

glycoside bond is slow or absent. If rotation was rapid, both ^{13}C - ^{13}C couplings would be more similar, representing an averaged value over a rotational itinerary.

Strategies similar to these have been extended to tri- and tetrasaccharides.^{55,56} The question of what effect the addition of new residues has on the conformations of existing *O*-glycoside bonds in a growing oligosaccharide is beginning to be addressed; we have observed one case where measurable changes in orientation occur.⁵⁶ It would be interesting to extend these studies to complexes of these oligosaccharides with receptor proteins through which they display their biological functions. Future studies will focus on questions related to solution conformations, further exploration of the effects of configuration and substitution on structure, and extensions to other complex systems, including oligo- and polynucleotides.

Concluding Remarks

This Account describes several studies that illustrate the value of stable isotopes as probes of chemical and biochemical reaction mechanisms and of the structures of complex biomolecules. While these studies focus on carbohydrates, the limiting factor in all studies with stable isotopes is the availability of compounds enriched at specific sites. Our studies were made possible by the development by the Los Alamos National Laboratory

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of methods for producing ^{13}C of 99+ % enrichment and our modification of the Kuhn method¹⁸ to introduce isotopes of carbon, hydrogen, and/or oxygen at various sites in a wide range of monosaccharides. Advances in NMR spectroscopy and mass spectrometry, as well as improved chemical and biochemical synthetic methods, point to an increasing role for stable isotopes in studies of the structures and reactivities of complex molecules, especially those of biological origin.

We have not discussed the use of carbohydrates enriched with stable isotopes for metabolic studies where they can be applied with apparent safety in humans and the environment, areas in which radioisotopes must be applied with caution. Although radioisotopes are more easily detected, they provide virtually no information about the chemical or physical nature of their molecular environment; they can report little but their presence.

The ability to selectively monitor an enriched atom(s) in a molecule as its chemical or physical environment changes is an important feature of studies with stable isotopes. Unlike other reporter groups, stable isotopes do not significantly perturb physical or chemical properties (with, of course, the exception of isotope effects), so the information they provide more reliably reflects reality. In our view, a substantial initial investment in developing synthetic methods was repaid many-fold by the ease with which complex questions of mechanism and structure could be addressed. In other systems, similar investments can be expected to pay similar dividends.

ESR Investigations of Molecular Cation Radicals in Neon Matrices at 4 K: Generation, Trapping, and Ion-Neutral Reactions

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The cations of most neutral parent molecules are radicals and hence amenable to ESR (electron spin resonance) study. However, their highly reactive nature, short lifetimes, and generation difficulties have been major obstacles to overcome in applying ESR and other spectroscopic methods to the major categories of cation radicals. Our experimental emphasis over the past several years has been to apply the rare gas (principally neon) matrix-isolation method to repre-

sentative and fundamentally important types of cation radicals which cannot be readily investigated by other approaches.¹ For the smaller cations, the ESR assignment is more definitive and detailed comparisons with *ab initio* theoretical calculations are possible. The ability to generate, characterize, and manipulate molecular ions in matrices or during the condensation process has enabled the study of ion-neutral reaction types which have previously eluded spectroscopic monitoring. Numerous questions concerning the chemistry of the upper atmosphere, interstellar clouds, and high-energy environments could be probed by this ion-neutral reaction approach described in this Account.

Open-shell cations are especially difficult to study in the gas phase by high-resolution microwave spectroscopy or LMR (laser magnetic resonance), and nuclear

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hyperfine interaction has been determined for only a few diatomics, H_2^+ ,² CO^+ ,³ HF^+ ,⁴ HCl^+ ,⁵ HBr^+ ,⁶ OH^+ ,⁷ and SH^+ ,⁸ and only one triatomic or larger cation, H_2O^+ ,⁹ although rapid progress is being made in this important area. Of these radical cations studied in the gas phase, two have also been investigated by ESR in neon matrices at 4 K, namely, $^{13}\text{CO}^+$ and H_2O^+ .¹⁰ The close agreement in the nuclear hyperfine parameters indicates that neon matrix shifts (2–3%) are quite small for such radical ions—an important matrix property that has also been demonstrated for numerous neutral radicals. Neon-matrix ESR spectra of ions is simpler to analyze than comparable gas-phase spectroscopy since at 4 K all transitions originate from ground-state energy levels. Obtaining sufficient numbers of ions for detection in matrices is also more controllable than in the gas phase since the deposition or sample collection time can be extended if necessary.

In a series of pioneering matrix ESR experiments, Kasai demonstrated in the late 1960s that atomic ion radicals (Mn^+ , Cd^+ , and Cr^+) and anions could be produced by the in situ visible-light photolysis of matrix-isolated neutral atoms and appropriate electron-acceptor species.¹¹ Mossbauer spectroscopy has also been applied to the study of atomic ions in rare gas matrices.¹² These earlier experiments on atomic cations and the highly innovative matrix approaches developed by Andrews,¹³ Jacox,¹⁴ Bondybey,¹⁵ Miller,¹⁵ and others for vibrational and electronic spectroscopic studies of charged species provided critical avenues of approach for our initial ESR matrix investigations of molecular cation radicals. A distinction has been made between isolated ions and chemically or coulombically bound types.¹³ All of the examples of cation radicals presented in this report are of the "isolated" type where cations and anions are trapped in different matrix cages separated by a large number of matrix atoms. The experimental methods we have employed for generating isolated cations have also produced isolated anion radicals including F_2^- ,¹⁶ Cl_2^- , HCl^- , HF^- , DF^- .¹⁷ Several chemically bonded anion radicals have been studied by rare-gas matrix ESR such as M^+C_2^- ,¹⁸ M^+HCl^- ,¹⁹ M^+Cl_2^- ,²⁰ M^+O_2^- ,²¹ M^+O_3^- ,²² and M^+SO_2^- ,²³ (where M^+ is

a metal ion), but apparently no previous matrix ESR results have been reported for isolated anion radicals except for a few examples in the earlier studies by Kasai.¹¹

