tagged phenyl radical product ion formed by UVPD is re-isolated and subjected to CID to produce an RDD spectrum. The primary benefit of RDD is rich fragmentation for a greater degree of structural information than CID alone. For example, RDD allows the identification of branching positions in isomeric lipids (Figure 1.18b,d).\textsuperscript{201,202}
Figure 1.18: Derivatisation of isomeric lipids as iodobenzyl esters.

(a, c) 266 nm UVPD spectra exhibiting primarily I\(^{•}\) loss, (b, d) RDD spectra enabling isomer discrimination. Figure from Reference 201.
1.8 Gas-Phase Distonic Ions

Formation of ions with site-specific radicals presented in the preceding section are examples of distonic ions. By contrast to canonical radical ions, distonic ions are radical ions in which the charge and radical sites are not co-localised on the same atomic centre. Distonic ions can arise from the ionisation of biradicals, zwitterions or ylides. Interest in distonic ions intensified upon the discovery of Yates et al. that distonic ions are in some cases more stable than their analogous conventional isomers. Because of the insulation between charge and radical sites, the two moieties react largely independent of one another.

Distonic ions have been extensively investigated in the gas phase via mass spectrometry. The technologies described in Section 1.7, when applied to carefully designed molecular scaffolds, enable the production, isolation and characterisation of pure populations of distonic ions with well-defined radical sites.

1.8.1 Formation

1.8.1.1 Distonic Radical Cations

Radical cations of a distonic nature were first postulated by Gross and McLaugherty in 1971 whilst investigating the ion-molecule reaction between cyclopropane radical cation and ammonia in an ion cyclotron resonance (ICR) cell. By means of electronic structure calculations, Bouma et al. later demonstrated that the \(^{1}\text{CH}_2\text{OH}_2^+\) distonic cation of methanol is significantly more stable than the conventional isomer \(\text{CH}_3\text{OH}^{++}\). The same research group subsequently investigated the formation of these isomers in the gas phase. Whilst \(\text{CH}_3\text{OH}^{++}\) is obtained by electron ionisation of methanol, the distonic isomer is observed in the gas phase by collisional activation of the ethylene glycol radical cation (Scheme 1.10). The two isomers exhibit different CID spectra, thus confirming the presence of two distinct radical ion species.
1.8 Gas-Phase Distonic Ions

**Scheme 1.10:** Formation of methyleneoxonium radical cation from ethylene glycol.

Kenttämaa and co-workers developed a general synthesis of distonic aryl radicals in an ICR dual cell.\(^{209-211}\) This procedure (Scheme 1.11) involves electron ionisation of multiply halogenated benzenes which undergo a gas-phase ion/molecule reaction with nucleophilic pyridine. A distonic radical cation is produced in a rare example of bond homolysis by low-energy CID, or by UVPD at 266 nm.\(^{197}\) Kirk et al., similarly employed UVPD to cleave the C–I bond of iodobenzene substituted with a 4-\(N,N,N\)-trimethylamino fixed positive charge tag.\(^{198}\)

**Scheme 1.11:** Charge-tagged phenyl radicals from pyridine and diiodobenzenes.

### 1.8.1.2 Distonic Radical Anions

Gas-phase radical anions with separated charge and spin sites were first synthesised by Harrison and Jennings\(^{212}\) in 1976 by the reaction of acetone with atomic oxygen radical anion (O\(^{-}\)) in an ICR mass spectrometer (Scheme 1.12). It must be noted that this product ion is not truly distonic, due to cross-conjugation. It was not until 1988 that the term ‘distonic’ was applied to radical anions.\(^{213}\)

**Scheme 1.12:** Formation of distonic radical anions from O\(^{-}\) and acetone.
Nonetheless, the reaction of $O^-$ with organic molecules is an effective strategy for synthesising a wide array of radical anions, including distonic ions.\textsuperscript{214} The power of mass spectrometry is such that if unwanted side-reactions do occur, the desired radical anion can still be isolated free from interferences of different masses. Guo and Grabowski\textsuperscript{215} reacted $O^-$ with benzene to form $o$-benzyne, which was allowed to subsequently react with $CO_2$ in a flowing afterglow apparatus, forming a negatively charge-tagged phenyl radical (Scheme 1.13).

![Scheme 1.13: Reacting benzene with O\textsuperscript{-} and CO\textsubscript{2} addition yields o-dehydrobenzoate.](image)

An alternate route to $o$-dehydrobenzoate is through oxidative decarboxylation of phthalate dianions by collisional activation (Scheme 1.14). The meta and para isomers are accessible from the appropriate di-acid. This route was first proposed by Siu \textit{et al.},\textsuperscript{216} and subsequently expanded by Kass and co-workers to include various dicarboxylates using ESI, or EI/CI of the equivalent diesters.\textsuperscript{217,218}

![Scheme 1.14: CID of dicarboxylates yields distonic radical anions via decarboxylation and concomitant electron loss.](image)

The oxidative decarboxylation strategy is advantageous compared to the use of $O^-$ due to its regioselectivity, ease of precursor preparation, and a lack of interfering side-reactions. Blanksby \textit{et al.} have subsequently shown that this strategy is also applicable to rigid cycloalkanes. ESI of 1,4-cyclohexanedicarboxylic acid or 1,3-
adamantanedicarboxylic acid yields dianions that undergo single oxidative decarboxylation under CID, producing distonic radical anions.\textsuperscript{219-221} The latter was also prepared by CID of a Barton ester derivative, which undergoes N–O bond cleavage and decarboxylation of the nascent oxycarbonyl radical (Scheme 1.15).\textsuperscript{220}

\begin{equation}
\text{Scheme 1.15: Two precursors for preparing adamantyl distonic anions by CID.}
\end{equation}

As shown in previous sections, UVPD is an attractive alternative to CID for increasing selectivity of radical ion formation from certain precursors. Kirk et al. complemented their positive ion study of phenyl radicals by employing 266 nm UVPD to cleave the C–I bond of deprotonated 4-iodobenzoic acid, thus forming a distonic anion. CID of the same precursor does not yield distonic ion products.\textsuperscript{198}

Electron photodetachment from alkyl dianions $\text{O}_2\text{C–(CH}_2\text{)_n–CO}_2^–$ has been extensively studied by photoelectron spectroscopy.\textsuperscript{222,223} Balancing the energy input by the photon and the kinetic energy of the detected electron yields the electron binding energy. Wang and co-workers\textsuperscript{223,224} postulated that the observed dependence of electron binding energy on $n$ (related to the distance between the charge sites) is a result of a reverse Coulombic barrier, which must be overcome to detach an electron. The importance of this finding led Kirk et al. to study the ionic products of the same reaction using UVPD mass spectrometry.\textsuperscript{225} Upon irradiation of the precursor dianion M$^2^–$, the
primary photodetachment product $M^-$ is not observed, instead subsequent decarboxylation yields an observed $[M – CO_2]^+$ product ion.

In this work, we consider the possibility of a simpler route to distonic radical ions. That is, electrospray ionisation of stable aminoxyl radicals substituted with remote Brønsted acid or base motifs, removing the challenging requirement of gas-phase radical synthesis entirely.

### 1.8.2 Characterisation

Confirming the distonic nature of the formed radical ion is critically important. Electronic structure calculations play a central role in comparing the energetics of radical ion isomers, and there are a number of experimental mass spectrometric techniques available to verify the findings of such surveys.

Selective isotopic labelling of precursor ions (e.g., $^2$H, $^{13}$C, $^{18}$O) will lead to a mass shift in the product ion. Deuterium-labelling experiments by Nibbering et al. confirmed the structure of the product ion obtained by the reaction of acetone and O$^-$ (Scheme 1.12). When acetone is replaced with 1,1,1-trideuterioacetone, the $m/z$ 56 product is shifted to $m/z$ 58, indicating that only two of the three deuterium atoms remain. That is, the two hydrogens were abstracted from different carbons.

Collisional activation of the conventional and distonic isomers can produce characteristic mass spectra. Wysocki and Kenttämaa compared the CID spectra of distonic and canonical radical cation isomers of small alcohols. Upon CID in a triple quadrupole, the conventional isomer of ethanol exhibits product ions arising from loss of CH$_3^+$ or H$^+$ (Figure 1.19a). The distonic ion is readily distinguished by a predominant ion at $m/z$ 28 due to the loss of water (Figure 1.19b). These results further suggest the two isomers do not inter-convert prior to dissociation.
1.8 Gas-Phase Distonic Ions

![Figure 1.19](image)

**Figure 1.19**: Product ion abundances as a function of collision energy from CID of canonical and distonic radical cations of ethanol. From Reference 227.

Comparing CID spectra in this way relies on definitive, distinct gas-phase syntheses of both (or all) radical ion isomers. Furthermore, analysis will be complicated if the canonical and distonic isomers are linked by an isomerisation barrier lower than the dissociation barrier. Wysocki and Kenttämaa discovered that CH$_3$CH$_2$CH$_2$OH•• and •CH$_2$CH$_2$CH$_2$OH$_2$• radical cations of propanol produce identical CID spectra in an ion trap mass spectrometer. They proposed that the lowest energy dissociation channel available to CH$_3$CH$_2$CH$_2$OH•• (H• loss) is higher in energy than the barrier to isomerisation to the distonic form and subsequent water loss.\(^{227}\)

Reactions between ions and volatile neutral molecules in the gas-phase have been central to mass spectrometry for synthesis of novel product ions (e.g., Scheme 1.11). Ion-molecule reactions can be employed as diagnostic tools for structural elucidation,\(^{228}\) including distonic ions.\(^{229,230}\) Unlike their canonical counterparts, distonic ions will typically exhibit reactivity similar to that of the neutral radical, by undergoing radical-radical combination, hydrogen abstraction, and alkene addition.

A principal example of distonic ion validation is the ion-molecule reaction of alkyl radical ions with dimethyl disulfide or dimethyl diselenide.\(^{231,232}\) Distonic ions abstract 'SCH$_3$, and are thus identified by a product ion with a mass 49 Da greater than
the precursor, or equivalently +95 Da upon abstraction of 'SeCH₃. Diagnostic reactions of distonic alkyl and aryl radicals with neutral molecules are summarised in Table 1.2. Proposed reaction pathways are validated by comparing the behaviour (e.g., CID) of the reaction product with standards. Conventional radical ions typically react via charge exchange with the listed reagents.

**Table 1.2: Reactions of distonic radical ions with neutral molecules.**

<table>
<thead>
<tr>
<th>Neutral Reagent</th>
<th>Reaction</th>
<th>Mass Shift (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrahydrofuran</td>
<td>H⁻ abstraction</td>
<td>+ 1</td>
</tr>
<tr>
<td>Acetone</td>
<td>H⁻ abstraction</td>
<td>+ 1</td>
</tr>
<tr>
<td>tert-butylisocyanide</td>
<td>'CN abstraction</td>
<td>+ 26</td>
</tr>
<tr>
<td>Allyl iodide</td>
<td>Allyl or I⁻ abstraction</td>
<td>+127</td>
</tr>
<tr>
<td>I₂</td>
<td>I⁻ abstraction</td>
<td>+127</td>
</tr>
<tr>
<td>Br₂</td>
<td>Br⁻ abstraction</td>
<td>+79 / 81</td>
</tr>
<tr>
<td>CBr₄</td>
<td>Br⁻ abstraction</td>
<td>+79 / 81</td>
</tr>
<tr>
<td>O₂</td>
<td>O₂ addition†</td>
<td>+ 32†</td>
</tr>
<tr>
<td>NO</td>
<td>NO addition</td>
<td>+ 30</td>
</tr>
</tbody>
</table>

Adapted from Reference 233. †Spontaneous peroxy radical decomposition is also observed.

**1.8.3 Probing Neutral Radical Chemistry**

Guo and Grabowski were the first to suggest that as distonic ions exhibit reactivity resembling that of corresponding neutral radicals, these ions are advantageous for examining the behaviour of their elusive non-ionic counterparts.⁵¹⁵ Shortcomings of conventional characterisation methods for neutral free radicals (Section 1.6) may thus be circumvented by investigating the corresponding distonic ion. A fixed charge remote from the radical permits isolation of a pure population of the desired radicals by mass spectrometry, and furthermore Coulombic repulsion eliminates adverse combination self-reactions. Careful experimental or computational assessment is required to evaluate how the presence of a charge perturbs radical behaviour. Kenttämaa and co-workers
have assessed the effect of positive and negative remote charge sites on the reactivity of the phenyl radical with neutral molecules, in terms of collision encounter probability, ion-molecule solvation, reaction exothermicity, and polar effects. They conclude that:

"the reactivity of distonic ions does provide a good qualitative model of radical reactivity, and there are many circumstances where the power and flexibility of mass spectrometric experiments and the illumination they can provide outweighs the kinetic perturbation of the charge site."\(^\text{233}\)

In other words, despite the influence of the charge on the nucleophilicity of the radical moiety, distonic ions react in a similar way to the neutral radical, and are therefore useful tools for the study of radical chemistry. Moreover, the role of the charge can be manipulated by varying its polarity and proximity to the radical site. Evaluating the influence of charge-tags in distonic ions is central to this research.

Carefully designed distonic radical ions with spatially and electronically insulated charge sites are therefore useful for extracting spectroscopic data,\(^\text{199}\) as well as relative rates\(^\text{198,233}\) and products\(^\text{221,234}\) of radical reactions. Such data can clarify unknown or ambiguous behaviour of the corresponding neutral, or can be compared to existing to neutral radical data to quantify the effects of the ion.

The relative ease with which stable neutral aminoxyl radicals may be characterised in the condensed phases (e.g., ESR) means that there has not been the same obligation to resort to distonic ions to study their reactivity. Therefore, little is known about the intrinsic gas phase energetics and reactivity of these radicals.
1.9 Project Aims and Scope

Aminoxyl radicals have widespread applications in polymer chemistry, and have therefore been extensively studied in the condensed phases. Their fundamental reactivity in the absence of polymer matrix or solvent effects however, has received scant attention. Understanding the intrinsic nature of these processes in the gas phase is essential to comprehending and predicting the uncertain behaviour of aminoxyl radicals in different chemical systems, including solutions and polymers. Furthermore, experimental gas-phase measurements are an essential tool for benchmarking the validity of complementary electronic structure calculations that can be used to elucidate reaction pathways and mechanisms.

This thesis aims to address the current paucity of information in this area by utilising the sensitivity and selectivity of tandem mass spectrometry to synthesise and characterise aminoxyl radicals with an ionic site remote from the radical moiety. The knowledge gained from such fundamental studies in the gas phase can subsequently be applied to unresolved problems concerning the role of aminoxyl radicals in polymer chemistry.

Furthermore, studying the formation and reactivity of aminoxyl radicals by mass spectrometry presents a convenient scaffold with which to assess the influence of a remote charge on radical energetics and reactivity. By studying the effect of charge polarity and proximity on radical behaviour, this thesis aims to critically evaluate the applicability of gas phase distonic ions as probes for neutral radical chemistry.
2. EXPERIMENTAL EVIDENCE FOR COMPETITIVE N–O AND O–C BOND HOMOLYSIS IN GAS-PHASE ALKOXYAMINES

This chapter has been submitted in its current form for peer-review publication.

Supporting Information is attached as Appendix A.


Author Statement

David L. Marshall performed all experimental research and wrote the manuscript with input from all authors. Electronic structure calculations were carried out by Anya Gryn’ova from Australian National University. This work would not have been possible without the intellectual input and support provided by Michelle L. Coote, Philip J. Barker and Stephen J. Blanksby.

Primary Supervisor Confirmation

I, Prof. Stephen J. Blanksby (Primary Supervisor), support and certify the above author statement.

Signature  
Date  
10/4/14
2.1 Abstract

The extensive use of alkoxyamines in controlled radical polymerisation and polymer stabilisation is based on rapid cycling between the alkoxyamine \((R^1R^2\text{NO}–R^3)\) and a stable aminoxyl radical \((R^1R^2\text{NO}^+)\) via homolysis of the labile O–C bond. Competing homolysis of the alkoxyamine N–O bond has been predicted to occur for some substituents leading to production of aminyl and alkoxyl radicals. This intrinsic competition between the O–C and N–O bond homolysis processes has to this point been difficult to probe experimentally. Herein we examine the effect of local molecular structure on the competition between N–O and O–C bond cleavage in the gas phase by variable energy tandem mass spectrometry in a triple quadrupole mass spectrometer. A suite of cyclic alkoxyamines with remote carboxylic acid moieties \((\text{HOOC–}R^1R^2\text{NO–}R^3)\) were synthesised and subjected to negative ion electrospray ionisation to yield \([M – H]^-\) anions where the charge is remote from the alkoxyamine moiety. Collision-induced dissociation of these anions yield product ions resulting, almost exclusively, from homolysis of O–C and/or N–O bonds. The relative efficacy of N–O and O–C bond homolysis was examined for alkoxyamines incorporating different \(R^3\) substituents by varying the potential difference applied to the collision cell, and comparing dissociation thresholds of each product ion channel. For most \(R^2\)-substituents, product ions from homolysis of the O–C bond are observed and product ions resulting from cleavage of the N–O bond are minor or absent. A limited number of examples were encountered however, where N–O homolysis is a competitive dissociation pathway because the O–C bond is stabilised by adjacent heteroatom(s) \((e.g., R^3 = CH_2F)\). The dissociation threshold energies were compared for different alkoxyamine substituents \((R^3)\) and the relative ordering of these experimentally determined energies is shown to correlate with the bond dissociation free energies, calculated by \textit{ab initio} methods. Understanding the
structure-dependent relationship between these rival processes will assist in the design and selection of alkoxyamine motifs that selectively promote the desirable O–C homolysis pathway.

**Keywords:** Tandem mass spectrometry, aminoxyl radical, alkoxyamine, bond homolysis
2.2 Introduction

 Hindered amine light stabilisers (HALS) are anti-oxidant additives employed to improve the durability and lifetime of polymer surface coatings in outdoor applications.\textsuperscript{76,235} Even at low concentrations, inclusion of HALS improves gloss and colour retention in pigmented coatings and provides a superior aesthetic for the lifetime of the product. Despite the clear advantages offered by their usage, the exact role of HALS in stabilising polymer coatings remains an active topic of discussion.\textsuperscript{77,79,81,82,89,90,93,236,237} Contemporary mechanisms for this anti-oxidant action invoke cycling of the amine functional group of the HALS between the alkoxyamine ($R^1R^2\text{NO}$–$R^3$), aminoxyl radical ($R^1R^2\text{NO}$', variously referred to as ‘nitroxide’ or ‘aminoxyl’), and aminyl radical ($R^1R^2\text{N}'$) forms (Scheme 2.1a).\textsuperscript{92} The stable aminoxyl radical scavenges deleterious polymer chain-based macroradicals ($R^3\text{•}$), which otherwise accelerate polymer degradation through chain scission. The combination product is an alkoxyamine containing the polymer fragment bonded directly to the nitroxide. Advantageously, aminoxyl radicals are subsequently regenerated from these alkoxyamines, and by-products of the reformation step are relatively inert. While direct cleavage of the O–C and N–O bonds directly connects the alkoxyamine with both the aminoxyl and aminyl radicals, under typical service conditions homolysis pathways are not energetically competitive with an alternative mechanism that involves a $\beta$-hydrogen elimination step and connects these intermediates within the catalytic cycle.\textsuperscript{92,94,238} Nevertheless, the impact of the $R^3$-substituent, which varies widely depending on the polymer substrate and breakdown mechanism, on the relative energetics of the O–C and N–O bonds remains of considerable interest.\textsuperscript{95}
2.2 Introduction

Scheme 2.1: (a) Polymer stabilisation activity of HALS by regenerative cycling of aminoxyl radicals involving β-hydrogen abstraction from an alkoxyamine and an aminyl radical intermediate; (b) Simplified mechanism of NMP, highlighting reversible O–C bond formation and homolysis.

Alkoxyamines are similarly central to the mechanism of nitroxide-mediated polymerisation (NMP) (Scheme 2.1b), a technique that produces materials with low polydispersity and controlled molecular weight. The success of NMP depends on control over the reversibility and rates of alkoxyamine formation and dissociation ($k_{\text{comb}}$, $k_{\text{diss}}$), as well as suppressing the significance of side-reactions, such as disproportionation. These factors in turn are dependent on the structure of both the nitroxide and the monomer. Driven by the importance of reversible O–C bond homolysis to both polymer synthesis and stabilisation, the molecular structural factors...
governing such a step in alkoxyamines are well characterised. Conversely, less attention has been paid to the unwanted corresponding N–O cleavage, which would result in the formation of highly reactive aminyl and alkoxyl radicals, detrimental to the activity of the catalytic cycle. Homolysis of the N–O bond in alkoxyamines is promoted when either or both of the resulting radicals are stabilised. For example, N–O bond homolysis is observed during degradation of indoline-based nitroxides, whereby the homolysis product is an aryl aminyl radical, and in thermolysis of alkoxyamines with aryl or acyl O-ether substituents. In a theoretical study of the competitive bond cleavage processes, Tordo et al. demonstrated that whilst semi-empirical computational methods reliably predict relative trends in the O–C bond dissociation energies (BDEs) of a series of alkoxyamines, higher level density functional methods are required to adequately describe the competition between the O–C and N–O cleavage processes. A more recent comprehensive theoretical study, using benchmarked high-level \textit{ab initio} methods, established certain alkoxyamine functionalities (\textit{i.e.}, $R^3$ in Scheme 2.1) promote N–O homolysis over O–C homolysis, due to anomeric stabilisation of the O–C bond. Gigmes and co-workers have suggested that “there may be a competition between (N)O–C and N–O(C) bond cleavage. The possibility and the extent of bond cleavage depend on the nature of the $R^3$ alkyl moiety bound to the O-atom of the nitrooxide function.” Testing this hypothesis experimentally for the intrinsic dissociation of alkoxyamines in the gas phase is the central aim of this work.

Tandem mass spectrometry is a well-established approach to investigate competing mechanisms of dissociation in gas-phase ions. If the charged moiety is largely fixed within the molecular scaffold and is isolated from the labile functional groups, then it is possible to observe charge-remote dissociation, which may be closely
related to thermolysis of analogous neutral species.\textsuperscript{180,255,256} It has previously been
demonstrated that alkoxyamines undergo charge-remote homolysis of the O–C
bond.\textsuperscript{122,257,258} For example, Oh et al. derivatised small peptides with alkoxyamines\textsuperscript{259–}
\textsuperscript{261} and subjected these to electrospray ionisation (ESI) to form ions with the charge sites
localised to amino acid residues and thus remote from the alkoxyamine. Subsequent
collision-induced dissociation (CID) of these ions resulted in almost exclusive O–C
bond homolysis, generating an alkyl radical which initiated further fragmentation of the
peptide ion. However, depending on the nature and proximity of the charge to the
alkoxyamine moiety, bond homolysis is not always selectively observed, and ions
resulting from charge-directed fragmentation may also be present.\textsuperscript{262,263} Building on this
understanding we have developed a molecular scaffold incorporating an alkoxyamine
functional group carrying a wide range of R\textsuperscript{3}-substituents and a remote negative charge
to experimentally investigate competition between charge-remote O–C and N–O
homolysis during low-energy CID. Further experimental and computational approaches
verify the structure-dependent energetics of the competing N–O and O–C homolysis
processes, and highlight the local structural requirements for N–O homolysis in
alkoxyamines.
2.3 Experimental

2.3.1 Materials

Aminoxyl radicals 4-carboxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-carboxy-TEMPO, 1) and 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3-carboxy-PROXYL, 2) were purchased from Sigma Aldrich (Sydney, Australia), and used without further purification. Hydrogen peroxide (Australian Chemical Reagents, Queensland, Australia) was used as a 40% (w/w) aqueous solution. Perdeuterated (D$_6$)-acetone was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Methanol employed for mass spectrometry was HPLC grade (Thermo Fisher Scientific, Melbourne, Australia) and used as received. All other materials for synthesis were purchased from Sigma Aldrich, and used as received.

2.3.2 Synthesis

Synthesis of alkoxyamines from nitroxide precursors and various alkyl radical sources has been widely documented.$^{264-269}$ Alkoxyamines (with the exception of 1c and 1e) were prepared according to the method of Schoening et al.,$^{270,271}$ whereby alkyl radicals are generated in situ from a ketone or aldehyde, copper (I) chloride, and hydrogen peroxide. In the presence of the nitroxide, nascent radicals are readily trapped, forming the desired alkoxyamines, listed in Scheme 2.2. This method was chosen for its simplicity, ready availability of reagents, and wide array of possible functionalities, with the exception of benzaldehydes, which do not generate phenyl radicals under these conditions. 1c was prepared by substituting TEMPO for 4-carboxy-TEMPO (1) in a literature method, refluxing overnight in cyclohexene.$^{272,273}$ Preparation of 1e was also based on adaptation of an existing method, refluxing 4-carboxy-TEMPO (1) and the radical initiator 1,1′-azobis(cyclohexanecarbonitrile) for 30 hours in methanol.$^{53}$ 4-$N,N,N,$-trimethylamino-TEMPO was prepared as the iodide salt according to the method
of Strehmel et al. Characterisation of all products by high-resolution mass spectrometry is provided as Supporting Information (Table S1).

<table>
<thead>
<tr>
<th>R³</th>
<th>(1)</th>
<th>(2)</th>
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<tbody>
<tr>
<td>CO₂H</td>
<td>CO₂H</td>
<td>CO₂H</td>
</tr>
<tr>
<td>O⁻</td>
<td>O⁻</td>
<td>O⁻</td>
</tr>
<tr>
<td>methyl</td>
<td>(a)</td>
<td>(b)</td>
</tr>
<tr>
<td>(c)</td>
<td>(d)</td>
<td>(e)</td>
</tr>
<tr>
<td>(f)</td>
<td>(g)</td>
<td>(h)</td>
</tr>
<tr>
<td>(i)</td>
<td>(j)</td>
<td>(k)</td>
</tr>
</tbody>
</table>

Scheme 2.2: Alkoxamines (1x) and (2x) examined in this study, based on 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2), where x is any R³ group (a-i).

2.3.3 Mass Spectrometry

Negative ion mass spectra were recorded with a QuattroMicro (Waters, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an ESI source and controlled by Micromass MassLynx software (version 4.1). Alkoxamines (Scheme 2.2) were diluted to ca. 5 μM in methanol, and infused directly into the ESI source at 5 μL min⁻¹. The capillary voltage was set to 3.0 kV, cone voltage 25 V, and source temperature 80 °C. Nitrogen was used as the drying gas, at a temperature of 110 °C, and flow rate of 320 L h⁻¹. In all collision-induced dissociation (CID) scans, ions of interest were selected in Q1, subjected to collisions with argon gas in Q2 at a pressure of 3.0 ± 0.1 mTorr, and the collision energy in the laboratory frame (E_lab) was varied from 2–25 eV. For product ion structural validation, MSⁿ spectra were recorded on an LTQ 2-dimensional linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA,
USA), and high-resolution MS and MS/MS spectra were acquired on a Waters Xevo G1 Q-ToF mass spectrometer.

Breakdown curves are obtained by plotting the normalised intensity of the product ion(s) of interest against the collision energy in the centre-of-mass frame ($E_{cm}$), where $E_{cm}$ is equal to $E_{lab}$ multiplied by the reduced mass of the colliding ion and neutral argon gas. Empirically, threshold behaviour was analysed by using a least-squares fitting criterion to fit sigmoid functions to the data, of the type shown in Equation 2.1

$$I_i(E_{cm}) = BR_i/(1 + e^{\left(\frac{E_{1/2,i} - E_{cm}}{b_i}\right)})$$

In Equation 1, $BR_i$ is the branching ratio of the product ion of interest (i), $E_{1/2,i}$ is the energy at which the function has reached half of its maximum value, and the parameter $b_i$ describes the steepness of the sigmoid curve. Furthermore, we define “dissociation threshold” as the energy at which the product ion abundance is equal to 5% of the total ion intensity (i.e., $I_i = 0.05$). As we are comparing fragmentation onsets and not quantitatively deriving energetic thresholds, we find that using the appearance energy definition of Schröder et al.,\cite{275,276} (i.e., using a linear extrapolation of the sigmoid curve at $E_{1/2}$ to the x-axis) does not significantly improve the quality of the data fit or the correlation with calculated thermodynamic quantities.

### 2.3.4 Computational Procedures

Standard $ab$ $initio$ molecular orbital theory and density functional theory calculations were carried out using Gaussian 09\cite{277} and MOLPRO 2012.1.\cite{278} Calculations were performed at a high level of theory, recently demonstrated to predict gas- and solution-phase bond dissociation energies and associated equilibrium constants to within chemical accuracy.\cite{95,279} Geometries of all species were fully optimised at the M06-2X/6-31+G(d) level of theory. For all species, full systematic conformational
searches (at a resolution of 60°) were carried out to ensure global, and not merely local, minima were located. Frequencies were calculated at this level and scaled by recommended scale factors.\textsuperscript{280} Improved energies for all species were calculated using a double layer ONIOM-type method. The core layer (including both the aminoxyl and carboxylic acid moieties) was calculated using composite high-level G3(MP2,CC)(+) level of theory,\textsuperscript{281} where (+) denotes inclusion of diffuse functions in a standard 6-31G(d) basis set. The full system was calculated with the M06-2X/6-31+G(d) method. This methodology has been shown to accurately predict the gas-phase energetics of aminoxyl and other free radical reactions.\textsuperscript{279,282} Entropies and thermal corrections at 25, and 80 °C were calculated using standard formulae for the statistical thermodynamics of an ideal gas under the harmonic oscillator approximation in conjunction with the optimised geometries and scaled frequencies.\textsuperscript{283}
2.4 Results and Discussion

Alkoxyamines are readily detected as protonated or alkali metal adduct ions by electrospray ionisation (ESI),\textsuperscript{263} matrix assisted laser desorption ionisation (MALDI),\textsuperscript{284,285} liquid extraction surface analysis (LESA),\textsuperscript{286} or desorption electrospray ionisation (DESI).\textsuperscript{121} However, in the absence of another basic moiety, protonation of an alkoxyamine nitrogen raises the O–C BDE by over 100 kJ mol\textsuperscript{-1}.\textsuperscript{287} Charge-driven dissociation mechanisms dominate upon CID of [M + H]\textsuperscript{+} or [M + Na]\textsuperscript{+} alkoxyamine ions, leading to predictable and readily assignable products in the resulting spectra,\textsuperscript{257,263} but no information is ascertained concerning the relative energies of the N–O and O–C bonds. Our first attempts to produce charge-remote alkoxyamines were based on 4-\textit{N,N,N}-trimethylamino-TEMPO, in order to sequester the charge on the piperidine fragment and promote homolysis. However, the CID spectra of such alkoxyamines (Supporting Information, Figure S1) feature predominantly protonated \textit{O}-alkyl acetone oxime fragment ions, and [M – 59]\textsuperscript{+} ions corresponding to loss of trimethylamine. The former dissociation is consistent with previous reports on the charge-directed dissociation of substituted piperidines.\textsuperscript{286} In the absence of the desired charge-remote dissociation of the alkoxyamine moiety, an alternative strategy was sought. Only limited examples in the literature describe the tandem mass spectrometric analysis of alkoxyamines upon negative ion electrospray ionisation.\textsuperscript{261} Commercially available nitroxides 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2) were found to be suitable scaffolds for producing [M – H]\textsuperscript{−} anions for the suite of TEMPO-based alkoxyamines designated \textbf{1a-i} and the PROXYL-series \textbf{2a, 2b} and \textbf{2h} (Scheme 2.2).
2.4 Results and Discussion

2.4.1 Dissociation of Methoxyamines

Methanolic solutions of alkoxyamines displayed in Scheme 2.2 exhibit abundant [M – H]⁻ ions upon negative ion ESI. These anions were isolated and subjected to CID in a triple quadrupole mass spectrometer using argon as the collision gas. Comparative CID spectra of methoxyamines 1a and 2a are displayed in Figure 2.1(a) and (b), respectively. The most abundant product ion in each example represents a loss of 15 Da from the precursor ion and is assigned to the neutral loss of a methyl radical (CH₃); a deviation from the ‘even-electron rule’.

