
7 Electron spin resonance

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1 Introduction

This may well be the last review on ESR of the second Millennium, and follows my last contribution to Annual Reports C, of three years ago.¹ Perhaps there should be an Electron Spin Resonance spectrometer, along with an NMR spectrometer, mass spectrometer and so forth, in the Millennium Dome at Greenwich, to signify the remarkable contribution to the understanding of Nature which has been provided by the technology of this concluding century.

Regarding ESR, specifically, the trends apparent when I compiled my last report¹ have continued, and lie in the areas of pulsed and high-frequency methods. It should be noted, however, that these activities are mainly ongoing in the non-UK parts of Europe, the United States and Japan. In the UK most workers remain entrenched in continuous wave X-band ESR techniques, which are of course still very powerful, and in finding applications of these methods, particularly to industrial problems, and in the biomedical field. It strikes me that ESR is increasingly becoming only one of a number of methods which are often used in concert to solve various structural problems, and perhaps will find its future role as a member of an analytical family, which includes NMR and other magnetic techniques, mass spectrometry, crystallographic methods, valence and core-ionisation techniques such as UPS, XAFS *etc.*

The National Centres are active: that in Cardiff for CW-ENDOR and in Manchester for multi-frequency CW-ESR; additionally via the Manchester route, is access to high-frequency ESR (I believe at 140 GHz) in the Physics Department at St Andrews University. Details of all these facilities may be found on the World-Wide-Web.

One suite of methods which are still undergoing rapid development concern the various forms of spectroscopy possible with polarised muons. I will discuss some of the advances (mainly in Chemistry, Biology and Environmental Science, since most of the results of this work are firmly to be found in Physics journals) later, but it is gratifying to note that the UK is making a very direct input into this, through the ISIS facility at the Rutherford Appleton Laboratory, near Oxford; the other major European centre where muons are available is the Paul Scherrer Institute, in Switzerland. I have just written a short and general article on the application of this approach to Chemistry,

which has appeared in *Chemistry in Britain*.²

Changes to the ESR discussion group of the Royal Society of Chemistry are that the tenures of the Secretary (Dr Chris Rowlands, Cardiff), and the Chairman (myself: Professor Chris Rhodes, Liverpool) have concluded; they are replaced, respectively, by Dr Damien Murphy (also Cardiff) and Dr David Lowe (John Innes Centre, Norwich).

Annual ESR conferences of the ESR group have been held in Edinburgh (1996), Lancaster (1997) and Manchester (1998), and the next (1999) will be held at the University of York.

I find that the subject matter largely sifts itself into broad areas of 'organic', 'inorganic', 'theoretical', 'instrumental' and 'biology and medicine', but there is increasingly so much overlap that my choices might sometimes appear arbitrary, and I have made them to try to capture the spirit, *i.e.* the main focus, of each article, which, for example, while revealing some hitherto unknown and important feature of the system being investigated, might demonstrate a novel technique that can later be used to solve other problems.

So, on with the review, which is essentially of the years 1996/1997 and of the earlier part of 1998. As usual, given the sustained high level of activity in the broad area encompassed by the label 'ESR' (or EPR) there are many papers omitted, as within the space available to me I have tried, for the benefit of the non-specialist, to illustrate this range—to show what ESR can do for *you*, be you a chemist, biochemist, materials or environmental (or any other kind of) scientist.

2 Instrumental and theoretical aspects

Techniques

Efforts continue in the field of ESR imaging, and I open with a consideration of a whole-body ESR imaging spectrometer, by Symons.³ He describes a CW ESR spectrometer which can record 3D images, operating at 250 MHz; its potential in whole-body imaging is discussed in terms of radicals of particular biological interest and of narrow-line transition metal complexes. This seems to illustrate the considerable problems that I alluded to in my last report,¹ namely regarding sensitivity, which have yet to be overcome, and it is concluded that, at present, it is necessary to use added radicals having the narrowest possible linewidths and a single-peak ESR spectrum, and that working in a pulse mode may prove to be essential for useful whole-body imaging. The possible added radicals include suitably substituted triarylmethyls with non-magnetic groups at the principal spin-bearing atoms, but the majority used are nitroxides.

There is another very exciting report which claims to be of the *in vivo* observation of bioradical metabolites in living animals—namely mice. What this is actually about is the detection of a stable 'nitroxide' in the buttocks or stomach region, following injection of nitrosobenzene into the animal. From these observations, and those from a model system containing nitrosobenzene and unsaturated fatty acids, it is concluded that one of the first possible targets for nitroso compounds *in vivo* may be regions of polyunsaturated fatty acid clusters in fat or in membranes. It is suggested that since such bioradicals may be detected, as generated from pollutants (or, I suppose,

xenobiotics, to use the term of toxicologists) *in vivo*, it may be possible to study their metabolism by 2D or 3D imaging. As a general method, however, this does rest upon the 'bioradical' metabolite being really quite stable.⁴

A radiofrequency ESR spectrometer has been described, which is modified from a Surrey Medical Imaging Systems NMR imaging console.⁵ This uses a PC with associated waveform controller cards to perform CW ESR; digital phase-sensitive detection is provided by a vector processor. The target-field designed magnet and gradients are driven by audio amplifiers, and the dual-channel broad-band RF bridge has a reflection homodyne configuration. Birdcage coils have been built to resonate near 200 MHz; importantly, spectra and also images of lossy samples have been acquired. The Dartmouth group⁶ have reported a new surface probe for low frequency *in vivo* EPR studies which was designed around a dielectric resonator that operates at 1.2 GHz. The resonator was surrounded by a silver coated brass shielding, which blocked the penetration of the field modulation, thus preventing a contribution from any intrinsic ESR signal from the dielectric material. A Q -factor of 3600 was obtained before loading, which is significantly higher than those Q -factors of *ca.* 1000 obtained with other kinds of surface probe. When used on animals or other biological samples, the factor drops to around 330, but this is still double that for other surface probes. It is proposed that the device would be especially useful for *in vivo* studies in which the animal has an irregular surface at the site of measurement and/or there is a significant air gap between the probe and the animal.

A Japanese group has shown that *in vivo* ESR-CT (ESR—computed tomography) may be done successfully on the brains of live rats using the spin probe, 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl, which can permeate the blood-brain barrier; this was introduced to the animal *via* an intraperitoneal injection. Using a rapid-scan ESR-CT system operating at 700 MHz, it was found that different regions of the brain showed differences in the rate of decay of the radicals, and by repeated injection of the probe, clear images of the brain were obtained.⁷

Two papers have appeared dealing with manipulative approaches to practically difficult ESR measurements. One,⁸ dealing with the fast sampling of biological tissues, involves using a tube-like stainless steel cutter, with which a cylindrical piece of tissue is dissected free; while holding the sample and cutter on a silicone rubber sample cutting base, the sample is transferred directly into the ESR tube, by pushing the tube into the device. The main features of this technique are its simplicity, rapidity, elimination of the necessity to manipulate frozen tissue, precise information on the sample volume, and the avoidance of the use of foreign compounds, such as spin traps. The method has successfully been used in the detection of radicals in rabbit spinal cord under conditions of ischemia, and can be readily modified for the manipulation of samples under an inert atmosphere. The second,⁹ reports a method for the measurement of translational diffusion constants in a variety of media, particularly 'soft solids' such as dough and other foodstuffs. Following preliminary work, it was found possible to improve upon the accuracy of the technique in two ways; both requiring an experimental measurement of the sampling profile of the resonant cavity. In the first procedure, the cavity profile is convolved with a trial Gaussian distribution and the result is compared with the experimental data obtained from the ESR measurements; the diffusion constant is then determined by graphical optimisation. The second method is a graphical one based on a knowledge of the experimental diffusion profile

and the cavity sampling profile. It is found that both methods give similar results, but the second one is generally simpler to apply.

For an aqueous medium, several days are required to acquire the necessary experimental data but this time can be shortened (or slower diffusion rates can be measured) by using a shielded resonant cavity.

Disc-shaped Whispering Gallery mode dielectric resonators were used to obtain ESR spectra of powder samples at 75 GHz. A mechanically and electronically controlled Gunn diode was used while the static magnetic field (B_0) was obtained by modifying a Bruker electromagnet.¹⁰ The samples were contained in polyethylene holders suitably designed so that the resonator could retain its original circular symmetry and the sample could remain as close as possible to the curved rim of the resonator. Q -Factors of the order of 10 000 were obtained at room temperature when a single crystal quartz was used for the disc resonator.

Pulsed methods

A new technique has been presented which uses a TRIPLE effect which is implicit in Mims pulsed ENDOR, and which allows the determination of the signs of hyperfine couplings with high sensitivity.¹¹ Since the effect is implicit in the Mims electron-spin-echo (ESE) ENDOR effect, it does not require using an additional radiofrequency field, as does normal TRIPLE, and thus yields the signs of hyperfine couplings with significantly higher sensitivity; in the presence of strong electron-spin-echo envelope modulation (ESEEM), the Mims ESE ENDOR technique provides complex intensity patterns that are immediately interpretable in terms of the relative hyperfine signs. The utility of the method is demonstrated by its application to revise the authors' original assignment of the solvent-derived OH_x ligand in the novel low-spin, nonheme iron enzyme, nitrile hydratase, from H_2O to OH^- . Schweiger¹² reports a technique which he terms 'inversion-recovery detected EPR', which is a field-swept ESR method, based on the detection of the first harmonic of the periodically oscillating transverse magnetisation. With this method, pure absorption spectra were obtained with signal amplitudes that depended on the strength of the probe field. The technique is particularly well suited to the measurement of broad lines and for separating overlapping spectra of paramagnetic species with different spin-lattice relaxation times.

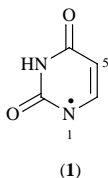
A practical route has been described for obtaining two-dimensional electron double-resonance spectra of radicals in disordered solids.¹³ It involves a narrow-band pulse excitation during a magnetic field step, combined with echo detection after a mixing time. Simulated spectra, which take into account distributions of correlation times, show the spectral features that can be determined with this technique. These simulations were improved over previous work by allowing for g -tensor fluctuations, which provide the main effect in determining the anisotropy of the electron spin-lattice relaxation. Data for nitroxide radicals in polycarbonate at 110 K are analysed and simulations show that this relaxation is caused by highly restricted librational motion with a distribution of correlation times having a mean of 0.1 μs and a width of -0.8 decades, in combination with a very narrow mode having a correlation time of 10 ps. Freed's group have studied slow reorientation motion of nitroxides in glassy media using 2D ESR.¹⁴ The contributions to the homogeneous relaxation time (T_2), from the overall and intramolecular motions are evaluated from the COSY spectra. In a 2D

ELDOR experiment, several mechanisms can lead to spectral diffusion, which leads to a broadening of the hyperfine auto-peaks with mixing time: these are termed 'spectral diffusion cross-peaks'. At higher temperatures, the principal mechanism for the formation of these cross-peaks is the slow molecular reorientation, which modulates the ^{14}N g - and hyperfine tensor components. A procedure is described for extracting a correlation time by monitoring the growth of the cross-peaks, which is in good accord with theory.

In an attempt to solve a practical problem, using time-resolved CIDEP along with conventional ESR and optical studies, an investigation was undertaken of the initial photochemical reactions of selected lignin model compounds. The resulting transient radicals may be regarded as models for intermediates in the photoyellowing of lignin containing mechanical pulps and papers.¹⁵ The theoretical background and experimental approaches pertinent to investigations using PELDOR (pulsed electron double resonance) have been reviewed.¹⁶ In PELDOR, the ESR spectrum is excited by two ESE pulses at one frequency, with an additional pumping pulse at a second frequency. It is the decay function $V(T)$ of the ESE signal, when the time interval between the first ESE pulse and the pumping pulse is varied which contains the information on the electron-electron dipolar couplings in the spin system. The kinetics of $V(T)$ decay depend strongly on distance, mutual orientation inside interacting spin pairs and on the spatial distribution of radicals throughout the sample. The distances between spins which were deduced by PELDOR varied between 15 and 130 Å, so this technique provides a powerful complement to conventional ESE in studying molecular distribution and diffusion processes.