The trapping of the $^{12,13}\text{CO}^+$ and $^{14,15}\text{NH}_3^+$ radicals in solid neon in 1982 was the first utilization of rare-gas matrices for the ESR investigation of molecular cations.^{1,24} Recent ESR studies have shown that NH_3^+ can be trapped in argon²⁵ and that transition-metal carbonyl cations can be generated in the heavier rare gases by γ -irradiation.^{26,27} Large organic cation radicals (relative to the sizes of radicals discussed in this Account) have been studied by ESR since 1979 in halogen-carbon matrices utilizing γ -irradiation.^{28,29} This Freon matrix procedure had been used previously for electronic spectroscopic studies of primarily aromatic cations.³⁰ ESR cation radical studies conducted in Freon and other more reactive media have been reviewed in recent articles and will not be covered in this account.³¹ These Freon matrix studies have yielded abundant and highly valuable information for the larger organic cation radicals.

For small highly reactive ion radicals, neon matrices have several advantages over Freons and other less inert media for ESR studies. The large ionization energy of neon (21.6 vs. 15.5 eV for argon) permits the trapping of cations having large electron affinities. Unsuccessful attempts to trap such small ions as CH_4^+ and H_2CO^+ in Freon matrices have been reported.^{32,33} Our experience has been that even argon matrices cannot trap molecular cation radicals having EA's above 10–11 eV. Neon's extreme chemical inertness, low polarizability, and large conduction band gap make it an ideal host for isolating highly reactive cation radicals.³⁴ The complications of extra hyperfine splittings caused by ion-matrix interactions do not occur for neon since practically none of its natural isotopes are magnetic. Freon and even krypton matrices are known to produce such extra hyperfine structure that in some cases can cause assignment problems or obscure useful information. Unresolved matrix hyperfine interactions and other effects produce ESR line widths in Freon matrices which are typically 20 times that observed in neon. The

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spectral quality we have observed for cation radicals in neon matrices has been sufficient for extracting all the magnetic parameters from a powder or randomly oriented sample with the help of sophisticated simulation programs and isotopic substitution. Typically, powder ESR spectra in other condensed media are not sufficiently resolved for such complete analyses. The limited temperature range (2–10 K) for neon compared to Freon matrices (2–150 K) is a disadvantage since motional averaging and other temperature-dependent effects can not usually be observed except in special cases. The necessity of 4 K operation and the high-vacuum conditions required cause the neon matrix method to be more time consuming and expensive than cation radical studies in Freon and other less inert matrices.

The class of purely inorganic radicals constitutes the least studied group of all molecular cation radicals in inert media. Except for the neon matrix results presented in this report, only two other inorganic cations have been studied by ESR, namely SnH_4^+ and N_2O_4^+ in Freon matrices.^{35,36} A convenient ESR summary of small inorganic cation and anion radicals trapped in frozen acid solutions, as defect centers in crystal lattices and other "reactive" media has been presented elsewhere.³⁷ The neon matrix isolation method coupled with the various ion-generation techniques discussed below is particularly well suited for nonvolatile compounds and should allow the study of numerous inorganic systems which have been previously inaccessible to ESR characterization.

Ion Formation and Matrix Trapping

Several ion-generation techniques have been made compatible with the formation of a solid neon matrix at 5 ± 1 K. The coupling of such high-energy generation conditions with the strict requirements of forming extremely delicate neon matrices only a few centimeters removed has required a considerable number of trial and error experiments. The number of isolated ion radicals required for reasonably intense ESR signals varies considerably depending upon the inherent A and g tensor anisotropy for each radical. Given the narrow line widths for neon samples we would estimate that roughly 10^{13} – 10^{14} ion radicals must be trapped as "free or isolated" ions in a matrix of $\sim 10^{20}$ neon atoms in order to obtain high-quality ESR spectra.

Matrix Apparatus. The basic design of the matrix isolation apparatus used with the various generation schemes described below is presented in Figure 1. A wide range of radical generation hardware can be accommodated, including high-temperature (>1000 K) vaporization flanges, a mass-selected ion source, an electron gun, a FAB (fast-atom-bombardment) source, laser sputtering, and vacuum UV resonance lamps for photoionization. For some depositions, two of these are required to operate simultaneously. The matrix target is moved vertically under high-vacuum conditions between the deposition position and the X-band ESR cavity by a simple hydraulic system. The matrix ap-

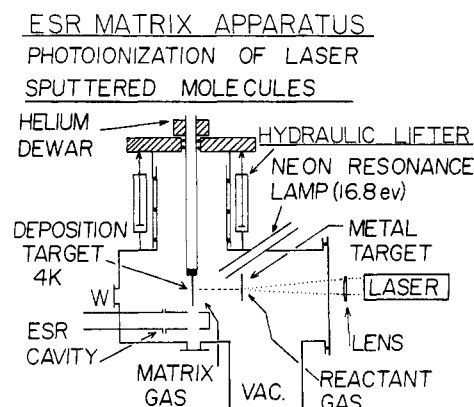


Figure 1. ESR apparatus for trapping ion radicals in neon matrices at 5 ± 1 K. Arrangement shown is for reactive laser sputtering generation of neutral and ion radicals as explained in the text. Neutrals are photoionized during matrix deposition with the 16.8-eV open-tube neon-resonance lamp.

paratus remains fixed in space while a 9-in. electromagnet ($3\frac{1}{2}$ -in. pole gap) mounted on tracks is rolled into position for recording ESR spectra. In addition to cryopumping, we have found that a high residual pumping capacity (500 L sec^{-1}) reduces scattering lost from molecular beams codeposited with the rare gas and facilitates the operation of the open-tube neon resonance lamp. A quadrupole mass spectrometer (0–500 amu) used for monitoring samples and background gases is permanently installed in the 4-in. pump-out line of the apparatus.