![Diagram](image)

**Figure 2.1**: ESI-MS/MS spectra \( (E_{\text{lab}} = 15 \text{ eV}) \) of alkoxyamine [M – H]⁻ ions. (a) 1a at \( m/z \) 214, (b) 2a at \( m/z \) 200, (c) \( (D_3\)-methyl)-2a at \( m/z \) 203.
To demonstrate that the loss of 15 Da arises solely from O–C homolysis and not by ejection of a methyl radical from the piperidine ring, selective replacement of hydrogen with deuterium on the methoxyamine moiety was undertaken by using $D_6$-acetone in the synthesis of 2a to form the isotopologue ($D_3$-methyl)-2a. Isolation and fragmentation of the [M – H]$^-$ ion at m/z 203, under the same experimental conditions, yields an abundant ion at m/z 185 representing a loss of 18 Da and ejection of $D_3$-methyl radical ($\cdot$CD$_3$) (Figure 2.1c). Importantly, no [M – H – 15]$^-$ ions are observed at m/z 188 suggesting the methyl loss pathways occurs exclusively via O–C bond cleavage. For further confirmation, CID experiments were repeated on a linear ion trap mass spectrometer with MS$^n$ capabilities. Upon isolation and collisional activation of the m/z 185 product ions from both 2a (Figure 2.1b) and $D_3$-2a (Figure 2.1c), the resulting spectra are identical, and moreover, identical to the MS$^2$ CID spectrum of the [M – H]$^-$ ion of a 3-carboxy-PROXYL (2) standard (Figure 2.2b). Similarly, the MS$^3$ spectrum of the m/z 199 product ion from 1a is identical to the MS$^2$ spectrum of 4-carboxy-TEMPO (1) (vide infra). The [M – H – 15]$^-$ product ions at m/z 199 and m/z 185 for 1a and 2a respectively, correspond to loss of a methyl radical exclusively via homolysis of the oxygen-carbon bond and are therefore assigned as the deprotonated aminoxyl radicals 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2), respectively.

Closer examination of the CID spectra in Figure 2.1 reveals further similarities in the precursor ion dissociation pattern. In Figure 2.1(a) and (b), additional ions constituting neutral losses of 30, 31 and 46 Da in both spectra indicate that the predominant fragmentation pattern is common to both the 6- and 5-membered ring alkoxyamines. In Figure 2.1(c), the corresponding losses from the deuterium-labelled precursor ion are increased by 3 Da (i.e., 33, 34 and 49 Da). That is, the product ions at
Results and Discussion

$m/z$ 170, 169 and 154 are observed in both Figure 2.1(b) and (c), indicating that none of these ions contain the methyl group from the methoxyamine.

The MS\(^2\) spectrum of $m/z$ 199 from authentic, deprotonated 4-carboxy-TEMPO (1) is identical to the MS\(^3\) spectrum of $m/z$ 199 from prior dissociation of 1a (Figure 2.2a). Collisional activation of $m/z$ 199 ions (either from standard 1 or prior dissociation of 1a) yields predominantly ions at $m/z$ 184, indicating that methyl radical ejection also occurs from the nitroxide radical. These data suggest that the ions observed at $m/z$ 184 in Figure 2.1(a) are likely to come from consecutive losses of methyl radicals rather than a single concerted loss of 30 Da. This stepwise process is illustrated in Scheme 2.3 and involves an initial loss of the methyl group from the methoxyamine to yield the nitroxide radical that subsequently ejects a methyl group from the piperidine ring. A plausible structure for the ion at $m/z$ 184 is therefore the nitrone 4-carboxy-2,2,6-trimethyl-2,3,4,5-tetrahydropyridine-\(N\)-oxide (Scheme 2.3a).

![Figure 2.2: CID spectra (\(E_{\text{lab}} = 20\) eV) of \([M - H]^+\) ions of authentic aminoxyl radicals.](image)

(a) 4-carboxy-TEMPO (1); (b) 3-carboxy-PROXYL (2), showing methyl radical loss to form nitrones at $m/z$ 184 and 170, respectively.
An analogous, stepwise methyl radical ejection process is proposed for the
dissociation of the 5-membered cyclic nitroxide 2 (Figure 2.2b). When the primary ions
(
m/z 185) from O–C homolysis of 2a are further interrogated by MS\(^3\), ions at m/z 170
are observed as a result of methyl radical ejection. A prominent ion at m/z 126 is also
observed corresponding to the loss of CO\(_2\) from ions at m/z 170 and suggesting the
carbanion structure as indicated in Scheme 2.3(b). Formation of this carbanion is
facilitated in the PROXYL scaffold by resonance stabilisation by the carbon-nitrogen
double bond. This contention is supported by the fact that, decarboxylation is not
observed directly from the precursor ion and likewise, CO\(_2\) loss is observed only to a
very minor extent (e.g., m/z 140, Figure 2.2a) upon the dissociation of the larger rings of
TEMPO-based series where similar stabilisation of the carbanion is not possible.

![Scheme 2.3](image)

**Scheme 2.3**: Dissociation of anionic alkoxyamines through competing charge-remote
O–C and N–O homolysis pathways.

(a) TEMPO-based alkoxyamines; (b) PROXYL-based alkoxyamines.
2.4 Results and Discussion

At high collision energies ($E_{\text{lab}} > 20 \text{ eV}$), additional low mass ions are observed in the CID spectra of both alkoxyamines 1a and 2a (Supporting Information, Figure S2). Putative structures for these ions are provided as Supporting Information (Scheme S1 and Table S2). Importantly, the presence of these ions in the CID spectra of the authentic nitroxides 1 (e.g., m/z 122, Figure 2.2a) and 2 (e.g., m/z 108, Figure 2.2b) implies that they are secondary dissociation products upon O–C homolysis of alkoxyamines. Thus the abundance of these minor ions are included when considering the total population of product ions arising from O–C homolysis at high collision energies.

In Figure 2.1, product ions at m/z 199 and 184 upon collisional activation of 1a (and equivalently m/z 185 and 170 for 2a) result from homolysis of the O–C bond. Conversely, ions at m/z 183 and 168 are the products of N–O bond cleavage. Homolysis of this bond results in loss of a methoxyl radical, and transient aminyl radicals are observed in low abundance at m/z 183 (1a), and m/z 169 (2a). As with the aminoxyl radical product ions, a further loss of 15 Da is also observed in the spectrum, and is likewise assigned to subsequent methyl radical ejection from the aminyl radical to form an imine (Scheme 2.3). Unlike stable aminoxyl radicals, authentic aminyl radicals are not readily prepared and isolated in the gas phase so CID spectra of known standards were not available for comparison. However, when the primary ions at m/z 183 from N–O homolysis of 1a are isolated in a linear ion trap, product ions at m/z 168 are observed in the MS$^3$ spectrum, arising from facile demethylation of the aminyl radical; even when no additional collisional activation is applied. No significant low mass secondary product ions are identified from further isolation and fragmentation of ions at either m/z 183 or 168. When the equivalent primary ions (m/z 169) from N–O homolysis of 2a are analysed by MS$^3$, ions at m/z 154 are similarly observed, indicating subsequent
demethylation. An additional ion at \( m/z \) 110 is also observed, putatively assigned to decarboxylation of \( m/z \) 154 ions, and resonance stabilised by the carbon-nitrogen double bond. Like the dissociation via O–C homolysis, decarboxylation is not observed directly from the precursor ion.

The major product ions observed upon collisional activation of methoxyamine 1a are summarised in Scheme 2.3(a). Importantly, product ions at \( m/z \) 199 and \( m/z \) 184 are a consequence of O–C homolysis, and \( m/z \) 183 and \( m/z \) 168 are the result of N–O homolysis. Equivalent mechanisms are proposed in Scheme 2.3(b) for the fragmentation of the pyrrolidine-based alkoxyamine 2a.

### 2.4.2 Effect of O-Ether Functionality (R³)

Product ions attributed to either N–O or O–C homolysis, as summarised in Scheme 2.3, do not contain the original O-ether fragment (R³). CID spectra of additional TEMPO-based alkoxyamines 1b-1i, each with a different R³ functionality, were also examined (Table 2.1). In each case, the same product ions were observed as for 1a, with the spectra only differing in peak intensities. For example, the CID spectra of 1b, 1g, 1h, and 1i (Figure 2.3) all show features at \( m/z \) 168, 183, 184 and 199 identical to those of 1a (Figure 2.1a). In a similar way, most of the product ions observed for the methoxyamine 2a (Figure 2.1b) are also observed in the CID mass spectra of the PROXYL-based alkoxyamines 2b and 2h (Table 2.1). Significantly, the major product ions arising from dissociation of both series of alkoxyamines do not contain the R³ substituent (full suite of MS/MS spectra are provided as Supporting Information, Figure S3).
2.4 Results and Discussion

Figure 2.3: Negative ion CID spectra ($E_{lab} = 20$ eV) of alkoxyamines.

(a) benzyloxyamine 1b; (b) $n$-butyloxyamine 1g; (c) fluoromethoxyamine 1h; (d) (1-acetyloxy)ethoxyamine 1i, each exhibiting product ions at $m/z$ 184, 183, and 168.
### 2.4 Results and Discussion

**Table 2.1**: Relative product ion abundance in CID spectra of 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2) [M – H]⁻ ions ($E_{\text{lab}} = 15$ eV).

<table>
<thead>
<tr>
<th># ($R^3$)</th>
<th>[M – H]⁻</th>
<th>m/z 199</th>
<th>m/z 184</th>
<th>m/z 183</th>
<th>m/z 168</th>
<th>Other ions m/z (% abundance)</th>
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<tbody>
<tr>
<td>1a (CH₃)</td>
<td></td>
<td>76.6</td>
<td>100.0</td>
<td>6.8</td>
<td>1.5</td>
<td>10.1</td>
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<tr>
<td>1b (CH₂Ph)</td>
<td></td>
<td>3.9</td>
<td>100.0</td>
<td>2.5</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1c (cyclohex-2-ene)</td>
<td></td>
<td>2.9</td>
<td>100.0</td>
<td>1.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1d (CH(CH₃)COOEt)</td>
<td></td>
<td>4.9</td>
<td>100.0</td>
<td>1.5</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1e (1-CN⁻C₆H₁₁)</td>
<td></td>
<td>23.5</td>
<td>100.0</td>
<td>7.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1f (¹C₄H₀)</td>
<td></td>
<td>100.0</td>
<td>66.2</td>
<td>1.1</td>
<td>n.d.</td>
<td>0.7</td>
</tr>
<tr>
<td>1g (⁶C₄H₀)</td>
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<td>100.0</td>
<td>78.1</td>
<td>4.5</td>
<td>0.9</td>
<td>6.2</td>
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2.4 Results and Discussion

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<td>6.0</td>
<td>36.4</td>
<td>182 (6.1); 138 (39.2); 49 (10.1)</td>
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<td>8.9</td>
<td>1.1</td>
<td>85.0</td>
<td>100.0</td>
<td>226 (78.6); 59 (54.2)</td>
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<th>m/z 170</th>
<th>m/z 169</th>
<th>m/z 154</th>
<th>Other ions m/z (% abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>23.4</td>
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<td>9.8</td>
<td>1.1</td>
<td>5.3</td>
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<tr>
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<td>100.0</td>
<td>3.7</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2h</td>
<td>100.0</td>
<td>1.2</td>
<td>30.0</td>
<td>14.8</td>
<td>69.2; 168 (1.8); 128 (8.2); 110 (3.3); 49 (25.9)</td>
</tr>
</tbody>
</table>

Each value is an average of 3 measurements, each comprising at least 50 scans, and normalised to the base peak in each spectrum.

n.d. = less than 0.2% relative to base peak.

† Isobutene loss from 1f (-56 Da)

‡ Concerted loss of HF and formaldehyde from 1h and 2h

# cis-Elimination from 1i producing either acetate anion (m/z 59) or [M – CH₃CO₂H]⁻ (m/z 226). See text for details.
Product ions not assigned to either N–O or O–C homolysis are observed in the CID spectra of 1h (Figure 2.3c), 2h, and particularly 1i (Figure 2.3d) to an extent not observed in other substrates. These ions may arise from even-electron dissociation of the precursor ion that is competitive with homolysis when the latter is energetically cumbersome. We speculate that the abundance of N–O homolysis product ions (m/z 168 and 183, Figure 2.3d) are inflated by a competing charge-remote cis-elimination of acetic acid that forms a vinyl-substituted alkoxyamine at m/z 226 (R¹R²NOCH=CH₂), which then undergoes further dissociation via N–O homolysis. When this ion is isolated within the linear ion trap and subjected to further collisional activation in an MS³ experiment, product ions at m/z 183 and m/z 168 are observed in high abundance. Due to this competing even-electron dissociation, 1i is excluded from further discussion on homolysis trends in alkoxyamines.

Based on the product characterisation conducted for 1a, the product ion at m/z 199 in all spectra in Figure 2.3 is assigned as 4-carboxy-TEMPO (1), arising from homolysis of the oxygen-carbon bond, and loss of a carbon-centred radical. More generally, homolysis of the oxygen-carbon bond is a major fragmentation process in the CID of charge-remote alkoxyamines, with a particularly high efficacy in substrates that produce a stabilised alkyl radical (e.g., a benzyl radical from 1b and 2b, an allyl radical from 1c, or an α-carbonyl radical from 1d, 1e). These results are consistent with the observation of selective O–C bond homolysis upon collisional activation of peptide ions modified with a TEMPO-CH₂Ph linkage, structurally similar to 1b. Conversely, homolysis of the oxygen-carbon bond is suppressed in 1h and 2h, due to anomeric stabilisation of the bond by hyper-conjugation from the adjacent heteroatom, and thus ions resulting from N–O homolysis are observed in high abundance from these precursors.
2.4 Results and Discussion

2.4.3 Dissociation Thresholds of Alkoxyamine Ions

The data set presented in Table 2.1 demonstrate that the alkoxyamine substituent, $R^3$, influences the relative efficacy of N–O bond homolysis against O–C bond homolysis, which manifests as varying abundances of the ion pairs: $m/z$ 168 and 183 for the former, and $m/z$ 184 and 199 for the latter (Scheme 2.3). To explore this effect, the energy acquired by each precursor ion was varied by adjusting the potential difference between the ion source and the collision cell ($E_{\text{lab}}$) of the triple quadrupole mass spectrometer. The normalised abundance of O–C and N–O homolysis product ions were then plotted as a function of applied energy in the centre-of-mass frame ($E_{\text{cm}}$) such that dissociation thresholds (the energy required for formation of 5% product ion abundance, $E_{5\%}$) could be compared between N–O and O–C bonds in a single substrate, and between different substituted alkoxyamines.

For the TEMPO-based alkoxyamines 1a – 1g, cleavage of the O–C bond was found to be the preferred homolysis channel. For example, the aforementioned ions at $m/z$ 199 and 184 (and at higher energies, $m/z$ 122 and 106 – Figure 2.2a) are major product ions in the CID spectrum of methoxypiperidine 1a, when $E_{\text{cm}} > 1.5$ eV ($E_{5\%}$), whereupon dissociation of the precursor ion becomes significant (Figure 2.4). The abundance of product ions reaches a maximum at approximately 3.0 eV where they constitute ca. 82% of the total ion population. Product ions from N–O homolysis at $m/z$ 183 and 168 are observed only at low abundance, accounting for ca. 18% of the total ion population, even at the highest energies utilised, with a higher dissociation threshold of 2.3 eV.
2.4 Results and Discussion

Figure 2.4: Relative abundance of product ions arising from N–O and O–C homolysis of methoxypiperidine (1a) as a function of collision energy ($E_{cm}$).

The relative dissociation thresholds are rationalised by comparing the calculated free energies of the two bonds. Our results computed at 298 K are given in Figure 2.5 below, while Table S3 in the Supporting Information demonstrates that the qualitative trends in these bond dissociation free energies are largely independent of temperature. In neutral alkoxyamines, O–C homolysis is preferential to N–O homolysis for all $R^3$ except 1h, 2h and 1i due to anomeric stabilisation of the O–C bond. The preference for direct O–C cleavage is minimal for those $R^3$ units that produce relatively non-stabilised carbon-centred radicals upon homolysis, e.g., $^{t}$CH$_3$ and $^{t}$Bu$^{•}$ as in 1a, 1f and 2a. However, the most striking feature of Figure 2.5 is the effect of deprotonation on these bond homolysis energetics. There is generally no appreciable difference in the N–O bond energetics between neutral and anionic alkoxyamines; however, O–C bonds are significantly weakened toward homolytic cleavage by deprotonation of the remote carboxylic acid moiety. This is due to a combination of two factors: (i) conventional polar effect of the negative charge destabilising alkoxyamines and stabilising the forming aminoxyl via their corresponding charge-separated resonance contributors, $R^1R^2NO^{–}R^3^{3+}$ and $R^1R^2N^{–}O^{+}$, respectively; and (ii) additional Coulombic stabilisation
of aminoxyl radicals by remote negative charges arising from their enhanced polarisability.\textsuperscript{279,282} As a result, the thermodynamic preference ($\Delta \Delta G_{298}^\circ$) for O–C cleavage in deprotonated alkoxyamines is further enhanced by up to 20 kJ mol$^{-1}$ compared to their neutral counterparts. This observation is true for all $R^3$ moieties studied herein, and thus trends in the effect of the $R^3$ moiety are representative of the trends in neutral species.

**Figure 2.5:** Calculated free energies (kJ mol$^{-1}$) of O–C and N–O bond homolysis in anionic (−) and neutral (H) alkoxyamines at 298 K.

Breakdown curves for the alkoxyamines \textbf{1b} – \textbf{1e} are shown in Figure 2.6(a). Homolysis of the O–C bond occurs at a significantly lower threshold energy ($E_{5\%} = 0.5 – 0.7$ eV) compared to methoxyamine \textbf{1a} ($E_{5\%} = 1.5$ eV). In these substrates, almost quantitative conversion from alkoxyamine to aminoxyl radical is observed, even below collision energies of 2.0 eV. Furthermore, O–C homolysis is highly selective, with no N–O homolysis product ions detected at $m/z$ 168 or 183 for any of the precursors \textbf{1b} – \textbf{1e}. Compared to the alkyl ethers (\textit{e.g.}, \textbf{1a}, \textbf{1f}, \textbf{1g}), which exhibit a modest
thermodynamic preference for homolysis of the O–C bond, substrates 1b – 1e contain much weaker O–C bonds ($\Delta G_{298} < 120 \text{ kJ mol}^{-1}$), and a greater selectivity ($\Delta\Delta G_{298} > 60 \text{ kJ mol}^{-1}$) for O–C cleavage (Figure 2.5). These observations are consistent with the presence of $R^3$-substituents that stabilise the resulting alkyl radical for 1b – 1e.

![Figure 2.6: (a) Selective O–C homolysis in alkoxyamines that release a stable radical upon dissociation. (b) N–O homolysis is the dominant fragmentation mechanism upon CID of fluoromethoxyamine (1h), compared to methoxyamine (1a). Conversely, the alkoxyamine containing an adjacent heteroatom (1h) exhibits remarkably different fragmentation behaviour. In Figure 2.6(b), the O–C homolysis product ion abundance from the CID of 1h (and 1a, for comparison) are plotted with open shapes, whilst the abundance of corresponding N–O product ions are denoted with filled shapes. In 1h, there is competition between the two pathways much like the alkyl substituted alkoxyamine 1a, however in this case N–O homolysis is the dominant pathway, and products of O–C homolysis are observed in minor abundance. Despite this, selectivity is not as pronounced as in 1b – 1e. Furthermore, higher experimental]
threshold energies are required for dissociation of the N–O bond: 1.8 eV for 1h and 2.3 eV for 1a, compared with 1.5 eV for O–C homolysis in 1a. These observations are rationalised by again considering the relative N–O and O–C bond energies in these ions (Figure 2.5). In deprotonated 1h, the free energy required to cleave either the N–O (163.9 kJ mol$^{-1}$) or O–C bonds (183.6 kJ mol$^{-1}$) at 298 K is higher than the energy requirement for cleavage of the O–C bonds in substrates that preferentially dissociate via O–C homolysis (75-135 kJ mol$^{-1}$).

Within the current experiment it is also possible to compare homolytic dissociation not only as a function of O-ether alkoxyamine substituent, but also as a function of ring size, by comparing the breakdown curves of 6-membered cyclic alkoxyamines with the equivalent 5-membered rings. In Figure 2.7, breakdown curves show the normalised abundances of ions arising from O–C homolysis of TEMPO-based 1a, 1b, 1h (denoted by open shapes), as well as PROXYL-based 2a, 2b and 2h (filled shapes). Compared to TEMPO-based alkoxyamines, PROXYL-based alkoxyamines exhibit a greater overall abundance of product ions at a given collision energy input, and similarly a lower O–C dissociation threshold (e.g., 1.2 eV for 2a and 0.5 eV for 2b, compared with 1.5 eV for 1a and 0.7 eV for 1b). Both O–C and N–O bonds are systematically weaker in 5-membered PROXYL-based alkoxyamines, compared with equivalent 6-membered TEMPO-based alkoxyamines, resulting in more facile dissociation. For the methyl and benzyl substituents, the calculated O–C bond dissociation free energy is 10-13 kJ mol$^{-1}$ lower in PROXYL substrates compared to TEMPO (Figure 2.5), consistent with the observation of lower experimental threshold energies. When the alkoxyamine contains a fluoromethyl moiety, the O–C bonds of 1h and 2h are approximately isoenergetic, thus their dissociation thresholds are similar.
Figure 2.7: Breakdown curves comparing O–C homolysis in TEMPO- and PROXYL-based alkoxyamines with CH₃, CH₂Ph, and CH₂F substituents.

By combining all of the obtained experimental and computational data from both TEMPO and PROXYL-based alkoxyamines, the utility of this method for experimentally deriving relative thermodynamic quantities can be evaluated. There is a good correlation ($R^2 = 0.82$, Supporting Information Figure S4) between experimentally obtained O–C threshold energies and calculated gas phase free energies of O–C homolysis. The fit spans an energy range of over 150 kJ mol$^{-1}$, from substrates with weak O–C bonds due to the formation of stable carbon-centred radicals upon dissociation (e.g., 1b-1e), to those with anomeric stabilisation of their O–C bonds (1h). However, the experimental dissociation threshold values are systematically over-estimated with respect to the calculated bond dissociation free energies (slope = 0.6, intercept = 62 kJ mol$^{-1}$). This may in part be caused by the arbitrary choice of a 5% product ion abundance to represent the dissociation threshold. More likely, this offset indicates a significant kinetic shift,$^{290,291}$ which is the excess energy required to produce detectable dissociation of an ion within the experimental timeframe, and scales with increasing vibrational degrees of freedom. That is, the observed dissociation threshold is
only an upper limit to the true thermochemical value. Moreover, when N–O homolysis is energetically competitive with O–C homolysis, a competitive shift may inhibit formation of higher-energy products, and as such are detected only at energies above the actual thresholds. Finally, these experiments were conducted on a commercial triple quadrupole mass spectrometer, and therefore the initial energy distribution and the kinetic energy of the ion population are not adequately controlled. This stands in contrast to guided ion beam mass spectrometry, wherein these parameters are precisely controlled and thus the resulting data can be used to derive accurate thermochemical quantities. Despite these limitations, for the aims of the current work, the experimentally derived dissociation thresholds have provided an excellent means to explore relative trends in bond energies and to test computational predictions.
2.5 Conclusions

A suite of novel alkoxyamines was prepared based on the aminoxyl radicals 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2). CID of [M – H]⁻ anions in a triple quadrupole mass spectrometer produced radical fragment ions, which arise from charge-remote homolysis of either the O–C or N–O bonds. The relative abundance of these ions is dependent on the O-alkyl functionality. These systems thus allowed the first direct experimental comparison of the competition between O–C and N–O homolysis in alkoxyamines. An exception to this behaviour was observed for alkoxyamine 1i, in which a charge-remote electrocyclic rearrangement was competitive with homolysis.

Breakdown curves were utilised to experimentally compare dissociation thresholds. The majority of alkoxyamines exhibit an O–C dissociation threshold lower than their corresponding N–O dissociation threshold, consistent with their relative calculated bond dissociation free energies. Only in the presence of a fluoromethyl substituent (1h and 2h) was N–O cleavage unequivocally observed as the dominant dissociation mechanism. Calculations further reveal that – compared with neutral scaffolds – O–C bonds are weakened in the presence of a negative charge on the TEMPO or PROXYL ring by ca. 20 kJ mol⁻¹. However, the effect is consistent across the range of scaffolds studied, and thus the observed structural trends are also representative of neutral alkoxyamines.

In controlled radical polymerisation and polymer stabilisation, the O-ether substituent (R₃) is dependent on the radicals produced by the growing or degrading polymer substrate. For example, benzyloxyamines (1b and 2b) are models for the alkoxyamines derived from polystyrenes, whereas carbonyl-containing substrates (1c and 1i) represent the capture of model polyester-derived radicals. It is clear from the presented data that direct N–O homolysis is typically not competitive with the
prevailing O–C homolysis. Only in the presence of an adjacent heteroatom would N–O homolysis be expected to dominate. Therefore, aminyl radicals and secondary amines observed in the degradation of alkoxyamines at normal service temperatures (up to 80 °C) are likely not formed directly from N–O bond homolysis, but by alternative processes, such as those outlined in Scheme 2.1(a).\textsuperscript{92,94}

2.6 Acknowledgements

The authors acknowledge the generous financial assistance provided by the Australian Research Council (ARC) through the Centre of Excellence for Free Radical Chemistry and Biotechnology (CE0561607) and Discovery grant (DP120102922) schemes, an ARC Future Fellowship (to M.L.C.), an Australian Postgraduate Award (to D.L.M.), and allocations of supercomputing time on the National Facility of the Australian National Computational Infrastructure.
APPENDIX A

SUPPORTING INFORMATION TO CHAPTER 2:
Experimental evidence for competitive N–O and O–C bond homolysis in gas-phase alkoxyamines

Figure S1: CID spectra ($E_{\text{lab}} = 20$ eV) of $N,N,N$-trimethylamino-TEMPO-based alkoxyamines: (a) methoxyamine ($R^3 = \text{CH}_3$); (b) $t$-butyloxyamine ($R^3 = t\text{C}_4\text{H}_9$). Product ions arising from bond homolysis are not observed.
Figure S2: CID spectra ($E_{lab} = 25$ eV) of methoxyamines: (a) 4-carboxy-TEMPO–CH$_3$ (1a); (b) 3-carboxy-PROXYL-CH$_3$ (2a).

Scheme S1: Proposed dissociation mechanisms to account for minor product ions observed in the CID spectra ($E_{lab} = 25$ eV) of: (a) 4-carboxy-TEMPO–CH$_3$ (1a); (b) 3-carboxy-PROXYL-CH$_3$ (2a).
Table S1: Accurate mass data acquired by high-resolution MS of alkoxyamine ions using leucine-enkephalin as the lockmass. See Scheme 2.2 for the structure of each ion.

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<th>Experimental m/z</th>
<th>Molecular Formula</th>
<th>Calculated m/z</th>
<th>m/z Error (ppm)</th>
</tr>
</thead>
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<td>1a</td>
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<td>-</td>
<td>-</td>
</tr>
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Table S2: Product ion masses acquired by high-resolution MS/MS of methoxyamine precursor ions (1a) and (2a), using leucine-enkephalin as the lockmass. For structural assignments, see Scheme S1 above.

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<th></th>
<th>Experimental m/z</th>
<th>Molecular Formula</th>
<th>Calculated m/z</th>
<th>m/z Error (ppm)</th>
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Figure S3: Representative collision-induced dissociation spectra ($E_{\text{lab}} = 15$ eV) of all alkoxyamines examined in this study (see Table 2.1).
Figure S4: Dissociation threshold energies ($E_{cm}$) plotted against BDFEs calculated by ab initio methods. The trend line is calculated by a linear regression least squares analysis of the O–C data only; N–O bond data points are shown only for reference.
**Table S3:** Full set of calculated homolysis energetics (entropies are given in J mol$^{-1}$ K$^{-1}$, all other parameters – in kJ mol$^{-1}$).

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<th>Species$^a$</th>
<th>Bond$^b$</th>
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<td></td>
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*Appendix A*
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See Scheme 2.2 of the main text for notations; \(^{(H)}\) stands for the neutral carboxylic acid group (COOH), \((-)\) stands for the anion (COO\(^{-}\)).
Table S4: Contributions to the free energies of all species (entropies in J mol\(^{-1}\) K\(^{-1}\), thermal corrections and Gibbs free energies in Hartrees).\(^a\)

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### Appendix A

|     | c1_no        | h1_n         | c1_n         | h2_no        | c2_no        | h2_n         | c2_n         | a_c          | a_o          | b_c          | b_o          | c_c          | c_o          | d_c          | d_o          | e_c          | e_o          | f_c          | f_o          | g_c          | g_o          | h_c          | h_o          | i_c          | i_o          |
|-----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|     | 519.5040     | -670.87996   | 565.9803     | 0.0218374    | -670.89137   | 506.3094     | -632.18294   | 550.0591     | 0.0206588    | -670.87996   | 499.8935     | -596.27666   | 534.0269     | -670.89137   | 489.9705     | -595.73439   | 545.1198     | 0.0211028    | -670.87996   | 500.0591     | 0.0206588    | -670.87996   | 543.0151     | 0.0206588    | -670.89137   | 565.9803     | 0.0218374    | -670.89137   |
|     | 0.0161054    | -670.87996   | 565.9803     | 0.0218374    | -670.89137   | 0.0156483    | -632.18294   | 0.0153602    | -631.64910   | 0.0143758    | -596.27666   | 0.0155255    | -632.18294   | 0.0143758    | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   |
|     | 0.0161054    | -670.87996   | 565.9803     | 0.0218374    | -670.89137   | 0.0156483    | -632.18294   | 0.0153602    | -631.64910   | 0.0143758    | -596.27666   | 0.0155255    | -632.18294   | 0.0143758    | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   | 0.0141535    | -631.64910   |

\(^a\) Calculated using M06-2X/6-31+G(d) method; \(^b\) Notations correspond to Scheme 2.2, ‘h’ in the beginning stands for the protonated state of the carboxylic group, ‘c’ in the beginning – for the deprotonated COOH, ‘_no’ denotes aminoxyl radicals, ‘_n’ denotes the corresponding aminyl radicals, ‘_c’ denotes carbon-centred radicals and ‘_o’ – their alkoxy- analogues.
Appendix A

Table S5: Contributions to the electronic energies of all species (in Hartrees).

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\*Notations correspond to Scheme 2.2, ‘h’ in the beginning stands for the protonated state of the carboxylic group, ‘c’ in the beginning – for the deprotonated COOH, ‘_no’ denotes aminoxyl radicals, ‘_n’ denotes the corresponding aminyl radicals, ‘_c’ denotes carbon-centred radicals and ‘_o’ – their alkoxy- analogues, ‘core’ denotes the core systems used in ONIOM approximation; \(^b\) ZPVEs are calculated using M06-2X/6-31+G(d) method.
3. **GAS-PHASE THERMOCHEMISTRY OF DISTONIC AMINOOXYL RADICAL ANIONS**

The research described in this chapter formed a part of the following peer-reviewed publication:


**Author Statement**

David L. Marshall performed all experimental research and assisted with manuscript preparation. Electronic structure calculations were carried out by Anya Gryn’ova from Australian National University. This work would not have been possible without the intellectual input and support provided by Michelle L. Coote and Stephen J. Blanksby.

**Primary Supervisor Confirmation**

I, Prof. Stephen J. Blanksby (Primary Supervisor), support and certify the above author statement.

Signature: [Signature]

Date: 10/4/14
“Electrospray ionization (ESI) is great. It can be used to gently transfer just about anything, except perhaps small fluffy animals, into the gas phase in an ionized state.”

O. M. Hamdy & R. R. Julian


3.1 Introduction

3.1.1 Background

Previously, alkoxyamines bearing a remote carboxylate anion were subjected to collision-induced dissociation (CID) tandem mass spectrometry, in order to probe the competition between homolytic dissociation of the nitrogen-oxygen and oxygen-carbon bonds. An intriguing finding of this study was that O–C bond dissociation free energies (BDFEs) were calculated to be systematically lower in the presence of the carboxylate anion, compared to analogous neutral species. Thus, the structural trends uncovered in anionic systems are representative of those in neutral alkoxyamines, but systematically offset by ca. 15-20 kJ mol$^{-1}$ (see Figure 2.5).

Here we aim to provide experimental verification of this prediction and to further probe the scope of the phenomenon. Consider the theoretical thermodynamic cycle shown in Scheme 3.1. Homolysis of the oxygen-carbon bond in deprotonated 4-carboxy-1-methoxy-2,2,6,6-tetramethyl piperidine (I) – thus forming distonic radical anion (II) – was experimentally investigated by a dissociation threshold measurement in the previous chapter. Calculations revealed that the O–C BDFE is higher in the equivalent neutral molecule (III). That is, formation of neutral radical (IV) by homolytic cleavage of the oxygen-carbon bond is less favoured upon protonation of anion (I) to form its conjugate acid (III). Clearly, by the means detailed previously, it is
not possible to directly determine experimental BDFEs in neutral molecules, as it is a fundamental requirement of mass spectrometry that molecules carry a charge.