In another study, the secondary quenching reactions of the 2-hydroxypropan-2-yl radical, produced by photolysis of pyrazine, quinoxaline and *p*-benzoquinone in 2-propanol, were investigated using time-resolved ESR. The time profiles of the transient ESR signals of pyrazinyl and 2-hydroxypropan-2-yl radicals were measured by FT-ESR and analysed in detail in terms of a reaction scheme involving the primary hydrogen abstraction reaction followed by the quenching reaction. It was found that half of the signal intensity of the pyrazinyl radical arises from polarization transfer accompanying 2-hydroxypropan-2-yl quenching by pyrazine.¹⁷ The kinetic isotope effect has been studied for the abstraction of the unique tertiary H atom in 2-propanol (and perdeuteriated 2-propanol) by hydrogen and deuterium atoms, using a pulse-radiolysis/ FT-ESR method.¹⁸ The rate constants were determined from the effective spin-spin relaxation times for the free induction decay of hydrogen or deuterium atom lines, by varying the 2-propanol concentration. It was found that the isotope effect favoured abstraction of the lighter isotope for both H and D atoms as the abstracting species. Also from the Leipzig group come FT-ESR studies of the photoreduction of 9,10-anthraquinone-1,5-disulfonate, using amines as the reductant.^{19,20} With 2,2,6,6-tetramethylpiperidine,¹⁹ studied on both nanosecond and microsecond timescales (using time-resolved optical and ESR measurements) it was concluded that the primary electron transfer from the amine to the photoexcited quinone triplet state generates solvent separated radical ion pairs which then diffuse apart. The radicals are spin polarised (CIDEP) by both the triplet and geminate radical pair mechanisms; because of steric hindrance, the recombination reaction is suppressed and, therefore, the spin dynamics are dominated by spin polarization, relaxation and spin exchange. With triethylamine,²⁰ the semiquinone radical anions and the triethylamine radical

cations could be detected at pH 11 in Coulomb-coupled radical pairs, in addition to the separated semiquinone radical anions; at pH 14, the aminoalkyl radical could be detected as the decay product of the amine radical cation. From the same group is a report of pyrimidine radicals (from thymine, uracil and 6-methyluracil) also generated from electron transfer to the triplet anthraquinone-2,6-disulfonate.²¹ Again, the pyrimidine radical cations decay, by deprotonation at the N(1) position, to the neutral pyrimidine-1-yl radical. In all cases, the spin density is highest at the 1,5 positions of **1**, similar to the radical cations themselves, as determined from a previous matrix-isolation study.²²



Spin labelling

A good start to this section is a review entitled 'What do probe molecules monitor in lipid bilayers?'²³ The thread of this is that although probe molecules are widely used in studies of the molecular order and rotational dynamics of hydrocarbon chains in a lipid membrane, no explicit link has been established between the behaviour of the probe molecules and the lipid structure. In this evaluation, the authors use Monte Carlo dynamics methods to monitor the behaviour of both the probes and the lipid molecules. From the wealth of information provided by these dynamical simulations, the orientational distribution functions and time correlation functions, whose decay behaviour is determined by rotational motions, are evaluated. The simulations indicate that the presence of rigid probe molecules in the membrane structure does not perturb the order and dynamics of the lipid molecules themselves. The simulations reveal, furthermore, that the probe molecules are confined to the free-volume cavities in the bilayer. Within the boundaries of these cavities, the probes exhibit only fast rotational motions, while large angular excursions are only possible upon rotation of the entire cavities; the compound motion model can be considered a formal implementation of this concept. The model provides a consistent description of the calculated CW-ESR spectra and the time-resolved fluorescence anisotropy decays, in addition to the time correlation functions calculated from the Monte Carlo trajectories.

In other words, the use of probe molecules is fine for the study of membrane lipids, as is generally assumed, which is reassuring!

Back to ESR imaging, but concentrating on the probe rather than the instrumentation, I note a report of a novel spin probe which consists of a TEMPO moiety bonded to a dextran, which apparently has a lifetime around 30 times longer than that for corresponding simple six-membered piperidine based nitroxides: actually, a half-life of 30 min, *in vivo*, which is the longest reported so far.²⁴ It is interesting that the dextran-bound probe was not as stable as some other probes towards reduction by ascorbate; thus its stability is due to its slow absorption into the tissues where nitroxide probes are generally quenched by metabolism. An ESR image of the head domain of a

mouse was obtained only after an intravenous injection of the probe into the tail vein. Moving towards advances of theory, the dynamics of spin-labelled side-chain groups in macromolecules have been revealed by a novel spectral simulation method.²⁵ The approach is based on the fact that every ESR spectrum corresponds to the existing stationary stochastic process of angular reorientation of the nitroxide group, relative to the macromolecule to which it is attached. Slow isotropic motion may be described by Brownian diffusion, while the nature of the fast stochastic process of the spin label is unknown, and the only way to describe it is by comparing it with some other, well defined, model process.

Quantum chemistry

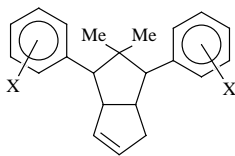
Reiger has calculated atomic hyperfine-coupling parameters for the transition metals. The dipolar parameters for transition metal nd and $(n + 1)p$ orbitals and isotropic hyperfine parameters for $(n + 1)s$ orbitals were computed from SCF–Hartree–Fock–Slater atomic orbitals. The parameters are found to depend strongly on electronic configuration (particularly on the number of d electrons) and empirical formulae are given which allow the calculation of the parameters, given an assumed configuration.²⁶

Onto the organic front comes more interesting work by Guerra.^{27,28} In the first paper are reported ^{13}C and ^{29}Si coupling constants obtained from theory which reproduce very well the experimental data in the permethylcyclotetrasilane radical anion. The radical is computed to have a π -type $^2\text{A}_{2u}$ electronic configuration, in contrast to the σ -type configuration determined from an ENDOR study, on the basis of the small ^{29}Si hyperfine anisotropy thereby measured. The components of the hyperfine tensor are computed to be small, since the unpaired electron is predominantly delocalised into the $\text{Si}(3p)$ outer-valence atomic orbitals; on this basis, the calculation gives 99.5 MHz^2 , to be compared with the limits set by the ENDOR study of $30\text{--}100\text{ MHz}^2$. I covered Guerra's theoretical studies of substituent effects in alkyl and silyl radicals last time,¹ which very convincingly demonstrate that the central atom isotropic hyperfine coupling cannot be taken alone to determine the geometry of the radical centre, as is often done, because there is an implicit electronic effect, whereby more electronegative substituents increase the coupling even where there is no change in geometry: *e.g.* for substituted methyl radicals, although the bond angles at the carbon centre were computed to be nearly the same, the ^{13}C coupling varies between 150 and 270 G for $(\text{RO})_3\text{C}^\bullet$ and CF_3^\bullet radicals. Guerra has now turned his attention to α -substituted vinyl radicals,²⁸ in which the effect of α -substituents on the structures, inversion barriers and ^{13}C α -atom isotropic couplings is seen; there are, unfortunately, almost no experimental data for the latter with which to make a comparison, but given the good agreement which Guerra's methods seem to achieve in the alkyl²⁹ and silyl³⁰ series, the values should be taken seriously. Somewhat surprisingly, the calculations predict that the degree of bending at the central atom decreases slightly with increasing electronegativity of the substituents, whereas the activation energy to inversion and the ^{13}C coupling both decrease markedly as the substituent becomes less electronegative; the linear structure appears favoured for the (trimethylsilyl)vinyl radical, in accord with spectroscopic evidence.^{31,32}

I could leave this until the section on 'Organic Radical Cations', and will mention it

again there, but regarding current activity in 'quantum chemistry', is the considerable application of the Density Functional Theory, which seems to work very well for the calculation of hyperfine couplings in organic radicals and radical cations. Through its agency, it has been established that the electronic ground state of the hexamethyl (Dewar) benzene radical cation is 2B_2 ,³³ in accord with my original assignment, yielding coupling constants in very good agreement with the experimental value.³⁴ Using a combination of density functional and *ab initio* methods, Nguyen *et al.* have examined the formation of the $\text{CH}_2\cdot\text{CH}_2\text{CH}=\text{NH}_2^+$ distonic radical cation from ionisation of cyclopropylamine and allylamine.³⁵ It is concluded that, on ionisation, cyclopropylamine undergoes a barrier-free ring-opening to the distonic species, while allylamine rearranges by a 1,2-H-shift; since the calculation indicates that $\text{MeCH}=\text{CHNH}_2^+$ should also be formed in the latter case, the lack of its observation experimentally is explained in terms of a matrix effect which stabilises, preferentially, the distonic form.

I conclude this discussion with two examples of quantum chemistry applied to biological problems. In one, semiempirical methods (AM1) have been used to provide detailed insight into the geometry and electronic structure of the tyrosyl radical, in regard to its importance in many electron transfer processes in biology, formed by oxidation of tyrosine. The geometry of the radical is found to differ considerably from the unoxidised amino-acid; anisotropic hyperfine coupling constants are calculated for comparison with ESR and ENDOR data for the radical.³⁶ Using density functional theory, the geometries, spin density distributions and hyperfine coupling constants in possible radical products from the radiolysis of guanine were calculated.³⁷ Notably, the calculated couplings for the guanine radical anion differ from those assigned experimentally; the conclusions, however, are not firm, and as is usual in such cases, it is suggested that 'further experimental and theoretical work is desirable'. It is, after all, a complicated but very worthy problem, especially given the enormous body of experimental work, to understand definitively the effect of radiation in DNA damage.

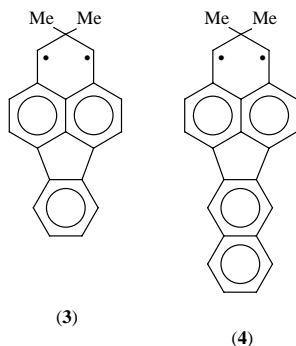


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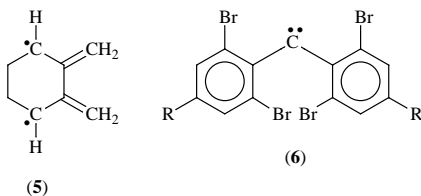
3 Triplet states and biradicals

The *D* parameters for the triplet 1,3-biradicals **2** ($X = 3,5\text{-OH}, 3,5\text{-NH}_2, m\text{-OH}, m\text{-CN}, p\text{-Cl}, p\text{-NO}_2$ etc.) are readily determined by ESR spectroscopy,³⁸ and reflect sensitively the electronic effects of the substituents on the α -spin density in the corresponding benzyl-type monoradicals. Since they are not subject to polar substituent effects, as are the α -proton hyperfine couplings in the free benzyl radicals,³⁹ the opportunity is offered to investigate strongly spin-delocalising substituents such as NO_2 and NR_2 or even charged groups like NH_3^+ and O^- . Indeed, the novel '*AD* scale' provides a

sensitive mechanistic probe of electronic effects in radicals through changes in the spin distribution which arise from spin delocalisation effects; the concept has been extended to heteroatomic π -systems, *i.e.* pyridines, furans and thiophenes. It is proposed that the elucidation of electronic effects in triplet biradicals could provide the necessary basis for the design of high-spin polyradicals for use as organic ferromagnets. Should be worth some research grants!



The biradicals **3** and **4** have been generated photochemically in rigid organic glasses, from two separate precursors. A singlet ground state was found for **3**, while **4** has a triplet ground state and represents the first example of a new class of high-spin molecules, being the first Kekulé hydrocarbon with a triplet ground state. The remarkable differences between **3** and **4** are the result of a different ordering of the lowest singlet and triplet states of the two.⁴⁰ Magnetic susceptibility and magnetization measurements were made on the biradical, 2,3-dimethylenecyclohexane-1,4-diyl **5**, which is a conformationally restricted tetramethylenecyclohexane; a theoretical curve for a model in which singlet and triplet states are in equilibrium was fitted to the data. In contrast with a previous interpretation of the ESR signal intensities for **5**, it is concluded that the singlet and triplet states are nearly degenerate ($2|J/k_b| < 1 \text{ K}$).^{41a}



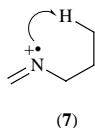
A series of 2,2',6,6'-tetrabromodiphenylcarbenes (**6**; R = Br, Me, *tert*-butyl) were investigated by ESR.^{41a} This study showed that the introduction of *tert*-butyl groups at the *para* positions resulted in a dramatic increase in the lifetime of **6**, to the extent that a triplet carbene with a lifetime of several minutes, in solution at room temperature, has been observed for the first time. Curiously, when R = Me there is a marked decrease in the lifetime; various factors are considered which might influence the reactivity of this highly sterically congested carbene, but I wonder whether intramolecular H-atom transfer from the Me groups provides an efficient mode of decay in the latter case.