Photoionization. Both single and multiple photon ionization methods have been used in these matrix studies. An open-tube resonance lamp utilizing a continuous neon flow of 3 sccm and powered by a microwave generator irradiates the sample during the matrix deposition process. The predominant output of this source occurs at 16.8 eV, which is well above the ionization energy of neutral molecules and atoms. The lamp is a 9-mm o.d. quartz tube whose open end is located 5 cm from the deposition target. The active part of the discharge extends to the tube's open end, and the lamp is typically operated at 50-W forward and 2-W reflected power.

Electron Bombardment. Ion generation by electron bombardment (EB) offers more experimental flexibility than photoionization since both the electron flux and energy can be varied conveniently over wide ranges. A homemade hot filament type electron source with a single draw-out control grid is mounted in the matrix apparatus in place of the neon resonance lamp (see Figure 1). To overcome matrix charging effects, we have found it necessary to electrically isolate the 4 K matrix deposition target made of copper and maintain it at the same positive potential as the electron control grid. Overcoming the negative surface potential, which has been shown to be in the 25–35-V range, allows the electrons to ionize neutrals right at or just below the matrix surface. A detailed account of EB for producing matrix isolated cation radicals has been presented in the N_2^+ report.³⁸ The ESR spectra of $^{13}\text{CO}^+$, H_2O^+ and N_2^+ shown in Figures 2 and 3 were obtained by the EB matrix technique with an electron energy of 50 eV. For all cases studied thus far, the ESR spectra of cation

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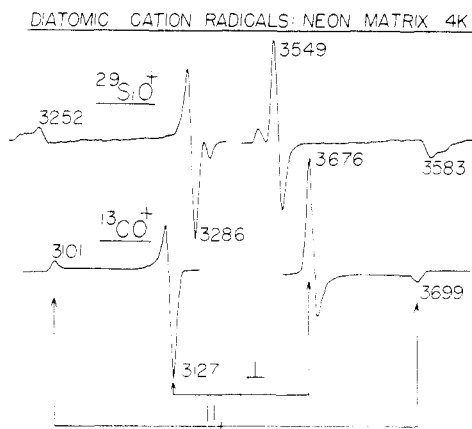


Figure 2. ESR spectra of the isovalent cation radicals, $^{13}\text{CO}^+$ and $^{29}\text{SiO}^+$, trapped in a neon matrix at 5 ± 1 K are shown. In both cases, the nuclear hyperfine pattern ($I = 1/2$) consists of a very anisotropic doublet for the parallel (\parallel) and perpendicular (\perp) directions relative to the bond axis. The magnetic field corresponding to the free spin value (g_e) is 3420 G.

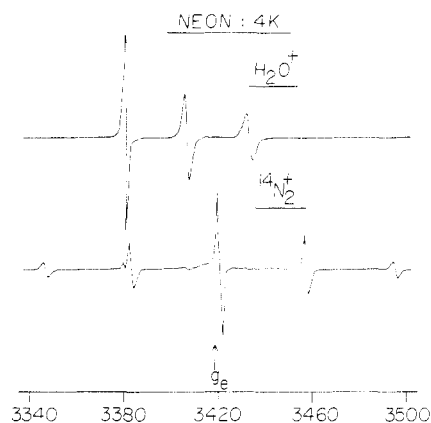


Figure 3. Neon matrix ESR spectra for H_2O^+ and $^{14}\text{N}_2^+$ generated by electron bombardment. Motional averaging effects are responsible for the isotropic nature of the spectra. The relatively large positive g ($g = 2.0093$ (3)) shift for H_2O^+ is apparent. The magnetic field corresponding to g_e is 3418. The $^{14}\text{N}_2^+$ quintet pattern results from two equivalent $I = 1$ nuclei; very weak H_2O^+ signals are barely detectable on the N_2^+ spectrum.

radicals generated by EB have been equivalent to photoionization results, although in some cases more intense ESR signals could be achieved with EB. Earlier matrix studies employed EB for the formation of negative ions, but no cations were identified.^{39,40}

Reactive Laser Sputtering. In addition to conventional high-temperature (>1000 K) vaporization,⁴¹ nonvolatile neutrals such as metal hydrides, fluorides, and nitrides can be generated by reactive laser sputtering. The reactant gas is passed over the metal target which is undergoing sputtering from focused laser pulses (see Figure 1). The highly reactive neutral intermediates generated by this process are ionized by photoionization or EB while being deposited into neon matrices at 4 K. The AlF and BF molecules have been generated by conventional high temperature methods and by reactive laser sputtering. The observed ESR spectra of their radical cations have been shown to be identical for these two independent sources (see Figure

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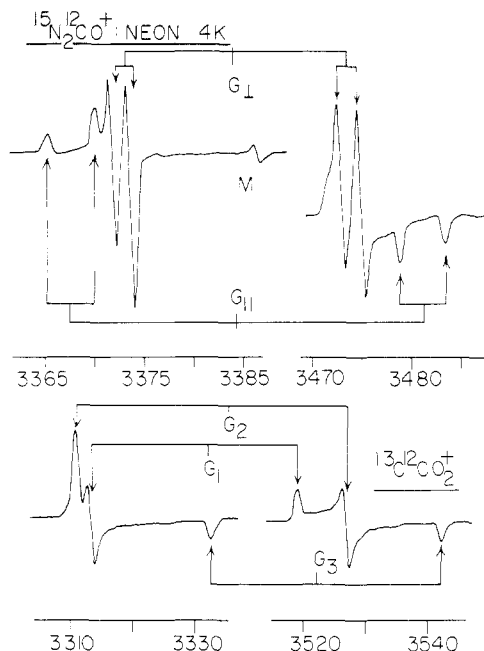


Figure 4. ESR spectra for the molecular cation radicals $^{15}\text{N}_2\text{CO}^+$ and $^{12,13}\text{C}_2\text{O}_2^+$. These unusual molecules were generated by ion-neutral reactions ($\text{CO}^+ + \text{N}_2$ and $\text{CO}^+ + \text{CO}$) during the neon matrix deposition process. Large ^{15}N ($I = 1/2$) and ^{13}C ($I = 1/2$) nuclei hfi is readily apparent as well as the inequivalency of the ^{15}N atoms in $^{15}\text{N}_2\text{CO}^+$. The lowest field methyl quartet line is denoted "M" in the upper spectrum. The magnetic field position of g_e is 3422 G.