The BDFEs in Scheme 3.1 are linked by Hess’ Law to the gas phase acidities (GPAs) of the radical and closed-shell species. IUPAC defines gas-phase acidity ($\Delta G_{\text{acid}}$) as the negative of the Gibbs energy change for the heterolysis of the generic acid $A^-\text{H}$ into a proton and the conjugate base $A^-$.\(^1\)

\begin{align*}
\text{Scheme 3.1:} & \text{ Thermodynamic cycle relating BDFEs of anionic and neutral alkoxyamines to GPAs of the radical and non-radical forms.} \\
\text{Each of the reactions in Scheme 3.1 can be described in terms of their free energy changes (3.1 – 3.4):} \\
\end{align*}

\begin{align*}
\text{BDFE}_{\text{(anion)}} &= \Delta G_{f(\text{II})} + \Delta G_{f(\text{CH}_3)} - \Delta G_{f(\text{I})} \quad (3.1) \\
\text{BDFE}_{\text{(neutral)}} &= \Delta G_{f(\text{IV})} + \Delta G_{f(\text{CH}_3)} - \Delta G_{f(\text{III})} \quad (3.2) \\
\text{GPA}_{\text{NOR}} &= \Delta G_{f(\text{III})} - \Delta G_{f(\text{H}^+)} - \Delta G_{f(\text{I})} \quad (3.3) \\
\text{GPA}_{\text{NOR'}} &= \Delta G_{f(\text{IV})} - \Delta G_{f(\text{H}^+)} - \Delta G_{f(\text{II})} \quad (3.4)
\end{align*}
Subtracting Equation (3.1) from Equation (3.2) gives the difference in BDFEs between anionic and neutral alkoxyamines (Equation 3.5); that is, the previously calculated value of 15–20 kJ mol\(^{-1}\). Similarly, subtracting Equation (3.3) from Equation (3.4) gives the difference in GPAs (Equation 3.6).

\[
\Delta BDFE = \Delta G_{f(IV)} - \Delta G_{f(III)} - \Delta G_{f(II)} + \Delta G_{f(I)} \quad (3.5)
\]

\[
\Delta GPA = \Delta G_{f(IV)} - \Delta G_{f(III)} - \Delta G_{f(II)} + \Delta G_{f(I)} \quad (3.6)
\]

Crucially, the difference in BDFEs in the homolysis of neutral and negatively charged alkoxyamines is exactly equal to the difference in GPAs in the presence or absence of an aminoxyl radical. Thus, if the gas phase acidities of carboxylic acids (III) and (IV) can be experimentally determined, so too can the difference in homolysis free energies. Such an indirect measurement of thermochemical data has documented precedents. In fact, the thermodynamic cycle depicted in Scheme 3.1 is analogous to the indirect determination of R-H bond dissociation energies (BDEs) by measuring the proton affinity of R-H (Section 3.1.2), the electron affinity of R' (e.g., by photoelectron spectroscopy) and the ionisation potential of a hydrogen atom. (Scheme 3.2).\(^{15}\) Suitable experimental methods are therefore required for determining the gas-phase acidities of carboxylic acids (III) and (IV).

![Scheme 3.2](image)

**Scheme 3.2:** Indirect BDE determination by a negative ion cycle involving the proton affinity of R-H, the electron affinity of R', and the ionisation energy of R'.
3.1.2 Determining Gas-Phase Acidities

3.1.2.1 Equilibrium Methods

Suppose one has an acid HA of unknown acidity, and many reference acids HB_i, for which reliable thermochemical data is available. Multiple methods exist for deriving accurate gas-phase acidity data for HA with respect to HB_i. If the electron affinity of A^- and BDE of HA are known, \Delta H_{acid}(HA) can be determined by applying the thermocycle in Scheme 3.2, and further converted to \Delta G_{acid}(HA) by consideration of entropy. Alternatively, gas-phase acidities can be established by examining proton-transfer in the ion-molecule reaction equilibrium:

$$A^- + HB_i \rightleftharpoons HA + B_i^-$$ (3.7)

The equilibrium constant (K) for the proton transfer reaction (3.7) is found from the ratio of the forward and reverse reaction rates (k_{forward} and k_{reverse}), or from the equilibrium concentrations, and is related to gas-phase acidity by Equation (3.8):

$$K = \frac{k_{forward}}{k_{reverse}} = \frac{[A^-][HB_i]}{[HA][B_i]} = e^{-\Delta G_{acid}/RT}$$ (3.8)

where [X] is the concentration of species X, R is the universal gas constant, and T is the temperature (in Kelvin). Measurements of the forward and reverse reaction rates are typically carried out in ion cyclotron resonance (ICR) mass spectrometers,^295,296 flowing afterglow instruments,^297-299 or high pressure mass spectrometers.\(^300\) Common to each of these experiments, neutral reference reactant HB_i is present (up to ca. 10 Torr in the case of the latter method), either in the ion source where A^- is produced, or seeded into the bath gas downstream from the source. Excess neutral HB_i increases ion-molecule reaction rates by several orders of magnitude over ion discharge on the walls of the source, flow tube, or ICR cell, and furthermore allows measurements to be made under pseudo first-order conditions. In customised apparatus, variable temperature
3.1 Introduction

measurements enable determination of enthalpic and entropic components from the Gibbs free energy by the Van’t Hoff equation.\textsuperscript{302}

3.1.2.2 Threshold Collision-Induced Dissociation

Equilibrium methods necessitate the availability of volatile, high purity samples and reference species. Cluster dissociation methods eliminate this requirement by the power of tandem mass spectrometry to isolate the ion of interest. A proton- or metal-bound heterodimer comprising the conjugate bases of an unknown and a reference species (e.g., \( A^- \cdot X^+ \cdot B_i^- \), where \( X = \text{H or metal} \)) is prepared in the ion source region, and isolated by the first mass analyser. Threshold guided ion beam mass spectrometry – pioneered by Armentrout and co-workers – is the principal method of determining accurate thermochemical data from gas phase cluster ions.\textsuperscript{175,293} In all threshold measurements, ion formation is determined as a function of externally applied energy. The required energy is supplied by interaction with an electron or photon, or by CID with an inert gas. Reaction cross sections and product branching fractions are measured as a function of applied collision energy. As true thresholds are not experimentally accessible, the enthalpy difference between competing reaction channels is found by modelling reaction cross sections near threshold using statistical rate theory to account for the energy-dependent product branching ratio.\textsuperscript{303,304} Threshold collision-induced dissociation (TCID) has been successfully applied to the determination of proton affinities,\textsuperscript{305} gas phase acidities,\textsuperscript{306,307} and alkali metal-ion affinities\textsuperscript{303,304} of small organic molecules, by competitive CID of the appropriate heterodimer anion of the conjugate bases.

Although modelling cross sections with statistical rate theory may be considered a disadvantage, there are multiple advantages of TCID over equilibrium measurements. Because of the large accessible dynamic range, the acidity of the unknown need not be
similar to that of the reference species (within *ca.* 50 kJ mol\(^{-1}\) is acceptable). Moreover, as the pressures of the neutral acids need not be determined, a more diverse array of ion complexes can be formed, particularly from non-volatile or reactive components.

3.1.2.3 The Kinetic Method

An alternative cluster dissociation approach that does not require specialised guided ion beam apparatus is the kinetic method, first developed by Cooks and co-workers in the late 1970s. The kinetic method was initially employed on a sector-based mass-analysed ion kinetic energy (MIKE) spectrometer,\(^{308,309}\) and subsequently a triple quadrupole instrument,\(^{310}\) both coupled to chemical ionisation sources. The latter study was the first reported use of the kinetic method to determine the relative gas-phase acidities of carboxylic acids, building on earlier studies that examined the relative basicity of alkyl amines.

As the name implies, the kinetic method is a procedure for making relative thermochemical measurements by examining the relative dissociation rates of a precursor cluster ion comprising the compound of interest and a reference compound\(^{311}\) (e.g., A\(^-\)···H\(^+\)···B\(^-\) for determining gas phase acidities), and in this regard is similar to TCID. Furthermore, in both experiments, the observed product branching fractions are highly sensitive to the relative acidity, but may suffer from further fragmentation at high energies. Like all relative methods, uncertainty in the reference values can be a substantial contributor to the total error of such experiments.\(^{312}\)

The kinetic method has gained much popularity since its inception. Experiments are fast, easily applicable to a wide array of mass spectrometer configurations with no specialised apparatus required, sensitive to small energy changes, and generally agree with values obtained by other methods.\(^{311}\) Coupling to contemporary ESI sources provides a convenient source for determining the thermochemistry of a large array of
3.1 Introduction

organic, inorganic, and even biomolecular ions,\textsuperscript{313-315} by circumventing some of the volatility and purity requirements of equilibrium measurements.

Ideally, tandem mass spectra in kinetic method experiments exhibit only the two constituent monomer ions ($A^-$ and $B_i^-$), with an abundance ratio sensitive to the choice of reference compound. For example, in Figure 3.1, a cluster ion ($m/z$ 133) comprising acetate and propionate is prepared by chemical ionisation, mass-selected, and subjected to CID. The resulting spectrum is simple to interpret qualitatively, as a higher abundance of propionate ions relative to acetate ions is a consequence of the greater acidity of propionic acid relative to acetic acid (the corollary being the propionate anion is a weaker base). By using multiple reference compounds, the acidity of the unknown can be bracketed between reference values. This work also demonstrated that the product ion branching ratio is dependent on input energy and the pressure of target gas in the collision cell.\textsuperscript{310} As the system acquires more internal energy, selectivity is lost and the ratio of fragment ion abundances approaches unity.

\textbf{Figure 3.1}: MS/MS spectrum of a negatively charged proton-bound heterodimer comprising acetate and propionate. From Reference 310.

In the simplest quantitative application of the kinetic method, entropic effects are assumed to cancel upon cluster ion dissociation, and competitive cleavage of the
weak bonds occur with rates (and therefore ion abundances) dependent on the enthalpic
differences between the alternate dissociation pathways. As these originate from a
common precursor ion, the difference in product states controls reaction rates \(k_i\), and
the natural logarithm of the ratio of product ion abundances is proportional to the
difference in acidity:

\[
\ln \left( \frac{k_1}{k_2} \right) = \ln \left( \frac{[A^+]}{[B_i]} \right) \approx \frac{\Delta(\Delta G_{acid})}{RT_{eff}} \approx \frac{\Delta(\Delta H_{acid})}{RT_{eff}} \tag{3.9}
\]

where \(T_{eff}\) is the ‘effective temperature’ parameter. The effective temperature accounts
for the non-Boltzmann population of ions sampled in the experiment,\(^{316}\) and is a
superposition of both experimental (observation timescale) and molecular (activation energies) parameters, and hence will vary from one experiment to another, even when
consistent instrumental settings are applied. Using classical kinetic rate theory for
unimolecular dissociation, Ervin\(^{317}\) derived an expression for effective temperature:

\[
T_{eff} \approx \frac{\Delta E_{avg}}{R(s-1)[((2\nu r)^{1/(s-1)}-1]
\]

Equation 3.10 demonstrates that the effective temperature is proportional to the average
well depth of the cluster ion (\(\Delta E_{avg}\) in Figure 3.2), and inversely proportional to
molecular size through \(s\), the number of degrees of freedom. Furthermore, the reaction
frequency (\(\nu\)) and experimental time window (\(r\)) also influence the effective
temperature. Determining the effective temperature for a particular cluster is
complicated. Armentrout suggests the inclusion of substantial uncertainties (50%) to
adequately represent the uncertainty.\(^{318}\) When (3.9) is re-written as (3.11):

\[
\Delta(\Delta G) = \Delta G_2 - \Delta G_1 = RT_{eff} \ln \left( \frac{[A^+]}{[B_i]} \right) \tag{3.11}
\]
the consequences of such uncertainty are easily predicted. For a typical effective temperature of 500 K and an ion abundance ratio of 10:1, a relative acidity of 9.6 kJ mol\(^{-1}\) is obtained. Even a 50% error in \(T_{\text{eff}}\) yields an uncertainty of only ± 5 kJ mol\(^{-1}\), which is comparable in magnitude to that obtained by other methods.\(^{311}\)

The successful application of Equation 3.9 depends on several key assumptions holding true. Firstly, that reverse activation energies are negligible, as shown in the potential energy diagram of dimer dissociation (Figure 3.2). Secondly, that the isolated cluster ions \(A^- \cdots H^+ \cdots B^-\) consist of an isomerically pure population, as isomers exhibit varying proton affinities, thus affecting the observed product ion ratio.\(^{319}\) Indeed, such differences can be used to identify and distinguish isomers.\(^{320}\)

\[
\begin{align*}
\Delta E &= \Delta_{\text{avg}} E - \Delta \Delta E/2 \\
\Delta \Delta E/2 &= \Delta_{\text{acid}} H(R_2\text{OH}) - \Delta_{\text{acid}} H(R_1\text{OH}) \\
\Delta_{\text{acid}} H(R_1\text{OH}) &= \Delta_{\text{acid}} H(R_2\text{OH}) \\
\Delta_{\text{avg}} E &= \Delta_{\text{avg}} E + \Delta \Delta E/2
\end{align*}
\]

**Figure 3.2:** Schematic potential energy diagram for the dissociation of a proton-bound dimer of alkoxide anions \(R_1O^-\) and \(R_2O^-\). Taken from Reference 317.

As an example, when linear alkyl carboxylates are paired with acetate, all fall on a straight line when the natural logarithm of ion abundance ratio is plotted against literature proton affinity values, however formate does not.\(^{310}\) The apparent deviation
from linearity arises due to formation of higher energy hydrogen-bonded conformers of the precursor complexes.\textsuperscript{321} Isomerisation barriers along the dissociation pathway affect the branching ratios between the two product ion channels. The final assumption underpinning Equation 3.9 is negligible entropy differences between the competing dissociation pathways (\textit{i.e.}, $\Delta \Delta S = 0$). This assumption is generally valid for structurally similar molecules, hence the selection of appropriate reference compounds is crucial.

\subsection*{3.1.2.4 Extended Kinetic Method}

From the examples provided above, assumptions involving constant entropic contributions to dimer dissociation are clearly not always valid. Indeed, the use of this approximation in circumstances where it is not applicable has made the kinetic method the target of substantial criticism.\textsuperscript{318,322} Wu and Fenselau\textsuperscript{323} – and subsequently Wenthold and Squires\textsuperscript{312} – were among the first to realise that the kinetic method is still applicable in cases of non-zero entropic contributions to dimer dissociation. An unknown of interest is paired with a series of structurally similar reference compounds that are not necessarily similar to the unknown. In this case, entropy effects are non-zero, but consistent for each dimer across the range of reference compounds, and thus a plot of $\Delta \Delta H_{\text{acid}}$ against the natural logarithm of the observed abundance ratio still yields a straight line.

Importantly, Fenselau and co-workers\textsuperscript{324-326} – as well as Wesdemiotis \textit{et al.}\textsuperscript{327,328} – recognised that if the multiple-reference kinetic method experiment was repeated with multiple collision energies (different $T_{\text{eff}}$), entropic effects are not just accounted for, but calculable. This variation has become known as the extended kinetic method, and was subsequently built upon by Cooks and co-workers.\textsuperscript{329} Plotting the natural logarithm of the ion abundance ratio against the proton affinity of Bi yields a $y$-intercept of $-\left[\Delta H_A - T_{\text{eff}}\Delta(\Delta S)\right]/RT_{\text{eff}}$ and a slope of $1/RT_{\text{eff}}$ (Figure 3.3). CID at different collision energies
enables isolation of the enthalpic $[\Delta H_A]$ and entropic $[\Delta (\Delta S)]$ contributions from the different slopes and intercepts. Subsequently plotting $[\Delta H_A - T_{eff}\Delta (\Delta S)]/RT_{eff}$ (the negative of the y-intercept) against $1/RT_{eff}$ (the slope) yields $\Delta H_A$ from the slope and $-\Delta (\Delta S)/R$ from the y-intercept of this second plot (Figure 3.4).

**Figure 3.3:** Multiple collision energy extended kinetic method plot for peptide Gly$_4$.

The natural logarithm of the observed abundance ratio is plotted against the proton affinity of each reference amine. Taken from Reference 324.

**Figure 3.4:** Second extended kinetic method plot for the tetrapeptide Gly$_4$.

The slope of each line in Figure 3.3 ($1/RT_{eff}$) is plotted against the negative of its y-intercept. The slope of this plot equals the proton affinity of Gly$_4$. From Reference 324.
3.1 Introduction

The coefficient of determination (R^2) from the second extended kinetic method plot (e.g., Figure 3.4) always equals 1, implying that the slope and intercept of the first plot are not independent. Armentrout developed a statistical procedure to remove this covariance.\textsuperscript{322} Rather than plotting \(\ln(k_A/k_B)\) versus \(\Delta H(B_i)\), the x-axis is replaced by the difference between \(\Delta H(B_i)\) and the average proton affinity of all references \(\Delta H_{\text{avg}}(B_i)\) (Figure 3.5). Once again, each collision energy produces a different slope ((1/\(RT_{\text{eff}}\)) and y-intercept (\(-[(\Delta H_A - \Delta H_{\text{avg}}(B_i))/RT_{\text{eff}} + \Delta \Delta S/R])\), which are plotted against each other (Figure 3.6). The slope ((\(\Delta H_A - \Delta H_{\text{avg}}(B_i)\)) and intercept (\(\Delta S(B_i)/R - \Delta \Delta S/R\)) of this second plot yield the enthalpic and entropic terms, respectively.

\textbf{Figure 3.5}: Statistical extended kinetic method plot for determining the gas-phase acidity of tryptophan, using substituted benzoic acids and multiple collision energies. From Reference 314.
3.1 Introduction

![Second extended kinetic method plot for tryptophan](image)

**Figure 3.6:** Second extended kinetic method plot for tryptophan. The slope and y-intercept yield the deprotonation enthalpy and entropy. From Reference 314.

A critical evaluation of the extended method has concluded that it “is definitely superior to the standard kinetic method”\(^3\) for the determination of accurate gas-phase thermochemical data. However, even the most rigorous analysis by the statistical extended kinetic method does not spare the technique from systematic and random error. Moreover, the thermodynamic quantities of reference species may not be precisely known, and thus contribute uncertainty to the final result. However, applying the kinetic method to the determination of relative thermodynamic quantities is advantageous as it is expected that many sources of such error will cancel.

### 3.1.3 Aims

In this chapter, the kinetic method is used to evaluate relative and absolute gas-phase acidities of carboxylic acids in the presence and absence of a remote aminoxyl radical in the same molecular scaffold. Unequivocal experimental evidence for the lowering of gas-phase acidity with the incidence of a distant radical is demonstrated and reinforced by complementary electronic structure calculations. In light of the interplay between a negative charge and radical stability, potential implications for the use of distonic radical anions as models for neutral radical reactivity are evaluated.
3.2 Experimental

3.2.1 Materials

Aminoxyl radicals 4-carboxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-carboxy-TEMPO, 1) and 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (3-carboxy-PROXYL, 2) were purchased from Sigma Aldrich (Sydney, Australia), and used without further purification. Hydrogen peroxide (Australian Chemical Reagents, Queensland, Australia) was used as a 40% (w/w) aqueous solution. All other materials for synthesis and reference compounds for kinetic method studies were purchased from Sigma Aldrich, and used as received. Methanol employed for mass spectrometry was HPLC grade (Thermo Fisher Scientific, Melbourne, Australia) and used as received.

3.2.2 Synthesis

Synthesis of alkoxyamines (1a-c, 2a-c, Scheme 3.3) from the corresponding aminoxyl radicals (1) and (2) is reported in Section 2.3.2.

![Scheme 3.3: Alkoxyamines (1a-c) and (2a-c)](image)

3.2.3 Mass Spectrometry

Mass spectra were recorded with a QuattroMicro (Waters, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an electrospray ionisation source. All mass spectra represent the accumulation of at least 100 scans. Samples were diluted individually to ca. 5-20 μM in methanol. Equal volumes of two solutions were mixed.
and infused directly into the source at $5 \text{ µL min}^{-1}$, to form proton-bound hetero-dimers. The capillary voltage was set to 2.9 kV, cone voltage 25 V, and source temperature 80 °C. Nitrogen was used as the drying gas, at a temperature of 110 °C, and flow rate of 320 L h$^{-1}$. In all collision-induced dissociation (CID) scans, argon was used as the collision gas at a pressure of $3.0 \pm 0.1$ mTorr. Collision energy in the laboratory reference frame ($E_{\text{lab}}$) was varied from 5-20 eV, and normalised to centre of mass reference frame energies ($E_{\text{cm}}$) by the relationship:

$$E_{\text{cm}} = E_{\text{lab}} \times \frac{m_{\text{Ar}}}{m_{\text{Ar}} + m_{\text{ion}}}$$

where $m_{\text{Ar}}$ and $m_{\text{ion}}$ are the masses of the argon collision gas and the isolated ion, respectively.

### 3.2.4 Computational Methods

Standard *ab initio* molecular orbital theory and density functional theory calculations were carried out as described in Section 2.3.4. Further detailed computational procedures and benchmarking studies are available in the literature and associated supporting information.$^{279,282}$
3.3 Results and Discussion

3.3.1 Single Reference Kinetic Method

Aminoxyl radicals with a remote acid moiety are readily observed as \([M - H]^-\) ions upon electrospray ionisation (ESI). By adding a corresponding reference partner containing the same acid moiety, proton-bound dimers of radical and non-radical species are prepared in a straightforward manner. Figure 3.7 highlights the formation of proton-bound dimers comprising 4-carboxy-TEMPO (1, \(m/z\) 199) and benzoic acid (\(m/z\) 121) when the two are combined in methanol and subjected to ESI.

![Figure 3.7](image)

**Figure 3.7:** ESI mass spectrum of benzoic acid and 4-carboxy-TEMPO (1) in methanol. Hetero- and homo-dimers are observed in minor abundance.

Figure 3.8 illustrates the utility of applying the kinetic method to proton-bound dimer ions. 4-carboxy-TEMPO (1) is firstly combined with benzoic acid as a reference acid \((\Delta H^\circ_{\text{acid}} = 1423 \pm 12 \text{ kJ mol}^{-1})\).\(^{302}\) As shown in Figure 3.8(a), CID of the proton-bound heterodimer at \(m/z\) 321 produces only the two constituent anions: namely benzoate (\(m/z\) 121) and deprotonated 4-carboxy-TEMPO (\(m/z\) 199). By applying Equation 3.9, even without the exact value of the effective temperature parameter, the greater abundance of 4-carboxy-TEMPO ions compared to benzoate ions indicates that the latter anion has a greater proton affinity. That is, when comparing the corresponding
3.3 Results and Discussion

conjugate acids, 4-carboxy-TEMPO (1) exhibits a greater gas-phase acidity than benzoic acid. Hence, upon CID of the dimer, the proton preferentially bonds to benzoate, and 4-carboxy-TEMPO is detected as a free anion. By contrast, the spectrum acquired upon dissociation of a dimer comprising the analogous methoxyamine (1a) and benzoic acid shows an inverted abundance ratio (Figure 3.8b). That is, benzoic acid is the more acidic of the two components making up the dimer.

![Figure 3.8](image)

**Figure 3.8:** Representative CID spectra ($E_{\text{lab}} = 10$ eV) of proton-bound dimers comprising benzoic acid (BA) as a reference compound.

(a) 4-carboxy-TEMPO (1); (b) 4-carboxy-TEMPO–CH$_3$ (1a).

Based on previous experiments on similar geometry instruments$^{310,331}$ an effective temperature of 600 K ($\pm$ 300 K) is initially assumed. Applying Equation 3.9 to the observed ion abundance ratios of Figure 3.8 with this value of $T_{\text{eff}}$ results in ‘apparent affinities$^{331}$ of 1411 $\pm$ 13 kJ mol$^{-1}$ for 4-carboxy-TEMPO radical (1), and 1430 $\pm$ 13 kJ mol$^{-1}$ for alkoxyamine 4-carboxy-TEMPO–CH$_3$ (1a). Strictly speaking, these apparent acidity values are related to $\Delta G_{\text{acid}}$, as this method does not account for
3.3 Results and Discussion

entropy. Assuming these entropic effects are both minor and relatively consistent (*vide infra*), these relative affinities determined from comparing intensity ratios should reflect the true $\Delta H_{\text{acid}}$ ordering. Encouragingly, these data stand in excellent agreement with calculated $\Delta H_{\text{acid}}$ values (using the G3(MP2)-RAD method) of 1414 kJ mol\(^{-1}\) and 1432 kJ mol\(^{-1}\) for (1) and (1a), respectively (at 298 K).\(^{279}\) Moreover, the difference between these values is consistent with the 20 kJ mol\(^{-1}\) difference predicted from previous calculations, and confirmation for the applicability of the thermodynamic cycle in Scheme 3.1. There are, however, limitations to this single reference analysis. The uncertainty in both the effective temperature and the gas-phase acidity of benzoic acid is reflected in the relatively large uncertainties in the results.

3.3.2 Multiple Reference Kinetic Method

A more thorough kinetic method analysis involves the use of multiple reference acids, in order to determine the magnitude of the effective temperature parameter. A series of structurally similar reference species is required to satisfy the requirements of the kinetic method, ensuring consistent, non-zero entropy terms. For this purpose, a series of substituted benzoic acids with known thermodynamic quantities are selected to act as reference species. These acids were carefully chosen to cover a suitably wide acidity scale ($\Delta H_{\text{acid}}^\circ = 1377$ kJ mol\(^{-1}\) for 3-nitrobenzoic acid, up to $\Delta H_{\text{acid}}^\circ = 1437$ kJ mol\(^{-1}\) for 4-aminobenzoic acid)\(^{301}\) to encompass the predicted gas-phase acidities of both radical and non-radical functionalised carboxylic acids.

Representative spectra from this multiple-reference analysis of 4-carboxy-TEMPO (1) are presented in Figure 3.9. Panel (a) is the spectrum resulting from CID of a proton-bound dimer comprising (1) and 3-methoxybenzoic acid ($\Delta H_{\text{acid}}^\circ = 1420$ kJ mol\(^{-1}\)).\(^{301}\) As 3-methoxybenzoate ($m/z$ 151) is the least abundant of the two product ions in the spectrum, it follows that it exhibits a higher proton affinity than deprotonated 4-
3.3 Results and Discussion

carboxy-TEMPO. Panel (b) is the analogous spectrum of (1) with 4-chlorobenzoic acid ($\Delta H_{\text{acid}} = 1404 \text{ kJ mol}^{-1}$) and in contrast to the spectrum shown in Figure 3.9(a), exhibits the inverse abundance ratio of aminoxyl radical to reference product ions. That is, 4-chlorobenzoic acid is the more acidic species of the two dimer constituents. On combining these data, the enthalpy of deprotonation of (1) lies between 1404 kJ mol$^{-1}$ and 1420 kJ mol$^{-1}$.

![Figure 3.9](image)

**Figure 3.9:** CID spectra ($E_{\text{lab}} = 10 \text{ eV}$) of proton-bound dimers comprising 4-carboxy-TEMPO (1) and (a) 3-methoxybenzoic acid; (b) 4-chlorobenzoic acid.

Proton-bound dimers containing 4-carboxy-TEMPO (1) and eight other reference benzoic acids (Table 3.1) were also prepared, and subjected to collision-induced dissociation at a consistent collision energy ($E_{\text{cm}} =1.10 \pm 0.04 \text{ eV}$). The resulting data are summarised in Figure 3.10, with each data point representing a single spectrum. Following the Armentrout modification to the kinetic method,$^{322}$ x-axis values are the difference between the deprotonation enthalpy of each reference acid $\Delta H(B_i)$ and the average deprotonation enthalpy of all reference species $\Delta H_{\text{avg}}(B_i)$. Reference acids more acidic than 4-carboxy-TEMPO fall in the upper left quadrant of the plot (e.g., 4-chlorobenzoic acid, Figure 3.9b), whilst those with greater proton affinities (e.g., 3-
methoxybenzoic acid, Figure 3.9a) lie in the lower right quadrant. As in Figure 3.5, the slope of plot in Figure 3.10 is inversely proportional to the effective temperature (slope \(=-1/RT_{\text{eff}}\)). The effective temperature is determined to be 558 K for this combination of ions and instrument conditions, in reasonable agreement with the earlier assumed value of 600 K, particularly if Armentrout’s suggestion of 50% uncertainty in \(T_{\text{eff}}\) is accepted.

**Table 3.1:** Reference benzoic acids for kinetic method analysis, their deprotonation enthalpies, and applied collision energy.

<table>
<thead>
<tr>
<th>Reference Compound</th>
<th>(\Delta H_{\text{acid}}^\circ) (kJ mol(^{-1}))</th>
<th>(E_{\text{lab}}) (eV)(^\dagger)</th>
<th>(E_{\text{cm}}) (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>1423 ± 12</td>
<td>10</td>
<td>1.11</td>
</tr>
<tr>
<td>2-chlorobenzoic acid</td>
<td>1402 ± 9</td>
<td>11</td>
<td>1.11</td>
</tr>
<tr>
<td>3-chlorobenzoic acid</td>
<td>1402 ± 9</td>
<td>11</td>
<td>1.11</td>
</tr>
<tr>
<td>4-chlorobenzoic acid</td>
<td>1404 ± 9</td>
<td>11</td>
<td>1.11</td>
</tr>
<tr>
<td>3-methoxybenzoic acid</td>
<td>1420 ± 9</td>
<td>11</td>
<td>1.12</td>
</tr>
<tr>
<td>2-methylbenzoic acid</td>
<td>1419 ± 9</td>
<td>10</td>
<td>1.07</td>
</tr>
<tr>
<td>4-aminobenzoic acid</td>
<td>1437 ± 9</td>
<td>10</td>
<td>1.06</td>
</tr>
<tr>
<td>4-formylbenzoic acid</td>
<td>1392 ± 9</td>
<td>11</td>
<td>1.13</td>
</tr>
<tr>
<td>3-nitrobenzoic acid</td>
<td>1377 ± 9</td>
<td>11</td>
<td>1.08</td>
</tr>
</tbody>
</table>

\(\dagger E_{\text{lab}}\) is restricted to integer input

**Figure 3.10:** Multiple reference, single collision energy variant of the kinetic method, applied to 4-carboxy-TEMPO (1).
3.3 Results and Discussion

3.3.3 Collision Energy Dependent Extended Kinetic Method

Repeating this series of nine different reference benzoic acids at nine different collision energies ($E_{	ext{lab}} = 5 – 25$ eV) yields a series of plots shown in Figure 3.11(a), each with a unique slope ($-1/RT_{	ext{eff}}$) and $y$-intercept ($-[(\Delta H_A - \Delta H_{\text{avg}}(B_i))/RT_{	ext{eff}} + \Delta \Delta S/R])$.

Equivalent data for methoxyamine (1a) are presented in Figure 3.11(b). Both complete data sets are summarised in Table 3.2. For the methoxyamine, only eight reference acids are used to construct the kinetic method plot. As 3-nitrobenzoic acid has a significantly lower proton affinity than (1a) ($\Delta \Delta H_{\text{acid}} > 45$ kJ mol$^{-1}$), the abundance of the latter ion ($m/z$ 214) upon collisional activation of the dimer is difficult to detect above the spectral baseline. The data presented in Table 3.2 are subsequently used to construct the second kinetic method plot for both (1) and (1a), with each slope plotted against the negative of the corresponding $y$-intercept (Figure 3.12). To reiterate, the slope of this plot is related to the deprotonation enthalpy of the unknown relative to the average of all references ($\Delta H_A - \Delta H_{\text{avg}}(B_i)$), and the $y$-intercept gives the entropic term ($\Delta S(B_i)/R - \Delta S_A/R$). As illustrated in Figure 3.12, the data for the two carboxylic acids are distinctly different.

Figure 3.11: Kinetic method plots at multiple collision energies.

(a) 4-carboxy-TEMPO (1); (b) 4-carboxy-TEMPO-CH$_3$ (1a). See also Table 3.2.
3.3 Results and Discussion

Table 3.2: Slope and intercepts of multiple reference kinetic method plots (Figure 3.11) repeated at various collision energies.