4 Organic radicals

As last time, I have divided this section into organic radicals in solid and in liquid environments, in view of the different approaches involved in their study using ESR methods.¹

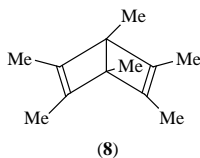
Organic radicals in solids

Radical cations. During the review period the 14th International Conference on Radical Ions was held in Uppsala in 1996; the proceedings of this are published in a special two volume issue of *Acta Chemica Scandinavica*. Not all the speakers, however, managed to write up their talk for this, and should either of the conference organisers read this article, I apologise to them for not finding the time to produce my contribution! I will cover, *inter alia*, selected papers from these proceedings. We did, however, publish this work, on imine radical cations, later, but in a different journal.⁴² This really follows a number of studies which we had made on radical cations of imines, $\text{Ph}_2\text{C}=\text{N}-\text{R}$ and $\text{PhCH}=\text{NR}$, formed by radiolysis in halocarbon matrices at low temperatures. In this study we were concerned with the propensity for imine radical cations, with alkyl substituents (R) of carbon chain length greater than three atoms, to rearrange to their distonic (spin-charge separated) isomers by intramolecular H-atom transfer from the carbon chain to the initially ionised N-atom. In all cases, mainly the spectrum of the distonic ion was observed, with little contribution from the intact primary structure: where there were γ -H-atoms present, rearrangement via the five-centre transition structure **7** was favoured; 1,2- and 1,3- H-atom shifts were not found, and when the γ -carbon was blocked by methyl groups, it was the 1,6- rather than the 1,4- shift which occurred.

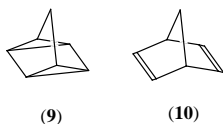


Studies of alkane radical cations continue to reveal novel features, for instance those of the 1,2,3-trimethylcyclohexane isomers, which were stabilised in various γ -irradiated halocarbon matrices at 4-77 K. At 4 K the ESR spectra are dominated by contributions from an asymmetrically distorted structure with the unpaired electron localised mainly in the C1-C2 bond. On increasing the temperature, a reversible change occurs in the lineshape of the spectra from the two symmetrical isomers. Using a two-site jump model to reproduce this behaviour, the effect can be rationalised in terms of an interconversion between two such distorted structures, each being the mirror image of the other. The Arrhenius plot associated with the process is clearly non-linear towards the low temperature region, and the experimental data were also modelled by postulating that another electronic state, with higher symmetry, becomes populated at higher temperatures. In this way, the spectral changes can be accounted for by a three-site jump model which couples the thermally activated two-site jump process ($E_a = 0.137 \text{ kcal mol}^{-1}$) with a dynamic equilibrium between the asymmetric ground state and a symmetrical structure $0.058 \text{ kcal mol}^{-1}$ higher in energy. The

energy barrier to pass from the distorted to the symmetrical structure was determined as $0.085 \text{ kcal mol}^{-1}$.⁴³ Recent work on alkane radical cations has been reviewed,⁴⁴ and it is found that for alkanes such as propane, norbornane, *n*-pentane (and some methyl substituted versions), which though not having degenerate HOMOs are distorted in a direction which is unique to each system, and is regardless of the matrix in which they are isolated; however, the extent of the distortion is matrix dependent.⁴⁴ The mechanism of the deformation was attributed to a pseudo-Jahn–Teller effect assisted by matrix interactions. The oft proposed twist-distortion of the ethene radical cation, which has only recently been shown conclusively,⁴⁵ was similarly explained.



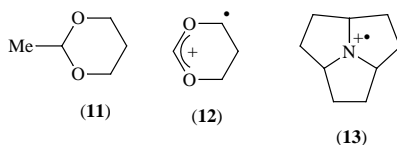
In the section on ‘Quantum Chemistry’ I mentioned the hexamethyl (Dewar) benzene **8** radical cation, which, nearly a decade after its initial investigation by ESR,³⁴ is apparently still of interest. Using the Density Functional Theory,³³ groups in Uppsala and Stockholm have undertaken a collaborative study of **8**⁺. The saga of experimental investigations of this species may be found in the references cited therein, but calculations at various levels demonstrate that the electronic ground state of **8**⁺ is ²B₂, with ²A₁ lying *ca.* $2.5 \text{ kcal mol}^{-1}$ higher in energy. Calculations on related systems are also reported, namely the pentamethylbenzene and pentamethylbenzyl trifluoroacetate radical cations, which have been proposed as alternative assignments to **8**⁺ from other ESR studies.^{1,33} Clark⁴⁶ has reported a combination of *ab initio* and density functional calculations which provide insight into the rearrangement of the quadricyclane **9** radical cation to the norbornadiene **10**⁺ structure. Various levels of interpretation regarding the principles governing free radical electrocyclic reactions are given, and are applied to this system.



In another study⁴⁷ it is shown that radical cations, generated either by photo-induced electron transfer in solution or by radiolytic oxidation in the channels of the zeolite NaZSM-5 may be deprotonated, giving rise to neutral radicals. It is concluded that deprotonations in solution are not efficient, because they occur in competition with intramolecular or other bimolecular reactions; deprotonations in the zeolite are more efficient, because of the insulation of the radical cation molecule from other influences.

Shida's group report a novel reaction of an acetal, 2-methyl-1,3-dioxacyclohexane **11**, whose radical cation undergoes an initial fragmentation which releases a methyl radical; however, this is then able to abstract a hydrogen atom from the resulting dioxacarbenium ion, giving the radical cation **12**.⁴⁸ In a study of the antioxidant

2,6-di-*tert*-butyl-4-methylphenol⁴⁹ the deprotonation of its radical cation was dem-

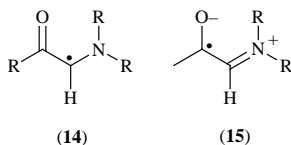


onstrated in a glassy matrix of freon-113 and 1-chlorobutane, using both ESR and optical methods. The azatriquinane radical cation **13** that was formed radiolytically is a $\text{CF}_2\text{ClCFCl}_2$ matrix at 77 K.⁵⁰ A prominent feature of its ESR spectrum is the ^{14}N hyperfine anisotropy, which broadens the ± 1 hyperfine components. The isotropic ^{14}N coupling of 25 G shows that the radical centre is pyramidal, and the large (40 G) couplings to the three methine protons show that the three methine C–H bonds eclipse the density axis of the nitrogen-centred half-filled orbital.

Studies relevant to biological systems. This section, probably not surprisingly, concerns studies of radiation damage to DNA. One report considers the role of superoxide in elevating iron levels, which in turn accelerate DNA damage.⁵¹ From the ESR spectra of whole cells, it is demonstrated that the level of free iron in superoxide-stressed cells greatly exceeds that of unstressed cells. Apparently, bacterial iron storage proteins were not the major source of free iron, since superoxide also increased the free iron levels in mutants lacking these storage proteins. It is striking that both growth defects and DNA damage induced by superoxide stem from its ability to damage a subset of iron–sulfur clusters. Two other papers consider the role of the hydration layer in radiation damage to DNA. Sevilla's group find that the induction of base damage products in γ -irradiated DNA, which was hydrated to between 2.5 and 32.8 molecules of water per molecule of nucleotide, and investigated by GC/MS depends on the degree of hydration. At low concentrations (< 13), the highest yields of products consistent with those radicals observed in low-temperature ESR studies were found. It is argued that conformational changes induced by hydration, and an increase in attack by radicals formed from the radiolysis of bulk water, are responsible for this dependence.⁵² In a spin-trapping study,⁵³ aqueous solutions of DNA were γ -irradiated at 77 K, then thawed. Three sets of experiments were carried out to confirm the generation of OH^\bullet radicals in the hydration layer of DNA and to decide whether these in fact induce strand-breaks and alterations to the base units. PBN–OH spin-adducts were found in DNA–PBN solutions, but not in aqueous PBN alone: this suggests that reactive free radicals were produced in the hydration layer of DNA and were efficiently scavenged by PBN, but that unreactive OH^\bullet radicals were produced in the free ice-phase. Taken with results from electrophoresis, it was concluded that OH^\bullet radicals generated in the hydration layer of DNA did not induce strand-breaks, but did modify the base units.

A very high-frequency (245 GHz) ESR study of the primary free radicals formed in randomly oriented DNA, and its nucleotides, has been made.⁵⁴ The great strength of this method is the resolution of overlapping signals from mixtures of organic radicals with only small differences in their g -factors, and which therefore cause interpretative ambiguity at X-band. The most clear-cut evidence was for the radical cation of

guanine, with both the cytosine and thymine radical anion being discernible; the guanine cation was found in all irradiated DNA samples, but its concentration was decreased in frozen aqueous solutions, while the contribution of the thymine anion was diminished by increasing hydration level. These workers consider that the results validate their proposal that the properties of hydrated DNA and DNA in frozen solutions are different, although the X-band spectra show almost no differences.

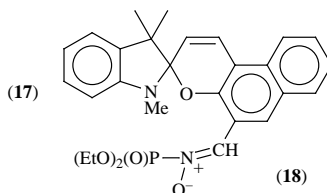
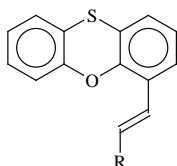
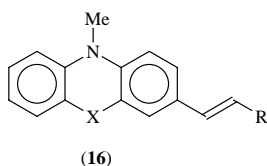


Radicals in liquids

The phenomenon of 'captodative stabilisation' is still being investigated, as in a study of α -amino- α -methyl radicals, $14 \leftrightarrow 15$, by ESR.⁵⁵ No coalescence temperature for the rotation of the two N-Me groups was found at temperatures below that at which the radicals decomposed, but from known coalescence temperatures and rotational barriers of substituted methyl radicals a rotational barrier of $>17 \text{ kcal mol}^{-1}$ was estimated for the C-N bond. From a thermochemical and correlation analysis of ESR hyperfine couplings with resonance stabilisation energies for these, and related substituted methyl radicals, it was determined that the captodative effect provided an additional stabilisation energy of $10.8 \text{ kcal mol}^{-1}$.

Eberson and Persson have determined a practical scale for measuring the acidity of media in which radical cations might be generated for ESR studies.⁵⁶ The basis of this is that 1,4,5,8-tetramethylnaphthalene undergoes a rapid rearrangement, first to 1,3,5,8-tetramethylnaphthalene and then to other tetramethylnaphthalene isomers on treatment with Brönsted and/or Lewis acids, whereas its radical cation is stable. By measuring the rate constant for the initial rearrangement, a scale of Brönsted/Lewis acidity of certain solvent-oxidant combinations commonly used to generate radical cations in solution for ESR studies, can be established. The results show that the reactivity falls in the order $\text{AlCl}_3 \gg \text{CF}_3\text{SO}_3\text{H} > \text{H}_2\text{SO}_4 > \text{H}_3\text{O}^+ \gg \text{CF}_3\text{COOH} = \text{SbCl}_5$; SbCl_5 does not catalyse the rearrangement but instead acts as a chlorinating agent. Eberson has also shown the utility of 1,1,1,3,3,3-hexafluoropropan-2-ol as a solvent in which radical cations may be stabilised, and has used this as a medium in which to study the photolysis of quinones (benzoquinone, duroquinone, anthraquinone and tetrafluorobenzoquinone), containing 6% trifluoroacetic acid.⁵⁷ The ESR spectra were assigned to the radical cations of the corresponding hydroquinones, in the first three cases, but the protonated semiquinone in the case of tetrafluorobenzoquinone; these are formed via a one-electron process involving the excited quinone as the reducing agent. Also in hexafluoropropan-2-ol have been studied radical cations of dibenzofuran and some of its methyl derivatives, and of bidibenzofurans; for the dibenzofuran and its 2-deuteriated isotopomer, a matrix isolation study was carried out, since it proved too reactive to measure in solution. The chemistry and electronic states of these intermediates are discussed.⁵⁸

I include one paper on radical addition to fullerenes. It is found that in the addition of MeS^\cdot to C_{70} , which has an ellipsoidal surface, three out of a possible five regioisomeric adduct radicals were detected:⁵⁹ each is characterised by a 1:3:3:1 quartet pattern, arising from coupling of the unpaired electron with the three equivalent protons of the MeS^- group; in contrast, all five possible isomers were observed from addition of MeO^\cdot radicals, each being characterised by a single line since the splitting from the MeO^- protons is too small to be resolved. The initial flurry of papers dealing, more generally, with radical addition to fullerenes were reviewed in my last report.¹

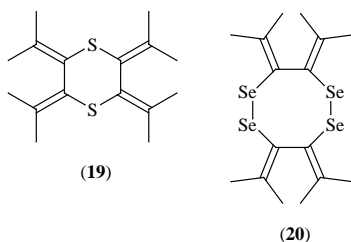


An ESR/ENDOR study has been made of some redox-active compounds, in which the donor and acceptor subunits are linked by a vinylenic group.⁶⁰ 10-Methylphenothiazinyl, 10-methylphenoxazinyl and phenoxathiinyl were used as electron-donor groups, whereas 9,10-anthraquinon-2-yl groups were employed as acceptors. The push-pull substituted stilbenes **16** ($\text{X} = \text{O}, \text{S}$; $\text{R} = \text{Ph}$, 2-anthraquinonyl) and **17** ($\text{R} = \text{Ph}$, 2-anthraquinonyl) were made by the Wittig method from the heterocyclic aldehyde and the anthraquinonyl or phenyl phosphonium salts. Electron attachment leads to configurationally stable radical anions, and is rationalised in terms of the electron distribution established by the ESR/ENDOR measurements. Another group report the synthesis of a photochromic spin-trap, with the structure **18**.⁶¹ The aim appears to be to have a spin-trap (radical scavenger) with a similar photochromic structure to materials in commercial use, which are also based around the spiro[indolinenaphthoxazine] unit, and which might retard the photochemical deterioration of these materials by scavenging the free radicals which are implicit in their photodegradation. The spin-trapping ability of **18** towards various types of radical was investigated.