5).⁴² In some instances, such as AlH^+ , we have observed matrix-isolated ions by laser sputtering alone.⁴³ Similar experiments have also utilized fast-atom neutral bombardment (FAB) for generating reactive neutrals and ions for matrix ESR studies.⁴⁴ The production of refractory neutrals, including MoN (4Δ) for matrix ESR study, by ion-sputtering techniques has been reported.⁴⁵⁻⁴⁹

The Trapping Process. The detailed mechanism of the ion-trapping process with the above generation methods is not completely understood, but we have demonstrated that both cations and anions are trapped as isolated ions in the rigid neon lattice, which prevents molecular or even atomic diffusion as long as the lattice temperature is maintained below 8–10 K. The most likely anions produced from background impurity gases are either closed-shell molecules such as OH^- or radical anions not having the proper electronic ground state for ESR detection in rare-gas matrices like N_2^- or O_2^- ($X^2\Pi$).⁵⁰ To obtain experimental evidence supporting this ion formation mechanism and to ascertain that free or isolated anions are present, we have intentionally

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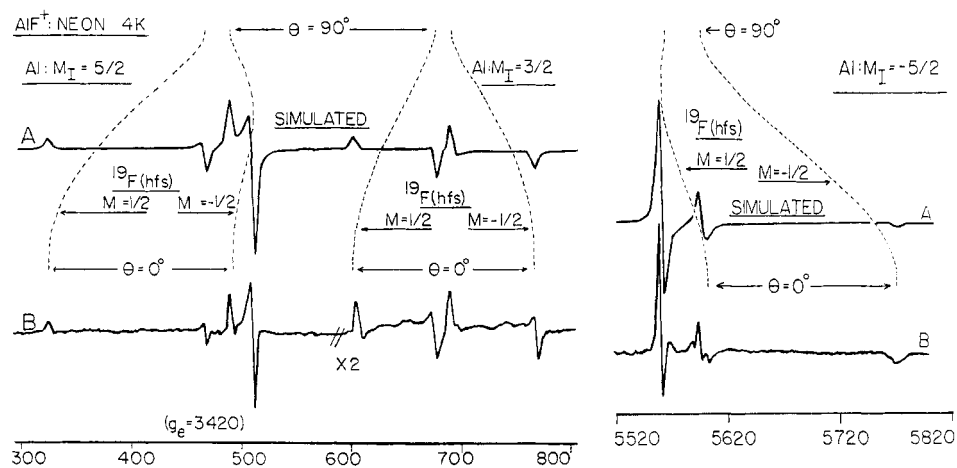


Figure 5. Two lowest field and the highest field Al hyperfine lines for the $\text{AlF}^+ 2\Sigma$ radical. Highly anisotropic ^{19}F hfs is apparent for each M_I value of Al. The dashed curves give H_{Res} vs. θ between the $\theta = 0$ or the parallel limit and $\theta = 90^\circ$, the perpendicular limit. Note that strong "extra" transitions are observed at intermediate angles at the positions predicted by the H_{Res} vs. θ plots. The simulated spectra and H_{Res} vs. θ results were obtained from an exact diagonalization treatment of the 24×24 spin determinant. See ref 42.

doped the neon matrix gas with trace amounts of an electron acceptor species such as $\text{F}_2(\text{g})$ whose anion is known to have an $X^2\Sigma$ ground state.⁵¹ This F_2 experiment and others involving the intentional introduction of an electron-acceptor species have produced excellent quality ESR spectra of isolated anion radicals.¹⁶ The arguments for classifying these as "isolated or free" ions rather than chemically bonded ion pairs in the same matrix cage (i.e., $\text{M}^+\text{Cl}_2^{-20}$) are based on the following considerations. No ^{19}F hyperfine splitting is observed on the various cation signals also present in the same matrix sample even though the atomic hf parameters for ^{19}F are extremely large. The observed ESR line widths are small, less than one gauss for some lines. No metal atoms with low-ionization energies are present in the matrix to produce stable ion pairs.

Ion-Neutral Reactions. Possibly, the most interesting and useful application of these matrix-ion methods is the study of ion-neutral reactions which are extremely difficult to characterize by other spectroscopic procedures. By carefully controlling the concentration of neutrals deposited into neon matrices under ionizing conditions, we have been able to generate and identify several ion-neutral product radicals not previously reported: $\text{CO}^+ + \text{CO} \rightarrow \text{C}_2\text{O}_2^+$;⁵² $\text{N}_2^+ + \text{CO} \rightarrow \text{N}_2\text{CO}^+$ and $\text{N}_2^+ + \text{N}_2 \rightarrow \text{N}_4^+$.⁵³ Under high-dilution conditions, ESR signals are observed only for the reactant ions (N_2^+ or CO^+). As reactant concentrations are gradually increased on a series of separate depositions, the reactant ion signals decrease while product ion radical ESR signals grow in. Formation of such ion products probably occurs during condensation when an ion and a neutral happen to be trapped in the same matrix cage. Given the experimental flexibility to produce nonvolatile metal atoms and ions with the matrix equipment described above, we plan to study several types of metal ion codeposition reactions with small reactant molecules.