<table>
<thead>
<tr>
<th>$E_{cm}$ (eV)</th>
<th>Slope ($y$-intercept)</th>
<th>Slope ($y$-intercept)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>-0.238 (0.930)</td>
<td>-0.140 (2.381)</td>
</tr>
<tr>
<td>0.8</td>
<td>-0.220 (0.902)</td>
<td>-0.131 (2.298)</td>
</tr>
<tr>
<td>1.1</td>
<td>-0.216 (0.898)</td>
<td>-0.135 (2.325)</td>
</tr>
<tr>
<td>1.3</td>
<td>-0.208 (0.920)</td>
<td>-0.127 (2.239)</td>
</tr>
<tr>
<td>1.6</td>
<td>-0.195 (0.829)</td>
<td>-0.131 (2.221)</td>
</tr>
<tr>
<td>1.9</td>
<td>-0.186 (0.727)</td>
<td>-0.134 (2.338)</td>
</tr>
<tr>
<td>2.1</td>
<td>-0.183 (0.692)</td>
<td>-0.137 (2.326)</td>
</tr>
<tr>
<td>2.4</td>
<td>-0.176 (0.634)</td>
<td>-0.138 (2.458)</td>
</tr>
<tr>
<td>2.7</td>
<td>-0.173 (0.603)</td>
<td>-0.144 (2.473)</td>
</tr>
</tbody>
</table>

Figure 3.12: Extended kinetic method plot for 4-carboxy-TEMPO (1) and 4-carboxy-TEMPO–CH$_3$ (1a). Note the split $y$-axis either side of the vertical line.

Adding the slope of each series to the average deprotonation enthalpy of all references used (1408.4 kJ mol$^{-1}$ for (1) and 1412.4 kJ mol$^{-1}$ for (1a), as 3-nitrobenzoic
3.3 Results and Discussion

acid was not used with the latter) provides final $\Delta H_{\text{acid}}$ values of $1414 \pm 9$ kJ mol$^{-1}$ for the aminoxyl radical 4-carboxy-TEMPO (1), and $1428 \pm 9$ kJ mol$^{-1}$ for alkoxyamine 4-carboxy-TEMPO–CH$_3$ (1a). Moreover, relating the y-intercepts to the entropic term, we find $\Delta \Delta S_{\text{acid}}$ is less than 3 J mol$^{-1}$ K$^{-1}$, and thus deprotonation enthalpies should be well represented by apparent affinities even in the simple applications of the kinetic method.$^{333}$ These data are in agreement with aforementioned calculated $\Delta H_{\text{acid}}$ (298 K) values of $1414$ kJ mol$^{-1}$ and $1430$ kJ mol$^{-1}$ respectively, as well as single reference and bracketing experiments. That an ostensibly remote aminoxyl radical should decrease the $\Delta H_{\text{acid}}$ of a carboxylate is consistent with the hypothesis of lower oxygen-carbon BDEs in the presence of a carboxylate anions (Scheme 3.1).

3.3.4 Relative Gas-Phase Acidities

Absolute thermodynamic quantities are certainly desirable, and obtainable through a rigorous application of the kinetic method with multiple reference compounds and entropy corrections. However, the overlapping uncertainty in the data brought about by the effective temperature parameter, ion abundance measurements, and imprecision of available reference data necessitates that exact ordering of acidity scales be clarified by relative measurements.$^{314}$ Experimental and modeling errors tend to cancel when determining an energy difference rather than an absolute energy. Moreover, in the context of the present work, the quantity of interest is the relative gas-phase acidity of carboxylic acids in the presence or absence of a distant aminoxyl radical.

To further demonstrate that an aminoxyl radical decreases the proton affinity of a remote carboxylate, each of the studied acids are paired with every other acid to generate all combinations of proton-bound dimer. This experimental set includes aminoxyl radicals 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2), and their corresponding methyl, benzyl and fluoromethyl alkoxyamines (1a – 1c and 2a – 2c,
3.3 Results and Discussion

Scheme 3.3). Representative spectra acquired upon CID of such dimers are displayed in Figure 3.13. The applied collision energy was moderated to ensure the ion abundance ratio was not skewed by homolytic dissociation of alkoxyamines into aminoxyl radicals.

Firstly, it is clear from Figure 3.13(a–c) that our earlier finding is qualitatively reinforced: in all cases the distonic radical anion exhibits a greater ion abundance than the paired alkoxyamine, due to its lower proton affinity. This result is independent of $R^3$ substituent (cf. spectra (a) and (b)) and ring size (spectra (b) and (c)).

![Scheme 3.3](image)

**Figure 3.13:** Representative CID spectra ($E_{lab} = 10$ eV) of proton-bound dimers comprising combinations of aminoxyl radicals and alkoxyamines.

(a) 1 & 1a; (b) 1 & 1b; (c) 2 & 2b; (d) 1 & 2; (e) 1a & 1b; (f) 2a & 2b. Precursor ions are denoted by an arrow.
Secondly, proton-bound dimers comprising two different aminoxy radicals are prepared and subjected to CID (Figure 3.13d). The greater abundance of 3-carboxy-PROXYL anions (2) compared to 4-carboxy-TEMPO ions (1) suggests that the closer proximity of the radical to the acid moiety further decreases the proton affinity of the former. Finally, when two different alkoxyamines of the same ring size series are paired and dissociated (spectra (e) and (f)), the ion abundance ratio is not unity as might be expected from the similarity of spectra (a) and (b). Such a result indicates that the alkoxyamine substituent (R³) influences the proton affinity of remote carboxylate anions, however the magnitude of the effect is small compared to the impact of a radical. Thus, relative proton affinities follow the order 1a > 1b ≈ 1 (and similarly 2a > 2b ≈ 2). That is, the difference in proton affinity between acids bonded to an aminoxy radical and benzyl substituted alkoxyamine is smaller than those bound to a radical and methoxyamine, which is in agreement with computational predictions.²⁸²

Expanding the result set to include all amalgamations of aminoxy radicals (1) and (2), and their corresponding alkoxyamines (1a – 2c), relative ΔHacid values for every combination are determined by again applying Equation 3.9. Based on results in Section 3.3.2, entropic factors are sufficiently negligible to be ignored (i.e., ΔΔHacid = ΔΔGacid), and that our previous estimate of 600 K for the effective temperature is appropriate for this combination of molecules and instrument parameters. Experimental results are presented along the x-axis of Figure 3.14, and plotted against the corresponding calculated ΔΔHacid values (G3(MP2)-RAD, 298 K, gas phase) for each pair of acids. The incidence of both positive and negative results merely reflect the arbitrary ordering of acids in Equation 3.9 (i.e., [ΔHacid,1a − ΔHacid,1b] = −[ΔHacid,1b − ΔHacid,1a]). The largest differences are obtained when a dimer consists of an aminoxy radical and alkoxyamine, whereas small differences are obtained for braces of radicals
and smaller still for alkoxyamine pairs. Excellent agreement is obtained between experimental and theoretical data, with a mean absolute deviation below 2 kJ mol⁻¹.

![Figure 3.14](image)

**Figure 3.14:** Calculated $\Delta H_{\text{acid}}$ differences in carboxylic acids with remote aminoxyl radicals and alkoxyamines, compared to experimental measurements.

Despite the well-documented limitations of the kinetic method, we have been able to employ several variations of this technique to derive absolute and – perhaps, more importantly – relative enthalpies of deprotonation for a series of carboxylic acids covalently bonded to remote aminoxyl radicals or even-electron alkoxyamines. Critical findings are summarised in Table 3.3. Most importantly, the presence of an aminoxyl radical unequivocally increases the acidity of carboxylic acids (or decreases the basicity of the corresponding anion), compared to those substituted by alkoxyamines.

**Table 3.3:** Relative $\Delta H_{\text{acid}}$ for aminoxyl radical (1) and alkoxyamines (1a) and (1b), obtained by the kinetic method, compared to computational results.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta H_{\text{acid}}$ (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Calculated</strong>†</td>
</tr>
<tr>
<td><strong>Pairs</strong></td>
<td><strong>Single Reference</strong> (3.3.1)</td>
</tr>
<tr>
<td><strong>Multiple Reference</strong> (3.3.3)</td>
<td></td>
</tr>
<tr>
<td>1:1a</td>
<td>-18.2</td>
</tr>
<tr>
<td>1:1b</td>
<td>-13.5</td>
</tr>
<tr>
<td>1a:1b</td>
<td>4.7</td>
</tr>
</tbody>
</table>

†Calculations performed using the G3(MP2)-RAD method.²⁷⁹
3.4 Conclusions

Multiple variations of the kinetic method were employed to determine relative and absolute thermochemical quantities for carboxylic acids covalently linked to aminoxyl radicals and closed-shell alkoxyamines. The results obtained by different variants of the kinetic method were internally consistent, and moreover, in good agreement with high-level theoretical data.

The combined experimental and computational results presented in this work suggest that the presence of a stable aminoxyl radical decreases the basicity of distant anions by as much as 20 kJ mol\(^{-1}\). Based on the thermodynamic cycle illustrated in Scheme 3.1, the corollary of such a finding is that an ostensibly remote anion moiety lowers homolytic bond dissociation energies elsewhere in the molecule, even in the absence of a through-bond interaction. The results of this investigation are consistent with the hypothesis of decreased oxygen-carbon BDEs in the presence of a remote negative charge, postulated in Chapter 2.

The results presented in this work imply that the reactivity of a radical can be manipulated by reversible protonation and deprotonation of a remote acid/base moiety remote from the radical itself. Employing distonic negative ion models in mass spectrometry may be compromised by a systematic offset of radical stability compared to neutral systems. This offset is in addition to the effects described by Kenttämaa and co-workers in their evaluation of distonic ions as probes for radical reactivity.\(^{233}\) Judicious selection of distonic ion model is therefore advised, as is thorough experimental and computational assessment of the charge site involvement.
4. EXPERIMENTAL EVIDENCE FOR THE ORIGIN AND SCOPE OF LONG-RANGE STABILISATION IN DISTONIC RADICAL ANIONS

The work in this chapter is being prepared for peer-review publication.


Author Statement

David L. Marshall performed all experiments and wrote the manuscript with input from all authors. Electronic structure calculations were carried out by Anya Gryn’ova from Australian National University. This work would not have been possible without the support provided by Michelle L. Coote, Philip J. Barker and Stephen J. Blanksby.

Primary Supervisor Confirmation

I, Prof. Stephen J. Blanksby (Primary Supervisor), support and certify the above author statement.

Signature

Date
4.1 Introduction

4.1.1 Background

Recent computational studies undertaken by Gryn’ova and Coote\textsuperscript{279,282} have further investigated the origin and scope of the long-range stabilising interaction between an aminoxyl radical and a remote carboxylate anion revealed experimentally in the preceding chapter by a kinetic method study. The first significant finding from this computational exploration was the variances in BDE and proton affinity encountered thus far are not unique to aminoxyl radicals. Although the magnitude of the difference varied, the effect was maintained throughout a diverse library of radicals, examples of which are shown in Figure 4.1. In each case, the radical is structurally separated and electronically insulated from the anion by the molecular framework. The switching effect was most pronounced when the radical is stabilised, for example by steric crowding, delocalisation (Figure 4.1h), or captodative effects in peptide backbone radicals (Figure 4.1g). Conversely, the variance was largely quenched in non-stabilised alkoxy and particularly alkyl radicals (Figure 4.1c,i).

![Figure 4.1](image)

**Figure 4.1:** M06-2X/6-31+G(d) calculated differences (kJ mol\(^{-1}\)) in R–H and R–CH\(_3\) BDEs between neutral and deprotonated carboxylic acids. Data from Reference 282.
Furthermore, there nothing exceptional about the carboxylate anions employed in the previous work. Calculations revealed radical stabilisation persisted in the presence of a variety of anions, including sulfates and phosphates. The effect increased with more localised, basic anions (less acidic conjugate acids) such as alkoxides.

Intriguingly, theoretical calculations further revealed the stabilisation phenomenon in distonic aminoxyl and peroxyl radical anions is associated with a remarkable rearrangement of electronic structure. The rearrangement is illustrated in Figure 4.2 for 4-carboxy-TEMPO (1). In the radical anion form, the unpaired electron does not reside in the HOMO.\textsuperscript{279} That is, the SOMO is not the HOMO, seemingly in violation of the Aufbau principle. According to single- and multi-reference calculations carried out at multiple levels of theory, the frontier occupied orbitals are supplied by the anion. Further evidence for this proposition comes from the theoretical gas-phase oxidation of 4-carboxy-TEMPO (Figure 4.2). Neutral 4-carboxy-TEMPO is reversibly oxidised to an oxoammonium cation. Conversely, the corresponding anion is oxidised to a neutral triplet biradical by electron loss from the doubly occupied HOMO, rather than removing the unpaired electron to form a closed-shell zwitterion.

![Figure 4.2: Oxidation products and MCSCF(9,5)/6-31+G(d) molecular orbital configurations of (1), exhibiting SOMO-HOMO converted and conventional orbital occupation. Adapted from Reference 279.](image-url)
4.1 Introduction

So-called SOMO-HOMO conversion has previously been reported in molecules containing a stable radical and a donor source of high-energy doubly-occupied orbitals, typically a sulfur-containing unsaturated heterocycle such as tetrathiafulvalene.\textsuperscript{334,335} Energetic ordering of orbitals cannot be manipulated by external stimuli in these systems, which limits their characterisation and practical applications. Conversely, upon protonation of the currently investigated distonic radical anions, ‘traditional’ orbital occupancy is restored, and radical stabilisation is quenched.

It must be stressed that SOMO-HOMO conversion is not observed in all distonic radical anions: conversion requires a sufficiently stable radical such as an aminoxyl, aminyl or peroxyl. Nor is SOMO-HOMO conversion the primary cause of the studied BDE and proton affinity variations. Indeed, some reported examples of SOMO-HOMO converted neutral radicals with high energy HOMOs (\textit{e.g.}, carbenes) exhibit no stabilisation at all.\textsuperscript{282} This is understandable, as molecular orbitals are merely mathematical descriptors of chemical behaviour with no physical origin. Moreover, the stabilisation effect persists when the anion is replaced by a negative point charge, which bears no orbitals. Gryn’ova and Coote have proposed that the stabilisation afforded to distonic radical anions is a result of two superimposed phenomena. The first is a traditional through-space polar effect between the remote charge and a permanent dipole on the radical moiety that persists in the absence of \(\pi\)-conjugation (or indeed, any bonding at all). For the studied anionic systems, this effect acts on both aminoxyl radicals and alkoxyamines, as illustrated in Scheme 4.1. In the presence of a remote negative charge in the 4-position of the piperidine ring, the polar resonance contributor (A) of the alkoxyamine is destabilised by a Coulombic interaction, and resonance form (D) of the aminoxyl radical is stabilised.\textsuperscript{111} Cumulatively, these two effects reduce the O–C bond energy. One would therefore expect a positive charge to act in the opposite
direction; namely, to increase the O–C BDE by stabilising charge-separated contributor (A) and destabilising resonance form (D). Indeed this approach has been pursued by Edeleva and co-workers, who incorporated proton-acceptor sites onto an aminoxyl radical scaffold as a means to control the dissociation rate of alkoxyamines (and therefore the polymerisation rate) in nitroxide-mediated polymerisation.\

\[ \text{Scheme 4.1: Decreasing O–C alkoxyamine BDEs by a remote negative charge is partly assigned to Coulombic destabilisation of alkoxyamine polar resonance contributor (A), and stabilisation of a polar aminoxyl radical resonance form (D).} \]

In addition to the conventional polar effect described above, computational results further predict that a component of the stabilisation is retained in radicals with no significant polar resonance contributor, such as aminyl radicals or diallyl-substituted carbon-centred radicals. Thus, there exists a second phenomenon that also promotes stabilisation of distonic anions. Even in the absence of permanent dipoles, a remote negative charge lowers the energy of a molecule, because attractive charge-nuclei Coulombic forces outweigh the charge-electron repulsion, resulting in an induced dipole. Such effects are generally negated in the course of a chemical reaction. However, charge-electron repulsion is minimised when electrons are delocalised, and are thus able to more effectively move away from the negative charge, compared with when they are restricted by localisation in a bond. This effect does not cancel out where the delocalisation of an unpaired electron is different between reactants and products, as is the case with resonance stabilised radicals (greater polarisability). Therefore, this
represents an extremely general background polar effect by which remote negative charges influence delocalised radicals. This additional stabilisation can be enhanced or diminished by a superimposed ‘traditional’ polar interaction.

4.1.2 Aims

The consequences of these theoretical predictions are experimentally verifiable using the same mass spectrometric methods employed in Chapters 2 and 3, so long as the requisite precursors can be prepared in the gas phase. To reiterate, stabilisation of distonic aminoxyl radical anions has multiple manifestations; (i) decreased oxygen-carbon BDEs in precursor alkoxyamine ions, or (ii) lowered proton affinities of anions in the presence of a radical. In the following sections, threshold measurements of alkoxyamine dissociation and kinetic method studies on the relative basicity of anions in the presence or absence of an aminoxyl radical are investigated. The results will shed new light on the influence of charge carrier, polarity, and location on the stabilisation of aminoxyl radicals.
4.2 Experimental

4.2.1 Materials

Aminoxyl radicals 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-hydroxy-TEMPO, 3), and 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino-TEMPO, 4), were purchased from Sigma Aldrich (Sydney, Australia), and used as received. Perdeuterated (D₄) methanol was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). For further information, refer to Section 3.2.1

4.2.2 Synthesis

4.2.2.1 4-substituted 2,2,6,6-tetramethylpiperidine-N-oxyl radicals

Along with commercially available aminoxyl radicals (1-4), additional 4-substituted 2,2,6,6-tetramethylpiperidine-N-oxyl radical derivatives (5-9, Scheme 4.2) were also prepared. Typically, crude products were of sufficient purity to be amenable to mass spectrometric analysis without additional purification.

Scheme 4.2: Aminoxyl radicals (1-9) examined in the present study.

The acidic proton removed to form [M – H]⁻ anions (or [M – K]⁻ for salt 8) by ESI is shown in red. 4-amino-TEMPO (4) is detected only in positive ion mode.
4.2 Experimental

4.2.2 4-sulfonatoxy-2,2,6,6-tetramethylpiperidine-N-oxyl (5)

To a stirring solution of 4-hydroxy-TEMPO (3) (172 mg, 1 mmol) in CH₂Cl₂ at 0 °C under nitrogen, an equimolar amount of chlorosulfuric acid trimethylsilyl ester (150 µL in 3 mL CH₂Cl₂) is added slowly over approximately 30 minutes. Stirring is continued overnight and the reaction mixture is allowed to warm gradually to ambient temperature. A yellow precipitate is collected by filtration and washed with CH₂Cl₂. Orange-yellow crystals are obtained (158 mg, 63%) Unusually, M⁻ (m/z 252) ions are predominantly observed in the negative ion ESI mass spectrum, which was also reported by Strehmel and co-workers. The [M – H]⁻ ion (m/z 251) is present in minor abundance. High-resolution negative ion ESI-MS for [M – H]⁻: expected 251.0827 (C₉H₁₇NO₅S⁻), found 251.0828 (0.4 ppm).

4.2.2.3 4-(((N-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)oxy)carbonyl)cyclohexane-carboxylic acid (6)

To a stirring solution of 1 mmol (172 mg) 4-hydroxy-TEMPO (3), 1.1 equivalents (227 mg) of dicyclohexylcarbodiimide, and 1 equivalent (172 mg) of 1,4-cyclohexanedicarboxylic acid in anhydrous CH₂Cl₂, 0.1 equivalents (12 mg) of 4-(dimethylamino)pyridine are added. Stirring is continued overnight at room temperature. Water (20 mL) is added to the reaction mixture, and extracted three times with ethyl acetate. After the dicyclohexylurea by-product is removed by filtration, combined organic fractions are dried over anhydrous Na₂SO₄. Solvent is removed under vacuum, and a crude orange powder is obtained (140 mg, 43%). High-resolution negative ion ESI-MS for [M – H]⁻: expected 325.1889 (C₁₇H₂₇NO₅⁻), found 325.1878 (-3.4 ppm).
4.2 Experimental

4.2.2.4 4-(2,2,6,6-tetramethylpiperidine-N-oxyl)-yl esters (7-8)

Synthesis of esters (7-8) follows the same procedure for that of (6), replacing 1,4-cyclohexanedicarboxylic acid with the appropriate acid (terephthalic acid and potassium 4-sulfobenzoate, respectively). High-resolution negative ion ESI-MS for [M – H]⁻: (7) expected 319.1427 (C₁₇H₂₁NO₅⁻), found 319.1420 (2.2 ppm); (8) expected 355.1090 (C₁₆H₂₁NO₆S⁻), found 355.1095 (1.4 ppm).

4.2.2.5 Alkoxyamine synthesis

Synthesis of methyl and benzyl alkoxyamines (1a-b, 2a-b, Scheme 3.3) from the corresponding aminoxyl radicals (1) and (2) is reported in Section 2.3.2. Methyl and benzyl alkoxyamines were also prepared based on aminoxyl radicals (3-9, Scheme 4.2), using the same copper(I)-catalysed reaction. Although 4-amino-TEMPO (4) is commercially available, alkoxyamines based on this scaffold are firstly prepared from 4-acetamido-TEMPO. Subsequent amide hydrolysis by refluxing in aqueous KOH yields the desired alkoxyamine product. Generally, yields of alkoxyamines based on (5-8) were improved by first preparing alkoxyamines (3a-b) from 4-hydroxy-TEMPO (3) by a standard literature method,²⁷⁰ and subsequent derivatisation at the 4-position as described in Section 4.2.2.1. Performing the synthesis in the opposite order (i.e., adding the O-ether functionality to aminoxyl radicals 5-8) leads to breakdown of the 4-position substituent under the conditions required for alkoxyamine formation.

Sulfonamide scaffolds (9) and (9a) were kindly donated by Professor Steven Bottle (Queensland University of Technology, Australia).
4.2 Experimental

4.2.3 Mass Spectrometry

As in Chapter 3, mass spectra were recorded with a QuattroMicro (Waters, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an electrospray ionisation source. High-resolution MS spectra were acquired on a Waters Xevo G1 Q-ToF mass spectrometer, equipped with an ESI source. For further information see Section 3.2.3.

4.2.4 Computational Methods

Standard \textit{ab initio} molecular orbital theory and density functional theory calculations were carried out using the methodology described in Section 2.3.4. For large systems such as those based on scaffolds (5-8), gas-phase BDEs were calculated using a two-layer ONIOM method, where \((\text{CH}_3)_2\text{NO}–\text{R}\) was employed as the core layer and treated at the G3(MP2)-RAD(+) level of theory, while R(O)MP2/6-311+G(3df,2p) or R(O)MP2/6-31+G(2df,p) methods were used for the full system.

Detailed computational procedures and benchmarking studies are available in the literature and associated supporting information.\textsuperscript{279,282}
4.3 Results and Discussion

4.3.1 Manipulating Spin-Charge Separation

In the absence of a traditional polar contribution, the induced charge-quadrupole interaction is predicted to exert a \(1/r^3\) distance-dependent BDE difference between anionic and neutral alkoxyamines, where \(r\) represents the distance between the charge and forming aminoxyl radical site.\(^{282}\) In alkoxyamines and aminoxyl radicals with polar resonance forms, this effect is not experimentally separable from standard polar effects, which exhibit a \(1/r\) dependence.\(^{279}\) The effect of distance on alkoxyamine BDEs is demonstrated in Figure 4.3 for a theoretical homologous series of \(\text{OOC}-(\text{CH}_2)_n\text{TEMPO}–\text{CH}_3\) anions. At small radical-anion separations (large \(1/r\)), the stabilisation effect is magnified compared to more distant anions. In the extreme case (\(n = 10\)), a remote anion has a minimal effect on bond strength, and the alkoxyamine BDE closely resembles a neutral system (dashed horizontal line at 232 kJ mol\(^{-1}\)).

![Figure 4.3](image_url)

**Figure 4.3:** Calculated M06-2X/6-31+G(d) O–C BDEs of 4-carboxy-TEMPO–CH\(_3\) homologues with \(n = 0–10\). Spin-charge separation \(r\) is based on extended chain conformers. Adapted from Reference 279.
4.3 Results and Discussion

4.3.1.1 O–C Bond Homolysis Thresholds

Experimentally preparing an homologous series of $^{\text{-}OOC-(\text{CH}_2)_n-\text{C(O)O-TEMPO-CH}_3}$ anions was considered, to systematically vary $r$ by adding $n$ methylene units to the linker. In this series the separation is difficult to determine with certainty during an experiment due to conformational flexibility, which is deliberately restricted in the calculations. Furthermore, upon isolation and collision-induced dissociation of the $n = 2$ homologue (prepared from succinic anhydride and 4-hydroxy-TEMPO–CH$_3$, 3a), product ions ascribable to homolysis are not observed (Figure 4.4). The predominant product ion observed is attributed to cyclisation of the flexible chain.

![Figure 4.4](image)

**Figure 4.4**: CID spectrum ($E_{\text{lab}} = 20$ eV) of $^{\text{-}O_2C-(\text{CH}_2)_2-C(O)O-\text{TEMPO-CH}_3}$, in which homolysis is not observed.

Rigid spacers provide finer control over the spin-charge separation ($r$), compared to flexible alkyl linkers with many conformations caused by free rotation about each C–C bond. However, this restriction does limit the number of substrates available, compared with the possibility of merely adding successive methylene groups to an alkyl chain linker. A novel benzyl-substituted alkoxyamine separated from the carboxylate by a rigid cyclohexyl scaffold (6b) was synthesised, ionised by ESI, and subjected to CID. Like the equivalent TEMPO (1b) and PROXYL (2b)
benzyloxyamines, under low energy CID this benzyloxyamine (6b) dissociates exclusively via homolysis of the oxygen-carbon bond. Increasing the complexity of the molecule by adding the linker does not introduce new ions into the CID spectrum in the threshold energy region. Only with increasing collision energy ($E_{cm} > 2.2$ eV) are additional ions observed, such as a weakly abundant ion at $m/z$ 171, (deprotonated 1,4-cyclohexanedicarboxylic acid). The normalised abundance of product ions assigned to O–C cleavage (aminoxyl radical 6 and second generation product ions) are plotted as a function of centre-of-mass collision energy in Figure 4.5. Included for comparison are the data for 4-carboxy-TEMPO–CH$_2$Ph (1b) and 3-carboxy-PROXYL–CH$_2$Ph (2b) that were described in Chapter 2. Dashed lines are sigmoidal lines of best fit to the recorded data points (Section 2.3.3). Each plot approaches quantitative conversion to aminoxyl radical product ions around 2.0 eV. However, the breakdown curve of charge-remote alkoxyamine (6b) consistently tracks below 4-carboxy-TEMPO–CH$_2$Ph (1b), which is in turn beneath 3-carboxy-PROXYL–CH$_2$Ph (2b). That is, more energy is required to induce fragmentation in a given proportion of (6b), indicating a stronger O–C bond than in (1b) or (2b). Similarly, the 5% dissociation threshold of (6b) is higher than either of the previously studied compounds (Table 4.1). The ordering of these data are consistent with the increased separation ($r$) between the charge and the forming radical site in (6b) relative to (1b), and (2b).

**Table 4.1:** Calculated charge-radical separations and dissociation thresholds

<table>
<thead>
<tr>
<th></th>
<th>1b</th>
<th>2b</th>
<th>6b</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$ (Å)</td>
<td>5.7</td>
<td>4.9</td>
<td>7.8</td>
</tr>
<tr>
<td>$E_{5%}$ (eV)</td>
<td>0.70</td>
<td>0.52</td>
<td>0.85</td>
</tr>
</tbody>
</table>

$^\dagger$Lowest energy conformation (B3LYP/6-31+G(d)) of the forming aminoxyl radical
4.3 Results and Discussion

Figure 4.5: Abundance of O–C homolysis product ions from CID of remotely-charged benzylxoyamines. Threshold region is magnified in the inset.

4.3.1.2 Anion Basicity

Given that nearby anions exert a greater stabilisation on aminoxyl radicals than more remote ions, the corollary of this result (by Scheme 3.1) is that a proximate radical exerts a greater influence on the lowering of an anion’s proton affinity, compared to a more distant radical. Previously described kinetic method results have already uncovered preliminary evidence to support the distance-dependence hypothesis. When the proton-bound dimer of 4-carboxy-TEMPO (1) and 3-carboxy-PROXYL (2) is subjected to CID (Figure 3.13d), the resulting product ion abundance exhibits preferential formation of the latter ion, which has a smaller charge-radical separation.

Encouraged by this preliminary result, the suite of target compounds was expanded to include the rigid cyclohexyl-spacer (6) between the aminoxyl radical and carboxylate anion. In a similar experiment to the pairing of PROXYL- and TEMPO-based carboxylates, Figure 4.6(a) shows the spectrum obtained upon CID of a proton-bound dimer comprising (1) and (6). The greater abundance of (1) in the spectrum is consistent with a lower proton affinity than (6), as predicted for the acid with a
proximate radical moiety. Applying the kinetic method equation with an effective
temperature of 600 K to the observed peak intensity ratio yields a difference in proton
affinity ($\Delta \Delta H_{\text{acid}}$) of 7 kJ mol$^{-1}$. The experiment was repeated at multiple collision
energies to account for potential variations in effective temperature. Whilst the
abundances of product ions vary relative to the abundance of precursor ions, the
abundance of aminoxyl radicals (6) relative to (1) remains consistent, indicating that the
collision energy variations in the centre of mass frame have only a minor influence on
the effective temperature parameter. Furthermore, the B3LYP/6-31+G(d) optimised
genometry of (6) exhibits a spin-charge separation of 7.8 Å ($1/r = 0.128$ Å$^{-1}$). The
experimentally derived proton affinity of 7 kJ mol$^{-1}$ (relative to 1) is in agreement with
the theoretical results in Figure 4.3 that predict a relative proton affinity of 6 kJ mol$^{-1}$
for the $n = 2$ homologue, which has a similar spin-charge separation ($r = 8.0$ Å).

![Figure 4.6](image_url)

**Figure 4.6:** CID spectra of proton-bound dimers illustrate the effect of charge-radical
separation on relative proton affinity.

(a) aminoxyl radicals (1) and (6); (b) aminoxyl radical (6) and benzyloxyamine (6b).
4.3 Results and Discussion

To obtain the spectrum in Figure 4.6(b), the cyclohexyl-linked distonic aminoxyl radical anion (6) is paired with its corresponding benzyl alkoxyamine (6b) and subjected to CID at a laboratory frame collision energy of 18 eV. The predominant product ion observed is distonic ion (6), indicating a lower proton affinity. However, the calculated difference in proton affinity (ΔΔH\text{acid}) between the two components is only 5 kJ mol\(^{-1}\), compared with 14-18 kJ mol\(^{-1}\) derived from the analogous spectra in Figure 3.13(a-c) (see also Table 3.3). This result is qualitatively consistent with the more distant radical having a lesser effect on the proton affinity of (6), compared with (1) and (2). However, the 5 kJ mol\(^{-1}\) difference recorded is smaller than that predicted theoretically (ca. 12 kJ mol\(^{-1}\)) for a separation of 7.8 Å.

Together, these data confirm that the proximity of the anion does influence the magnitude of how far the O–C BDE deviates from the analogous neutral BDE. Negative ions close to the aminoxyl radical moiety significantly weaken its bonds with carbon-centred radicals in alkoxyamines, whereas distant anions exert a lesser influence.

4.3.2 Effect of Charge Carrier

For a given spacing between the charge and radical sites, the chemical nature of both the radical and the anionic moiety is also predicted to affect the magnitude of stabilisation. The greatest difference between anionic and neutral radical stability occurs with a combination of an initially stable radical and a strongly basic anion (weakly acidic conjugate acid).\(^{282}\) Conversely, the effect is muted for stable, delocalised anions.

4.3.2.1 Homolysis Thresholds

To test the relationship between anion basicity and radical stability, TEMPO-based alkoxyamines bearing an O-benzyl (Bz) substituent were synthesised with various acidic moieties in the 4-position of the piperidine ring. The [M – H]\(^-\) ions of each benzyloxyamine were subjected to CID in a triple quadrupole mass spectrometer.
4.3 Results and Discussion

Representative CID spectra are depicted in Figure 4.7. Like model compound 4-carboxy-TEMPO–CH₂Ph (1b), all precursor ions (5b – 8b) exhibit a characteristic loss of 91 Da, attributed to cleavage of the O–C bond and the formation of the corresponding aminoxyl radical (5 – 8). Subsequent loss of a methyl radical (–15 Da) is observed in most cases. Importantly, selectivity is retained and no product ions associated with the 4-position substituent are observed in the threshold energy region.