There are two papers which illustrate that the detection of magnetic nuclei in low natural abundance can be extremely useful in structural investigations, although it is generally a difficult task, particularly for cases like ^{33}S , ^{17}O , ^{13}C , which require at least that the radicals are relatively quite stable. A good example is the radical anion of 2,6-dithioxobenzo[1,2-*d*:4,5-*d'*]bis[1,3]dithiole-4,8-dione, from which not only the primary satellites are detectable, but also the secondary ones, i.e. the satellites-of-the-satellites! Presumably due to modulation of the anisotropic hyperfine tensors of these nuclei, by Brownian motion, the satellite lines are found to show marked linewidth asymmetry among the multiplet components of each group, from which the signs of the isotropic hyperfine couplings can be determined.⁶² Another group have studied radical cations derived from a dithia[6]radialene **19** and a tetraselena[8]radialene **20**. From the magnitude of the ^{33}S coupling in $\mathbf{19}^{+\cdot}$ it is suggested that there is restricted delocalisation within the 2,3-dithiatetramethyl-butadiene unit, and the slow electron transfer from one part of the molecule to the other was ascribed to steric effects. For the

radical cation of **20**, it was shown that only two of the four selenium atoms are involved, in accord with a slow electron transfer rate between the two divinyldiselenide moieties.⁶³

Highly fluorinated radicals continue to provide some interest, as in an ESR/



ENDOR investigation of the reaction between perfluorohypofluorites and perfluoroalkenes, in which secondary and tertiary radicals are formed either by addition of a fluorine atom, or a perfluoroalkyl radical to the alkene double bond. Long range couplings, even from δ -¹⁹F nuclei.⁶⁴ A group in Moscow has reported the perfluoro-1,2-di-*tert*-butylvinyl radical, which is highly stable in inert media. It was formed by direct fluorination of perfluoro-di-*tert*-butylacetylene, or by addition of the perfluoro-*tert*-butyl radical to perfluoro-*tert*-butylacetylene in perfluorodiamyl ether.⁶⁵

I conclude with a problem that involves both solid and liquid state studies, namely the structure of imidazole radical cations. An ESR/ENDOR study has been made of the 4,5-diphenylimidazole radical cation, which is a highly delocalised radical with significant spin density on the phenyl groups, which are twisted with respect to the imidazole ring.⁶⁶ The authors claim that their data match more closely the reported ESR spectrum of the 1-methylimidazole radical cation in solution,⁶⁷ by which I presume that they mean that the couplings are all small, in contrast with a solid state study of this cation,⁶⁸ which appeared to show large, and anisotropic, ¹⁴N couplings; these average to isotropic couplings of *ca.* 10 G. They suggest that there may be a matrix effect which changes the imidazole ring structure, but I think the matter may be worth investigating further with some specifically deuteriated imidazoles.

Environmentally relevant studies

A spin-trap ESR method was developed and calibrated for the measurement of the rate constants of the hydrated electron with some important water pollutants, namely dichloromethane, trichloromethane, tetrachloromethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,2-dibromoethane, trichloroethylene, 1,2-dichlorobenzene, bromobenzene, 2,4-dichlorophenol, acetone, benzaldehyde and benzonitrile.⁶⁹ The purpose of the study is to obtain information of relevance to the photodegradation of such pollutants in aqueous media. Moving onto some work proposed to be relevant to the chemistry of the troposphere, are two studies^{70,71} from the Sussex–Bologna collaboration, which investigated the nature of radicals formed by hydrogen abstraction from VOCs (volatile organic compounds), as are released from natural sources (e.g. pine trees). Since the chemistry of the troposphere is dominated by reactions of OH[•]

radicals, these studies which use instead photochemically generated alkoxy radicals should reveal parallel radical products, arising from H-atom abstraction. Specifically, the first⁷⁰ deals with reactions of *tert*-butoxy radicals with terpenes: α -pinene, β -pinene, α -terpinene, car-2-ene, car-3-ene and limonene; in each case, the radical is, as expected, a substituted cyclohexenyl or cyclohexadienyl radical. In the second,⁷¹ a series of cycloalk-2-enones (ring = 5–7 members) was studied in which H-atom abstraction occurred preferentially from the 4-position, other than in cases where this site was blocked by methyl substitution, in which case abstraction from the alternative site α to the carbonyl group occurred.

This will be covered in the next section, on ‘Muon Spectroscopy’, but given its ‘Environmental’ context, I mention it now. My group has developed a method for studying the dynamics of radicals on surfaces, which uses positive muons as a radioactive label for them, developed initially to aid our researches on the behaviour of free radicals in zeolites,^{72–74} to determine the adsorption behaviour and dynamics of pollutant derived radicals on environmental particulate matter. The significance of this is that there is growing awareness that many atmospheric processes, including the degradation of pollutants, in fact occur on the surfaces of airborne particles, and in atmospheric aerosols generally. Additionally, at the ground level, small particles (e.g. PM10s, with a particle size less than 10 μm) can be toxic via inhalation, not only in their own right, but also because they can transport adsorbed toxins, e.g. to the deep lung where they are very harmful. So far, we have studied the adsorption and dynamics of the 1,1-dichloroethyl radical (as derived from the solvent 1,1,1-trichloroethane) on mineral particulates⁷⁵ and cyclohexadienyl (derived from benzene) on carbon particulates, since both the latter are significant products in vehicle emissions.⁷⁶

Muon spectroscopy

I have shortened this title rather, from ‘muon spin rotation spectroscopy’, since this is only one of the many techniques available using muons as magnetic probes of particular systems. Much information can be found in the proceedings of the International Conferences on Muon Spin (Relaxation, Rotation and Resonance), as is all encompassed by the label MuSR, the last being held in Japan in 1996;⁷⁷ the next will be hosted in Switzerland in 1999. The main advantages of muons are that they can be implanted into almost any sample to act as a probe: they are magnetic, being ‘spin-one-half’ particles, but they are also radioactive, and decay on a microsecond timescale which actually sets the timescale of the processes they can measure. In molecules, a positive muon takes the place of a proton and acts as a ‘tag’ for it, both in investigations of reaction kinetics of the radical, and as a ‘spin-probe’ in studies of its motional behaviour.

Workers in this area should probably stress this more, but the techniques using muons actually provide information equivalent to ENDOR and TRIPLE, since they are mostly resonance experiments but which do not require additional radiofrequency fields. So Transverse-Field MuSR is equivalent to ENDOR and yields the nucleus (muon) coupling directly, while that of Avoided Level Crossing (ALC) yields the magnitudes of the couplings from other nuclei, and also their relative signs (like TRIPLE does). Radiofrequency experiments are possible, however, and so the analogies with more conventional magnetic resonance become more obvious.

The response of the muon to motional effects allows it to be used as a (very-tiny!) spin-label with which to investigate intramolecular motion, and adsorption and diffusion processes on surfaces. This is because it has a mass of only one-ninth that of a (normal) proton, and it is sensitive to modulation of both isotropic and dipolar hyperfine interactions. Since the detection methods employed are those of single particle counting, these techniques are incredibly sensitive.

In order to do such experiments, it is necessary to obtain access to one of the muon facilities around the world, particularly the Paul Scherrer Institute (PSI; Switzerland), the ISIS facility at the Rutherford Appleton Laboratory (RAL; UK); TRIUMF (Canada) and KEK (Japan); my group works mainly at PSI and ISIS.

There are very good reviews available about muon methods: the books on the subject by Walker⁷⁸ and by Roduner,⁷⁹ along with articles on applications of ALC in chemistry generally,⁸⁰ and specifically in studying radicals on surfaces;⁸¹ I have recently written a short article intended for a general Chemical or Biochemical audience.²

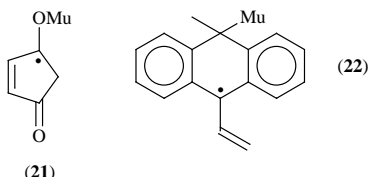
Given this introduction, I now survey some of the more recent developments which have taken place, predominantly in applications to chemistry, and I apologise for having to be highly selective in this. One approach which is showing great promise to chemistry is Longitudinal Field Muon Spin Relaxation (LF-MuSRx), in which, as the title suggests, the relaxation of muon spins in labelled radicals, maintained in an external magnetic field applied longitudinal to the direction of the muon beam, is monitored as a function of temperature. The basis of this method is that when a molecular motional frequency approaches that of the dominant spectral transition in the muon-electron coupled system, there is an increase in the muon spin-relaxation rate λ which reaches a maximum when the frequencies are equal.^{82,83} Since $\lambda = 1/T_1$, this maximum corresponds to a T_1 minimum, familiar in NMR and ESR spectroscopy, where a transition frequency is matched by that of some motional process and the relaxation mechanism finds its maximal efficiency. The first clear success in molecular studies was provided by Jayasooria *et al.*, who have determined the barriers to rotation of the phenyl groups in the series of compounds Ph_4X , where $\text{X} = \text{C}, \text{Si}, \text{Ge}, \text{Pb}$, which are found to decrease as the C-X bond lengthens;⁸⁴ the method has also been successful in determining activation parameters for the ring-rotation in ferrocene and related compounds,⁸⁵ these being in good agreement with those obtained from NMR and neutron scattering measurements.

One study of the motional dynamics in solid C_{60} (labelled as the radical Mu-C_{60}) has been reported,⁸⁶ in which correlation times and hence activation parameters were determined, again, broadly in agreement with neutron scattering measurements. Following our work using TF-MuSR to determine activation parameters for the adsorption and diffusion of hydrocarbon radicals in zeolites,^{72,73} in which motional correlation times were extracted from the temperature dependent linewidths, we have explored the application of LF-MuSRx technique in the study of cyclohexadienyl radicals in benzene loaded samples of zeolite X,⁷⁴ exchanged with group 1 and group 2 cations. The technique is found very effective, and, in contrast with TF-MuSR, can discriminate between radicals at different adsorption sites by virtue of their differing motional behaviour.