Details of this matrix approach for studying ion-neutral reactions have been presented in the C_2O_2^+ report where six different isotopic product ions were used to confirm the spectral assignment.⁵² ESR spectra for isolated $^{13}\text{CO}^+$ and the ion-neutral product ion, $^{12,13}\text{C}_2\text{O}_2^+$, are shown in Figures 2 and 4, respectively. The geometry of C_2O_2^+ has been determined jointly from these ESR measurements and theoretical calculations (CI) to be planar (OCCO) with a CCO bond angle of approximately 141° and C-C and C-O bond lengths of 1.58 and 1.14 Å, respectively. The unpaired electron is delocalized over the entire molecular framework as revealed by nuclear hyperfine structure. Figure 4 presents the ESR spectrum of $^{15}\text{N}_2\text{CO}^+$ in neon at 4 K. The doublet of doublets hyperfine pattern for the resolved g components shown clearly indicates the inequivalency of the two $^{15}\text{N}(I = 1/2)$ atoms.

Photobleaching. The photobleaching of matrix-isolated ions has been described previously for ESR and electronic and vibrational matrix studies.^{1,54,55} ESR studies of the ions discussed in this report show that low-intensity visible light photolysis of a deposited matrix sample can eliminate ESR signals of the trapped ion but does not affect the intensity of most neutral radicals. Visible photons are not sufficiently energetic to break most bonds but can photoionize the anion traps whose ionization energies are in the 1-3-eV range. The electrons produced travel throughout the solid lattice and neutralize all types of cation radicals in a nonselective manner. The photobleaching effect is an extremely important tool in developing ion generation methods especially for the production of new molecular cation radicals. Intensity changes in the ESR spectrum before and after visible light photolysis dramatically indicate which signals can be associated with ion radicals. This is an extremely important capability since numerous unknown neutral radicals are often produced under high-energy deposition conditions.

Ion Radicals Investigated

Table I lists the molecular cation radicals reported by our group along with their magnetic parameters and generation techniques employed. In addition to these,

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Table I
Molecular Cation Radicals Studied by ESR in Neon Matrices at 4 K
 nuclear hyperfine interaction, A tensor, MHz

cation radicals	g tensor		magnetic nuclei		generation methods ^a
	g_{\parallel}	g_{\perp}	A_{\parallel}	A_{\perp}	
$^{12}\text{C}^{16}\text{O}^+$, $^{13}\text{C}^{16}\text{O}^+$ ^b	2.0010 (2)	1.9993 (2)	1665 (2)	1527 (2)	photoion, EB, FAB, CB
$^{12}\text{C}^{17}\text{O}^+$ ^c	$g_{\text{iso}} = 2.0004$ (2)		47.6 (9)	-51.5 (6)	
$^{14}\text{N}_2^+$, $^{15}\text{N}_2^+$ ^d			$A_{\text{iso}} = 104.1$ (6)		photoion, EB, CB, FAB, MPI
$^{27}\text{Al}^{19}\text{F}^+$ ^e	2.0015 (5)	2.0000 (5)	2893 (8)	2782 (6)	laser vap. with photoion;
			473 (5)	90 (5)	thermal vap. with photoion and EB
AlH^+ / AlD^+	2.0018 (3)	1.9996 (3)	1685 (2)	1537 (2)	reactive laser sputtering
			447 (2)	440 (2)	
			68 (1)	66 (1)	
$^{11}\text{BF}^+$, $^{10}\text{BF}^+$ ^f	2.0012 (3)	2.0004 (3)	1784 (1)	1727 (1)	laser vap. with photoion;
			410 (1)	152 (1)	thermal vap. with photoion
$^{28}\text{Si}^{16}\text{O}^+$, $^{29}\text{Si}^{16}\text{O}^+$ ^h	2.0012 (2)	2.0000 (2)	597 (1)	578 (1)	thermal vap. with photoion;
			-924 (1)	-733 (1)	thermal vap. with EB
$^{26}\text{Mg}^+$	2.0016 (3)		$A = 620$ (1)		thermal vap. with in-situ
	[2.0006 (4) (argon)] ^l		[$A = 595$ (1) (argon)]		laser MPI and photoion
$^{111}\text{Cd}^+$, $^{113}\text{Cd}^+$ ^k	2.0005 (6)		[$A = 14,385$ (5) (argon)] ^l		FAB
	[2.0006 (2) (argon)] ^l		[$A = 15,048$ (5) (argon)]		
H_2O^+ , $\text{H}_2^{17}\text{O}^+$, D_2O^+ ^m	$g_{\text{iso}} = 2.0093$ (3)		$ A_{\text{iso}} = 73.7$ (6)		photoion
HDO^+			$ A_{\text{iso}} = 11.5$ (3)		
			$ A_{\text{iso}} = 83.5$ (6)		
$^{14}\text{NH}_3^+$, $^{15}\text{NH}_3^+$ ^b	2.0032 (2)		54.9 (6)		
			76.8 (6)		
			77.1 (6)		
C_2O_2^+ , $^{13}\text{C}_2\text{O}_2^+$, $^{12}\text{C}^{13}\text{CO}_2^+$,	$g_x = 2.0034$ (2)		$A_x = 577$ (1)	$A_y = 606$ (1)	$A_z = 583$ (1)
$\text{C}_2^{17}\text{O}_2^+$, $\text{C}_2^{16,17}\text{O}_2^+$,	$g_y = 2.0019$ (2)		$A_x = \sim 0$	$A_y = 74$ (1)	$A_z = \sim 0$
$^{12,13}\text{C}_2^{16,17}\text{O}_2^{+n}$	$g_z = 1.9912$ (2)		$A_x = 363.4$ (6); $A_y = 376.7$ (6); $A_z = 376.3$ (6)		
$\text{H}_2^{12}\text{CO}^+$, $\text{H}_2^{13}\text{CO}^+$ ^o	$g_x = 2.0069$ (2)				
	$g_y = 2.0015$ (2)				
	$g_z = 2.0025$ (2)		$A_x = -123.3$ (6); $A_y = -104.5$ (6); $A_z = -98.4$ (6)		
$^{12}\text{CH}_4^+$ ^p	$g_{\text{iso}} = 2.0029$ (3)		$A_{\text{iso}} = 153.6$ (6)		photoion, EB
$^{12}\text{CH}_2\text{D}_2^+$ ^p	$g_{\text{iso}} = 2.0029$ (3)		$A_{\text{iso}} = 341$ (1)		photoion, EB, FAB,
			$A_{\text{iso}} = -6.2$ (2)		laser MPI
					photoion, EB