**Figure 4.7**: CID spectra ($E_{lab} = 20$ eV) of various anionic benzyloxyamines
(a) sulfate (5b); (b) cyclohexylcarboxylate (6b); (c) benzoate (7b); (d) phenylsulfonate (8b) The O–C bond cleaved in each precursor ion is indicated by a dotted line.

The normalised abundance of product ions attributed to O–C homolysis from these precursor ions are plotted as a function of collision energy in the breakdown curves shown in Figure 4.8. Complete conversion of the even-electron alkoxyamine precursor ion population to aminoxyl radicals is achieved at or before 2.0 eV. The breakdown curve of sulfate-tagged benzyloxyamine (5b) exhibits remarkable similarity
4.3 Results and Discussion
to the control 4-carboxy-TEMPO-Bz (1b) across the energy range, with dissociation
thresholds of 0.71 eV and 0.69 eV, respectively. On the other hand, the breakdown
curves of benzoyloxyamines (7b) and (8b) do not track each other, despite differing only
by the replacement of phenylcarboxylate with phenylsulfonate as the charge carrier.
Sulfonate (8b) has a higher threshold (0.83 eV), which – independent of charge site
effects – is consistent with a more remote charge (r = 12.1 Å). Whilst the separation in
carboxylate (7b) is of comparable size (r = 11.9 Å), this compound exhibits a threshold
approximately equal to that of 4-carboxy-TEMPO-Bz (0.69 eV). This discrepancy
would appear to indicate an unexpectedly large charge-tag effect, however the same is
not observed in the comparison of carboxylate and sulfate. Moreover, when the
dissociation threshold for each of the ions in Figure 4.8 – as well as the cyclohexyl
linker (6b) – are plotted against their calculated O–C BDEs (using an ONIOM
approximation to G3(MP2)-RAD(+)), a straight line links all data points except (7b),
which is a clear outlier and is not applied to the linear fit (Figure 4.9).

Figure 4.8: Abundance of O–C bond homolysis product ions observed upon CID of
anionic benzoyloxyamine precursors. Threshold region is magnified in the inset.
4.3 Results and Discussion

**Figure 4.9**: Experimental dissociation thresholds of charge-tagged benzyloxyamines compared to calculated BDEs.

In order to further expand the test set, a series of charge-tagged methoxyamines (1a – 9a) were also prepared to ensure that the observed effects are not unique to the TEMPO–CH₂Ph moiety. Resulting CID spectra are displayed in Figure 4.10. The predominant product ion observed is the corresponding aminoxyl radical (1 – 9) as a result of O–C bond homolysis. As explored in Chapter 2, N–O cleavage is also observed in CID of methoxyamines, due to the closer relative bond energies compared to benzyloxyamines. Moreover, absolute O–C BDEs are also higher in methoxyamines, and therefore these ions are susceptible to energetically-competitive even-electron dissociation (e.g., CO₂ loss from phenylcarboxylates 7 and 7a in Figure 4.10d).

Alkoxide 3a was also studied, and [M – 15]⁻ is indeed observed upon CID. However, its appearance is concomitant with multiple other ions, which increases the complexity of spectral interpretation. Upon isolation of the alkoxide with no applied collision energy, adduct ions including [M + 44]⁻ are observed, which is consistent with previous reports of addition of adventitious CO₂ to gas-phase alkoxides. These supplementary ions are likely to be the source of additional product ions upon CID.
4.3 Results and Discussion

Figure 4.10: CID spectra ($E_{\text{lab}} = 20$ eV) of selected charge-tagged methoxyamines (1a – 9a), exhibiting predominantly formation of aminoxyl radicals (1 – 9).

†Subsequent loss of methyl radical from the aminoxyl radical (–30 Da). ‡N–O cleavage followed by demethylation (–46 Da).

Figure 4.11: Abundance of O–C bond homolysis product ions observed upon CID of anionic methoxyamine precursors. Inset: magnified view of threshold region.
4.3 Results and Discussion

The abundance of O–C homolysis product ions are subsequently plotted against collision energy in Figure 4.11. Due to the competing N–O homolysis mechanism, the maximum abundance of aminoxyl radical product ions is at most 80%, and lower still for certain anions. Similar to benzyloxyamines, methoxyamines with sulfate (5a) and carboxylate anions (1a) directly attached to the piperidine ring exhibit comparable behaviour, with dissociation thresholds of 1.41 eV and 1.38 eV, respectively. An even lower threshold (1.21 eV) is observed for the methoxyamine with a proximate sulfonamide anion (9a). Furthermore, the unexpected behaviour of the phenylcarboxylate anion in the benzyloxyamine study (7b) is not repeated in the analogous methoxyamine (7a), which would seem to rule out any unaccounted charge-tag decomposition (e.g., oxidative decarboxylation) in the former. The breakdown curve profile of (7a) closely resembles the structurally similar phenylsulfonate (8a); both exhibiting dissociation thresholds greater than 1.5 eV. Because of similar charge-radical separations in these two ions, differences in dissociation profile are likely due to the chemical makeup of the anion itself.

Within these test sets, it is difficult to isolate the small anion effect on radical stabilisation from the dominant overlaid distance-dependence. Furthermore, for a defined distance, the switching magnitude is predicted to vary less than 10 kJ mol\(^{-1}\) for a suite of common oxygen-, sulfur-, and carbon-centred anions covering a broad basicity range. Radical stability is therefore less sensitive to the type of anion, compared to the distance between the two moieties. However, anions of various chemical natures all impart a net stabilisation on remote radicals, even in the absence of \(\pi\)-conjugation between the moieties, thus weakening the O–C bond compared to neutral alkoxyamines.
4.3.2.2 Kinetic Method

The influence of a radical on anion basicity was also explored by comparing proton affinities using the kinetic method. From the available suite of compounds, the most significant radical stabilisation is predicted to be exerted by a strongly basic alkoxide anion. When methanolic solutions of either 4-hydroxy-TEMPO (3) or its corresponding methoxyamine (3a) are subjected to negative ion electrospray ionisation, [M – H]⁻ ions are observed and were found to be isolable by MS/MS scans. Water adduct ions (+18 Da) and addition of CO₂ to form carbonate ions (+44 Da) are also observed. An additional ion at [M – H + 32]⁻ is also observed in the full ESI mass spectrum. Upon replacement of methanol solvent with the isotopologue D₄-methanol, this ion shifts upwards in mass by 4 Da. This ion is therefore assigned as the ion-molecule complex of the alkoxide and methanol. Formation of such an ion is convenient for applying the kinetic method to alkoxides. CID spectra of alkoxides (3) or (3a) complexed with methanol are displayed in Figure 4.12.

![Figure 4.12](image_url)

**Figure 4.12:** CID spectra ($E_{lab} = 10 \text{ eV}$) of alkoxides complexed with methanol.
(a) Aminoxyl radical (3); (b) alkoxyamine (3a). Precursor ions denoted by an arrow.
4.3 Results and Discussion

In both spectra, the predominant product ion observed is the TEMPO-based alkoxide, which subsequently adducts to water or adds to carbon dioxide. Neither spectrum exhibits any evidence for formation of methoxide ($m/z$ 31), nor potential adducts thereof (e.g., methyl carbonate, $m/z$ 75). The absence of methoxide ions indicates that the proton affinity of methoxide ($1599 \pm 3$ kJ mol$^{-1}$) is significantly greater than the studied alkoxides, and thus $\Delta(\Delta H_{acid})$ for these ion pairs is too great to be measured. Attempts to generate proton-bound dimers using alcohols with lower proton affinities were stymied by incompatibility with ESI, or isobaric interferences (e.g., isopropoxide and acetate).

The influence of an aminoxyl radical on the proton affinity of remote anions including alkoxides is determined by a relative measurement, avoiding the need for reference compounds. In a typical experiment similar to that described in Sections 3.3.4 and 4.3.1.2, an aminoxyl radical and alkoxyamine with the same charge-tag were combined in a common methanol solution, and infused into the mass spectrometer. The resulting proton-bound hetero-dimer was isolated and subjected to CID; with the relative ion abundances indicating the ordering of the two anions’ proton affinities.

CID spectra for alkoxides (3 and 3a), sulfates (5 and 5a), and sulfonamides (9 and 9a), are shown in Figure 4.13. Regardless of anion type, upon CID of each proton-bound dimer, the observed ion abundance ratio favours the radical anion. That is, the proton affinity of alkoxide, sulfate, and sulfonamide anions is decreased by the presence of a remote aminoxyl radical, relative to an even-electron alkoxyamine. Without repeating a full multiple-reference kinetic method with entropy correction, it is difficult to determine absolute thermochemical quantities, and thus compare the magnitude of $\Delta \Delta H_{acid}$ to calculated values.
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Figure 4.13: CID spectra ($E_{\text{lab}} = 10$ eV) of proton-bound dimers comprising aminoxyl radicals and methoxyamines of: (a) alkoxides; (b) sulfates; (c) sulfonamides.

Despite using the same instrumental parameters throughout, the effective temperature parameter also depends on properties of the ion itself, and thus may not be constant between different anion classes. Even so, the fact that the distonic aminoxyl radical anion is the most abundant ion in each CID spectrum qualitatively supports the hypothesis that a remote aminoxyl radical has a stabilising effect on various organic anions, by decreasing their proton affinities.

4.3.3 Effect of Charge Polarity

It is apparent that negative charges stabilise radicals by traditional polar effects and a hitherto under-appreciated long-range electrostatic charge-quadrupole interaction. In both cases, the extent of stabilisation depends on the charge carrier and location. In the presence of a positive charge, induced charge-quadrupole stabilisation in aminoxyl radicals is countered by a reversal in the contribution from polar resonance forms (Scheme 4.1). That is, the radical experiences a net destabilisation from a positive
charge (higher O–C BDE in precursor alkoxyamine). Similar to the anionic cycle presented in Scheme 3.1, by constructing an analogous thermodynamic cycle for the model proton acceptor 4-amino-TEMPO (4), the relative bond dissociation free energies are equivalent to relative gas-phase basicities (Scheme 4.3). Consequently, a greater O–C bond dissociation free energy in the presence of a positive charge must also be manifest as a greater gas phase basicity of alkoxyamine (I) compared to aminoxyl radical (II) (in enthalpy terms, a greater O–C BDE implies the alkoxyamine will exhibit a larger proton affinity). Once more, there are two options for testing this hypothesis: employing the kinetic method to examine the relative gas phase basicity of alkoxyamines and aminoxyl radicals, or using threshold measurements to evaluate the effect of a positive charge on O–C BDEs in alkoxyamines.

\[ \Delta G_{O-C(\text{neutral})} \]

\[ \Delta G_{\text{base(NOR)}} \]

\[ \Delta G_{\text{base(NO)}} \]

\[ \Delta G_{O-C(\text{+})} \]

\[ \Delta G_{O-C(\text{neutral})} \]

\[ \Delta G_{\text{base(NOR)}} \]

\[ \Delta G_{\text{base(NO)}} \]

\[ \Delta G_{O-C(\text{+})} \]

**Scheme 4.3**: Thermodynamic cycle linking relative BDFEs in neutral and cationic alkoxyamines to relative gas-phase basicity of amines in the presence or absence of an aminoxyl radical.

A series of 4-substituted TEMPO-based methoxyamines were synthesised to study the effect of a positive charge on the homolysis behaviour of alkoxyamines. Figure 4.14 features the spectra obtained upon CID of the [M + H]\(^{+}\) ions of trial
4.3 Results and Discussion

TEMPO–CH₃ derivatives. Spectrum (a) features a predominant ion at m/z 88, assigned to protonated O-methyl acetone oxime. The 3 Da shift of this ion to m/z 91 when the methoxyamine is selectively deuterated (Figure 4.14(b)) is consistent with this assignment. Moreover, this dissociation pathway is also observed in earlier studies on the charge-directed dissociation of substituted piperidines. A second major product ion is observed at m/z 142 in both spectra (a) and (b), representing a loss of 46 Da from the molecular ion (49 Da from the D₃-isotopologue). Given that this product does not contain the methoxyamine moiety, this ion is attributed to proton-mediated N–O bond cleavage, followed by subsequent ejection of a methyl radical.

Figure 4.14: CID spectra (E_{lab} = 20 eV) of methoxyamine [M + H]^+ ions.
(a) 4-hydroxy-TEMPO–CH₃ (3a); (b) 4-hydroxy-TEMPO–CD₃ (D₃-3a); (c) 4-oxo-TEMPO–CH₃; (d) 4-acetamido-TEMPO–CH₃; (e) 4-amino-TEMPO–CH₃ (4a); (f) 4-amino-4-carboxy-TEMPO–CH₃.

To quell the proton’s influence on fragmentation, 4-position substituents on the piperidine ring were varied to incorporate moieties with greater Brønsted basicity.
4.3 Results and Discussion

However, these same ions at $m/z$ 88 and $[M - 46]^+$ are also observed in the resultant spectra (Figure 4.14c–f), indicating that dissociation is largely independent of the 4-position functionality. Evidently, the anticipated product ions resulting from O–C bond cleavage are not observed in any of the derivatives. Thus, the presence of a mobile proton quenches O–C homolysis either by increasing the bond energy, and/or introducing charge-driven, energetically accessible dissociation pathways. To eliminate the mobile proton entirely, two novel fixed charge alkoxyamines were prepared, featuring a quaternary nitrogen either in the 4-position of the piperidine ring, or on the alkoxyamine motif. The CID spectra of these ions are presented in Figure 4.15.

![Figure 4.15: CID spectra ($E_{lab} = 20$ eV) of M$^+$ ions of fixed charge alkoxyamines.](image)

(a) 4-NMe$_3$-TEMPO–CH$_3$; (b) TEMPO–CH$_2$-NMe$_3$.

An ion at $m/z$ 88 assigned to $O$-methyl acetone oxime is again observed as the base peak in Figure 4.15(a). $[M - 59]^+$ ions present in both spectra are assigned to loss of trimethylamine associated with the charge tag. Therefore, even-electron dissociation pathways once again prevail, and product ions resulting from homolysis are not observed in either spectrum. Positively charged TEMPO-based alkoxyamine ions –
including those with sequestered fixed charge moieties – do not undergo O–C bond homolysis under CID conditions.

Although homolysis is not observed, this study showed that alkoxyamines based on 4-amino-TEMPO (4) could be successfully prepared and detected as [M + H]^+ ions upon ESI. Although thus far in this work the kinetic method has been employed to study the relative proton affinity of carboxylate anions, it is equally applicable to the study of positive-charged species, such as proton-bridged amines. Successful application of the kinetic method requires a pure population of isomers, and thus protonation must occur exclusively at the 4-position primary amine, rather than the aminoxyl/alkoxyamine nitrogen. The lower basicity of alkoxyamines compared to amines in the condensed phase suggests that this may be the case. Protonated alkoxyamines typically exhibit a pK\textsubscript{a} between 4.2 and 4.4, and aminoxyl radicals are even less basic. By contrast, the pK\textsubscript{a} of primary ammonium ions (R-NH\textsubscript{3}^+, the conjugate acids of amines) is greater than 10 (e.g., for CH\textsubscript{3}CH\textsubscript{2}-NH\textsubscript{3}^+, pK\textsubscript{a} = 10.8).}

Figure 4.16 illustrates the effect of polarity on the CID spectra of proton-bound dimers containing an aminoxyl radical and a methoxyamine. Figure 4.16(a) shows the proton-bound dimer of 4-carboxy-TEMPO (1) and 4-carboxy-TEMPO–CH\textsubscript{3} (1a) (reproduced from Figure 3.13a). The greater abundance of the former ion upon CID is a consequence of its lower proton affinity. Depicted in Figure 4.16(b) is the spectrum obtained upon CID of the proton-bound dimer comprising 4-amino-TEMPO (4) and 4-amino-TEMPO–CH\textsubscript{3} (4a). The intensity ratio of product ion peaks is inverted relative to spectrum (a); the abundance of alkoxyamines (m/z 187) is greater than that of aminoxyl radicals (m/z 172). The reasoning for formation of an additional product ion at m/z 173 is unclear. Although m/z 172 [M + H]^+ and m/z 173 [M + H\textsubscript{2}]^+ are both observed in the full ESI mass spectrum of 4-amino TEMPO (4), the m/z 173 ion cannot be a
4.3 Results and Discussion

component of a proton-bound dimer of $m/z$ 358, without a partner of $m/z$ 186. The existence of such a partner is not apparent in the full ESI mass spectrum. Potentially, the positive charge destabilises the aminoxyl radical to such an extent that it becomes an active hydrogen-atom scavenger. Even if the ions at $m/z$ 172/3 are assumed to be related, their summed abundances remains less than the abundance of alkoxyamines ($m/z$ 187). This result must be a consequence of the lower proton affinity of the amine in the presence of the aminoxyl radical (greater GPA of the conjugate acid). These data support the purported destabilisation of aminoxyl radicals’ polar resonance contributor (Scheme 4.1) in the presence of a positive charge. This effect is manifest as a shift in the nitrogen coupling constant (related to spin density) during ESR analysis.\textsuperscript{113}

Figure 4.16: Changing the charge-tag polarity inverts the product ion abundance ratio when proton-bound dimers are subjected to CID.

(a) carboxylic acids (1) and (1a); (b) primary amines (4) and (4a).

This effect has recently been exploited to design pH-sensitive nitroxide-mediated polymerisation agents. Multiple basic groups incorporated into the nitroxide structure are protonated at low pH, destabilising the polar aminoxyl radical resonance form, and thus the radical overall. This polar effect results an increased O–C bond
energy, and consequently slower decomposition of the precursor alkoxyamine.\textsuperscript{336} Nonetheless, it is important to realise that the effect of protonation on radical stability is significantly smaller in magnitude and acts over a shorter distance than the effects observed in negatively charged species that also exhibit charge-quadrupole stabilisation and associated SOMO–HOMO conversion.
4.4 Conclusions

In this work, the influence of the nature and proximity of a charge-carrying group on radical stabilisation (and similarly the effect of a radical on anion basicity) was examined by two tandem mass spectrometric techniques; collision-induced dissociation thresholds, and the kinetic method. The magnitude of such stabilisation can be as much as 20 kJ mol$^{-1}$, but decays with increasing separation between charge and radical moieties. Nonetheless, a minor effect is still observed in some form over distances beyond 10 Å, even in the absence of through-bond interactions.

The effect of the anion moiety on radical stability was also examined, although it was difficult to distinguish from the overlaid distance-dependence. The choice of charge-tag moiety is an important factor when using threshold dissociation methods to compare radical reactivity, even when the two moieties are spatially distant. As the switching effect is quenched by highly stabilised anions, the long-range electrostatic interaction and associated orbital conversion is not retained in polar solvents whereby anions are solvated. Thus there is no pH-dependent switching of radical stability. This theoretical prediction has recently been experimentally verified using an ESR technique, which found practically indistinguishable hydrogen-atom transfer equilibrium constants for COO$^-$–TEMPO and COOH–TEMPO.$^{344}$

Complementary electronic structure calculations have identified the effect as a long-range electrostatic interaction, associated with (but not caused by) rearrangement of electronic structure. These calculations have extended the scope of this effect beyond aminoxyl radicals. More localised radicals experience less of a shift upon deprotonation, which may explain why N–O bond energies are not significantly perturbed by an anion (Figure 2.5). Such predictions are more difficult to verify experimentally due to the complexity of producing and isolating such reactive radical species.
5. Photodissociation of TEMPO-MODIFIED PEPTIDES: NEW APPROACHES TO RADICAL-DIRECTED DISSOCIATION OF BIOMOLECULES

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Supporting Information is attached as Appendix B.

Author Statement

David L. Marshall performed all research with the assistance of Christopher S. Hansen, and wrote the manuscript with input from all authors. This work would not have been possible without the support of Han Bin Oh, Adam J. Trevitt, and Stephen J. Blanksby.

Primary Supervisor Confirmation

I, Prof. Stephen J. Blanksby (Primary Supervisor), support and certify the above author statement.

Signature

Date

10/4/14
5.1 Abstract

Radical-directed dissociation of gas phase ions is emerging as a powerful and complementary alternative to traditional tandem mass spectrometric techniques for biomolecular structural analysis. Previous studies have identified that coupling of 2-[(2,2,6,6-tetramethylpiperidin-1-oxyl)methyl]benzoic acid (TEMPO-Bz) to the N-terminus of a peptide introduces a labile oxygen-carbon bond that can be selectively activated upon collisional activation to produce a radical ion. Here we demonstrate that structurally-defined peptide radical ions can also be generated upon UV laser photodissociation of the same TEMPO-Bz derivatives in a linear ion trap mass spectrometer. When subjected to further mass spectrometric analyses, the radical ions formed by a single laser pulse undergo identical dissociations as those formed by collisional activation of the same precursor ion, and can thus be used to derive molecular structure. Mapping the initial radical formation process as a function of photon energy by photodissociation action spectroscopy reveals that photoproduct formation is selective but occurs only in modest yield across the wavelength range (300 – 220 nm), with the photoproduct yield maximised between 235 and 225 nm. Based on the analysis of a set of model compounds, structural modifications to the TEMPO-Bz derivative are suggested to optimise radical photoproduct yield. Future development of such probes offers the advantage of increased sensitivity and selectivity for radical-directed dissociation.
5.2 Introduction

In the field of proteomics, radical-based tandem mass spectrometry of peptides and proteins is a powerful alternative tool to collision-induced dissociation (CID). Whilst their speed and simplicity cannot be disputed, “slow-heating” methods\textsuperscript{173} such as CID or infrared multi-photon dissociation (IRMPD) generally promote even-electron fragmentation of the peptide or protein ion, and used in isolation may not provide full sequence coverage or complete characterisation of labile post-translational modifications (PTMs).\textsuperscript{184,345} Compared to CID or IRMPD, electron capture dissociation (ECD)\textsuperscript{185,346-348} and electron transfer dissociation (ETD)\textsuperscript{186,349} of multiply-charged peptide/protein cations typically produce spectra with greater sequence coverage and PTM localisation, due to extensive, non-selective fragmentation.\textsuperscript{186,187} Electron-induced dissociation (EID) of both protonated\textsuperscript{191,350} and deprotonated\textsuperscript{351} singly charged peptide ions also elicits additional radical driven sequence coverage information complementary to that derived from collisional activation, without modifying the initial charge state.

The increasing importance of radical ions in biomolecular mass spectrometry has motivated development of chemical methods for introducing radical sites into peptide/protein ions that are applicable to a wider range of mass spectrometer configurations. For example, CID of ternary metal/peptide complexes generate peptide radical cations,\textsuperscript{352} which produce \textit{al/x-} or \textit{c/z-type} peptide backbone fragments and notable side chain losses upon subsequent activation.\textsuperscript{353-357} Covalent attachment of a free radical precursor to peptides is another pathway to produce peptide radical ions, by introducing a labile bond that is susceptible to homolysis. Free-radical initiated peptide sequencing (FRIPS) methods offer the particular advantage of a well-defined initial radical site, thus fragmentation can be induced at specific amino acid residues. For
example, collisional activation of \( N \)-nitroso- and \( S \)-nitroso-derivatives of tryptophan and cysteine-containing peptides produce aminyl and sulfenyl radicals through bond homolysis with loss of nitric oxide. Subsequent activation of these radical ions initiates radical-driven fragmentation along the peptide backbone and thus yields a range of diagnostic product ions.\(^{358-362}\)

Chemical modification of the \( N \)-terminus of the peptide or protein is another example of derivatisation for FRIPS. For instance, upon CID, peroxycarbamates\(^ {363} \) and azo moieties\(^ {364} \) are susceptible to homolytic dissociation forming nitrogen- and carbon-centred radicals, respectively. Collisional activation of such radical ions results in side-chain and backbone cleavage with extensive sequence coverage. Lee et al. introduced 2-[(2,2,6,6-tetramethylpiperidin-1-oxyl)methyl]benzoic acid (TEMPO-Bz, Scheme 5.1) as a free radical precursor bound to peptides through the \( N \)-terminus or at the \( \varepsilon \)-amine of lysine residues,\(^ {259-261} \) and is the tagging group employed in the present study. Collisional activation of the precursor peptide ions initiates homolytic cleavage of the labile NO–C bond, promoted by the remarkable stability of the released aminoxyl radical. Subsequent collisional activation of the peptide radical ions yields peptide backbone fragments and side-chain losses with extensive sequence coverage. Abundant formation of \( a/x \)-and \( c/z \)-type ions suggests that radical-driven peptide backbone dissociation is the major fragmentation pathway, similar to the electron-based methods.

Ultraviolet photodissociation (UVPD) has also been used extensively for peptide characterisation and sequencing.\(^ {365} \) The identity and yield of photoproduct ions depends on many aspects, including the incident photon energy, chemical modifications to the peptide,\(^ {366} \) ion charge-state, laser fluence, overlap between the incident light and the ion ensemble, absorption cross-section, and the number of pulses with which the ion ensemble is irradiated.\(^ {367} \)
5.2 Introduction

Scheme 5.1: Peptide-TEMPO-Bz structure used in the present study.

Activation of the derivatised peptide by either (a) CID (shown previously), or (b) PD results in homolytic cleavage of the O–C bond to yield a peptide radical ion.

The complexity of laser-based structural characterisation is increased for large molecules as bond dissociation can be mediated by the redistribution of excess energy among many vibrational degrees of freedom.\textsuperscript{368} The energy required for radical formation through bond homolysis can thus exceed the intrinsic bond dissociation energy. In order to form protein radical ions in the gas phase by UV irradiation, it is therefore necessary to induce dissociation on a time scale faster than competing processes, including intramolecular vibrational energy redistribution (IVR). IVR extends the lifetime of the activated ion population and provides opportunity for non-dissociative removal of excess energy \textit{via} collisional cooling. One such example is electron photodetachment from polyanions.\textsuperscript{369} Peptide radical anions are formed by efficient UV excitation of bound $\pi^* \rightarrow \pi$ electronic transitions within aromatic amino acids, followed by crossing to unbound electronic states, leading to electron detachment on a timescale that is competitive with IVR.

Photolysis of suitable photolalible precursors is an alternative way to efficiently produce a radical site for peptide sequencing. For example, upon irradiation with 266 nm photons, iodine atom loss from aryl iodide-modified peptide ions is observed as the major process in the mass spectrum.\textsuperscript{190,370,371} Kirk \textit{et al.} recently demonstrated the
wavelength dependence of iodine atom loss from iodosylated tyrosines by photodissociation (PD) action spectroscopy, highlighting the influence of charge state on the efficacy of photoproduction formation.\textsuperscript{372} Compared with CID, photodissociation of aryl iodides facilitates the production of radical sites without complications due to isomerisation.\textsuperscript{197,373} Strategies that utilise UV radiation and a photolabile radical precursor show promise for peptide characterisation. High photoproduction yields,\textsuperscript{374} the availability of tunable laser sources, and avoiding the low-mass cut-off inherent in ion trap-based analysis\textsuperscript{375} offer an opportunity for enhanced peptide sequencing coverage over collisional and electron-based tandem mass spectrometric methods. Photodissociation can access site-specific radical ion product channels, whereas collisional activation of the same precursor ion yields less selective fragmentation, a mixture of isomers, or no radical-directed dissociation at all.\textsuperscript{376,377}

In the present study, we have explored the application of UVPD to TEMPO-Bz modified peptides and assessed the suitability of this strategy for FRIPS. Here we show that absorbed UV radiation induces homolytic cleavage of the labile carbon-oxygen bond in the TEMPO-Bz derivatives. Thus generated, the peptide radical ions can be further activated by CID to fragment the peptide backbone, resulting in sequence ions and diagnostic side chain losses. In this study, the factors influencing the efficacy of photodissociation are explored and strategies to enhance the selectivity and sensitivity of this approach are discussed.
5.3 Experimental

5.3.1 Materials

Methanol (HPLC grade), acetic acid (99%, AR grade), and ammonium acetate (AR grade) were obtained from Ajax Fine Chemicals (now part of Thermo Fisher Scientific, Sydney, Australia). All other materials were obtained from Sigma Aldrich (Sydney, Australia). The synthesis of TEMPO-Bz conjugated peptides from commercially available 2-methylbenzoic acid methyl ester has been reported previously. Peptide solutions of kinetensin (IARRHPYFL), bradykinin (RPPGFSPFR), and YGGFMRF were prepared at ca. 10 – 20 μM, in 1:1 methanol:water solvent mixtures, with the addition of 0.1% acetic acid or 0.1% ammonium acetate to facilitate the formation of positive and negative ions, respectively.

Synthesis of model compounds 1-(benzyloxy)-2,2,6,6-tetramethylpiperidine-4-carboxylic acid (1); 4-(((1-(benzyloxy)-2,2,6,6-tetramethylpiperidin-4-yl)oxy)carbonyl)cyclohexanecarboxylic acid (2); and 4-(((1-(benzyloxy)-2,2,6,6-tetramethylpiperidin-4-yl)oxy)carbonyl)benzoic acid (3) was undertaken based on appropriate adaptation of literature procedures. Briefly, 1 was prepared by the addition of phenylacetaldehyde, hydrogen peroxide and copper (I) to 4-carboxy-TEMPO (1a). 4-hydroxy-TEMPO-Bz is prepared from 4-hydroxy-TEMPO in the same manner and is subsequently esterified with the appropriate difunctional acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). Desired [M − H]− anions of (1-3) are produced without additional purification by diluting aliquots of the reaction mixture to a concentration of approximately 5 μM in methanol, and subjecting these solutions to electrospray ionisation (ESI). A summary table listing all peptides investigated in this study and the charge states that were subjected to photodissociation is provided as Supporting Information (Table S1).
5.3 Experimental

5.3.2 Mass Spectrometry

Photodissociation experiments were performed using a Thermo Fisher Scientific LTQ linear quadrupole ion trap mass spectrometer (San Jose, CA, USA) modified for both fixed-frequency, and tunable laser photodissociation experiments. Briefly, the aforementioned peptide solutions are infused into the mass spectrometer through the ESI source with instrument parameters similar to those described previously. The TEMPO-Bz modified peptide ions are mass-selected, isolated, and thermalised (ca. 307 K) in the helium buffer gas (2.5 mTorr helium). The ion ensemble is then irradiated with a single pulse from either a fixed-frequency (266 nm) or tunable (300 – 220 nm) nanosecond laser system, the ion trap is scanned out and a PD mass spectrum is recorded containing signals for both photoproduct ions and the intact precursor ion population. In a photodissociation action spectroscopy experiment, the photodissociation yield is calculated as a fraction of the total ion population from this mass spectrum, the laser is then tuned to the next wavelength and the process repeated. For each action spectrum, the photoproduct yields are corrected by a power spectrum (acquired offline), and plotted as a function of photon wavelength.

Fixed-frequency experiments are performed using the 4th harmonic (266 nm) of a Nd:YAG laser (Continuum Minilite, Santa Clara, USA), delivering a 5 ns, 4 mJ pulse on demand. Tunable photodissociation experiments employ an optical parametric oscillator (OPO) fitted with a frequency-doubling unit (versaScan and uvScan, GWU-Lasertechnik, Erftstadt, Germany) pumped by the 3rd harmonic (355 nm) of a QuantaRay INDI Nd:YAG laser (Spectra-Physics, Santa Clara, USA). This provides a pulsed, nanosecond photon source, tunable across the range 2500 – 220 nm, delivering ca. 2 mJ per pulse in the UV. This laser operates at 10 Hz and is carefully shuttered to ensure that a single pulse enters the ion trap at the correct stage of each MS cycle.
5.4 Results and Discussion

5.4.1 Photodissociation of Derivatised Peptides

Electrospray ionisation of TEMPO-Bz peptide solutions produced singly and multiply charged ions, with the relative abundance of charge states dependent on ion source parameters, solvent composition, and the number of acidic and basic amino acid residues in the peptide. CID spectra resulting from the isolation and activation of [M + nH]^{+} ions (where n = 1, 2) of bradykinin-TEMPO-Bz are displayed in Figure 5.1. Prior to activation, no product ions are observed from either precursor ion. The carbon-oxygen bond energy in the TEMPO-Bz motif is estimated to be only 110 kJ mol^{-1} and thus upon CID, the bond between the aminoxyl oxygen and the benzyl carbon is preferentially cleaved to form an abundant radical ion (m/z 589 and m/z 1177 for doubly and singly charged ions, Figure 5.1(a) and (c), respectively). The resulting alkyl radical initially formed at the benzylic position remains covalently tethered to the N-terminus of bradykinin (cf. Scheme 5.1). Additional product ions present in low abundance are attributed to spontaneous, radical-mediated backbone cleavage (e.g., c_{5}^{+}) or amino acid side chain losses (e.g., -100 Da, from arginine) from the primary radical product (vide infra). When the isolated precursor ions are irradiated with a single, λ = 266 nm laser pulse (henceforth PD_{266}), the doubly and singly protonated ions (Figure 5.1(b) and (d), respectively) undergo TEMPO loss through carbon-oxygen bond homolysis. Despite the perceived selectivity, the yield of desired radical ions is much lower in the PD_{266} spectra (less than 4% of the precursor ion abundance), compared to the almost complete conversion achieved by CID. In the PD_{266} experiment, the relative abundance of the open-shell product ion is higher from the singly protonated charge state, compared to the doubly charged species.
5.4 Results and Discussion

Figure 5.1: (a) CID (collision energy = 20); or (b) PD266 of protonated TEMPO-modified bradykinin ions, selectively yield TEMPO loss.