The significance of this work to Environmental chemistry was alluded to in the previous section, but we have also used the LF-MuSRx method in the determination

of the adsorption and diffusion of 1,1-dichloroethyl radicals ($\text{MuCH}_2\text{CCl}_2\cdot$) on particulate matter, such as is present in the atmosphere, namely silica and kaolin, as models of wind-blown dusts, and which can provide a surface on which pollutants such as 1,1,1-trichloroethane may be degraded. For relatively high loadings of 1,1-dichloroethene (which is used to form the dichloroethyl radicals), we find an activation energy of *ca.* 9 kJ mol by both this and TF-MuSR methods, and for both kinds of particulate.⁷⁵ We have also measured the motional behaviour of cyclohexadienyl radicals (spin-labelled benzene) on carbon powder using both LF and TF methods: both find a fraction with an activation energy to surface motion of *ca.* 5 kJ mol⁻¹; importantly, the LF-MuSRx method reveals an additional more strongly adsorbed fraction ($E_a = 22 \text{ kJ mol}^{-1}$), which is invisible to TF-MuSR.⁷⁶

It is proposed that the radical $\text{PhCHOMu}\cdot$, formed by muonium addition to benzaldehyde, may be used as a molecular probe of solvation effects and of cation–molecule interactions in a series of cation-exchanged zeolites (X); by using substituted benzaldehydes, the system reveals the nature of electronic effects in radicals.^{87,88} There is less research now on purely structural details of organic radicals using muons, than was once the case, but there are a few novelties, which I feel are worth including here. As reported last time,¹ Percival and co-workers have succeeded in producing a radical with a negative muon coupling, $\text{Me}_3\text{SiCHMu}\cdot$, since the muon is disposed in a position α to the spin-bearing carbon atom.⁸⁹ For carbonyl adducts, $\text{R}_2\text{C}\cdot\text{O}-\text{Mu}$, the coupling could in principle be either positive or negative, depending on the sweep of the out of plane amplitude of the muon, but is positive in all cases known so far; however, on the basis of the temperature dependence of the muon coupling in **21**, its sign is established as being negative.⁹⁰ This is a consequence of the electronic push–pull character of the $\text{MuO}-\text{C}\cdot-\text{C}=\text{C}=\text{O}$ system, which increases the ‘double-bond character’ of the $\text{MuO}-\text{C}$ unit, and reduces the out of plane amplitude of the muon, so it does not excurse from the region of negative coupling. A related study is of the muonium adduct of biacetyl;⁹¹ this has a coupling of only 2 MHz at 298 K, but the temperature dependence establishes that it is still of positive sign. We had originally speculated that the small coupling might be due to an intramolecular H-(Mu)bond involving the two oxygen atoms in an *s-cis* geometry, but on theoretical grounds, and also with the example of **21** in mind (where this geometry is impossible, but the muon is still confined close to the radical plane), we now believe that it is also due to an electronic ‘push–pull’ effect: *i.e.* $\text{MuO}-\text{C}\cdot-\text{C}=\text{O}$. One other surprise is that while muonium addition to the vinyl group, leaving a spin-delocalised benzyl-type radical, is strongly favoured for styrene and vinylnaphthalenes, for 9-vinylanthracene, the expected 9-anthrylethyl radical, 9-anthryl- $\text{CH}\cdot\text{CH}_2\text{Mu}$, is not formed, but instead the ring adduct **22**.⁹² Adducts of nitroalkanes, $\text{RN}(\text{O})\text{OMu}\cdot$, have also been characterised, and the temperature dependences of the, positive, muon couplings were rationalised in terms of the calculated N-OMu torsional barriers.⁹³



One aspect which is important, because it takes MuSR into the biological field in a

sense that utilises its unique character, is the study of thiyl radicals (RS[•]) in non-aqueous media.^{2,94,95} Thiyl radicals are implicit in the protective mechanism of cells against damage by free radicals, but partner a duplicity in that they are themselves reactive species, so that once a thiol (SH) has scavenged, say, an oxygen radical, an RS[•] radical is 'at loose', and is a rather reactive species. These studies reveal the conformational geometries of (alkyl) RS[•] radicals and, moreover, demonstrate that their reactivity towards H-atom abstraction from lipids is higher, by a factor of 3–4, than previous pulse-radiolysis studies (which use aqueous media) would indicate, the tenet thus being that MuSR studies are more relevant to understanding membrane-type systems (which are, of course, non-aqueous). The corollary, however, is that the rates of reaction for thiyl radicals with radical scavengers such as glutathione(SH) and dietary supplements such as β -carotene are similarly enhanced by a factor of 3–4 in non-aqueous media, so Nature is well aware of these reaction rates!

David Walker has published a comprehensive article dealing with muonium reactions in solution which focuses on kinetic isotope effects of muonium and protium as isotopic hydrogen atoms.⁹⁶ Explanations for these, which may be either positive or negative, are proposed: broadly, muonium is slightly more nucleophilic, and so adds more quickly to electron deficient sites than does protium; on the other hand, muonium abstracts hydrogen atoms more slowly than protium does, in consequence of differential zero point energy effects in the transition state. Reactions in micellar media are more complex yet: I have reprints of all David's recent work in this area, which he was kind enough to send me, and which are included in the references cited in this article.⁹⁶ Similarly, I asked Brian Webster to send me any of his recent work, which provides a theoretical aspect that is of great value in structural assignments of radicals formed in muon studies, for instance in the assignment of the paramagnetic species formed by irradiation of elemental sulfur with positive muons.⁹⁷ These calculations suggest that the muonated sulfanyl radical (MuS[•]) is a likely candidate. Other papers by Brian offer theoretical predictions regarding the muonated formyl radical MuCO[•],⁹⁸ and the muonated ethyl radical MuCH₂CH₂[•].⁹⁹

5 Inorganic and organometallic radicals

Small molecules

We open with a truly archetypal example, the dihydrogen radical anion (H₂^{•-}): identified in solid *para*-hydrogen which had been exposed to ionising radiation at 4.2 K. The resulting centre feature showed a narrow 1:1 doublet, which was centred on $g = 2.007$, and with a splitting of 12.4 G.¹⁰⁰ Having focused rather more on muon and other matters over the last few years, than solid-state ESR, which was my mainstay before then, I am reminded, abruptly, of the tremendous challenge of solid-state work, where there are many examples of two interpretations of the same spectrum—but, of course with entirely different chemical conclusions, since the assignment of an ESR spectrum to a particular radical (paramagnetic) species depends absolutely on its analysis. That understood, here is a perfect example: the discussion of the original paper is in terms of a zero-field splitting from a triplet state encompassing (H₂^{•+}•⁻) pairs, but this paper proposes that these features are the central doublet (from

second-order splitting) of $\text{H}_2^{\cdot-}$ radical anions. If this is correct, the real g -value becomes 2.0033, and the proton splitting, estimated from the 12.4 G, second-order splitting, is 203 G for two equivalent protons; but the outer ± 1 hyperfine features were not detected originally, although a personal communication says that they have, apparently, now been detected. It is worth recalling that the 'wing' regions of ESR spectra, particularly in the solid state, often yield very definitive information.

On a similar topic, regarding the irradiation of (quantum) solid *para*-hydrogen, is the observation of lines corresponding to a hyperfine splitting of 203 G, and, as above, is thus from the $\text{H}_2^{\cdot-}$ radical anion. It is suggested that electron bubbles assumed previously in irradiated solid hydrogen are probably, in fact, the $\text{H}_2^{\cdot-}$ ions observed here.¹⁰¹ dare I suggest that one observation can be extremely helpful to another? Prompted by this assignment, another group report *ab initio* calculations of the g -factors for $\text{H}_2^{\cdot-}$.¹⁰² Interestingly, they predict that the g -value shifts are only small, and remain close to that for the free H-atom, which does not support Symons' estimate of the g -value of 2.0033; but probably there is simply a calibration error, as the hyperfine evidence that the species detected in irradiated solid hydrogen is $\text{H}_2^{\cdot-}$ is very compelling.

Symons has reviewed his work, carried out over many years, on small inorganic radicals: these include atoms, H, N, O, Ag; diatomics, OH, NO, $\text{O}_2^{\cdot-}$; AB_2 species, $\text{CO}_2^{\cdot-}$, NO_2 , HCO , O_3^- etc.; AB_4 centres, PO_4^{2-} , PO_4^{4-} , PF_4 , etc. Matrix effects on these species are described and explained, and aspects of reaction mechanisms in which they participate are discussed.¹⁰³ He has also reviewed, mainly his, work on three-electron bonded σ^* -species, which may be formed by electron capture and electron loss; examples are drawn from homonuclear species A–A, which generally are the more stable, and heteronuclear species, A–B.¹⁰⁴ He discusses S–S (anions and cations), H–halogen radical anions, P–P cationic radicals, C–halogen and N–halogen radical anions where the unpaired electron occupies the antibonding C(N)–halogen σ -orbital, the latter being the more stable. Sevilla reports that there is a specific interaction of the chlorine atom with water, also in the manner of a localised three-electron bond, on the basis of ESR results and *ab initio* calculations.¹⁰⁵ A series of isolated chlorine–water radical species consisting of hydrated chlorine atoms, as well as chloride ions interacting with hydroxyl radicals were considered, but the best agreement with the experimental results was found for chlorine atom–water interactions: $\text{H}_2\text{O}-\text{Cl}(\text{H}_2\text{O})_n$, in which 60% of the spin-density resides on the Cl atom. Full optimisation of the $\text{OH}^{\cdot}-\text{Cl}^-$ aquated systems shows that strong ion dipole forces overcome weaker $\sigma-\sigma^*$ interactions and result in full spin-localisation on the hydroxyl radical.

Metal atoms

Mile and Sillman have reviewed ESR studies of small metal clusters, including Na_3 , Na_2Li , Au_2Ag , Cu_3 , Ag_3 , Au_3 , Li_3 and Na_2K_5 ; the last has the pentagonal bipyramidal structure seen for homonuclear septamers.¹⁰⁶ The studies of metal atom chemistry from the Canadian group continue, and they report the isolation of small metal complexes in cyclohexane and adamantane matrices, as studied by X- and Q-band ESR. The spectra show no evidence of either g - or hyperfine-anisotropy, so the free atoms are not perturbed appreciably. It is found that trimers, pentamers and/or septamers are not formed with Rb and Cs under experimental conditions which gave

Li_3 , Li_5 , Na_3 and K_7 : in solid cyclohexane, only colloidal metal particles were produced, with a mean diameter of $< 100 \text{ \AA}$.¹⁰⁷ In another paper is reported ENDOR studies of both 'naked' and hydrated triatomic lithium clusters, isolated in an adamantane matrix.¹⁰⁸ Essentially the couplings obtained agree quite well with those previously deduced from ESR measurements; for the hydrated cluster, there was a reduction in the hyperfine coupling consistent with the transfer of spin-density onto the coordinated water molecules of 20%.

A series of formyl-type radicals have been prepared, MSiO , where $\text{M} = \text{Cu}, \text{Ag}, \text{Au}$ or V , by laser ablation of the metal and solid SiO_2 during condensation in neon or argon matrices at 2–4 K.¹⁰⁹ The ESR parameters were compared with those for the corresponding monocarbonyls. $\text{Cu}(\text{SiO})$ is found to have 71% metal s -character in the SOMO, and is similar to $\text{Cu}(\text{CO})$. $\text{Ag}(\text{SiO})$ gave the distinct ESR spectrum of a linear molecule, with 74% metal s -character, in contrast to the non-bonded $\text{Ag}(\text{CO})$. $\text{Au}(\text{SiO})$ is non-linear, in contrast with the linear $\text{Au}(\text{CO})$. $\text{V}(\text{SiO})$ has a quartet ground state, whereas $\text{V}(\text{CO})$ is a sextet; this is ascribed to increased bonding of the metal d -electrons with SiO . Knight has reported some novel gallium hydrides, formed by the reaction of laser ablated gallium atoms with H_2 and CH_4 , into which they insert, giving mixtures of the isotopomers of GaH_2 and HGaCH_3 ; these radicals were isolated in neon matrices. *Ab initio* calculations with a large uncontracted basis set and CI with all single excitations from the spin-RHF configuration gave good agreement with the experimental results and were in support of the free atom comparison method of interpretation of the hyperfine interactions. A comparison with related aluminium-centred radicals was also made.¹¹⁰ The same group have also made a study of the monoxides of Al, Ga and In , again by isolation in solid neon, using laser ablation of the metals, the atoms of which react with $^{16}\text{O}_2$ or $^{17}\text{O}_2$,¹¹¹ while studies on the isotomeric forms of C-B-C ¹¹² and BH_4 ¹¹³ were also reported.

6 Metalloproteins

Iron-sulfur clusters continue (of course, not surprisingly, given their enormous importance in living systems) to be a topic of active investigation, as is illustrated by the following two examples. The iron protein component of nitrogenase is a homodimeric protein with a bridging $[\text{4Fe-4S}]$ cluster and two nucleotide binding sites, one on each sub-unit. One paper presents evidence that the $[\text{4Fe-4S}]$ cluster domain of the nitrogenase Fe protein functions as a hinge region between the two nucleotide binding domains, participating in the cooperative binding of two nucleotides. Alanine residues at position 98 (located near the $[\text{4Fe-4S}]$ cluster) of the *Azotobacter vinelandii* Fe protein were exchanged by means of site-directed mutagenesis to valine (V) and glycine (G), and the resulting altered proteins were purified and characterised. While the wild-type and A98G Fe proteins were found to bind two nucleotides (MgATP or MgADP) with strong cooperativity, the A98V protein was found to bind one nucleotide with no obvious cooperativity. Results from proton NMR, ESR and CD spectra, along with Fe chelation rates, were all consistent with binding of a single nucleotide to the A98V protein inducing a partial conformational change.¹¹⁴ The five cytosines closest to the carboxyl terminus of human ferrochelatase have been individually mutated to serine, histidine or aspartate residues to identify the protein ligands to the

[2Fe–2S] cluster.¹¹⁵ Mutations of cysteines at selected positions (403, 406, 411) all resulted in an inactive enzyme that failed to assemble the [2Fe–2S] cluster as judged by whole-cell ESR studies. In contrast, mutation of the cysteines at positions 360 and 395 to serines did not affect the enzymic activity, and the resulting enzyme assembled a [2Fe–2S] cluster that was indistinguishable, spectroscopically, from the wild-type enzyme. The results indicate that three of the conserved cysteines in the 30-residue C-terminal extension of mammalian ferrohcalatase are involved in ligating the [2Fe–2S] cluster.