^a Photoion—photoionization at 16.8 eV with open tube neon resonance lamp; EB—electron bombardment (20–100 eV); FAB—fast-atom bombardment (neon, 3–10k eV); CB—cation beam; MPI—multiphoton ionization (laser); Vap.—vaporization; RLS—reactive laser sputtering. ^b Reference 1. ^c Reference 24. ^d Reference 38. ^e Reference 42. ^f Reference 43. ^g Reference 42. ^h Reference 41. ⁱ Reference 56. ^j Reference 57. ^k Reference 44. ^l Reference 11. ^m Reference 10. ⁿ Reference 52. ^o Reference 64. ^p Reference 61.

unambiguous neon-matrix ESR spectra have been obtained for the following paramagnetic cations and anions that will be analyzed and compared to theoretical calculations in future reports: F_2O^+ , $^{14}N_4^+$, $^{15}N_4^+$, $^{14}N_2^{12}CO^+$, $^{15}N_2^{13}CO^+$, $^{14}N_2^{13}CO^+$, $F_2^{12}CO^+$, $F_2^{13}CO^+$, PH_3^+ , $^{13}CH_4^+$, CD_4^+ , $^{13}CH_3D^+$, CHD_3^+ , CH_3F^+ , CH_3CHO^+ , $^{13}CH_3^{13}CHO^+$, CH_3CDO^+ , $C_6F_6^+$; F_2^- , HF^- , DF^- , $H^{35}Cl^-$, $H^{37}Cl^-$, $^{35}Cl_2^-$, $^{35,37}Cl_2^-$, F_2CO^- and $F_2^{13}CO^-$.

Very interesting results have also been achieved for the magnesium cluster cation radicals, Mg_N^+ ($N = 2-6$), which is one of the first such series investigated by any spectroscopic method.⁵⁶ Nuclear hyperfine patterns for the 10% natural abundance of ^{25}Mg ($I = 5/2$) occurring in these clusters allow molecular assignments to be made. The Mg_2 molecule with a zero formal bond order is bound only by van der Waals forces but Mg_2^+ missing one antibonding electron has a bond order of one half. The unpaired electron in Mg_2^+ ($X^2\Sigma$) occupies a MO composed primarily of 3s orbitals on each magnesium atom. A_{iso} for the atomic ion is 213 G in neon compared to 221 G in argon,⁵⁷ while A_{iso} for $^{25}Mg^+$ in $^{25}Mg_2^+$ is 103 G in argon. Similar A values for two different matrices provide additional evidence that absolute matrix shifts are small for isolated ions.

In these initial studies where emphasis had to be placed on the development of independent-matrix-generation schemes, small ions were selected for study that have high natural abundances of magnetic isotopes or ones amenable to multiple isotopic substitution to confirm the spectral assignment through their characteristic nuclear hyperfine splitting patterns. However, most any ion regardless of size can be studied by these generation and trapping methods. The availability of ab initio CI or other reliable theoretical calculations of the expected nuclear hfi (A tensor) is extremely valuable in determining the reasonableness of a particular assignment and in interpreting the measured ESR parameters in terms of electronic structure information. Such rigorous comparisons between calculated and observed one-electron properties also assist in the development of theoretical methods as they apply to a wide range of physical properties.

Experiment vs. Theory

The simple free-atom-comparison method (FACM) has been applied in most all ESR investigations to obtain electron structure information.³⁷ Although it is clearly recognized that FACM is an approximate procedure, there has not been a systematic effort to determine its accuracy nor to identify radical types where its application would be inappropriate. It is difficult to estimate the accuracy of orbital occupancies obtained from FACM in an LCAO description of the unpaired electron since such information is not usually available from other independent experimental methods. Also, the lack of nuclei hfi from all the atoms in the various radicals studied by ESR has prevented internal consistency checks in most cases. In our studies of simple diatomic neutral and cation radicals we have tried to evaluate FACM results in the following manner. The complete wave function is obtained from state of the art theoretical calculations (ab initio CI with millions of configurations). The integrity of the wave function

calculated in the usual manner using the energy criterion is determined by using it to compute values of A_{iso} and A_{dip} which can be compared directly with experimental results. Once it is established that the calculated ψ yields A values within 10–15% of the observed (not a trivial exercise), a Mulliken type population analysis involving reduction to a minimum basis set is conducted on the theoretical ψ to obtain valence orbital occupancies for the unpaired electron.⁴¹ These theoretical populations, obtained from a "tested" ψ , are then compared with results of the FACM procedure illustrated in the next paragraph.