(a) CID of [M + 2H]^{2+} ions; (b) PD266 of [M + 2H]^{2+} ions; (c) CID of [M + H]^{+} ions; (d) PD266 of [M + H]^{+} ions. Numbers represent the mass difference after TEMPO loss, and the letter in parentheses indicates the amino acid involved.

Except for the y_{8}^{+} ion observed in the CID spectrum of derivatised bradykinin (Figure 5.1a), characteristic b-type and y-type product ions from the intact modified peptide, typically observed from collisional activation of protonated peptide ions,^{380,381} are notably minor in both the CID and PD266 spectra. Although an intact b_{1}^{+} ion is not observed in Figure 5.1(a), a radical ion is present corresponding to formation of a b_{1}^{+}
ion after TEMPO loss, indicating that loss of a TEMPO radical via homolysis is the preferred dissociation process. Additional $b$-type and $y$-type ions are present following PD$_{266}$ of the derivatised bradykinin but are of too low abundance to be readily identified at the magnifications shown in Figure 5.1(b) and (d). Notably however, when the abundance of $b$-type and $y$-type ions are considered relative to the abundance of the [$M + xH − TEMPO]^{x+}$ ion (rather than the precursor ions as displayed in Figures 1b and 1d) similar peak ratios are found for PD$_{266}$ as CID spectra (see Supporting Information, Figure S1a). Indeed, some comparable $b$-type and $y$-type ions are observed in the PD$_{266}$ spectrum of unmodified bradykinin (i.e., in the absence of the TEMPO-Bz tag, see Supporting Information, Figure S1b).

Activation of [M + 3H]$^{3+}$ TEMPO-modified bradykinin ions ($m/z$ 445) by CID also produces ions attributed to TEMPO loss at $m/z$ 393 (see Supporting Information Figure S2). However selectivity is not retained, and the CID spectrum is dominated by ions with higher mass-to-charge ratios, indicating charge reduction fragmentation mechanisms prevail over the desired radical formation by homolysis of the oxygen-carbon bond. Likewise, irradiation of the [M + 3H]$^{3+}$ ions by PD$_{266}$ does not result in TEMPO loss. This may be rationalised by considering that after the basic arginine residues, the final protonation site is likely to be the piperidinyl nitrogen of the TEMPO-Bz moiety. Upon protonation of the piperidinyl nitrogen, homolysis of the alkoxyamine NO–C bond is suppressed relative to even-electron rearrangement processes under CID,$^{263}$ and we infer that analogous processes prevail upon photodissociation. Similar observations were made for other peptides when the number of charges approached or exceeded the number of basic side chains available, placing an upper limit on the charge states for which CID and PD$_{266}$ can produce the desired radical ions.$^{382}$
Advantageously, the TEMPO-based FRIPS methodology is also applicable to the study of negative ions.\textsuperscript{261} Negative ion mass spectrometry provides structural information for proteomics that is both confirmatory and complementary to that obtained in the study of positive ions. Furthermore, for peptides with acidic residues or phosphorylation, negative ion mass spectrometry may be preferred for increased sensitivity. By contrast with positive ions, only a few studies have considered the formation and fragmentation of site-specific peptide radical anions with the aim of peptide sequencing and characterisation.\textsuperscript{377,383} Singly and multiply deprotonated TEMPO-Bz peptide anions [M – H]\textsuperscript{−} and [M – 2H]\textsuperscript{2−} were produced by ESI, depending on the number of acidic residues available in the peptide. For example, isolation and PD\textsubscript{266} of kinetensin dianions produces the spectrum shown in Figure 5.2(a). It is clear from the presence of [M – 2H]\textsuperscript{2−} product ions at m/z 1442 that electron detachment competes with TEMPO photodissociation. A putative biradical anion at m/z 1286 arises from a combination of both electron detachment and bond homolysis processes. For singly charged anions (m/z 1443, Figure 5.2b), selective loss of TEMPO is observed, producing a radical anion at m/z 1287. Electron detachment is also prevalent for the singly charged anions, forming unobservable neutral products, and reducing the total ion signal by ca. 10% when compared with a spectrum obtained with the laser beam blocked. Like the protonated species, higher photoproduction ion abundance is observed for the lower charge state, specifically 1.6% for singly charged kinetensin anions, compared to 0.2% for the dianions. Unlike the previously published CID spectrum,\textsuperscript{261} diagnostic sequence ions were not found to be abundant following UV irradiation of the precursor anions.
5.4 Results and Discussion

Figure 5.2: Mass spectra obtained upon isolation and PD\textsubscript{266} irradiation of deprotonated TEMPO-Bz modified kinetensin ions: (a) [M – 2H]\textsuperscript{2–}; (b) [M – H]\textsuperscript{–}.

Despite the comparably high selectivity for NO–C bond homolysis observed by both activation methods, the production of peptide radical ions through TEMPO loss (cf. Scheme 5.1) is less prevalent for PD\textsubscript{266} than for CID. The efficacy of the latter can also be easily modulated, by adjusting the activation energy applied to the trapped ions. The modest yield from PD\textsubscript{266} irradiation was found to be consistent for all peptides studied, with a small improvement in PD\textsubscript{266} photoproduct yields for singly charged ions, compared to doubly charged ions. The maximum raw photoproduct ion abundance observed was \textit{ca.} 10.5\% relative to the precursor ion, for singly protonated YGGFMRF. By comparison, the radical photoproduct yield of the equivalent [M + 2H]\textsuperscript{2+} ion was only 0.2\%, and 2.3\% for the [M – H]\textsuperscript{–} ion (see Supporting Information, Figure S3).
5.4 Results and Discussion

5.4.2 Characterisation of Radical Ions

Despite overall low PD yields, sufficient radical photoproduction ions were produced to enable further interrogation through MS" experiments in the linear ion trap, and allow comparison of the radical ions initially formed by CID and PD

Such ions are matched by comparing CID/CID (MS^3) spectra to the equivalent PD/CID (MS^3) spectra. For example, [M + 2H]^{2+} ions of modified YGGFMRF (m/z 576) are subjected to CID and PD to form radical ions (m/z 498) via loss of TEMPO. Upon subsequent isolation and collisional activation, CID/CID and PD/CID spectra were acquired. These spectra are compared in Figure 5.3(a) and Figure 5.3(b), respectively, and exhibit the same product ions at similar relative abundances. The highest intensity product ion peak is a doubly charged c_6 ion, which can only feasibly be generated by radical-driven processes. Hydrogen atom transfer is proposed to explain the radical mediated backbone cleavage and side chain losses observed in FRIPS, and the relationship to peptide structure. Independent of charge state, hydrogen atom transfer from the amino acid side chain to the benzylic radical results in a peptide-based radical with a closed-shell o-methylbenzamide modification at the N-terminus. Subsequent β-cleavage of the peptide-based radical yields a/x- and c/z-type ions. Side chain losses are also observed, with diagnostic mass losses identifying specific amino acid residues, such as the 61 Da loss (CH_3SCH_2•) specific to methionine (Figure 5.3b). Noticeably, an array of b- and y-type ions are also observed in both MS^3 spectra. As there are more protons than basic arginine residues, conventional mobile-proton-assisted (rather than radical-driven) backbone dissociation is responsible for the formation of these ions. Based on the similarities between the two MS^3 spectra, the radical ion species formed by both CID and PD activation methods are identical. Therefore, PD/CID may be used to obtain the same structural information as CID/CID, albeit with a lower radical ion abundance.
As a further example, the PD\textsubscript{266}/CID spectrum of doubly charged bradykinin is shown in Figure 5.3(c). Predominantly \textit{a}-type ions are observed, along with \textit{b}-, \textit{c}-, \textit{y}-, and \textit{z}-type ions, as well as radical-driven side chain losses associated with arginine. This MS\textsuperscript{3} spectrum shares several features with a result obtained by Sun \textit{et al}., whereby doubly protonated bradykinin radical ions were generated through PD\textsubscript{266} of a non-covalent complex, and subsequently subjected to CID.\textsuperscript{203} Fragmentation is preferentially observed at aromatic phenylalanine residues, producing \textit{a}\textsubscript{5} and \textit{a}\textsubscript{8} ions. As a consequence of cleavage between phenylalanine and serine, \textit{c}\textsubscript{5} and \textit{z}\textsubscript{4} ions are also observed. The proline effect governs the formation of abundant \textit{y}\textsubscript{8} ions. However, compared to YGGFMRF, \textit{b}-type and \textit{y}-type ions are otherwise suppressed in bradykinin, due to the sequestration of charge on multiple arginine residues. Like bradykinin, the PD\textsubscript{266}/CID spectrum of kinetensin (IARRHPYFL, Figure 5.3d) is dominated by \textit{a}-type ions, and fragmentation of the side chain of the first amino acid from the \textit{N}-terminus, namely a 29 Da loss ($\text{CH}_3\text{CH}_2\cdot$) from isoleucine. Other radical-driven side chain losses from tyrosine and arginine are also observed. Comparing with the equivalent CID/CID spectra for bradykinin and kinetensin (Supporting Information, Figure S4 and Figure S5, respectively) confirms that the radical ion species initially produced by CID and PD\textsubscript{266} are essentially equivalent, and thus provide the same structural information. Importantly, these results confirm that the structure of radical ions produced from TEMPO-Bz derivatives of peptides are identical regardless of the activation method used to generate them. The sensitivity and thus the utility of PD\textsubscript{266} irradiation is hampered however by the low photoproduct yields; typically less than 5\% of the precursor ion abundance.
5.4 Results and Discussion

Figure 5.3: MS$^3$ spectra obtained by CID of [M + 2H – TEMPO]$_{2+}$ radical ions.

(a) YGGFMRF, generated by CID; (b) YGGFMRF, generated by PD$_{266}$; (c) bradykinin, generated by PD$_{266}$; (d) kinetensin, generated by PD$_{266}$. Major peptide sequence ions and radical mediated side chain losses are assigned, with the identified amino acid in parentheses. The precursor ion in each spectrum is marked with an asterisk (*).
5.4 Results and Discussion

5.4.3 Photodissociation Action Spectroscopy of Derivatised Peptides

To investigate the possibility of improving the radical photoproduct yield by varying the incident photon energy, PD action spectra are acquired across a wavelength range of 300 – 220 nm in one nanometre increments. Photofragmentation is not observed when the wavelength is tuned further to the red (\(\lambda > 300\) nm), consistent with previous observations of the photolysis of the TEMPO-Bz moiety.\(^{386}\) Plotting the power-corrected photodissociation yield of the \([\text{M} + 2\text{H} – \text{TEMPO}]^{2+}\) product ion from the \([\text{M} + 2\text{H}]^{2+}\) ions of both bradykinin and kinetensin against the photon wavelength results in the PD action spectra shown in Figure 5.4. Representative photodissociation mass spectra for both TEMPO-Bz modified peptides are provided as Figure S7 and Figure S8. The maximum photodissociation yield for bradykinin occurs around 227 nm, and for kinetensin around 232 nm. Changes in PD action spectrum profile may be used as indicators for variations in the primary or secondary structure of small peptides.\(^{372}\) Differences in the PD action spectra between kinetensin and bradykinin are qualitatively attributed to the different number and type of aromatic residues (chromophores) in the peptide, and their proximity to the \(N\)-terminus, where the dissociation occurs. Although it must be noted that the absolute photoproduct yields acquired with different lasers may not be directly comparable, the low photodissociation yield at longer wavelengths correlates with low photoproduct yields observed using the fixed-frequency 266 nm laser (maximum raw photoproduct abundance \(ca. 2\%\) for doubly charged peptide ions). Based on these data, 266 nm does not correspond to the ideal photon energy to efficiently induce dissociation of the NO–C bond in TEMPO-modified peptides with more efficient conversion to the radical ions achieved near 230 – 225 nm (representing an increase in yield of \(ca. 20-50\) fold).
5.4 Results and Discussion

Figure 5.4: Photodissociation action spectra exhibiting the relative yield of \([M + 2H - TEMPO]^{2+}\) photoproducts from TEMPO-Bz modified bradykinin and kinetensin \([M + 2H]^{2+}\) ions as a function of wavelength.

Proteins as large as ubiquitin, modified to contain an iodotyrosine residue, produce abundant radical ions upon PD\(_{266}\) (at a similar fluence to that employed herein), through homolysis of the aryl carbon-iodine bond on an excited state surface,\(^{200,387}\) which is known to occur on the sub-picosecond timescale following excitation.\(^{379}\) By contrast, although the photon energies employed herein \((300 \text{ nm} = 399 \text{ kJ mol}^{-1})\) exceed the NO–C bond dissociation energy in TEMPO-Bz \((\text{ca. } 110 \text{ kJ mol}^{-1})\), the excess energy does not result in the formation of abundant photoproduct ions \textit{via} homolysis. The modest abundance of peptide radical ions by PD may be a result of poor absorption cross-section, or a large population of the ions redistributing their excess energy \textit{via} non-dissociative relaxation pathways (\textit{e.g.}, collisional cooling). Photodissociation of the NO–C bond in the TEMPO-Bz moiety therefore warranted further investigation.

The formation of \(y_8^+\) ions \((m/z \ 904)\), observed in the CID spectra of both unmodified\(^{388}\) and TEMPO-Bz modified bradykinin (Figure 5.1a), also proceeds upon ultraviolet excitation of the latter. Formation of \(y_8^+\) ions as a function of wavelength exhibits a similar profile to that of the TEMPO loss product (see Supporting
Information, Figure S6), which suggests that both products are formed by similar processes following photo-excitation. Dissociation to $y_n^+$ ions is known to be facilitated by vibrational excitation on the electronic ground state surface.\textsuperscript{389} Therefore, TEMPO loss through oxygen-carbon bond dissociation also likely occurs following excitation and internal conversion to a vibrationally-excited electronic ground-state. Previous work has suggested, that the branching ratio between excited state fragmentation and ground state fragmentation is variable, depending on the peptide sequence and conformation, charge state, and charge site.\textsuperscript{390,391} In some cases, internal conversion is so dominant that excited state-specific fragmentation is completely quenched;\textsuperscript{389} this appears to be the case here. We surmise therefore, that UV photodissociation of the derivatised peptides kinetensin and bradykinin is occurring on their respective ground electronic states. Differences in their photoaction spectra (Figure 5.4) thus reflect variation in absorption profile, resulting from differences in primary and secondary structure, rather than participation of any particular excited-state dissociation mechanisms.

\subsection*{5.4.4 Photodissociation of Model Compounds}

In order to better understand -- and potentially improve -- the apparently poor efficiency of NO–C photodissociation in the TEMPO-Bz system, model compounds (1-3) were synthesised and subjected to PD\textsubscript{266} irradiation (Figure 5.5). Isolation and photodissociation of the $[M-H]^-$ anions formed from 4-carboxy-TEMPO-Bz (1) selectively produce radical anions at $m/z$ 199 in modest yield, through cleavage of the NO–C bond and neutral loss of the benzyl radical moiety (Figure 5.5a). Further interrogation of the $m/z$ 199 ions by CID resulted in an $MS^3$ (PD\textsubscript{266}/CID) spectrum identical to: (i) the $MS^3$ (CID/CID) spectrum produced by successive CID steps from the same precursor ion (1); and (ii) the CID spectrum obtained from the $[M-H]^-$ anion
formed by ESI of an authentic sample of 4-carboxy-TEMPO (1a) (see Supporting Information, Figure S9). Although much smaller in size than the studied peptides, photodissociation of the NO–C bond in this system indicates that the chromophore present on the TEMPO-Bz linker is also able to initiate homolysis. Therefore, peptides without aromatic residues may also be amenable to photodissociation using this tagging group. With this prototype system, the photoproduct yield is ca. 3% relative to the precursor ion and is similar in magnitude to the maximum PD\textsubscript{266} efficiency observed for the TEMPO-modified peptides. A similarly modest photoproduct yield (ca. 4%) is also observed when, for compound (2), a cyclohexyl linker is added between TEMPO-Bz and the carboxylate charge carrier (Figure 5.5b). The TEMPO-Bz moiety does not undergo efficient bond homolysis at 266 nm, despite the stability of the putative distonic aminoxyl radical anion products (1a) and (2a).\textsuperscript{279} In the third model compound (3) an additional aromatic chromophore was installed at the 4-position of the piperidine ring. Compared with (1) and (2), the benzyl loss photoproduct yield from [M – H]\textsuperscript{−} anions formed from (3) is greatly enhanced, contributing over 50% of the total ion population, as illustrated in Figure 5.5(c). In addition to the major photoproduct arising from loss of the benzyl moiety (3a), a secondary product ion is also observed at \textit{m/z} 304, corresponding to subsequent loss of a methyl radical. Covalent attachment of an additional aromatic chromophore to the 4-position of the piperidine ring improves the photodissociation yield in the TEMPO-Bz motif more drastically than variation of chromophores on the peptide backbone, potentially through increased absorption cross-section, or by opening up more efficient pathways to NO–C bond homolysis on an excited state surface.\textsuperscript{392,393}
Figure 5.5: PD$_{266}$ of the [M – H]$^-$ anions formed from model TEMPO-Bz compounds (1-3) produce the respective aminoxyl radicals (1a-3a).

(a) 1-(benzyloxy)-2,2,6,6-tetramethylpiperidine-4-carboxylic acid (1); (b) 4-(((1-(benzyloxy)-2,2,6,6-tetramethylpiperidin-4-yl)oxy)carbonyl)cyclohexanecarboxylic acid (2); and (c) 4-(((1-(benzyloxy)-2,2,6,6-tetramethylpiperidin-4-yl)oxy)carbonyl)benzoic acid (3).
5.5 Conclusions

Photodissociation of TEMPO-Bz conjugated peptide ions were carried out in a modified linear ion trap mass spectrometer coupled to two laser systems. Despite modest yields of radical photoproducts, further MS" interrogation confirms the structural identity of the formed radical to be the same as that afforded by CID of the same precursor ion, yielding a similarly informative mass spectrum. Wavelength dependence of the photodissociative radical formation step by action spectroscopy reveals a maximum photoproduct yield around 230 nm, contingent on the charge state and peptide sequence.

The results from photodissociation of model systems suggests that the proximity of additional chromophores improves the efficiency of photodissociation and is consistent with prior observations in both polymers and peptides. Although the peptides investigated here incorporate aromatic residues, different peptide structures elicit only minor changes in the photodissociation profile. Based on the data in Figure 5.5, we speculate that an additional chromophore installed within the TEMPO-Bz derivative would increase the radical photoproduct yield when coupled to peptides, due to more efficient energy transfer into the NO–C bond. Extensive dissociation in such systems may even lead to secondary radical-driven fragmentation of the peptide within a single activation step, providing sufficient diagnostic information for peptide sequencing. If realised this scenario could remove the need for subsequent collisional activation and thus avoid the low-mass cut-off inherent in the use of ion traps for CID. These insights will therefore inform future design of more photolabile derivatives that may further enhance the utility of radical-directed dissociation for proteomics applications.
5.6 Acknowledgements

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APPENDIX B

SUPPORTING INFORMATION TO CHAPTER 5:

Photodissociation of TEMPO-modified peptides: New approaches to radical-directed dissociation of biomolecules

**Table S1**: Peptides investigated in this study, and the charge states for which ions arising from TEMPO loss are observed upon photodissociation by 266 nm wavelength photons.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>( m/z )</th>
<th>([M - \text{TEMPO}]^{n+/-})</th>
<th>([M + H]^+)</th>
<th>([M + 2H]^{2+})</th>
<th>([M - H]^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>RPPGFSPFR</td>
<td>1177.4</td>
<td>589.2</td>
<td>1175.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetensin</td>
<td>IARRHPYFL</td>
<td>n.d.*</td>
<td>645.3</td>
<td>1287.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YGGFMRF</td>
<td>YGGFMRF</td>
<td>994.1</td>
<td>497.2</td>
<td>992.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II DRVYIHPF</td>
<td>n.d.*</td>
<td>582.3</td>
<td>948.9</td>
<td>n.d.*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P† RPKPQQFFGLM-NH₂</td>
<td>n.d.*</td>
<td>733.1</td>
<td>948.9</td>
<td>n.d.*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d.: Product ion not detected upon PD\textsubscript{266}

* Precursor ion containing TEMPO not detected in sufficient abundance for isolation and photodissociation.

† An additional ion at \( m/z \) 948 is also observed in the full ESI mass spectrum, due to TEMPO-Bz coupling at lysine and the N-terminus. This doubly charged ion also exhibits TEMPO loss upon PD\textsubscript{266} (\( m/z \) 870).
Figure S1: (a) Photodissociation spectrum of modified bradykinin [M + 2H]^{2+} ions, irradiated with a single laser pulse of 266 nm wavelength photons. The spectrum has been normalised to the abundance of [M + 2H – TEMPO]^{2+} ions (cf. Figure 5.1b, main text), exhibiting similar features as the CID spectrum of the same precursor ion (Figure 5.1a, main text). (b) Photodissociation spectrum of unmodified bradykinin (RPPGFSFPR) [M + 2H]^{2+} ions, irradiated with a single laser pulse of 266 nm wavelength photons, exhibiting formation of b_n^+ and y_n^+ ions in minor abundance.

Figure S2: Collision-induced dissociation of [M + 3H]^{3+} ions of TEMPO-modified bradykinin (RPPGFSFPR), highlighting loss of selectivity at high charge states, due to protonation of the piperidinyl nitrogen of the TEMPO-Bz moiety.
Figure S3: Charge-state dependence of radical ion photoproduct yields upon PD$_{266}$ of the TEMPO-modified peptide YGGFMRF: (a) [M + H]$^+$; (b) [M – H]$^-$; (c) [M + 2H]$^{2+}$. The photoproduct and neutral loss are indicated with a horizontal arrow. All spectra were acquired with the same laser fluence.
Figure S4: MS\(^3\) spectrum obtained following CID of \([M + 2H – TEMPO]^{2+}\) radical ions of bradykinin, generated by CID of doubly charged TEMPO-modified bradykinin ions (cf. Figure 5.3(c), main text). Major peptide sequence ions and radical mediated side chain losses are assigned, with the identified amino acid in parentheses. The precursor ion is marked with an asterisk (*).

Figure S5: MS\(^3\) spectrum obtained following CID of \([M + 2H – TEMPO]^{2+}\) radical ions of kinetensin, generated by CID of doubly charged TEMPO-modified kinetensin ions (cf. Figure 5.3(d), main text). Major peptide sequence ions and radical mediated side chain losses are assigned, with the identified amino acid in parentheses. The precursor ion is marked with an asterisk (*).
Figure S6: Photodissociation action spectrum of TEMPO-Bz modified bradykinin (RPPGFSFPR) deconvolved into the $[\text{M} + 2\text{H} - \text{TEMPO}]^{2+}$ and $y_8^+$ product ion signals.
Figure S7: Representative PD mass spectra at varying wavelengths used to compile the PD action spectrum of TEMPO loss from bradykinin [M + 2H]^{2+} ions (Figure 5.4, main text): (a) $\lambda = 225$ nm; (b) $\lambda = 250$ nm; (c) $\lambda = 275$ nm; (d) $\lambda = 300$ nm. Each spectrum is the accumulation of at least 50 MS scans. Note the increasing magnification required to observe the photoproduct radical ion ($m/z$ 589). Raw ion abundances are corrected for power variations over the wavelength range before compiling the PD action spectrum.
Figure S8: Representative PD mass spectra at varying wavelengths used to compile the PD action spectrum of TEMPO loss from kinetensin [M + 2H]^{2+} ions (Figure 5.4, main text): (a) $\lambda = 232$ nm; (b) $\lambda = 250$ nm; (c) $\lambda = 275$ nm; (d) $\lambda = 300$ nm. Each spectrum is the accumulation of at least 50 MS scans. Note the increasing magnification required to observe the photoproduct radical ion ($m/z$ 645). Raw ion abundances are corrected for power variations over the wavelength range before compiling the PD action spectrum.
**Figure S9:** (a) MS³ spectrum obtained following CID of [M – H – 91]⁻ radical ions (1a), resulting from CID of (1); (b) MS³ spectrum obtained following CID of [M – H – 91]⁻ radical ions (1a), resulting from PD266 of (1); (c) CID spectrum of [M – H]⁻ anions of standard (1a).
6. OXIDATION OF TEMPO DERIVATIVES

REVEALS MODIFICATIONS AT THE 1- AND 4-

POSITIONS

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Supporting Information is attached as Appendix C.

**Author Statement**

David L. Marshall performed all research together with Meganne Christian and Philip J.
Barker, and wrote the manuscript with input from all authors. Electronic structure
calculations were carried out by Anya Gryn’ova from Australian National University.
This work would not have been possible without the intellectual input and support
provided by Michelle L. Coote, Philip J. Barker and Stephen J. Blanksby.

**Primary Supervisor Confirmation**

I, Prof. Stephen J. Blanksby (Primary Supervisor), support and certify the above author
statement.

signature 10/4/14
6.1 Abstract

Potential pathways for the deactivation of hindered amine light stabilisers (HALS) have been investigated by observing reactions of model compounds - based on 4-substituted derivatives of 2,2,6,6-tetramethylpiperidine-\(N\)-oxyl (TEMPO) - with hydroxyl radicals. In these reactions, dilute aqueous suspensions of photocatalytic nanoparticulate titanium dioxide were irradiated with UV light in the presence of water-soluble TEMPO derivatives. Electron spin resonance (ESR) and electrospray ionisation mass-spectrometry (ESI-MS) data were acquired to provide complementary structural elucidation of the odd- and even-electron products of these reactions and both techniques show evidence for the formation of 4-oxo-TEMPO (TEMPONE). TEMPONE formation from the 4-substituted TEMPO compounds is proposed to be initiated by hydrogen abstraction at the 4-position by hydroxyl radical. High-level \textit{ab initio} calculations reveal a thermodynamic preference for abstraction of this hydrogen but computed activation barriers indicate that, although viable, it is less favoured than hydrogen abstraction from elsewhere on the TEMPO scaffold. If a radical is formed at the 4-position however, calculations elucidate two reaction pathways leading to TEMPONE following combination with either a second hydroxyl radical or dioxygen. An alternate mechanism for conversion of TEMPOL to TEMPONE \textit{via} an alkoxyl radical intermediate is also considered and found to be competitive with the other pathways. ESI-MS analysis also shows an increased abundance of analogous 4-substituted piperidines during the course of irradiation, suggesting competitive modification at the 1-position to produce a secondary amine. This modification is confirmed by characteristic fragmentation patterns of the ionised piperidines obtained by tandem mass spectrometry. The conclusions describe how reaction at the 4-position could be responsible for the gradual depletion of HALS in pigmented surface coatings.
and secondly, that modification at nitrogen to form the corresponding secondary amine species may play a greater role in the stabilisation mechanisms of HALS than previously considered.
6.2 Introduction

Nitroxyl radicals based on 2,2,6,6-tetramethylpiperidinyl-N-oxyl (TEMPO) are highly stable, isolable species. The addition of TEMPO to alkyl and acyl radicals occurs below the diffusion-controlled limit, but nonetheless these trapping reactions proceed more readily than dimerisation or other self-reactions of TEMPO. TEMPO derivatives are therefore used as spin labels for supramolecular complexes, reversible inhibitors for nitroxide mediated polymerisation, and as superoxide dismutase mimetics for the protection of biomolecules against oxidative stress. Similarly, derivatives of TEMPO can act as radical scavenging, anti-oxidant stabilisers for polymers, improving durability and aesthetic properties throughout their service lifetime. By using a colourless precursor to generate the TEMPO-based aminoxyl in situ, hindered amine light stabilisers (HALS) are widely used to prolong the lifetime of automotive coatings, bulk polymers, and thin films.

The predominant mode of HALS action is believed to involve activation of the 2,2,6,6-tetramethylpiperidine moiety (TEMP-X, X = H, alkyl, or ether, Scheme 6.1) to the TEMPO free radical, which subsequently scavenges otherwise reactive polymeric alkyl or acyl radicals to form an amino-ether (TEMPO-R). Upon further reaction with a peroxyl radical, the aminoxyl radical is regenerated, converting harmful propagating radical species to less reactive even-electron by-products, as in Scheme 6.1.

Scheme 6.1: A generalised stabilisation mechanism for HALS.
The exact nature of oxidation and propagation processes described by Scheme 6.1 remain an area of active discussion. However, little is known about the fate of active HALS molecules in-service. Testing HALS molecules for efficacy in surface coatings involves empirically monitoring aesthetic properties in HALS-doped coatings to demonstrate an extension of product lifetime in a specific application, compared to a control coating. Such investigations demonstrate that the benefits afforded by HALS are finite, as shown by the eventual discolouring, fading, or degradation of the stabilised polymer, with the decline in physical properties mirrored by a concomitant decrease in the concentration of available active HALS within the polymer over time. Current understanding is limited as to why HALS do not stabilise polymers catalytically and indefinitely, as might be expected from Scheme 6.1. Characterisation of deactivation mechanisms could be crucial in selecting appropriate HALS for specific applications.

In principle, there are several general mechanisms by which HALS molecules could be deactivated or decomposed. HALS have been shown to migrate into lower coating layers where they can no longer act as efficient stabilisers. Deactivation driven by the inherent basicity of HALS could occur by chemisorption onto activated particle surfaces within the polymeric matrix, particularly in highly pigmented coatings, or by acid/base interactions with the catalysts of acid-curing surface coatings. Chemical or photochemical decomposition of HALS can lead to the formation of non-oxidisable adducts whereby regeneration of the active aminoxyl is inhibited. Moreover, the deactivation of HALS need not depend on the modification of the aminoxyl radical. Oxidative decomposition can cause HALS fragments to be volatilised from the coating during high temperature processing or in-service. Low molecular weight methoxy- and hydroxy-piperidines were detected when ester-linked oligomeric HALS in polypropylene were subjected to ultraviolet (UV)
Similarly, after prolonged ambient exposure of polyethylene films, 4-amino-2,2,6,6-tetramethylpiperidine was detected as a decomposition product of HALS possessing a secondary amine linkage at the 4-position (R^4). In order to complement such studies, simplified chemical systems are employed in mechanistic studies of antioxidant action to elucidate plausible reaction pathways. For example, simple derivatives of TEMPO have been employed as putative models for studying chemical reactions at the active sites of HALS. Further studies, undertaken from a medicinal perspective, reported that 4-hydroxy-TEMPO (TEMPOL) is susceptible to modification at the 4-position, leading to the formation of 4-oxo-TEMPO (TEMPONE), proposed to occur via the mechanism shown in Scheme 6.2. In many currently utilised HALS, the active TEMPO moiety is anchored to a larger molecular weight scaffold at R^4, and thus such an elimination could lead to deactivation of HALS via volatilisation of the low mass TEMPONE fragment.

Scheme 6.2: Hydroxyl radical induced formation of TEMPONE from TEMPOL.

In this study we have undertaken a detailed experimental and theoretical investigation of the reactions of TEMPO and selected 4-substituted TEMPO derivatives (1-5, Figure 6.1) with hydroxyl radicals (HO•), with an aim to identify pathways that might lead to deactivation of these compounds, and thus by extension deactivation of HALS in polymer coatings. Hydroxyl radicals were generated by irradiating dilute aqueous suspensions of AEROXIDE® P-25 nanoparticulate titanium dioxide, providing a milder approach than direct photolysis or radiolysis of hydrogen peroxide solutions. The conditions also allow a greater opportunity to observe the evolution of the reaction
(and potential reaction intermediates) by both electron spin resonance (ESR) spectroscopy and electrospray ionisation mass spectrometry (ESI-MS), in contrast to traditional end-product analysis. Moreover, this method is of direct relevance to the conditions encountered by HALS in situ, as titanium dioxide is a common inorganic pigment present in polymer coatings. Both pigmentary and nanoparticulate grades of titanium dioxide accelerate the degradation of polymer films, with little benefit afforded by HALS due to their photocatalytic destruction.\textsuperscript{414} Perhaps the most astonishing demonstration of the photocatalytic destruction of durable surface coatings containing HALS is that caused by certain commercial sunscreen formulations, in which the presence of photocatalytic grades of titanium dioxide were confirmed spectroscopically.\textsuperscript{415} While not quantitative in nature, this combined experimental and theoretical study identifies potential mechanisms of TEMPO degradation and deactivation that are not accounted for by current descriptions of their major reactivity pathways (cf. Scheme 6.1). The identification of thoroughly characterised mechanisms for irreversible chemical deactivation of TEMPO derivatives, even if minor compared to well-established aminoxyl radical chemistry, provide important new understanding of long term HALS deactivation over the service lifetime of surface coatings.