The utility of the electron spin echo envelope modulation (ESEEM) method has been demonstrated in probing the active site structure of metalloproteins.¹¹⁶ Two examples are provided, with Cu^{II} and Co^{II} coordinated to imidazole, and it is shown how the measurement of nuclear quadrupolar and weak nuclear hyperfine interactions provides a basis for understanding how local changes in a protein structure are transmitted to metal binding sites. In another study, the mechanism of action of xanthine oxidase has been investigated,¹¹⁷ using ¹³C and ¹⁷O ENDOR of molybdenum(v) species, and by kinetic studies of exchange between oxygen isotopes. Three ESR-detectable species were studied: (i) very rapid, a transient intermediate in substrate turnover; (ii) inhibited, the product of an inhibitory side reaction with aldehyde substrates, and (iii) alloxanthine, a species formed by reaction of reduced enzyme with the inhibitor, alloxanthine. Estimates of Mo–C distances were made from the anisotropic components of the ¹³C couplings: these were 1.9 and 2.4 Å, respectively, in the inhibited and very rapid species, thus a Mo–C bond is strongly suggested in the inhibited species, presumably associated with side-on bonding to molybdenum of the carbonyl of the aldehyde substrate; for the very rapid species, a Mo–C bond is also deemed highly likely.

The selenium containing F420-reducing hydrogenase from *Methanococcus voltae* was anaerobically purified to a specific hydrogen-uptake activity of 350 U mg⁻¹ protein, as determined with the natural acceptor: the concentrated enzyme was used for ESR investigations. As isolated, the enzyme showed an ESR spectrum with principal *g*-values of 2.21, 2.15 and 2.01.¹¹⁸ Illumination of these samples at low temperatures led to an ESR spectrum with *g*-values of 2.05, 2.11 and 2.29, which are typical for [NiFe]hydrogenases in the active state. Spectra of samples enriched in ⁷⁷Se showed a hyperfine interaction between the spin at the nickel atom and the nuclear spin of one ⁷⁷Se atom before and after illumination: a 90° flip of the electronic *z*-axis is proposed to explain the hyperfine interaction in both states. The results demonstrate that the three-dimensional structures of the active sites in the selenium-containing F420-reducing and F420-non-reducing hydrogenases from *M. voltae* are highly similar and hence are not influenced by the unusual sub-unit structure of the latter enzyme. Oxidised samples containing either natural selenium or ⁷⁷Se were prepared from the F420-reducing and the selenium containing F420-non-reducing hydrogenase. Both enzymes exhibited ESR spectra typical for [NiFe]hydrogenases in the active 'ready' state. In contrast to the reduced form, no splitting of the nickel-derived signal due to the nuclear spin of ⁷⁷Se was observed in the oxidised state, indicating that the electronic *z*-axis is perpendicular to the Ni–Se direction.¹¹⁸

7 Transition metal ions

The first four papers in my survey under this heading concern the properties of transition metal ions present in zeolites. Firstly, we have a study of a copper(II) complex with water, measured in Cu-ZSM-5.¹¹⁹ By means of ESR and ENDOR, the detailed structure of a Cu complex with water molecules in this zeolite was determined: the complex has an axial distorted octahedral symmetry, in which the distances between the Cu ion and the protons within the water molecules are 2.54 and 2.69 Å. Accordingly, the H₂O molecules can be divided into two groups: two being at the longer distance are in the axial position, while the other four are equatorial with respect to the Cu ion. A group in Poland have used both ESR and ESEEM to study silver-coordinated hydroxymethyl radicals in different molecular sieves.¹²⁰ These radicals were produced by γ -irradiation of silver-exchanged SAPO-42, SAPO-5 and SAPO-11 and zeolite-A, loaded with methanol or ethanol: it was established that the doublet features, with splittings in the range 97–180 G, represented covalent Ag⁺-CH(R)OH⁺ radicals; the influence of the trapping cage size on the configuration of these organosilver radicals is also discussed. A very comprehensive study has been made of framework iron in ⁵⁷Fe-exchanged zeolites, using pulsed ENDOR at 95 GHz.¹²¹ Fe-sodalite (FeSOD), Fe-L (FeLTL), Fe-mazzite (FeMAZ) and Fe-ZSM5 (FeMFI) were investigated, where the ⁵⁷Fe was introduced during their synthesis. The echo-detected ESR spectra of all these zeolites show mainly the $|-\frac{5}{2}\rangle$ to $|-\frac{3}{2}\rangle$ transition, and accordingly the ENDOR spectra exhibit only two ⁵⁷Fe transitions at 67.8–68.8 and 39.0–39.6 MHz, corresponding to $M_S = -\frac{5}{2}$ and $-\frac{3}{2}$, respectively. From these frequencies, isotropic couplings of -29.0, -29.4, -29.4 and -29.4 MHz were derived for FeSOD, FeL, FeMAZ and FeMFI, respectively. Based on an earlier assignment of the $g = 2$ signal in FeSOD to Fe^{III} in tetrahedral framework sites, it is concluded that hyperfine couplings in the range -29.0 to -29.4 MHz are characteristic of Fe^{III} in zeolite frameworks. In contrast to X-band measurements, the W-band signals are free from second- and third-order contributions of the hyperfine and zero-field splitting (ZSF) interactions, and are thus significantly simpler to assign and interpret. The ZFS contributions caused excessive inhomogeneous broadening of the X-band ENDOR spectra of FeL, FeMAZ and FeMFI so as to be almost undetectable. All zeolites showed ENDOR signals from ²⁷Al and FeSOD also showed clear ²³Na ENDOR signals. This work demonstrates the power of high-field ENDOR in terms of resolution, signal assignment and ready spectral analysis.

Zeolite-rho was exchanged with Pd^{II}, which was then reduced to Pd^I by a thermal activation process.¹²² The reactions of this Pd^I with oxygen, water, methanol, ammonia, carbon monoxide and ethylene were studied using ESR and electron spin echo modulation (ESEM). Pd^I reacts with water to form Pd^{II}-O₂, indicating decomposition of the water. Equilibration with methanol results in a broad isotropic signal from small palladium clusters, while ESEM shows that the Pd clusters coordinate one molecule of methanol. Adsorption of ammonia produces a Pd^I complex containing four molecules of ammonia, according to the ¹⁴N splitting. Adsorption of carbon monoxide results in a Pd^I complex containing two molecules of CO, based upon the ¹³C superhyperfine coupling, while adsorption of ethene results in two new Pd^I species each of which coordinates one molecule of ethene. Finally,¹²³ I note a study of transition metal complexes with triazacyclononane in zeolites, formed by addition of the ligand to

samples of Mn^{2+} , Co^{2+} and Cu^{2+} exchanged zeolite Y, in which the zeolite provides a confined reaction space in which the cluster nuclearity and the oxygen binding may both be controlled.

One intriguing report is of ESR spectra from 'EPR-silent' species.¹²⁴ This actually refers to some manganese porphyrins which are, indeed, ESR-silent at X- and Q-band frequencies, but not at high (200–550 GHz) frequencies. The explanation is that the crystallites of these compounds orient in the high magnetic fields, and so their spectra show one main feature whose resonant field shifts linearly with the microwave quantum, with a value of Δ between 7 and 8 cm^{-1} .

With an equally intriguing title is a commentary by Symons, 'When is a radical not a radical?'¹²⁵ The purpose of this note is to point out that symmetrical Fe^{III} complexes are expected to have ESR spectra dominated by a symmetrical, narrow line close to the free-spin g -value, and that such features can be mistakenly identified as the spectra of stable organic radicals; this situation is a particular problem in studies of animal or plant tissue samples.

Superconductors

Interest in the area of superconductors continues, and I shall merely include a small number of papers which I hope illustrate some current trends. One reports the temperature dependence of the static magnetic susceptibility and ESR linewidth of some manganate perovskites, $\text{La}_{0.67}\text{Sr}_{0.33}\text{MnO}_3$ and $\text{La}_{0.62}\text{Bi}_{0.05}\text{Ca}_{0.33}\text{MnO}_3$, both of which are linear above T_c (238 K). It is argued that this linearity is most likely due to contributions from spin-phonon interactions; the results support the recent proposal that lattice effects are important in the magnetic properties of these oxides.¹²⁶ Japanese workers show that a tetravalent perovskite, SrPrO_3 , has an orthorhombic structure, with the distortion from the ideal cubic perovskite symmetry being much greater than for BaPrO_3 . Magnetic susceptibility measurements on SrPrO_3 do not show the existence of magnetic ordering down to 2 K, and is in contrast to the result for isomorphous BaPrO_3 , which shows an antiferromagnetic transition at 11.5 K. The ESR spectrum of the Pr^{4+} ion could be measured by doping it into an isomorphous SrCeO_3 which is diamagnetic, and lowering the temperature to 4.2 K: the spectrum is anisotropic, which corresponds to the deviation of the octahedral coordination symmetry around the Pr^{4+} ion, for which the g -values are discussed in terms of crystal field theory.¹²⁷

Other workers have turned their attention to the question of oxygen vacancies in BaTiO_3 .¹²⁸ By means of ESR, two types of Ti^{3+} -related centres may be identified in BaTiO_3 , which are assigned to $\text{Ti}^{3+}\text{-Vo}$ and to $\text{Ti}^{3+}\text{-Vo}$ associated with Na or K, respectively, and are supported by the observation of Ti hyperfine structure, which further reveals that electron capture has occurred at a single Ti site. This time from Hungary comes an article reporting an investigation of the resonance interaction between superconductors and a radiofrequency field, using a modified ESR spectrometer.¹²⁹ At T_c the amplitude of the response increased about 20–25 times, and the resonance frequency from the circuit from 1 to 3.5 MHz. The frequency *vs* temperature curve, *i.e.* the frequency and the resistance, first slowly and then sharply decreases near T_c . At temperatures below T_c (80 K) the frequency approaches a saturation, while the resonance interaction features a magnetic memory effect.

The ESR signal of Gd spin-probes (0.5 wt%) as well as the static normal state susceptibility of Eu-doped $\text{La}_{2-x-y}\text{Sr}_x\text{Eu}_y\text{CuO}_4$ reveal pronounced changes in the Cu magnetism at the structural transition from the orthorhombic to the low-temperature tetragonal phase for all nonsuperconducting compositions; both a jump-like decrease of CHI as well as the ESR data show an increase in the in-phase magnetic correlation length in the low-temperature tetragonal phase. From the Gd^{3+} ESR linewidth for specific Eu and Sr concentrations in the LTT phase the correlation length increases up to > 100 lattice constants and the fluctuation frequency of the CuO_2 spin system slows down to $10^{10}\text{--}10^{11} \text{ s}^{-1}$. However, there is not static order above *ca.* 8 K, in contrast to the LTT phase of Nd-doped $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$ with pinned stripe correlations.¹³⁰

The model systems FeMgBO_4 and FeMg_2BO_5 are characterised by one-dimensional magnetic exchange interactions along Fe^{3+} chains separated from each other by diamagnetic Mg^{2+} chains. The solid solution series $\text{Fe}_x\text{Ga}_{1-x}\text{MgBO}_4$ ($x = 0.55\text{--}1$) allowed the determination of the effect of diamagnetic Ga^{3+} dilution on their magnetic properties; site inversion between Fe and Mg sites cuts off the Fe^{3+} chains and produces a distribution of chain segments: the statistics of this distribution were calculated. The temperature dependence of the ESR intensity was described by thermal activation, averaging over a distribution of quadrupole-split rings, reflecting a distribution of Fe surroundings; below 10 K, magnetic interchain interactions cause distinct spin glass behaviour.¹³¹