Values of A_{iso} and A_{dip} for a given magnetic nucleus can be obtained in a straightforward manner from the measured nuclear hyperfine A tensors.

$$A_{iso} = \frac{8}{3}\pi g_e g_n \beta_e \beta_n \langle \delta(r) \rangle$$

$$A_{dip} = \frac{1}{2}g_e g_n \beta_e \beta_n \left\langle \frac{3 \cos^2 \theta - 1}{r^3} \right\rangle$$

All symbols have their standard meaning and the averages are over the spin density. The effects of L-I terms can be ignored in cases where the g values do not deviate significantly from g_e . For small radicals where d orbitals are not involved, the magnitudes of A_{iso} and A_{dip} reflect the amount of unpaired "s" and "p" character, respectively. FACM orbital occupancies are obtained by comparing experimental values of A_{iso} and A_{dip} with a set of atomic parameters for the free atom. For example, one obtains a C 2s character of 0.42 for the MO containing the unpaired electron in $^{13}CO^+$ by comparing the observed A_{iso} value of 1570 MHz for ^{13}C with the calculated A_{iso} value of 3780 MHz for a free carbon atom; a similar comparison for A_{dip} yields a C 2p_z character of 43%. Such FACM results will now be compared with theoretical populations for four related radicals.

$^{13}CO^+$, $^{29}SiO^+$. Extensive theoretical and experimental studies have been conducted on these two interesting isovalent cation radicals in an attempt to monitor bonding changes that occur as Si replaces C in a simple molecule.^{1,3,24,41} Based on a set of commonly used atomic parameters for the neutral ^{13}C and ^{29}Si atoms,⁵⁸ the FACM results for the valence s/p_z orbitals are 0.42/0.43 for $^{13}CO^+$ and 0.17/0.56 for $^{29}SiO^+$. These FACM populations indicate a large increase in p_z hybridization going from carbon to silicon which is not the expected trend between first- and second-row elements.

The theoretical s/p_z occupancies obtained from a Mulliken population analysis conducted on wave functions which yield "molecular" A_{iso} and A_{dip} values within 10% of the observed magnetic parameters are 0.51/0.41 for $^{13}CO^+$ and 0.30/0.27 for $^{29}SiO^+$. Note that the FACM and theoretical results show reasonably close agreement for CO^+ but significant disagreement for SiO^+ . Even if the atomic silicon parameters used in the FACM procedure were corrected for positive charge effects, there would still remain large discrepancies between the two methods. The theoretical populations for $^{29}SiO^+$ are consistent with the expected trend toward less hybridization in the second row.

It has been determined that FACM results differ from the theoretical populations because FACM does

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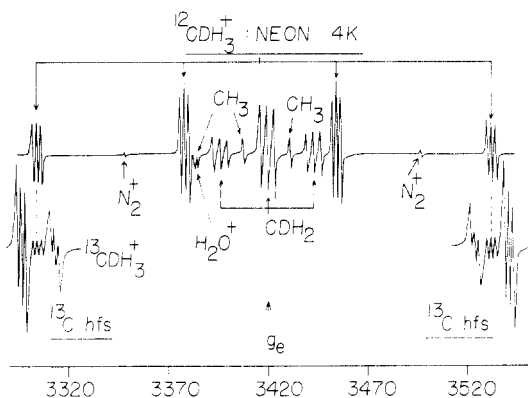


Figure 6. Neon matrix ESR spectrum of $^{12}\text{CDH}_3^+$ consisting of a widely spaced quartet resulting from 3 H atoms which appear equivalent because of dynamic Jahn–Teller effects. Each quartet line shows a D ($I = 1$) triplet nuclear hyperfine pattern. The lowest and highest field lines of $^{13}\text{CDH}_3^+$ are shown and reveal ^{13}C ($I = 1/2$) hyperfine doublets. The first and fifth lines of the $^{14}\text{N}_2^+$ quintet resulting from background impurities are marked.

not consider the involvement of core orbitals on silicon.⁴¹ It is not simple core polarization, but the effects on the core caused by unpaired valence overlap from the adjacent oxygen atom. The magnitude of this core–other valence overlap effect is primarily related to the unpaired density in the O $2p_z$ orbital lying along the bond axis. In CO^+ , the oxygen $2p_z$ character is considerably less than in SiO^+ and the 1s carbon core is not as susceptible as the 2s silicon core. Also, in CO^+ there is no inner 2p core.

AlF⁺, AlO. In radicals like AlF^+ , isoelectronic to SiO^+ , where spin density in the F $2p_z$ orbital is known experimentally to be small (<9%), the core–other valence overlap effect will not be large. In such cases of small spin density on the adjacent atom, FACM and theoretical populations should show closer agreement. Theoretical s/p_z results for Al in AlF^+ are 0.74/0.18 compared to 0.61/0.26, using FACM based on charge-adjusted atomic values of A_{iso} and A_{dip} .⁴² The FACM 3s population in AlF^+ based on A_{iso} for neutral Al is 0.72 which is practically identical with the theoretical value of 0.74. For the isoelectronic neutral radical AlO ,^{59,60} where the O $2p_z$ character has increased to 0.31, the core–other valence overlap effect is probably responsible for causing the FACM values of 0.17/0.38 to deviate significantly from the theoretical s/p_z values of 0.41/0.26. These results, although limited in the number of radicals represented, provide some guidance in knowing when to expect large errors in the FACM procedure for estimating orbital occupancies, assuming of course that the theoretical populations are more correct.

CH₄⁺, ¹³CDH₃⁺, CD₂H₂⁺. Another type of joint experimental–theoretical interpretation of radical ion ESR results has been illustrated in a preliminary report describing extreme isotopic and Jahn–Teller effects in CH_4^+ and CD_2H_2^+ .⁶¹ These investigations have been extended to all H/D isotopic combinations of the methane cation and one doubly labeled isomer, $^{13}\text{CDH}_3^+$, whose ESR spectrum along with $^{12}\text{CDH}_3^+$ is shown in Figure 6. Ionization of CH_4 , involving elec-

tron loss from the 3-fold degenerate t_2 orbital, can lead to three possible Jahn–Teller types of distortion for the resulting methane cation: C_{2v} , D_{2d} , and C_{3v} . Previous experimental studies have not been able to unambiguously assign the ground electronic state of CH_4^+ . The ESR results for CD_2H_2^+ are quite dramatic in showing the presence of two inequivalent groups of two H-atom positions which is consistent only with the C_{2v} ground-state assignment. Ab initio calculations yield A_{iso} values of -17 and 137 G for the two types of H atoms compared to experimental values of -15 and 122 G, respectively. The two H atoms with the small negative A_{iso} lie in the nodal plane of the p orbital on carbon which contains about 55% of the spin density according to ^{13}C A tensor results recently obtained in our laboratory; the other two hydrogens lie along a line parallel to the axis of this p orbital and their large positive A_{iso} value is consistent with symmetry-allowed mixing with this carbon p orbital.