\textbf{Figure 6.1}: TEMPO derivatives used as HALS model compounds.

(1) 2,2,6,6-tetramethylpiperidine-N-oxyl ‘TEMPO’; (2) 4-hydroxy-TEMPO ‘TEMPOL’; (3) 4-methoxy-TEMPO; (4) 4-carboxy-TEMPO; (5) 4-oxo-TEMPO ‘TEMPONE’.
6.3 Experimental

6.3.1 Materials

TEMPO derivatives and the corresponding piperidine analogues were purchased from Sigma Aldrich (Castle Hill, Australia), stored at 4 °C, and used without further purification. AEROXIDE® P-25 TiO₂ was a gift from Evonik (Essen, Germany). Deionised water (reverse osmosis, median conductivity 3-4×10⁻⁶ S m⁻¹) was employed throughout. Solvents employed for mass spectrometry (acetonitrile and methanol) were HPLC grade (Ajax Finechem, Taren Point, Australia) and used as received. Solvents used for thin layer chromatographic (TLC) analysis (ethyl acetate, hexane and triethylamine) were reagent grade (Ajax Finechem, Australia) and also used as received.

6.3.2 Photochemistry

Photolysis experiments were carried out in a custom-built reactor, with a 1 kW Xe arc lamp (Oriel Corporation, Stratford, CT, USA) producing a collimated parallel beam of 4.8 cm diameter. The system was equipped with a 10 cm pathlength infrared filter (water), a green glass filter passing only wavelengths above 375 nm, and a neutral density attenuation filter passing 30% of the incident light. The filtered beam entered a quartz reactor, containing a stirring suspension of P-25 TiO₂ (125 mg) and the TEMPO derivative 1-5 (100 μM) in 200 cm³ water. The reactor was enclosed within a dark box, fitted with an extractor fan for temperature control, and openings for sampling and stirrer control. Samples (ca. 1.5 cm³) were taken at regular time intervals, centrifuged, and the supernatant transferred to a quartz ESR flat cell (active volume 0.15 cm³). After the ESR measurement, the solutions were reserved for mass spectrometric analysis. Control experiments were undertaken whereby TEMPO solutions were mixed with TiO₂ maintained in darkness, and irradiated under identical experimental conditions for 3 hours in the absence of TiO₂.
6.3 Experimental

6.3.3 ESR Spectroscopy

ESR data were obtained on a Bruker ESP300E spectrometer (Bruker GmbH, Rheinstetten, Germany) operating in the X-band of the microwave spectrum, equipped with an NMR gaussmeter and frequency counter. Instrument settings were as follows: microwave power, 2.0 mW; modulation frequency, 100 kHz; modulation amplitude, 0.2 mT. Measurements were conducted in a climate-controlled laboratory at 20.0 °C ± 0.5 °C. For the solution mixing experiments, 5 cm\(^3\) of a 100 μM TEMPOL solution was placed in a small vial. A similar solution of TEMPO was added dropwise, with ESR spectra recorded after the addition of every drop until the relative concentrations were in the range similar to those observed in the photolysis experiments. The composite spectra were scaled to a similar intensity to the experimental spectra.

6.3.4 Mass Spectrometry and Chromatography

Positive ion mass spectra were recorded with a Waters QuattroMicro (Waters, Manchester, U.K.) triple quadrupole mass spectrometer equipped with an ESI source and controlled by Micromass MassLynx software (version 4.1). The aqueous solutions were diluted to ca. 15 μM in acetonitrile, and infused directly into the ESI source at 10 μL min\(^{-1}\). The capillary voltage was set to 3.5 kV, cone voltage 25 V, source temperature 65 °C and desolvation temperature 100 °C. Nitrogen was used as the drying gas, at a flow rate of 320 L h\(^{-1}\). In all collision-induced dissociation (CID) scans, argon was used as the collision gas at a pressure of 3 mTorr, and the collision energy was 15–25 eV, depending on the fragility of the isolated ion.

TLC separation of analytes in irradiated samples was performed on 2.5 cm x 7.5 cm glass backed normal phase TLC plates (silica gel 60 F\(_{254}\), Merck, Darmstadt, Germany). The plate was developed in a mobile phase of 1:1 ethyl acetate : hexane containing 1% (w/w) triethylamine. A basic permanganate staining solution was
prepared by addition of KMnO$_4$ (1 g), K$_2$CO$_3$ (7 g), and NaOH (0.1 g) to water (100 cm$^3$). The developed plates were dipped into the permanganate solution and then completely dried using a heat gun.

6.3.5 **Computational Procedures**

Standard *ab initio* molecular orbital theory and density functional theory calculations were carried out using Gaussian 09$^{277}$ and MOLPRO 2009.1$^{416}$. Calculations were performed at a high level of theory, recently demonstrated to predict solution-phase bond dissociation energies and associated equilibrium constants to within chemical accuracy.$^{95}$ Geometries of all species were fully optimised at the B3-LYP/6-31G(d) level of theory. For all species, full systematic conformational searches (at a resolution of 120°) were carried out to ensure global, and not merely local, minima were located. Frequencies were also calculated at this level and scaled by recommended scale factors.$^{417}$ Improved energies for all species were calculated using the G3(MP2)-RAD level of theory, a high level composite *ab initio* method that approximates URCCSD(T) calculations with a large triple zeta basis set using additivity approximations.$^{418}$ This method was recently shown to reproduce a large test set of experimental gas-phase BDEs to within chemical accuracy.$^{19}$

Entropies and thermal corrections were calculated using standard formulae$^{283}$ for the statistical thermodynamics of an ideal gas under the harmonic oscillator approximation in conjunction with the optimised geometries and scaled frequencies, and reaction Gibbs free energies were computed using the Gibbs fundamental equation. Free energies of solvation were computed using the polarised continuum model, PCM-UAKS,$^{419}$ also at the B3-LYP/6-31G(d) level. Geometries of all species were re-optimised in solution. Free energies of each species in water solution at 298.15 K were calculated as the sum of the corresponding gas-phase free energy and the obtained free
energy of solvation. The phase change correction term $RT(\ln V)$ was included in the energy of all species. Gibbs free energies of activation for the reactions involving a hydrogen transfer have been corrected for the effects of tunnelling using the standard formulae: $\Delta G^\ddagger_{\text{corr}} = \Delta G^\ddagger - RT \ln(\kappa(T))$, where $\kappa(T)$ is the tunnelling correction factor, $T$ is the absolute temperature, $R$ is the universal gas constant and $\Delta G^\ddagger$ is the Gibbs free energy of activation.\textsuperscript{420}
6.4 Results and Discussion

6.4.1 ESR Characterisation of TEMPO Derivatives

Initially, the ESR spectra of TEMPO 1 and derivatives containing at least one hydrogen at the 4-position (2-4) are characterised by fairly broad ESR lines as a result of the unresolved couplings to the methyl hydrogens. In contrast, the spectrum of TEMPONE 5 shows much sharper lines with well resolved $^{13}$C-coupling, due to the restrictions placed on the piperidine ring conformation by the ketone double bond. Typical g-values and coupling constants are given in Table 6.1, and show good agreement with the available literature data.

Table 6.1: ESR properties of aminoxyl radicals 1-5 in water at 293 K.

<table>
<thead>
<tr>
<th></th>
<th>$R^4$</th>
<th>$g_e$</th>
<th>$a_{14}N$ / mT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>2.00563</td>
<td>1.73</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>2.00570</td>
<td>1.71</td>
</tr>
<tr>
<td>3</td>
<td>OCH$_3$</td>
<td>2.00568</td>
<td>1.70</td>
</tr>
<tr>
<td>4</td>
<td>COOH</td>
<td>2.00566</td>
<td>1.71</td>
</tr>
<tr>
<td>5</td>
<td>=O</td>
<td>2.00562</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Microwave power 2.0 mW, modulation frequency 100 kHz, amplitude 0.2 mT.

6.4.2 Reaction Monitoring by ESR

While there is some debate about the exact role of free HO$^+$ and surface charge carriers (photo-generated electrons and holes) in TiO$_2$ photocatalysis, this approach provides mild conditions in which to monitor TEMPO oxidation. The conditions employed here were modelled on previous ESR experiments in which HO$^+$ production was monitored by either spin-trapping, or aminoxyl radical decay. By using low concentrations of TiO$_2$ and the TEMPO derivatives (1-5), combined with a
controlled irradiation regime to investigate purely photocatalytic reactions, extended periods of irradiation (up to 6 hours) were possible without unwanted photochemical side reactions. Direct absorbance and subsequent destruction of the aminoxyl moiety by photons of wavelength below 230 nm was excluded by the use of a green glass filter, transmitting only photons of wavelength greater than 375 nm. Under our conditions, no changes were observed in the spectrum when a standard solution of TEMPOL was maintained with TiO$_2$ in darkness, nor when irradiated in the absence of TiO$_2$.

ESR spectra are presented as a function of irradiation time for TEMPOL in Figure 6.2. The initial spectrum is shown in Figure 6.2(a). As the irradiation proceeds, asymmetry is observed after 30 minutes, indicative of a mixture of aminoxyl radicals. Distinctive features of a second radical begin to emerge after 70 minutes, particularly on the $M_I = -1$ line, as in Figure 6.2(b). After 150 minutes, these features of the emerging radical become clear (Figure 6.2c). The experiment continues until after 360 minutes the new radical is the only species present in the spectrum (Figure 6.2d). Alongside Figure 6.2(b) and (c), Figure 6.2(e) and (f) are the spectra obtained by gradual addition of TEMPONE 5 to a solution of TEMPOL without irradiation. Figure 6.2(g) is a spectrum of pure TEMPONE. These pairs of spectra confirm the nature of the radical mixture observed during the experiment to be TEMPOL and TEMPONE.

This general pattern of results was repeated for the irradiation of both 4-methoxy-TEMPO 3 and 4-carboxy-TEMPO 4, with the observed formation of TEMPONE concurrent with decreasing intensity of the parent aminoxyl radical. No other paramagnetic product species were identified. When either TEMPO 1 or TEMPONE 5 are exposed to the same conditions and subjected to the same analysis, no changes to the coupling constants are observed, the signal merely decays over time until no aminoxyl radicals are detectable after 3 hours.
6.4 Results and Discussion

Figure 6.2: ESR spectra of TEMPOL as a function of irradiation time with TiO₂.

(a) No Irradiation; (b) 70 minutes; (c) 150 minutes; (d) 360 minutes; (e, f) dropwise addition of TEMPONE to TEMPOL; (g) standard TEMPONE 5.

The total peak to peak signal intensity (ΔM₁ = −1 line) attributable to each of the TEMPO derivatives 2-4 as a function of time is plotted in Figure 6.3, along with the formation of TEMPONE 5. For clarity, only the first 3 hours of irradiation are shown. In all cases, the initial aminoxyl radical concentration is depleted (open shapes) and an increase in abundance of 5 is observed (corresponding filled shapes). Data for the formation of 5 are not present prior to 100 minutes, as the narrow peaks are obscured by the broader peaks of 2-4. The rate at which the aminoxyl radical signal diminishes follows the trend of 4 > 3 > 2 and is thus clearly a function of the substituent in the 4-
position of the piperidine ring \((R^4 = \text{CO}_2\text{H} > \text{OCH}_3 > \text{OH})\). This is broadly consistent with trend in redox potentials of these aminoxyl radicals \((e.g., \text{corrected } E^\circ(4) = 0.924 \text{ V versus } E^\circ(2) = 0.877 \text{ V})\)\(^{46}\) and, in line with previous studies, suggests that oxidation to the diamagnetic oxoammonium cation may be a major reaction pathway.\(^{429}\)

---

**Figure 6.3:** Photocatalytic consumption of TEMPO derivatives with concurrent formation of TEMPONE as a function of irradiation time.

The rate and abundance of TEMPONE 5 formation is similarly dependent on \(R^4\), and does not form at all in the absence of a substituent in the 4-position, as demonstrated by the irradiation of TEMPO 1. In every case, the yield of 5 is less than would be expected if all of the reactant had been transformed to this product, indicating that this is a minor pathway in the overall reaction scheme, while the bulk of the products are diamagnetic (see above). From the relative signal intensities of standard solutions of TEMPOL 2 and TEMPONE it can be estimated that TEMPONE formation from TEMPOL accounts for \(ca. 12\%\) of the theoretical yield. Similarly, for 4-methoxy-TEMPO 3, the formation of 5 is only \(ca. 6\%\) of the theoretical yield, and for 4-carboxy-TEMPO 4 the yield is less than 1%. While TEMPONE formation in these instances is
6.4 Results and Discussion

clearly a minor product, its observation is significant because only the hydroxyl radical driven conversion of TEMPOL to TEMPONE has previously been reported. Indeed, previous studies of HO\(^{•}\) reactivity with 4 did not report TEMPONE formation. Overall the differing yields of TEMPONE from precursors 2-4 indicate that \(R^4\) has a pronounced effect on the propensity for formation of this product. This observation is not correlated to the relative trend for the disappearance of the reactant (see above), perhaps unsurprisingly given that the latter is predominantly driven by modification of the aminoxyl moiety.

6.4.3 Characterisation of TEMPO Derivatives by ESI-MS

ESI-MS analyses were undertaken to provide complementary detection of diamagnetic products arising from photocatalytic degradation of TEMPO derivatives 2-4. It should be noted that under these conditions ESI-MS is not quantitative and cannot be used in this manner to provide a comprehensive mass-balance for the reaction. This is due to (i) the differences in ionisation efficiencies among the different species present (e.g., Table 6.2) and (ii) the possibility that extensive oxidative fragmentation may result in low molecular weight products that can be volatilised from the reaction vessel. Despite these stated limitations, ESI-MS is a well-recognised method for reaction monitoring, including those employing photocatalytic TiO\(_2\), and has proven particularly effective at identifying reaction intermediates.

Prior to irradiation, the initial positive ion ESI mass spectra of the aminoxyl radicals display a distribution of ions around the molecular mass M. As redox processes can play a role in electrospray ionisation, the observation of multiple ions in the molecular ion region can be attributed to oxidation and reduction of the aminoxyl moiety under positive ion ESI conditions. The \(M^+\) oxoammonium cation, \([M + H]^+\) protonated aminoxyl radical cation, and \([M + H_2]^+\) protonated hydroxylamine cation are
observed in varying abundances, with a strong dependence on the analyte, as well as the solvent and source conditions.\textsuperscript{155,157} Typical abundances of these ions obtained from ESI-MS of 1-5 are provided in Table 6.2. Collision-induced dissociation (CID) of these ions gave rise to fragmentation patterns similar to those obtained for larger N-ether HALS analogues previously reported.\textsuperscript{263} For example, one prominent product ion observed in the CID of the [M + H\textsubscript{2}]\textsuperscript{+} ions is protonated acetone oxime [(CH\textsubscript{3})\textsubscript{2}C=NHOH]\textsuperscript{+}, comprised of two hindering methyl groups and the piperidinyl nitrogen at m/z 74. This is illustrated for TEMPOL 2 in Figure 6.4(a).

**Table 6.2:** Mass-to-charge ratio and relative intensities (uncorrected for isotopic contributions) obtained from ESI-MS analysis of 1-5 in 4:1 CH\textsubscript{3}CN:H\textsubscript{2}O.

<table>
<thead>
<tr>
<th>R\textsuperscript{\textdagger}</th>
<th>M\textsuperscript{+}</th>
<th>[M + H]\textsuperscript{2+}</th>
<th>[M + H\textsubscript{2}]\textsuperscript{+}</th>
<th>[M – 14]\textsuperscript{+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>156.3 (13.0)</td>
<td>157.3 (84.5)</td>
<td>158.3 (56.6)</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>172.3 (38.9)</td>
<td>173.3 (14.8)</td>
<td>174.3 (100.0)</td>
</tr>
<tr>
<td>3</td>
<td>OCH\textsubscript{3}</td>
<td>186.3 (11.3)</td>
<td>187.3 (17.8)</td>
<td>188.4 (100.0)</td>
</tr>
<tr>
<td>4</td>
<td>COOH</td>
<td>200.3 (20.7)</td>
<td>201.3 (11.6)</td>
<td>202.3 (100.0)</td>
</tr>
<tr>
<td>5</td>
<td>=O</td>
<td>170.3 (16.3)</td>
<td>171.3 (16.8)</td>
<td>172.3 (100.0)</td>
</tr>
</tbody>
</table>

From Table 6.2 it is seen that an abundant ion, 14 Da below the molecular mass, is also observed in positive ion ESI-MS spectra of 1-5. CID of the [M – 14]\textsuperscript{+} peak of TEMPOL 2 at m/z 158 is shown in Figure 6.4(b), with the product ion at m/z 74 corresponding to protonated acetone oxime noticeably absent. Rather, fragmentation to an equivalent propan-2-iminium ion [(CH\textsubscript{3})\textsubscript{2}C=N=NH\textsubscript{2}]\textsuperscript{+} is observed 16 Da lower at m/z 58 suggesting that the loss of 14 Da from the precursor aminoxyl radical results from reductive formation of [M + H\textsubscript{2} – O]\textsuperscript{+}, rather than the isobaric [M + H – CH\textsubscript{3}]\textsuperscript{2+} radical ion. Indeed, the CID spectrum shown in Figure 6.4(b) is identical to that obtained from
the \([\text{M} + \text{H}]^+\) ion of an authentic sample of 4-hydroxy-2,2,6,6-tetramethylpiperidine (denoted 2H, data not shown). The presence of these piperidine ions at \([\text{M} – 14]^+\) could be due to their presence in the samples themselves or they might arise during the ESI process. To assess these possibilities, the carboxylate functionality enabled 4-carboxy-TEMPO 4 to also be detected as an \([\text{M} – \text{H}]^-\) ion in negative ion ESI. In this case, where the charge is maintained remote from the aminoxyl moiety, \([\text{M} – \text{H}]^-\) is the only peak observed in the molecular ion region at \(m/z\) 199, i.e., no \([\text{M} – \text{H} – 14]^–\) was observed. Furthermore, the \([\text{M} – 14]^+\) peak can be eliminated from the positive ion spectra entirely by changing solvent system from a mixture of acetonitrile and water to acidified methanol. Moreover, when the analytes 1-5 are developed by thin-layer chromatography, only one spot is observed, indicating that the additional \([\text{M} – 14]^+\) peak is most likely a consequence of the modification of the aminoxyl moiety by positive ion ESI, rather than solution processes. ESI-MS and CID analyses of derivatives 3-5 exhibited similar results as described above for TEMPOL 2. However, neither \(m/z\) 58 nor 74 are observed in the CID spectrum of 1 or 1H, suggesting that this fragmentation process is dependent on \(R^4\).

**Figure 6.4:** CID mass spectra \((E_{\text{lab}} = 15 \text{ eV})\) of selected ions arising from ESI-MS analysis of TEMPOL: (a) \([\text{M} + \text{H}_2]^+\); (b) \([\text{M} + \text{H}_2 – \text{O}]^+\).
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6.4.4 ESI-MS Analysis of Photo-oxidation Products

Multiple studies have reported on the non-radical products arising from the reaction of TEMPO derivatives with HO• under different conditions. Oxidation of the aminoxyl moiety to the oxoammonium cation was reported as one product, while another study reported reduction of the aminoxyl to the hydroxylamine, where the aminoxyl was recovered upon addition of potassium ferricyanide.412,413 These species are indistinguishable from the aminoxyl radical in positive ion ESI-MS, as all three oxidation states of the aminoxyl are present in the spectrum at M⁺, [M + H]⁺, and [M + H₂]⁺ (Table 6.2). With this in mind, ESI-MS spectra were concurrently taken during the course of the irradiation to complement the ESR data and provide additional insight into the nature of the non-radical oxidation products of the TEMPO derivatives. Figure 6.5 shows a series of ESI spectra of TEMPOL 2 when irradiated in the presence of TiO₂ over the same time course as the ESR analysis (Figure 6.2). Consistent with ESR findings, no changes are observed in the spectrum when TEMPOL is irradiated with UV light in the absence of TiO₂, or when mixed with TiO₂ in darkness. At no stage are peaks attributable to dimers observed at multiples of the molecular mass.408,427

The initial series of ions in Figure 6.5(a) are consistent with those reported for TEMPOL 2 in Table 6.2. During (Figure 6.5b) and following (Figure 6.5c) irradiation, the M⁺ and [M + H₂]⁺ ions at m/z 172 and m/z 174 (open shapes) respectively, are diminished and predominantly supplanted by an ion at m/z 170, along with an ion at m/z 156 and an increase in the relative abundance of the ion at m/z 158 (filled shapes). The appearance of the ion at m/z 170 and its companion at m/z 156 would appear to be consistent with the formation of TEMPONE 5, corroborating the ESR observations. The CID spectrum of m/z 170 however, is not an exact match with that obtained from the equivalent ion derived from ESI of an authentic sample of 5 (data not shown).
Figure 6.5: ESI-MS spectra of TEMPOL (4:1 CH$_3$CN:H$_2$O) in the presence of TiO$_2$, highlighting TEMPONE and piperidine oxidation products. (a) Pre-irradiation; (b) during irradiation; (c) post-irradiation.

This likely indicates the presence of a second, isobaric, product perhaps arising from dehydrogenation of the piperidine ring. Consistent with the ESR data, a putative TEMPONE ion at $m/z$ 170 was also observed as a reaction product from 3 and 4 (Supplementary Information Figure S1 & S2, respectively). In the absence of isobaric ions in both these cases the CID spectrum matches that of authentic 5. Thus, on the basis of both ESR and ESI-MS observations, the formation of TEMPONE upon reaction with HO$^*$ is not limited to 2. Rather, TEMPONE was detected following irradiation of any 4-substituted analogue 2-4. In contrast, neither of the product ions at $m/z$ 170 and $m/z$ 156 are observed by ESI-MS during the irradiation of TEMPO 1, just as no ESR signal attributable to 5 is observed in these experiments. This supports the conclusion that the 4-substituent is involved in the reaction mechanism of TEMPONE formation.
The [M + 16]$^+$ ion, denoted by a solid cross at $m/z$ 188 in Figure 6.5(b), is observed during the oxidation of TEMPOL 2 and an analogous [M + 16]$^+$ species is also observed for the reactions of 3 and 4 (Supplementary Information Figures S1 and S2, respectively). These observations are consistent with a net increase of one oxygen atom of mass 16 Da. Multiple additions of oxygen at 16 Da intervals were not observed at any point. The CID spectra of the [M + 16]$^+$ peaks exhibit characteristic losses of water (-18 Da) and R$_4^-$H (i.e., H$_2$O, CH$_3$OH or HCO$_2$H for 2, 3 and 4, respectively), indicating the presence of a hydroxyl moiety possibly the result of hydrogen abstraction followed by HO$^-$ addition as previously proposed.$^{413}$ While the position of the hydroxyl group is not certain, it is consistent with the 4,4-dihydroxylated species outlined in Scheme 6.2 for 2, however analogous hydroxylation at other sites cannot be excluded. Indeed calculations of the barriers for hydrogen atom abstraction indicate a preference for initial radical formation and thus possible hydroxylation at the 3-position and perhaps even the methyl moieties (see below).

Although the [M – 14]$^+$ ion at $m/z$ 158 (Figure 6.5, filled diamonds) was shown to form during positive ion ESI-MS of TEMPOL 2, it is unexpectedly observed to increase in abundance upon irradiation in the presence of TiO$_2$ despite a concomitant decrease in other signature ions at $m/z$ 172 and 174. Furthermore, when the irradiated solutions are analysed by TLC, a second spot is observed with a retention factor equal to the corresponding 4-hydroxy-2,2,6,6-tetramethylpiperidine 2H, whereas only the aminoxyl spot is observed prior to irradiation. CID of the [M – 14]$^+$ ions yield the same spectrum as pre-irradiation, indicating that 2H remains present. This suggests that while the ion at $m/z$ 158 in Figure 6.5(a) arises exclusively from ionisation of TEMPOL 2, two different sources contribute to this ion abundance in Figure 6.5(b) and (c) and thus authentic 2H is present in the irradiated solutions as an oxidation product. The contribution of the
6.4 Results and Discussion

latter dominates in Figure 6.5(c) and is consistent with ESR data suggesting a decreased concentration of TEMPOL 2. Figure 6.5 also reveals an increase in abundance of an ion at \( m/z \) 156 upon oxidation of TEMPOL under these conditions with this ion dominating the post-irradiation spectrum at the expense of TEMPONE at \( m/z \) 170 (Figure 6.5c). The CID spectrum of \( m/z \) 156 that arises from the irradiation of TEMPOL 2 is shown in Figure 6.6(a) and is identical to standard 4-oxo-2,2,6,6-tetramethylpiperidine 5H (Figure 6.6b). The presence of the characteristic iminium product ion at \( m/z \) 58 in both spectra confirms the presence of the secondary amine functionality in this ion. The formation of 5H in these experiments appears to be a result of conversion of TEMPOL 2 to TEMPONE 5 with subsequent deoxygenation (as described below).

![Figure 6.6: CID mass spectra (\( E_{\text{lab}} = 15 \text{ eV} \)) of \( m/z \) 156 ions arising from ESI-MS analysis of; (a) authentic 4-oxo-2,2,6,6-tetramethylpiperidine, and (b) irradiation of TEMPOL in the presence of TiO\(_2\).

Increased abundances of equivalent \([M-14]^+\) ions are also observed during the irradiation of derivatives 3-5, but the trend is most obvious for TEMPO 1, because of the absence of the competing pathway for formation of TEMPONE 5. Reduction of TEMPO 1 to the corresponding secondary amine 1H is the predominant feature of the ESI spectrum, resulting in an abundant ion at \( m/z \) 142. Although \([M-14]^+\) ions are
initially present in the mass spectra as a consequence of the ESI process, this combination of data infer that the 2,2,6,6-tetramethylpiperidines 1H-5H are diamagnetic products arising from photo-oxidation of 1-5, and moreover, that 5H is observed at m/z 156 from each of 2-4. This deoxygenation reaction is consistent with previous observations of the reduction of cyclic 5-membered aminoxyl radicals to amines when irradiated in the presence of TiO₂, and the detection of hindered piperidines from active HALS in weathered polymer coatings.¹²¹,⁴³³

The formation of piperidines from N-oxyl piperidines 1-5 during photooxidation in the presence of TiO₂ could arise via one or all of the mechanisms outlined in Scheme 6.3. Combination of an alkyl radical (R') with the aminoxyl oxygen results in an intermediate amino ether (Scheme 6.3a). Although the formation of aminyl radicals is often considered unlikely, for particular R groups it has been shown that cleavage of the N–OR bond is thermodynamically favoured over NO–R bond cleavage, releasing an aminyl radical rather than an aminoxyl.⁹⁵ The formed aminyl radical may then abstract an available hydrogen to form the secondary piperidine. Given the expected low concentration of suitable alkyl radicals under our experimental conditions this pathway is unlikely to dominate. Alternatively, addition of a peroxyl radical to the aminoxyl results in the formation of aminyl and alkoxyl radicals and molecular oxygen (Scheme 6.3b), however the first step in this process is slightly endothermic (8.5 kJ mol⁻¹) and has a significant energy barrier (72 kJ mol⁻¹).⁹³ Similarly, addition of HO' to the aminoxyl oxygen, with subsequent cleavage of the N–OR bond can result in formation of the secondary amine and releasing the hydroperoxyl radical (Scheme 6.3c), however our unpublished calculations indicate that the decomposition step in this process is significantly endoergic (146.7 kJ mol⁻¹ at 298 K). Further pathways might also be possible via the intermediary oxoammonium cations formed by electron
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transfer, but the precise mechanism of these is currently unclear.\textsuperscript{434,435} Such pathways may be favoured in aqueous media due to the stabilisation of the charged species afforded by solvation.\textsuperscript{93} While several mechanistic possibilities are suggested in the literature, there is no definitive explanation for secondary amine formation.

![Scheme 6.3: Potential formation mechanisms of N-H piperidines from aminoxyl radicals by alkyl, peroxyl, or hydroxyl radical addition to TEMPO derivatives.](image)

**Scheme 6.3**: Potential formation mechanisms of N-H piperidines from aminoxyl radicals by alkyl, peroxyl, or hydroxyl radical addition to TEMPO derivatives.

### 6.4.5 Investigating the Effects of 4-position Substituent

The observed oxidative modification at the 4-position of 2-4 warranted further investigation. The influence of this substituent on the adjacent carbon-hydrogen (H\textsuperscript{4}) bond dissociation enthalpy (BDE) – a parameter related to the stability of the intermediate carbon-centred radical resulting from the initial hydrogen abstraction – was compared to the C-H BDEs of the methylene ring hydrogens (H\textsuperscript{3}) and the hindering methyl hydrogens (denoted CH\textsubscript{3}). Calculations were undertaken using the G3(MP2)-RAD approach at 298.15 K and are presented in Table 6.3. Consideration of reaction temperature and bulk solvent does not appear to have any significant effect on the observed trends (see Table S1 in Supplementary Information). The data in Table 6.3 demonstrate that for the 4-substituted TEMPO derivatives 2-4, the C-H\textsuperscript{4} BDE is
significantly lower than the C-H BDEs of either the 3-position or in the hindering methyl groups. Furthermore, the C-H$_4$ BDE in the substituted analogues 2-4 is significantly lower (by at least 16 kJ mol$^{-1}$) than that in the archetypal TEMPO 1 system indicating stabilisation of the carbon-centred radicals resulting from H$_4$-abstraction. In contrast, the BDEs of the hindering methyl group hydrogens are consistent with those of TEMPO and are not significantly influenced by variations in the remote R$_4$ substituent. Similarly, C-H$_3$ BDEs are constant, except in the case of TEMPONE 5 (ca. 35 kJ mol$^{-1}$ lower than 1-4) due to the stabilising effect afforded by the conjugation with the neighbouring C=O bond.

**Table 6.3:** Gas phase C-H BDEs for TEMPO species (G3(MP2)-RAD, 298 K).

<table>
<thead>
<tr>
<th>R$_4$</th>
<th>BDE / kJ mol$^{-1}$</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H$_4$</td>
<td>H$_3$</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>1 H</td>
<td>412.4</td>
<td>412.1</td>
<td>422.5</td>
</tr>
<tr>
<td>2 OH</td>
<td>392.1</td>
<td>414.1</td>
<td>427.5</td>
</tr>
<tr>
<td>3 OCH$_3$</td>
<td>396.2</td>
<td>414.9</td>
<td>430.4</td>
</tr>
<tr>
<td>4 COOH</td>
<td>378.0</td>
<td>415.5</td>
<td>427.7</td>
</tr>
<tr>
<td>5 =O</td>
<td>–</td>
<td>377.9</td>
<td>428.0</td>
</tr>
</tbody>
</table>

Considering hydroxyl (HO$^\cdot$) and hydroperoxyl radicals (HO$_2^\cdot$) are likely reactive species arising from aqueous TiO$_2$ photocatalysis, the relevant BDE(HO$^\cdot$H) = 493 kJ mol$^{-1}$ and BDE(HOO$^\cdot$H) = 366 kJ mol$^{-1}$ were calculated at the same level of theory and at 298.15 K. Both computed values are in agreement with literature values.$^{16}$ Comparison of these values with the data in Table 6.3 demonstrates that abstraction of hydrogens by HO$^\cdot$ will be exothermic from all positions (*i.e.*, H$_4$, H$_3$ or CH$_3$). By contrast, abstraction of all hydrogens in the experimental compounds by HO$_2^\cdot$ would be
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thermodynamically disfavoured, consistent with our recent study of HO$_2^\cdot$ abstractions in other organic compounds.$^{13}$ Although H$^4$ generally has a lower BDE than either H$^3$ or CH$_3$ in the TEMPO compounds studied, these thermodynamic differences alone do not provide an explanation for the reaction at the 4-position observed by ESR and ESI-MS analysis. Therefore, a study of the transition states of hydrogen abstraction by HO$^\cdot$ was undertaken, and the resulting gas-phase activation energies are displayed in Table 6.4. The corresponding solution-phase data follow the gas-phase trends and are provided in Table S2 of the Supplementary Information.