8 Catalytic systems

Some of these papers might have been included in the section on 'organic radicals' in solids, under the 'radical cations' heading, but I have chosen to place them here as they feature in the aspect of those properties which determine how catalysts actually work, which is the theme of this section. So, I note a paper by the Linköping group, which refers to the stabilisation of the naphthalene radical cation in the zeolite H-ZSM5, which has been characterised using ESR, ENDOR and ESEEM techniques; for comparison, the cation was also measured in a solid CFCl_3 matrix.¹³² The *g*- and proton hyperfine tensors were found to be identical in these two media, within experimental error. The hyperfine tensors obtained for the radical cation of perdeuterionaphthalene were analogous to those for the protiated compound, scaled by the gyromagnetic ratio; the nuclear quadrupole interaction tensors of the deuterium nuclei were also determined by ESEEM. The same group has also reported studies of the motional dynamics of NO_2 adsorbed into mordenites.^{133,134} The temperature dependence of the ESR spectrum of NO_2 adsorbed into Na-mordenite was studied over the range 77–300 K, for which an analysis based on slow-motional ESR theory shows that the reversible change with temperature is caused by Heisenberg spin-exchange; the activation energy is 0.8–1.6 kcal mol^{-1} depending on NO_2 concentration, and it is believed that the NO_2 molecules interact with each other by diffusion in the zeolite channels.¹³³ A similar study was made of mordenite exchanged with other cations, and the ESR spectra were again analysed using the Heisenberg exchange model; the exchange rate was found to depend on the amount of water present in the zeolite and on the cation radius.¹³⁴

The topic of radical cation generation by H-zeolites which have been thermally

activated in an oxygen atmosphere was the subject of a comprehensive review, which takes a critical stance and militates against the conclusions of a number of published reports.^{135,136} Essentially, when organic substrates are introduced to such materials radical cations from either monomer (where this provides an energy minimum) or oligomer species, generally formed by proton catalysed reactions, are formed; activation in oxygen always gives a higher yield of the radical cations than, say, activation under vacuum, or under an inert atmosphere such as nitrogen. This much is clear, although there are many misassignments in the literature; however, there is controversy regarding the nature of the electron-capture (oxidizing) site. It has been suggested¹³⁷ that the site may be an Al-coordinated dioxygen centre with a very high electron affinity, and this has been proposed to explain the broad, underlying signal which is often observed in addition to the spectrum of the radical cation, on the basis of the expected range of *g*-values which would exist for a range of slightly differing local site structures. However, when strongly electron affinic species, such as dioxygen or sulfur dioxide, are co-adsorbed with the organic, clear signals from the radical anion are seen. It is proposed that the electron capture site does not merely catch a single electron (counter to the radical cation), but acts more as a conventional Lewis site and captures electrons in pairs, resulting in an ESR-silent centre which is located at extra-framework aluminium.^{138,139} The role of extra-framework aluminium in radical cation formation is demonstrated by the fact that only a weak radical cation signal is found with zeolites in which it has been removed by treatment with acids or by chelation with oxalic acid. This would imply that these extra-framework Al-sites are formed more readily on heating the zeolite in oxygen rather than by a simply thermal route. Another group¹⁴⁰ have shown that dealumination probably does not occur in H-ZSM5 simultaneously with framework dehydroxylation, and so framework Lewis sites may be formed. A two-step dehydroxylation mechanism is postulated which dominates the dehydroxylation and dealumination processes at different temperatures.

From the Argonne laboratory are two papers on radical ion formation, this time in γ -irradiated zeolites. Essentially, it is proposed that this radiolysis/ESR method may be used as a means to study the fundamentals of catalysis by zeolites, and that products of catalytic reactions are spin-labelled by conversion to their radical cations. Comparison of reactions occurring in H-ZSM5 and H-mordenite reveal the strong influence of shape-selectivity.¹⁴¹ Curiously, the *cis*-bent form of the acetylene radical *anion* has been observed in irradiated acetylene loaded H-ZSM5, but not in Na-ZSM5;¹⁴² I say curiously, because, surely, the anion would be expected to protonate in the H-zeolite? The corresponding radical cation is not stabilised by the zeolite matrix, but undergoes ion-molecule reactions, including the formation of the benzene radical cation. Benzene is also formed by the acid-catalysed trimerisation of benzene on H-ZSM5. It is stated that 'the evidence that acetylene (IP = 11.4 eV) is ionized places a new lower limit on the range of ionizable species in the radiolysed zeolite';¹⁴² however, it is more likely that the oligomeric species are formed by acid-catalysed routes, and being of sufficiently low ionization potential are subsequently ionized, as discussed in the general case.^{135,136}

9 Free radicals in biology and medicine

This continues to be an enormous area, and, as I mention in the Introduction, certainly

in the UK many chemists working with ESR have made stringent efforts to move into this area, which is rather more available funded than is free-radical chemistry. I am amazed, in fact, at how 'free radicals' have entered the public consciousness, even that of some of my relatives who do not have a science background: fortunately, for the first time ever, my family now feel that they have some clue as to what 'Chris does for a living'. This is, I think, simply that the term has become a buzz-word in advertising, where free radicals are the 'bad-guys' which are responsible for your looking prematurely old if you worship too much sunshine, and give you cancer and heart and other troubles: but there are (at suitable cost) various proprietary preparations to protect you from them; last time I visited a health shop the term 'Free Radicals/Antioxidants' graced many labels describing what was for sale! Moreover, the internet reveals that free radicals are, indeed, big business, particularly in America.

On the scientific front, ESR studies of very many systems flourish, with the aim to establish the role of free radicals in disease, mainly through the agency of spin-trapping. At the University of Lancaster in 1997, the ESR Group of the RSC ran its annual ESR Conference in conjunction with the International Spin-Trapping community: this combined conference was very well attended, and a number of advances in the method were presented, as may be seen in the section below. A chemical study has been made of C-2 glyceryl radicals which provides indications that lipids may be damaged *via* attack at the glyceryl function rather than just at the allylic groups on the hydrocarbon chain. In this work, it was found that the cleavage of the β -C-O bond proceeds rapidly if a hydroxyl group is present at the radical carbon centre, and a concerted elimination mechanism is suggested, involving heterolysis of the β -C-O bond.¹⁴³ We have investigated the reaction of allylic and β -acetoxy-alkyl radicals with vitamin E and vitamin K in non-polar media, to mimic the membrane environment, using muon spin rotation spectroscopy.¹⁴⁴ Our results show that both radicals react rapidly with both antioxidants: vitamin E providing an H-atom to the abstracting hydrocarbon radical, while vitamin K, with its naphthaquinone moiety, acts as an electron acceptor and forms the corresponding carbocation from the radical; in the latter case, there was no evidence for the assistance of the β -acetoxy group in the ionization of the radical, which might be expected to promote the formation of an incipient, bridging carbocation-like transition-state, and aiding the formation of the final bridged carbocation product. I feel it is worthy of note, regarding both these studies, that a process such as a heterolytic elimination of an acyloxy group might be more favourable in aqueous media, and is probably less significant to membranes; in our study in diethyl ether,¹⁴⁴ not only was there no evidence for carbocation stabilisation, or indeed radical stabilisation via a bridging acetyl group, but nor was the radical itself unstable to heterolytic elimination, at least on the μ s time-scale.

Various mechanisms have been proposed for the reaction between heme proteins and organic hydroperoxides, including a peroxidase-type mechanism and homolytic cleavage. One group of workers¹⁴⁵ have used ESR to investigate the formation of radicals in the hematin/*tert*-butyl hydroperoxide system, and have demonstrated the formation of peroxyl and alkoxy radicals, with methyl radicals being produced at elevated hematin concentrations. The mechanism is proposed to be an initial scission of the O-O bond, and although some *tert*-butylperoxyl radicals could be detected, the majority were methylperoxyl radicals formed by elimination of methyl radicals from *tert*-butoxyl radicals, and their subsequent combination with dioxygen. A group in

China have investigated fullerenols in their ability to trap oxygen radicals, and find their role to be most effective in scavenging both the hydroxyl radical and the superoxide radical anion; it is proposed that the fullerenols are a new type of radical scavenging and water soluble antioxidant.¹⁴⁶

Accurate measurements of radiation dose in bone are required to improve the dosimetry in systematic radiotherapy for osseous metastases (bone cancer). During irradiation, electrons become trapped in the crystalline component of the bone mineral (hydroxyapatite), and have a half-life of many years. At room temperature, the radiation-induced signal, with a g -value of 2.001 ± 0.001 , increased linearly with absorbed dose above a lower threshold of 3 Gy, up to doses of 200 Gy.¹⁴⁷

Hoffman's group have used their rapid freeze-quench technique to study the intermediate generated during the formation of the diferric cluster and the tyrosyl radical in *Escherichia coli* ribonucleotide reductase.¹⁴⁸ The ^{17}O ENDOR results have detected three exogenous oxygen atoms associated with the intermediate at 600 ms: one is derived from $^{17}\text{O}_2$, and has properties similar to those of a second, distinct, solvent derived oxygen. Cellular studies¹⁴⁹ have indicated that some Fe-S proteins, and the aconitases in particular, are targets for nitric oxide, and specifically, NO has been implicated in the intracellular process of the interconversion of active cytosolic aconitase containing a [4Fe-4S] cluster, to its apoform, which functions as an iron-regulatory protein. In a study of the reaction of NO with both mitochondrial and cytosolic aconitases, made by following enzyme activity and observing the formation of ESR signals not present from the initial reactants, it was found that enzyme inactivation is accompanied by the appearance of the $g = 2.04$ signals from [3Fe-4S] clusters and the $g = 2.04$ signal from a protein-bound dinitrosyl-iron-dithiol complex in the d^7 state. In addition, in the reaction of cytosolic aconitase, the transient formation of a thiyl radical, with $g_{\parallel} = 2.11$ and $g_{\perp} = 2.03$ was observed.

It makes sense that NO could combine with molecular oxygen to form the peroxy radical ONOO \cdot , but one ESR study designed to detect it managed, at best, to find only very small amounts, from which it was concluded that the radical is unlikely to be of major importance, of itself, in biological systems, in contrast with its one-electron adduct, the peroxy nitrite anion.¹⁵⁰

Spin-trapping

Since this is the means by which an enormous range of biological systems have been interpreted in terms of free radicals being fundamental to their mechanism, the technique and methodology of spin-trapping is of central importance. I have already alluded to the Lancaster ESR/spin-trapping conference, which focused mostly on the chemical aspects of the technique: there are some good examples, and I will begin with these. First, there remains the problem of spin-trapping nitric oxide (NO), for which a number of strategies have been proposed. For instance, there were two papers from John Walton's group in St Andrews which deal, respectively, with the reaction between NO and various functionalised alkenes and dienes,¹⁵¹ and the other which explores diazoketones as potential spin-traps for NO.¹⁵² It is found that NO does not add to alkenes or to conjugated dienes, but that their reactions are initiated by NO $_2$ addition to C=C bonds to produce β -nitroalkyl radicals which combine with nitric oxide to yield β -nitro-nitroso compounds, which trap radicals to afford mixtures of nitrox-

ides.¹⁵¹ 2-Diazocycloheptanone was found to trap NO to produce a mixture of radicals consisting mainly of the (*E*)-iminoxyl radical, a dialkyl nitroxide and an acyl nitroxide; mechanistic details are discussed.¹⁵² Gabr and Symons¹⁵³ have also continued their work on the reaction of NO with dienes. They found that addition of NO to dilute (*ca.* 10^{-4} mol l⁻¹) solutions of 2,3-dimethylbuta-2,3-diene gave at least two nitroxides: the major radical, with a half-life of *ca.* 30 min, had an ESR spectrum with a splitting of *ca.* 10 G from four protons; similarly, the major radical from 2,5-dimethylhexa-2,4-diene gave a major triplet showing no resolved proton coupling, but satellite features from ¹³C (*a* = 10 G) from two equivalent nuclei. Apparently these reactions occurred in the complete absence of NO₂, and while no radicals were observed when using pure NO₂, mixtures with NO gave strong signals from nitroxides. So, these findings are largely in support of Walton's conclusions.¹⁵¹ UV irradiation of nitrite ions in air-free NaOH solution, in the presence of the aci-anion of nitromethane, generates ESR spectra of [CH(NO₂)₂]²⁻ and [CH(NO₂)NO]²⁻ radicals, formed by the addition of NO₂ and NO to the initial anion.¹⁵⁴ The fact that the NO and NO₂ radicals originate from NO₂⁻ was confirmed by the ESR spectra observed during photolysis of ¹⁵N-labelled nitrite, which afforded the [CH(¹⁴NO₂)(¹⁵NO₂)]²⁻ and [CH(NO₂)(NO)]²⁻ spin-adducts. These results are consistent with the notion that the primary species generated during the UV photolysis of the nitrite anion in aqueous solution are hydroxyl and NO radicals.