Dynamic Jahn–Teller or fluxional behavior is observed for CH_4^+ which causes the H atoms to appear equivalent with an average A_{iso} value of 55 gauss. A similar occurrence is observed for CD_4^+ and the mixed H/D isotopic combinations exhibit weighted averages of the two different sites in the C_{2v} geometry. The complex dynamic effects occurring in the methane cation as revealed by these ESR matrix results at 4 K have been analyzed in a recent theoretical calculation of the ground-state potential-energy surface.⁶²

Concluding Remarks

Several ion-generation and ion–neutral reaction techniques for matrix-isolation ESR spectroscopy have been presented along with a summary of results for the first molecular cation radicals studied in rare-gas matrices. The methods allow the investigation of highly reactive and fundamentally important cations which are practically impossible to manage by other existing procedures. The wide variety of generation methods developed permits most any type of cation or anion radical to be studied, especially the class of nonvolatile inorganic cations. A method for studying ion–neutral reactions has been discussed.

The formation of unexpected and previously unreported small neutral radicals has made development of the various ion generation schemes particularly interesting and exciting. For example, in attempting to trap BH^+ in neon by the electron bombardment of B_2H_6 , the previously unreported HBBH species was detected by ESR and found to have a $^3\Sigma$ ground state as predicted by theoretical calculations.⁶³ Additional attempts to produce BH^+ by laser sputtering boron under $\text{H}_2(\text{g})$ flow and photoionization also failed but yielded instead the B_2 molecule, which has not been previously observed by ESR and whose ground state has been a subject of debate. These ESR results clearly establish B_2 as a $^3\Sigma$ radical with a small zero field parameter (D value) of approximately 0.14 cm^{-1} . A third example of an unexpected neutral radical was observed in the magnesium cation cluster work already described. A neutral magnesium cluster radical with a triplet elec-

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tronic ground state was observed; this is especially interesting since the Mg atom has the closed-shell configuration ($---3s^2$).

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Chemistry and Structure of Alcohol Dehydrogenase: Some General Considerations on Binding Mode Variability

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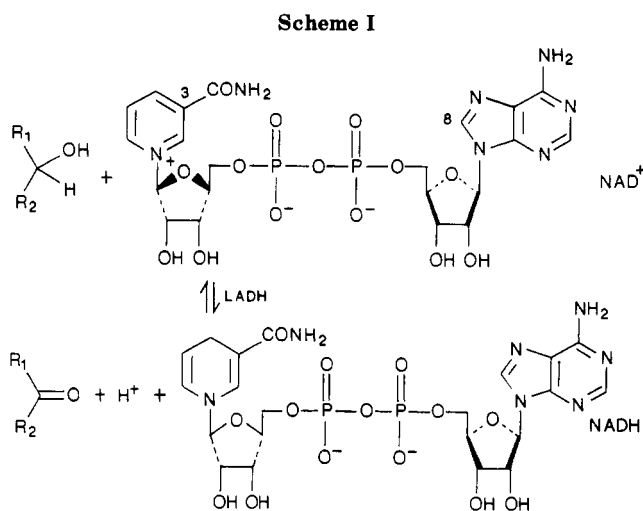
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Liver alcohol dehydrogenase (LADH) (E.C.1.1.1.1) provides a superb example of collaborative interaction between protein crystallographers and bioorganic chemists.¹ During the stages of the structural determination of its isoenzyme EE, the collaboration evolved from preparing compounds for use as tools in the structure determination to a stage where the enzyme structure itself was used to design molecules which might bind to the enzyme. In this Account we describe work involving the crystallography group of Dr. C. I. Bränden (Uppsala) and our own group in Strasbourg.²

Liver alcohol dehydrogenase belongs to the major pathway of alcohol metabolism³ (Scheme I). No other physiological role has been firmly established for this enzyme. The enzyme is a dimer composed of two identical subunits, each containing two zinc ions. One of these ions plays a structural role. The other, called "active site zinc", plays a role in the oxidation-reduction as a Lewis acid. It is coordinated to Cys-46 and -174 and His-67. Alcohol dehydrogenase belongs to the group of metal-containing NAD(P)⁺-dependent dehydrogenases, which also includes sorbitol dehydrogenase⁴ and glycerol dehydrogenase.⁵

The structure of LADH is presented in Figure 1.⁶ The two subunits interact through their nucleotide binding domains. The nucleotide binding domain presents general features which have been conserved in the evolution of the nucleotide-dependent enzymes.⁷ The coenzyme NAD(H) binds to the nucleotide binding domain and the nicotinamide ring extends towards the catalytic domain. This catalytic domain determines not only the catalytic events but also the substrate specificity. The presence of a large hydrophobic barrel explains why the enzyme acts on a great variety of ali-



phatic and cyclic ketones, aldehydes, and alcohols,⁸ represented in a diamond lattice.^{9,10} The fact that the

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(2) Additional collaborative efforts on other aspects of LADH have involved the groups of Prof. M. Zeppezauer (Sarrebruck) and Prof. B. V. Plapp (Iowa), but the present report will be focused on areas of interest to the Strasbourg group.

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