Table 6.4: Gas phase activation barriers (G3(MP2)-RAD, 298 K) for hydroxyl radical-induced hydrogen abstraction from 4-substituted TEMPO species.

<table>
<thead>
<tr>
<th>R$^4$</th>
<th>H$^4$</th>
<th>H$^3$</th>
<th>CH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>37.4</td>
<td>33.6</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>105.5$^a$</td>
<td>30.7</td>
</tr>
<tr>
<td>3</td>
<td>OCH$_3$</td>
<td>38.5</td>
<td>25.0</td>
</tr>
<tr>
<td>4</td>
<td>COOH</td>
<td>35.3</td>
<td>23.9</td>
</tr>
</tbody>
</table>

$^a$Approximate barrier only (see text below for further details).

The gas phase activation Gibbs free energies of CH$_3$ hydrogen abstractions from 1 and 2 were calculated to be ca. 25 kJ mol$^{-1}$, and are assumed to be approximately constant with changes to the remote R$^4$ substituent in both 3 and 4. H$^3$ abstraction barriers varied from ca. 24-30 kJ mol$^{-1}$ and were consistently lower for the substituted derivatives 2-4 than the corresponding activation energies for abstractions of H$^3$ from TEMPO 1 itself (ca. 34 kJ mol$^{-1}$). Conversely, the adjacent functional group (R$^4$) does not significantly lower the activation barrier of H$^4$ abstraction relative to TEMPO 1 with a barrier of ca. 35-38 kJ mol$^{-1}$ in all cases except 2. Whilst 2 appears to be a significant
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outlier, it should be noted that for this reaction, standard transition state optimisations for $H^4$ abstraction led to geometries in which the attacking $'OH$ instead interacts with the covalently bound 4-OH substituent. For this system, a series of constrained optimisations were performed. While the final ‘transition structure’ contains a single imaginary frequency consistent with $H^4$ abstraction, we expect that the resulting structure is not fully optimised and the reported barrier is thus likely to be significantly overestimated. Even so, in contrast to the thermochemistry (Table 6.3), calculation of the activation barriers of hydrogen abstraction (Table 6.4) suggests a clear kinetic preference for abstraction at $H^3$ and $CH_3$ over $H^4$. The increasing abstraction barrier heights in the order $CH_3 < H^3 < H^4$ can be attributed to polar inductive effects overriding the thermodynamic preference for the formation of $\alpha$-radicals and is consistent with previous computational and experimental observations of other systems.$^{436-438}$ Based on these calculations, it is plausible to conclude that the formation of TEMPONE 5 in the experiments described above does not arise from any preferential hydrogen abstraction at the 4-position. Indeed hydrogen abstraction at the 3-position and from the methyl groups is kinetically preferred, though given the generally low barriers abstractions at all positions are likely to take place to some extent. In order to rationalise TEMPONE formation, plausible reactions following formation of the carbon-centred radicals were considered in further detail. Although we do not exclude the possibility of $H^4$ abstraction from TEMPOL 2 based on the reported barrier, an alternate mechanism to TEMPONE formation unique to TEMPOL via an alkoxy radical is also considered below.

Under the conditions of the experiments described above hydroxyl radicals and dioxygen are both present with the latter estimated to be more concentrated by at least an order of magnitude.$^{427}$ Addition of hydroxyl radical to carbon-centred radicals arising
from H\textsuperscript{4}, H\textsuperscript{3} or CH\textsubscript{3} hydrogen abstraction from the 4-substituted TEMPO derivatives 2-4 would give rise to an even-electron alcohol. These isomeric species are all plausible intermediates and/or products of this reaction and could contribute to the observation of [M + 16]\textsuperscript{+} ions upon ESI-MS analysis of the reaction mixture (see above). Of these alcohols, only those arising from hydroxyl addition at the 4-position can reasonably give rise to formation of TEMPONE 5. Calculations for reactions arising from the addition of HO’ to an alkyl radical centred on the 4-position are summarised in Scheme 6.4(a). The corresponding solution-phase free energies follow the gas-phase results and are provided in Table S3 of the Supplementary Information.

These data indicate that the combination reaction of hydroxyl radical and HALS biradical is significantly exoergic, with free energies ranging from -316 kJ mol\textsuperscript{-1} for 4 to -364 kJ mol\textsuperscript{-1} for 2 relative to the nascent biradical in the gas phase. However, transition states for subsequent hydrolysis of the \(\alpha\)-hydroxy intermediates to form TEMPONE were located and represent significant kinetic barriers to this reaction, ranging from 121 kJ mol\textsuperscript{-1} for 3 to 282 kJ mol\textsuperscript{-1} for 4. While these barriers to direct hydrolysis mediated by a single water molecule may seem prohibitive, other pathways – especially those involving acid or base catalysis – may provide lower energy alternatives.

In contrast, addition of ground state molecular oxygen to the carbon centred radicals (Scheme 6.4b) was found to be mildly exoergic for 2 and 3 and negligibly exoergic for 4 due to the greater relative stability of the radical in the latter case (cf. Table 6.3). Significantly however, the barriers to unimolecular rearrangement of the peroxyl radicals to form TEMPONE were moderate in all cases, ranging from 56 kJ mol\textsuperscript{-1} for 2 to 82 kJ mol\textsuperscript{-1} for 3.
Scheme 6.4: Calculated gas-phase Gibbs free reaction energies and activation barriers (denoted by *) for TEMPONE formation from 4-substituted TEMPO species.

After formation of a biradical by H⁻⁴ abstraction, (a) the product from recombination with HO⁺ can rearrange to TEMPONE through a cyclic transition state with a water molecule to facilitate hydrogen transfer. (b) Alternatively, addition of O₂ to the biradical yields a peroxyl radical, which forms TEMPONE by intramolecular hydrogen transfer.

These calculations suggest that Scheme 6.4(b) provides a viable mechanism for the experimental observation of TEMPONE described above, consistent with previous proposals of carbonyl formation from α-hydroxy, α-methoxy, and α-carboxy peroxyl
6.4 Results and Discussion

It should be noted however, that O$_2$ addition to the intermediate biradicals is clearly reversible with the direct dissociation in the reverse direction entropically favoured over forward rearrangement to products. Therefore even with the greater abundance of O$_2$ compared to HO$^\cdot$ under the reaction conditions the irreversible trapping of the biradical as an alcohol is likely to be competitive as evidenced by ESI-MS observations of [M + 16]$^+$ ions.

For TEMPOL 2, an alternative pathway to TEMPONE 5, exclusive to this system, needs to be considered (Scheme 6.5). Abstraction of the hydrogen from the hydroxyl group itself would give rise to a nascent alkoxy radical that could then donate a hydrogen atom to dioxygen (or another acceptor) to form the carboxyl moiety directly. Calculated kinetic and thermodynamic parameters (see also Table S3) are in favour of this pathway – both steps are exoergic and the activation barrier of hydrogen abstraction is plausible under the experimental conditions. Thus, 5 is likely to form from 2 via an alkoxy radical rather than by thermodynamically reversible O$_2$ addition to an alkyl radical, a process which also involves a potentially prohibitive activation barrier to hydrogen abstraction. This alternate mechanism may also explain the higher yield for 5 arising from 2 compared to 3 or 4, as observed by ESR (Figure 6.3).

Scheme 6.5: Alternative pathway to TEMPONE via an alkoxy radical.

The alkoxy radical subsequently adds O$_2$ and eliminates HO$_2^\cdot$. Values correspond to gas-phase Gibbs free reaction energies (kJ mol$^{-1}$) and activation barriers (denoted by $^\ast$).
6.5 Conclusions

This work demonstrates the conversion of TEMPOL 2 to TEMPONE 5 from reaction with hydroxyl radicals generated by photocatalytic TiO$_2$ and further shows the broader applicability of this reaction to other 4-substituted TEMPO derivatives (3, 4). Several putative mechanisms for the formation of TEMPONE initiated by hydrogen abstraction at the 4-position have been investigated by high level \textit{ab initio} calculations and suggest both hydroxyl radical and dioxygen addition to the diradical intermediate may play a role in this conversion. A second TEMPONE formation mechanism unique to TEMPOL 2 \textit{via} an alkoxy radical was shown to be a plausible alternative to the peroxyl radical pathway. The quantitative loss of aminoxyl radicals observed by ESR during the oxidation (Figure 6.3) can be rationalised by ESI-MS observations of formation of a secondary amine at the 1-position. This is broadly consistent with previous estimates of the sites of reaction in TEMPO derivatives that suggest \textit{ca.} 40\% of reactive encounters result in reaction at the aminoxyl moiety with the remaining chemistry initiated by hydrogen atom abstraction at other sites.$^{435}$ It is further consistent with our own observations of the conversion of functionalised HALS to the corresponding secondary amine in polymer coatings$^{121}$ and detailed investigations of the mechanisms of this pathway and its consequences for the Denisov cycle (Scheme 6.1) are currently underway.

Experiments similar to those described herein with authentic HALS molecules are limited by their insolubility in aqueous media. It is not unreasonable however, to extend the current discussion to speculation of a plausible mechanism for HALS consumption in pigmented surface coatings, where TiO$_2$ is ubiquitously employed. Active HALS are recorded at highest concentrations in the top 30 $\mu$m of automotive clearcoats, near the air to polymer interface.$^{443}$ Coincidentally, this is the region of
highest photocatalytic activity in TiO\textsubscript{2}-pigmented materials, given the necessity of moisture and oxygen to photocatalytic oxidation. It is plausible that a transient encounter between photocatalytically generated HO\textsuperscript{•} and a HALS molecule may lead to formation of TEMPONE by elimination of the HALS backbone (added for the very purpose of thermal stability). The active HALS moiety would subsequently be volatilised from the coating at service temperatures. Given that the elimination described herein is not the major pathway of HALS activity, and furthermore, that HO\textsuperscript{•} production is suppressed by TiO\textsubscript{2} surface modifications in durable pigments, the chances of these encounters would be low. However, over the entire lifetime of the polymer it may provide a significant, irreversible pathway for depletion of these otherwise demonstrably prophylactic additives.

6.6 Acknowledgements

We acknowledge the generous allocation of time on the National Facility of the National Computational Infrastructure in Canberra, Australia, which is supported by the Australian Commonwealth Government. S.J.B. and D.L.M. acknowledge funding from the Australian Research Council (ARC) and BlueScope Steel (LP0775032). S.J.B and M.L.C are funded through the ARC Centre of Excellence for Free Radical Chemistry and Biotechnology (CE0561607). D.L.M. is the holder of an Australian Postgraduate Award, and M.L.C acknowledges an ARC Future Fellowship.

Supplementary Information Available (as Appendix C)

ESI-MS spectra for the irradiation of 3 and 4, analogous to Figure 6.5. Normalised energy profiles for TEMPONE formation from each of 2-4 (see Scheme 6.4 and Scheme 6.5). Optimised geometries in the form of GAUSSIAN archive entries, corresponding total energies, thermal corrections, zero-point vibrational energies, entropies, free energies and free energies of solvation.
APPENDIX C

SUPPORTING INFORMATION TO CHAPTER 6:
Oxidation of 4-substituted TEMPO derivatives reveals modifications at the 1- and 4-positions

**Figure S1:** ESI-MS spectra of 4-methoxy-TEMPO 3 (in 4:1 CH₃CN:H₂O) at various stages of irradiation in the presence of TiO₂. Oxidation products TEMPONE 5, piperidine 3H, and the intermediate 4,4-didubstituted species are highlighted. (a) Pre-irradiation; (b) during irradiation; (c) post-irradiation.
Figure S2: ESI-MS spectra of 4-carboxy-TEMPO 4 (in 4:1 CH₃CN:H₂O) at various stages of irradiation in the presence of TiO₂. Oxidation products TEMPONE 5, piperidines 4H and 5H, and the intermediate 4,4-didubstituted species are highlighted. (a) Pre-irradiation; (b) during irradiation; (c) post-irradiation.

It is noted that not all TEMPONE related ions generated by ESI of pure TEMPONE (Table 6.2) are observed in these spectra. This is not surprising given that the relative abundance of the different oxidation states will be influenced by the solution composition, which is different in each instance. What is unequivocal however is that the CID spectra of m/z 170 arising from oxidation of 3 and 4 are identical to the same ion obtained from ESI of pure TEMPONE.
**Table S1**: Calculated thermodynamic parameters of C–H bond homolysis in selected HALS in gas phase and in water solution at 298.15 K and 333.15 K.

<table>
<thead>
<tr>
<th>Bond</th>
<th>( \Delta S_{gas}^{298.15} ) J mol(^{-1}) K(^{-1})</th>
<th>( \Delta H_{gas}^{298.15} ) kJ mol(^{-1})</th>
<th>( \Delta G_{gas}^{298.15} ) kJ mol(^{-1})</th>
<th>( \Delta G_{gas}^{333.15} ) kJ mol(^{-1})</th>
<th>( \Delta G_{solv}^{298.15} ) kJ mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1-H(^4))(^*)</td>
<td>124.236</td>
<td>412.443</td>
<td>375.402</td>
<td>376.230</td>
<td>393.076</td>
</tr>
<tr>
<td>(1-H(^3))(^*)</td>
<td>129.017</td>
<td>412.144</td>
<td>373.677</td>
<td>374.515</td>
<td>392.607</td>
</tr>
<tr>
<td>(1-CH(_2)H)(^*)</td>
<td>120.902</td>
<td>422.488</td>
<td>386.441</td>
<td>387.260</td>
<td>405.203</td>
</tr>
<tr>
<td>(2-H(^4))(^*)</td>
<td>120.843</td>
<td>392.117</td>
<td>356.087</td>
<td>356.772</td>
<td>375.226</td>
</tr>
<tr>
<td>(2-H(^3))(^*)</td>
<td>129.764</td>
<td>414.047</td>
<td>375.357</td>
<td>376.160</td>
<td>396.463</td>
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<tr>
<td>(2-CH(_2)H)(^*)</td>
<td>121.563</td>
<td>427.539</td>
<td>391.295</td>
<td>392.156</td>
<td>410.015</td>
</tr>
<tr>
<td>(3-H(^4))(^*)</td>
<td>117.854</td>
<td>396.176</td>
<td>361.038</td>
<td>361.726</td>
<td>381.767</td>
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<tr>
<td>(3-H(^3))(^*)</td>
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<td>(3-CH(_2)H)(^*)</td>
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<td>422.034</td>
<td>386.004</td>
<td>386.819</td>
<td>404.390</td>
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<td>(4-H(^4))(^*)</td>
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<td>378.009</td>
<td>343.506</td>
<td>344.204</td>
<td>365.448</td>
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<tr>
<td>(4-H(^3))(^*)</td>
<td>125.765</td>
<td>415.467</td>
<td>377.970</td>
<td>378.820</td>
<td>398.072</td>
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<tr>
<td>(4-CH(_2)H)(^*)</td>
<td>122.126</td>
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<td>391.325</td>
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<td>410.213</td>
</tr>
<tr>
<td>(5-H(^4))(^*)</td>
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<td>342.548</td>
<td>343.265</td>
<td>361.018</td>
</tr>
<tr>
<td>(5-CH(_2)H)(^*)</td>
<td>123.961</td>
<td>427.978</td>
<td>391.019</td>
<td>391.892</td>
<td>410.326</td>
</tr>
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</table>
### Table S2: Calculated kinetic and thermodynamic parameters of hydrogen abstraction by HO\(^{•}\) from 1-5 in gas phase and in water solution at 298.15 K.

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Reaction</th>
<th>(\Delta S_{gas}^\dagger) J mol(^{-1}) K(^{-1})</th>
<th>(\Delta H_{gas}^\dagger) kJ mol(^{-1})</th>
<th>(\Delta G_{gas}^\dagger) kJ mol(^{-1})</th>
<th>(\Delta G_{solv}^\dagger) kJ mol(^{-1})</th>
<th>(\Delta S_{gas}) J mol(^{-1}) K(^{-1})</th>
<th>(\Delta H_{gas}) kJ mol(^{-1})</th>
<th>(\Delta G_{gas}) kJ mol(^{-1})</th>
<th>(\Delta G_{solv}) kJ mol(^{-1})</th>
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<tbody>
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<td>1.4</td>
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<td>-131.894</td>
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<td>42.994</td>
<td>20.137</td>
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</tr>
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<td>-124.997</td>
<td>-2.560</td>
<td>33.597</td>
<td>41.938</td>
<td>24.917</td>
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<td>31.426</td>
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<td>2.4(^a)</td>
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<td>105.499</td>
<td>103.264</td>
<td>15.674</td>
<td>32.585</td>
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<td>-109.186</td>
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<td></td>
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<td>-87.121</td>
<td>-88.167</td>
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</table>
Appendix C

3.4

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{N}^* \\
\text{O}
\end{array}
\xrightarrow{\text{OH}}
\begin{array}{c}
\text{H}_2\text{CO} \\
\text{H} \\
\text{O}
\end{array}
\xrightarrow{\dagger}
\begin{array}{c}
\text{H}_2\text{CO} \\
\text{H} \\
\text{O}
\end{array}
\rightarrow
\begin{array}{c}
\text{N} \quad 	ext{O}^* \\
\text{O}
\end{array}
\]

-128.838 0.370 38.465 43.050 15.420 46.228 \textbf{-102.403} -103.826

3.3

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{N}^* \\
\text{O}
\end{array}
\xrightarrow{\text{OH}}
\begin{array}{c}
\text{OCH}_3 \\
\text{O}
\end{array}
\xrightarrow{\dagger}
\begin{array}{c}
\text{OCH}_3 \\
\text{O}
\end{array}
\rightarrow
\begin{array}{c}
\text{N} \quad 	ext{O}^* \\
\text{O}
\end{array}
\]


4.4

\[
\begin{array}{c}
\text{O}_2\text{C} \\
\text{N}^* \\
\text{O}
\end{array}
\xrightarrow{\text{OH}}
\begin{array}{c}
\text{HO}_2\text{C} \\
\text{H} \\
\text{O}
\end{array}
\xrightarrow{\dagger}
\begin{array}{c}
\text{HO}_2\text{C} \\
\text{H} \\
\text{O}
\end{array}
\rightarrow
\begin{array}{c}
\text{N} \quad 	ext{O}^* \\
\text{O}
\end{array}
\]

-140.871 -5.699 35.321 37.770 12.505 32.052 \textbf{-119.692} -119.902

4.3

\[
\begin{array}{c}
\text{O}_2\text{C} \\
\text{N}^* \\
\text{O}
\end{array}
\xrightarrow{\text{OH}}
\begin{array}{c}
\text{CO}_2\text{H} \\
\text{H} \\
\text{O}
\end{array}
\xrightarrow{\dagger}
\begin{array}{c}
\text{CO}_2\text{H} \\
\text{H} \\
\text{O}
\end{array}
\rightarrow
\begin{array}{c}
\text{N} \quad 	ext{O}^* \\
\text{O}
\end{array}
\]

-140.335 -13.947 \textbf{23.921} 27.457 21.639 69.716 -84.753 -86.803

5.3

\[
\begin{array}{c}
\text{O}_2\text{C} \\
\text{N}^* \\
\text{O}
\end{array}
\xrightarrow{\text{OH}}
\begin{array}{c}
\text{O}_2\text{C} \\
\text{H} \\
\text{O}
\end{array}
\xrightarrow{\dagger}
\begin{array}{c}
\text{O}_2\text{C} \\
\text{H} \\
\text{O}
\end{array}
\rightarrow
\begin{array}{c}
\text{N} \quad 	ext{O}^* \\
\text{O}
\end{array}
\]

-134.735 -7.196 27.364 32.037 14.121 17.604 -120.043 -123.725

\textsuperscript{a} For reaction 2.4, standard transition state optimisations for H\textsuperscript{4} abstraction lead to geometries in which the attacking \textsuperscript{*}OH instead interacts with the covalently bound 4-OH substituent. A series of constrained optimisations were performed. While the final “transition structure” contains a single imaginary frequency consistent with H\textsuperscript{3} abstraction, we expect that the resulting structure is not fully optimised and the reported barrier is thus likely to be significantly overestimated.
### Table S3: Calculated kinetic and thermodynamic parameters of reactions I–IIIb for selected HALS in gas phase and in water solution at 298.15 K.

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<thead>
<tr>
<th></th>
<th>$\Delta S_{\text{gas}} \uparrow$</th>
<th>$\Delta H_{\text{gas}} \uparrow$</th>
<th>$\Delta G_{\text{gas}} \uparrow$</th>
<th>$\Delta G_{\text{poly}} \uparrow$</th>
<th>$\Delta S_{\text{gas}} \downarrow$</th>
<th>$\Delta H_{\text{gas}} \downarrow$</th>
<th>$\Delta G_{\text{gas}} \downarrow$</th>
<th>$\Delta G_{\text{solv}} \downarrow$</th>
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<tbody>
<tr>
<td>TEMPOL 2</td>
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<td></td>
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<td>I</td>
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<td></td>
</tr>
<tr>
<td>IIa</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IIb</td>
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<td></td>
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</tr>
<tr>
<td>IIIb</td>
<td>1.312</td>
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<td>55.868</td>
<td>60.458</td>
<td>187.644</td>
<td>61.769</td>
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<td>Alternative pathway to TEMPONE 5 exclusive to TEMPOL 2 via alkoxy radical (Scheme 5)</td>
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<td>4-methoxy-TEMPO 3</td>
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<td>IIb</td>
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<tr>
<td>IIIb</td>
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<td>323.911</td>
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<td>-156.668</td>
<td>-166.315</td>
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<td>4-carboxy-TEMPO 4</td>
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<td>I</td>
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<tr>
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228
Figure S3: Normalised energy profiles for H\textsuperscript{+} abstraction and subsequent TEMPONE formation via addition of either O\textsubscript{2} or HO\textsuperscript{*} to (a) TEMPOL 2 (including alternative pathway via alkoxy biradical) (b) 4-methoxy-TEMPO 3 (c) 4-carboxy-TEMPO 4.
Table S4: Contributions to the gas and solution-phase free energies of species at 298.15 K.

<table>
<thead>
<tr>
<th>Species</th>
<th>( E_{\text{gas}} ) (Hartrees)</th>
<th>( T_{c} ) (Hartrees)</th>
<th>ZPVE (Hartrees)</th>
<th>( S_{\text{gas}} ) (J mol K(^{-1}))</th>
<th>( G_{\text{gas}} ) (Hartrees)</th>
<th>( \Delta G_{\text{sol}} ) (water) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
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<td>'H</td>
<td>-0.50012</td>
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<tr>
<td>(1-H(^+))(^*)</td>
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<td>0.01328</td>
<td>0.24368</td>
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<td>450.624</td>
<td>-482.25209</td>
<td>-1.76</td>
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<tr>
<td>(1-CH(_2)H(^*))</td>
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The origins of the mass spectra of organic compounds were, for many years, regarded by chemists as a curiosity at best. Exploring them seemed far removed from anything that might qualify as chemistry.

The things that happen to a molecule in the mass spectrometer are in fact chemistry, not voodoo.

7.1 N–O and O–C Bond Homolysis in Alkoxyamines

This thesis has examined the chemistry of aminoxyl radicals and related even-electron alkoxyamines in the gas phase and in solution, primarily using tandem mass spectrometry. In this final chapter the most significant findings from this work are summarised and potential implications are discussed for the formation and fate of aminoxyl radicals in polymer formation and anti-oxidant stabilisation processes. The broader implications of the discovery of hitherto unrecognised stabilisation mechanisms in distonic radical anions are also examined and the suitability of such species as models for neutral radicals is considered.

7.1 N–O and O–C Bond Homolysis in Alkoxyamines

Fragmentation of gas phase $N$-alkoxyamines ($R_1R_2NOR_3$) bearing a remote negative charge was undertaken by collision-induced dissociation tandem mass spectrometry (Chapter 2). This study revealed that homolysis of the NO–C bond was the preferred fragmentation pathway for most alkyl substituents $R_3$, due to the well-known stability of the formed aminoxyl radical. In a limited number of substrates however, N–OC bond cleavage was observed as the predominant fragmentation pathway; in cases where the NO–C bond is stabilised through an anomeric interaction with an adjacent heteroatom (e.g., $R_3 = \text{CH}_2\text{F}$). These results agree with prior computational predictions of the relative bond dissociation energies in the analogous suite of neutral alkoxyamines.\textsuperscript{95}

It has previously been suggested that N–OC homolysis may be responsible for the observation of secondary amines from alkoxyamines in degrading polymer coatings containing these stabilisers.\textsuperscript{121} The results shown herein suggest that N–OC homolysis is unlikely to be competitive with NO–C homolysis for the range of alkoxyamine $R_3$-substituents expected in most polymer systems when considering the temperature range typically experienced by these coatings during service or cure. This finding is consistent
with more recent calculations suggesting an alternate mechanism to explain the formation of aminyl radicals that does not involve N–OC homolysis.\textsuperscript{94}

In light of these findings, it seems unlikely that cleavage of the N–OC bond is a significant contributor to the well-documented time-dependent decrease in the efficacy of HALS in polymer coatings. Alternative chemical processes – such as those proposed in Chapter 6 for the reaction of aminoxyl radicals with reactive oxygen species – may explain the apparently inevitable deactivation or removal of aminoxyl-based HALS from the matrix. The products identified in this latter study (Chapter 6) indicate that chemical changes away from the aminoxyl moiety may alter physical properties of the stabiliser (\textit{e.g.}, volatility), and thus are just as significant as reactions of the aminoxyl radical itself. These processes must be considered when designing new stabilisers for optimal performance in applications where different service conditions may be experienced by the coating.

### 7.2 Remote Negative Charges Stabilise Aminoxyl Radicals

The kinetic method was applied to the determination of relative and absolute gas-phase acidities of carboxylic acids in the presence and absence of remote aminoxyl radicals (Chapter 3). The results revealed a systematic decrease in proton affinity of carboxylates in the vicinity of an aminoxyl radical (\textit{ca.} 20 kJ mol\textsuperscript{-1}); the corollary of this result being consistently lower NO–C BDEs in anionic alkoxyamines compared to analogous neutral scaffolds. The findings of this experimental study were consistent with computational predictions, \textit{viz.} NO–C BDEs in alkoxyamines are lowered in the presence of a negative charge due to stabilisation of the aminoxyl radical afforded by a remote anion.

Further kinetic method studies presented in Chapter 4 uncovered the scope of this stabilisation effect on aminoxyl radicals, which is detectable even when the charge...
and radical are separated by distances of \( ca. \) 8 Å. At a set distance, the effect of an anion on radical stability is both greater in magnitude and opposite in sign to the polar effects exerted by a positive charge. The implication of these results is that an ostensibly remote anion decreases the homolytic bond dissociation energy of the alkoxyamine functional group located elsewhere in the molecule. This is due to additional stabilisation imparted onto the forming aminoxyl radical by the anion beyond simple polar effects.

Our findings suggest that the stabilisation of radicals by remote anions may have implications in the condensed phase, particularly in non-polar environments. Manipulating alkoxyamine NO–C BDEs though deprotonation of a remote acid functionality opens the possibility of designing polymerisation mediators responsive to external stimuli other than temperature. Brémond and Marque have demonstrated protonation of a pyridine unit on the alkoxyamine scaffold results in a 20-fold increase in the rate of NO–C homolysis, due to a destabilising polar effect on the alkoxyamine by a positive charge (Scheme 7.1). Our results suggest that incorporating a Brønsted acid onto the aminoxyl scaffold would be even more sensitive. As a 20 kJ mol\(^{-1}\) difference in gas-phase acidity represents more than 1000-fold shift in the equilibrium constant, the result would be an even larger change in dissociation rate upon deprotonation and anion formation.

**Scheme 7.1:** NO–C bond homolysis in alkoxyamines prompted by protonation of a remote pyridine moiety.
7.3 Long-Range Interactions of Negative Charges and Radicals

In Chapter 4, the scope of this discovery was extended to include anions other than carboxylates, all of which qualitatively exhibited the same stabilisation effects on remote aminoxyl radicals. Complementary computational work, recently published by Gryn’ova and Coote, showed that the stabilisation of a radical by a remote anion is not unique to aminoxyl radicals, and is the result of a long-range electrostatic interaction between an anion and any polarisable, delocalised radical. Associated with this effect, they predicted a rearrangement of electronic structure illustrated in Figure 7.1, in which the unpaired electron does not inhabit the highest-occupied molecular orbital (HOMO).

Figure 7.1: Interchangeable traditional (A) and SOMO/HOMO-converted (B) orbital occupancy in neutral and deprotonated 4-carboxy-TEMPO.

Included in the computational survey of distonic radical anions were alkylperoxyl radicals, which are predicted to experience a similar shift in reactivity as aminoxyl radicals upon deprotonation at a remote site. Negatively-charged alkylperoxyl radicals (Scheme 7.2) have been prepared and studied in the gas-phase by Kirk et al.
7.3 Long-Range Interactions of Negative Charges and Radicals

In light of our current findings and the generality of Gryn’ova and Coote’s proposal, both of these peroxyl radicals would be stabilised, and subsequent unimolecular or bimolecular reactivity of the peroxyl radical may be perturbed with respect to their neutral counterparts. By analogy with the TEMPO systems investigated herein, SOMO-HOMO conversion may also be observed in these peroxyl scaffolds.

Scheme 7.2: Distonic alkylperoxyl radicals previously studied in the gas phase.

The magnitude of the stabilisation effect demonstrated here is forecast to vary for different radical types. Specifically, it is largely suppressed in localised alkyl radicals, such as the precursors shown in Scheme 7.2, as these are not readily polarisable. For some time, Kenttämaa and co-workers have employed distonic ions to model the reactivity of the transient neutral phenyl radical. In a critical comparison of the effect of positive and negative charge tags on aryl radical reactivity, these authors conclude that ions of either polarity are equally good models for phenyl radicals, as both experience a similar kinetic perturbation due to polar effects. This result is consistent with the findings presented herein, as additional stabilisation to the phenyl radical is negligible due to its localisation, and therefore is subject only to (approximately equal and opposite) polar effects. More generally, our results suggest that distonic negative ions as probes for neutral radical reactivity may be compromised by a systematic offset when the radical exhibits significant delocalisation.
7.3 Long-Range Interactions of Negative Charges and Radicals

Judicious selection of distonic ion scaffold is imperative if comparisons to the neutral system are sought. In delocalised radicals, a positive ion may more closely replicate the neutral system, by minimising the additional long-range electrostatic interaction. Therefore, it would be interesting to compare the bimolecular and unimolecular reactivity of the distonic peroxy radical anions shown in Scheme 7.2 with analogous peroxy radicals carrying a fixed positive charge tag. Investigations of the rate of biomolecular reactions between these positive and negatively charge peroxy radicals may make it possible to quantify the extent of stabilisation in the anionic systems. Moreover, testing the hypothesis of unforeseen molecular orbital rearrangement is an attractive goal, employing negative ion photoelectron spectroscopy to interrogate the electronic structure of distonic aminoxyl or peroxy radical anions is one candidate approach to achieving this objective.

We subsequently consider the impact of these findings on the reactivity of radical anions in the gas phase in the context of radical-directed dissociation techniques, such as those employed in Chapter 5 for structural elucidation of peptides. There is already indirect evidence from Oh and co-workers to suggest that radical ion formation is far more efficient upon collisional activation of anionic scaffolds compared to protonated species.\textsuperscript{259,261} We speculate that this may in part be due to the production of distonic anions where the radical has migrated to the $\alpha$-carbons along the peptide backbone. Gryn’ova and Coote\textsuperscript{282} predict that distonic anions featuring a radical in such captodative positions (Figure 7.2a) will experience additional stabilisation, depending on the proximity of the charge.
7.3 Long-Range Interactions of Negative Charges and Radicals

![Chemical structure](image)

**Figure 7.2:** Biologically relevant protein (a) and peroxidised lipid (b) distonic radical anion scaffolds that may experience long-range stabilisation and associated SOMO-HOMO conversion.

Finally, the generality of the current findings suggests there may be broader implications for synthetic and biological radical chemistry. For example, peroxidation of a protein or lipid (Figure 7.2b) will produce peroxyl radicals on these scaffolds. Based on our results, the presence of such a radical will increase the acidity of nearby residues. Nature may exploit this change in reactivity as a part of more complex reactions, such as enzymatic catalysis.
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