There have been various phosphorylated spin-traps reported, which have the advantage of (i) stability, compared with standard non-phosphorylated versions, and (ii) that the ³¹P coupling furnishes an additional structurally sensitive parameter with which to discriminate between and identify different kinds of trapped radical. These have been developed in Marseilles from where the latest report that I came across is of six new spin-traps based around the PBN moiety. As is the case for other PBN type traps, the lipophilicity is markedly dependent on the structure of the aromatic moiety.¹⁵⁵ The first synthesis of PBN-¹⁵N, has been reported, which engendered a 50% increase in ESR sensitivity compared with the normal (¹⁴N) trap. The material was prepared using ¹⁵NH₄Cl as the nitrogen starting material in a four-step sequence, which produces 2-methyl-2-nitrosopropane (MNP), which is useful in the synthesis of many other PBN-type spin-traps.¹⁵⁶ Janzen's group have reported on the spin-trapping chemistry of acyl radicals using PBN and MNP, including benzoyl, acetyl and other alkylacyl, alkoxyacyl, aminoacyl and alkylaminoacyl adducts. All cases gave relatively long-range β-hydrogen hyperfine splittings ranging from 2.71 to 5.93 G in the PBN spin-adducts; the MNP adducts gave the well known 7–8 G nitrogen hyperfine splitting, with little else.¹⁵⁷

As a novelty in the free-radical chemistry of fullerene derived radicals, is a study of the stabilities and reactivities of radicals (*tert*-BuO)_{*n*}C₆₀[•] conveniently formed by addition of photochemically generated *tert*-butoxyl radicals to C₆₀.¹⁵⁸ Where *n* > 2 (and an odd number) these adducts are very persistent, and can be observed for as long as 9 days; reactions with dioxygen are slow and reactivities vary among the various adducts; however, NO reacts with them all much more rapidly. Among the products are a stable difullerene nitroxyl [(BuO)_{*n*}C₆₀]₂NO[•], but the butoxyl-fullerene radicals are not reactive towards conventional spin-traps, such as DMPO, PBN, MNP and nitrosobenzene.

A paper by Clarkson's group demonstrates the power of 95 GHz ESR in separating

the spectra from spin-adducts formed simultaneously in the same system. Information regarding molecular diffusion anisotropy in which high-frequency ESR is highly sensitive is also more readily obtained at 95 GHz; other advantages over conventional X-band ESR are also outlined.¹⁵⁹ It is shown that PBN is decomposed to NO by an Fe^{II} catalysed route which involves hydrolysis of PBN; a second pathway involves PBN–OH adduct decomposition. It is proposed that PBN is hydrolysed by Fe^{III} or is attacked by hydroxyl radical, leading eventually to a common transient species, *tert*-butyl hydronitroxide [*tert*-BuN(O[•])H], which is further oxidized to a NO source, *tert*-BuNO; the results are taken to imply that PBN may decompose to NO when used in biological models under conditions of oxidative stress.¹⁶⁰ Finally, I note two reports of work involving the use of mass spectrometry in the characterisation of spin-adducts.^{161,162} The first deals with free radical metabolites, while the second is of free radicals formed spontaneously in nitron-peracid reactions, the significance of which is that such peracids can be considered as models of biological peracids produced during lipid peroxidation: these acids were found to react spontaneously with spin-traps of the PBN and DMPO kind to produce significant amounts of nitroxides.¹⁶²

Free radicals in toxicology

I have a great personal interest in this matter, having just edited a book on it.¹⁶³ This (I only discovered at the end of the project!) appears to follow one with a similar title¹⁶⁴ 'Free Radical Toxicology', while mine is called 'Toxicology of the Human Environment – the Critical Role of Free Radicals'; they are completely different in their coverage, however. Probably also worth mentioning here is another volume on the 'Immunopharmacology of Free Radical Species',¹⁶⁵ in which is an article dedicated to the pros and cons of detecting free radicals *in vivo*, using different methods including the AMES test and also ESR and NMR measurements; it's not all 'cut-and-dried', basically!¹⁶⁶ The currently vexed role of β -carotene as a dietary supplement in preventing, or indeed *causing* (lung), cancer is well documented on the Internet, and I note one study which investigates the notion that α -tocopheryl radicals can be rejuvenated to their parent α -tocopherol by β -carotene: this study shows that since the kinetics of formation and decay of the α -tocopheryl radical are not affected by β -carotene, this cannot be the case.¹⁶⁷

It is proposed that reactive oxygen species may be involved in the mechanism of silicosis.¹⁶⁸ To investigate this, the authors studied the kinetic clearance of instilled nitroxides: antioxidant enzymes, superoxide dismutase and glutathione peroxidase and lipid peroxidation were also studied in whole lungs of rats exposed to crystalline silica (quartz) and sham exposed controls. Low-frequency (L-band) ESR spectroscopy was used to measure the clearance of TEMPO in whole rat lungs directly, and which followed first-order kinetics, but showing distinct differences in the rate for clearance between the diseased and sham exposed control lungs. Comparison of TEMPO clearance rates in the sham exposed controls and silicotic rats showed an oxidative stress in the rats exposed to quartz. Overall, this study demonstrates, for the first time, that silicotic lungs are in a state of oxidative stress and that increased generation of reactive oxygen species is associated with enhanced levels of oxidative enzymes and lipid peroxidation.

Russian workers report an ESR and spectrophotometric study of the denitration of

nitroheterocyclic drugs by liver homogenates from cytochrome-P450-induced mice.¹⁶⁹ This work extends a previous study on the mechanism of hepatic denitration of two nitroheterocyclic drugs, quinifuryl and nitracrine, in which the release of nitric oxide (NO) from these compounds can be accompanied by the formation of a nitroyl-heme complex. Pretreatment of mice with three inducers of cytochrome P450 (phenobarbital, clophen A50 and butylated hydroxytoluene (BHT)) increased the yield of the nitrosyl complex, which correlated with a rise in the cytochrome P450 content of mouse liver microsomes. In contrast, treating the animals with β -naphthoflavone decreased the complex yield while still increasing the P450 content. Based on the overall results, a possible mechanism of denitration for these drugs is given.

Due to the importance of all-*trans*-retinoic acid (RA) in the treatment of various dermatological conditions, and the wide distribution of prostaglandin H synthase (PGHS) in tissues, one group of workers have further examined the mechanisms involved in the hydroperoxide-dependent cooxidation of RA and its isomer, 13-*cis*-retinoic acid by PGHS. In this complex paper, ESR evidence is presented for the intermediacy of free radicals.¹⁷⁰ The human lens component, kynurenine, has been found to act as a photosensitiser which generates superoxide by electron transfer to molecular oxygen, on the basis of a spin-trapping study;¹⁷¹ this material also sensitises photo-oxidation of cysteine, NADH, EDAT, azide and ascorbate. Other workers show that aryl radicals are produced during the metabolism of arylhydrazines by microsomes.¹⁷² Only hydroxyl radicals were detected upon incubation of *p*-methylphenylhydrazine with rat liver microsomes and DMPO; however, both hydroxyl and aryl radicals were trapped when the experiment was repeated with *p*-(methoxymethyl)phenylhydrazine. In contrast, when either of these substrates was incubated with C50 cells, aryl and hydroxyl radicals were trapped. The intensity of the ESR signal from the spin-trapped aryl radicals parallel the extent of C8-arylguanine formation in DNA, and so it is likely that the aryl radical is the reactive intermediate responsible for C8-arylguanine formation. It is suggested that aryl radicals and C8-arylguanine adducts may be related to the genotoxicity of arylhydrazines and related materials that are oxidatively metabolised to arenediazonium ions, which precure aryl radicals, including arylalkyl nitrosamines, arylazo-compounds and triazenes.

It is shown that 4-hydroxytamoxifen is activated by horseradish peroxidase in the presence of DMPO and glutathione as a H-atom donor; since a species is detected which is characteristic of a glutathione thiyl radical.¹⁷³ Similar results were obtained with 4-hydroxytoremifene, but no radicals were produced with 3-hydroxytamoxifen: on-line LC-ESI MS analysis of the incubation products from 4-hydroxytamoxifen resulted in the identification of three products with a protonated mass of 773, consistent with the formation of dimers of 4-hydroxytamoxifen. It had previously been proposed that inhalation of fly ash (an emission source air pollution particulate) causes the *in vivo* production of free radicals, and a study was carried out to test this hypothesis.¹⁷⁴ Rats were instilled with either 500 μ g of fly ash or saline, and after 24 h the chloroform extract of their lungs was examined by ESR: a spectrum was obtained consistent with formation of a carbon-centred radical (as the 4-POBN adduct), while the signal was much weaker from rats which had only been administered the saline solution. It is concluded that the soluble metal fraction of the ash (V, Ni, Fe) is responsible for this effect.

In an investigation of peroxyxynitrite decomposition, again using spin-trapping, the

superoxide-adduct was detected, predominantly;¹⁷⁵ a combination of superoxide dismutase and catalase suppressed the formation of the adduct. The results demonstrate that peroxyntirite decomposition generates the superoxide radical anion, and since reaction of H_2O_2 with NO_2^- generates peroxyntirite, a pathway for conversion of H_2O_2 to superoxide via peroxyntirite is suggested.

10 Polymers

Again, there is a huge literature under this subject heading, of which I shall limit coverage to only the following few papers. In the presence of protons, nitroxide radicals cause polymerisation of pyrrole to poly(pyrrole) with the reduced (hydroxylamine) and oxidised (nitrosonium ion) form of the nitroxides incorporated into the polymer matrix.¹⁷⁶ Following electrochemical reduction of such poly(pyrrole) films, the ESR spectrum was found to depend strongly on the extent of the reduction and to reflect the ratio of nitroxide and polaron (cation) concentrations. Other workers have investigated the combined effect of NO_x and stress on the chain-scission and mechanical properties of oriented semi-crystalline nylon 6 fibres.¹⁷⁷ Oriented nylon 6 fibres were placed in an NO_x atmosphere and a sustained tensile load was applied to them: after exposure periods of 0–240 min the fibres were removed and pulled to failure in a tensile testing machine coupled with an ESR spectrometer. Free radicals produced by bond cleavage were detected, and their concentration provided an insight into the number of tie chains cleaved during the final fracture process and in the distribution in the loading of these chains connecting the crystalline region of the polymer. Elongation of the sample prior to the onset of free radical production was found to increase with increasing NO_x concentration, the magnitude of the sustained stress, and the duration of exposure to the aggressive atmosphere, while the number of free radicals produced during this final loading to failure decreased.

In a study of sodium intercalated poly(paraphenylene), a linear dependence of the ESR peak-to-peak linewidth was found when the material was intercalated to saturation, and is apparently characteristic of metallic behaviour.¹⁷⁸ The conversion of the precursor, a sulfonium derivative of poly(*p*-phenylene–vinylene) (PPV), into films of the polymer was studied by XPS and ESR methods. XPS revealed that the PPV films may contain impurities on their surface, depending on the nature of the precursor used, while ESR showed a progressive disappearance of the radicals formed on the sulfur sites upon heating: these complementary studies allowed the determination of the optimum rate for the thermal conversion process, to enhance the purity of the PPV for its use in LEDs.¹⁷⁹ Another study of PPV, using ENDOR, was made using stretch-oriented polymers.¹⁸⁰ Detailed spectral line shape analysis reveals that the spin-density extends over about four phenyl rings, while the maximum and second largest spin-densities reside on the carbons of the vinylene sites. These features correspond well with those calculated from the theoretical distribution of polarons in the case of finite electron-correlation, and correspond to the spin defects being trapped polarons, as thought previously. The polaron spin-distribution also reproduces well the photo-induced ESR spectra of PPV, showing that polarons are photogenerated in the polymer. A French group have introduced controlled amounts of Fe^{3+} into HCl doped poly(aniline), and find that the broadening of the ESR signal from mobile spins

by collisions with the fixed paramagnetic impurities may be used to determine the polaron mobility.¹⁸¹ Another report is of different kinds of poly(aniline) and poly(aminopyridine) derivatives:¹⁸² poly(aniline) derivatives with a stable radical group along with iodine doped or oxidised poly(aminopyridine) showed one or two half-field peaks in their ESR spectra due to the $\Delta m_s = \pm 2$ transition, in addition to one at free-spin. Finally, I note a paper by David Keeble on ESR characterisation of diamond films, published from Dundee University.¹⁸³ These diamond films are made by hot-filament chemical vapour deposition using methane concentrations in the range 0.25–5%. The bulk spin-concentration and the peak-to-peak linewidth for the characteristic $g = 2.0027$ signal were found to increase with increasing methane concentration, and the results show that the spectrum contains components due to paramagnetic defects related to non-diamond carbon phases within the films.

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