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Communication

An *in situ* electrochemical cell for Q- and W-band EPR spectroscopy

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ABSTRACT

A simple design for an *in situ*, three-electrode spectroelectrochemical cell is reported that can be used in commercial Q- and W-band (ca. 34 and 94 GHz, respectively) electron paramagnetic resonance (EPR) spectrometers, using standard sample tubing (1.0 and 0.5 mm inner diameter, respectively) and within variable temperature cryostat systems. The use of the cell is demonstrated by the *in situ* generation of organic free radicals (quinones and diimines) in fluid and frozen media, transition metal ion radical anions, and on the enzyme nitric oxide synthase reductase domain (NOSrd), in which a pair of flavin radicals are generated.

1. Introduction

EPR spectroscopy is a powerful technique for the study of electron transfer reactions because one-electron transfer must involve paramagnetic species. Redox generation of materials for EPR can be performed *ex situ* by chemical or electrochemical means followed by transfer to the spectroscopic cell. However, *in situ* electrochemical generation is preferable, giving access to less stable radicals. A comprehensive review of cell designs for *in situ* electrochemical EPR has been published by Compton and co-workers [1]. The vast majority of such cells are for X-band (ca. 9 GHz) spectroscopy, since this is the most common microwave frequency employed in continuous wave EPR, and have been developed for both static and flow (giving access to kinetic information) experiments and different resonator designs. However, in our electrochemical EPR work we have often found poorly resolved spectra from frozen solutions of electrochemically generated paramagnets at X-band due to the limited field resolution of the *g*-matrix. This motivated us to develop an *in situ* electrochemical cell for higher frequency EPR. In addition to enhanced field and orientation resolution of *g*-values, higher frequencies also offer advantages in, for example, detection of $S > 1/2$ species with large zero-field splitting, accessing faster motional dynamics, and higher sensitivity for small samples.

X-band resonators have ample space for design of three-electrode cells and hence proper control of potential and segregation of electrodes. Higher microwave frequencies present severe space limitations when using resonant cavities, scaling with the wavelength. Simple *in situ* electrochemical experiments have been described at Q-band using crude two electrode cells [2], but this gives no control over potential and is only really of use for simple single redox event species. Here we detail a simple three-electrode *in situ* electrochemical cell which can be used in commercial Q- and W-band spectrometers (ca. 34 and 94 GHz, respectively). The cell is suitable for variable temperature use, allowing chilling and/or freezing of the samples (during or post-electrolysis) and hence access to the anisotropic *g*-values. We illustrate the use of the cell with coordination chemistry, free radical and biological examples.

2. Results and discussion

Most EPR spectroelectrochemical cells (for X-band spectroscopy) are based on grid, coil or cylinder working electrodes, ensuring a high surface area and efficient electrolysis [1]. This is not possible for Q- and W-band (on commercial spectrometers with resonant cavities) because of the space restrictions: the inner diameter of the sample tubing is 1.0 and 0.5 mm for Q- and

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W-band, respectively. Hence, we have built a three-electrode assembly based on simple narrow wires (A–M Systems) as electrodes, using Teflon coated platinum (0.20 and 0.13 mm coated and uncoated diameters, respectively) and silver wires (0.18 and 0.13 mm coated and uncoated diameters, respectively) for working and quasi-reference electrodes [3], and a naked platinum wire (0.125 mm) as counter electrode (Fig. 1), which can be applied to standard EPR tubes.

The cells were constructed using standard quartz tubing, for use in Bruker Q-band (ER5106QT) and W-band cylindrical TE_{011} resonators for continuous wave EPR spectrometers.

The bottom 1 cm of the Teflon coated wires were stripped (using an Eraser International Ltd., RT2S fine wire stripper), and the electrodes staggered in vertical position as in Fig. 1. The working electrode is positioned lowest such that the redox product of interest is generated at the bottom of the tube and well separated from the counter electrode. There is very little diffusion of electrogenerated species within the tubes given their narrow diameters. The naked platinum wire counter electrode ensures a much greater surface area than the working electrode, while the Teflon coating on the working and reference electrodes prevents short-circuiting. The electrodes are soldered to narrow three-core microphone wire which is fed through the hollow sample rod, the top end of which can be sealed air tight by O-ring and collet (Figs. 1 and S1, see Supplementary information) hence allowing cryogenic work (see later). The potential was controlled with a portable microAutolab II potentiostat. For the materials studied spectra were visible after a few minutes of electrolysis, but spectra were measured after ca. 20 min to ensure efficient generation.

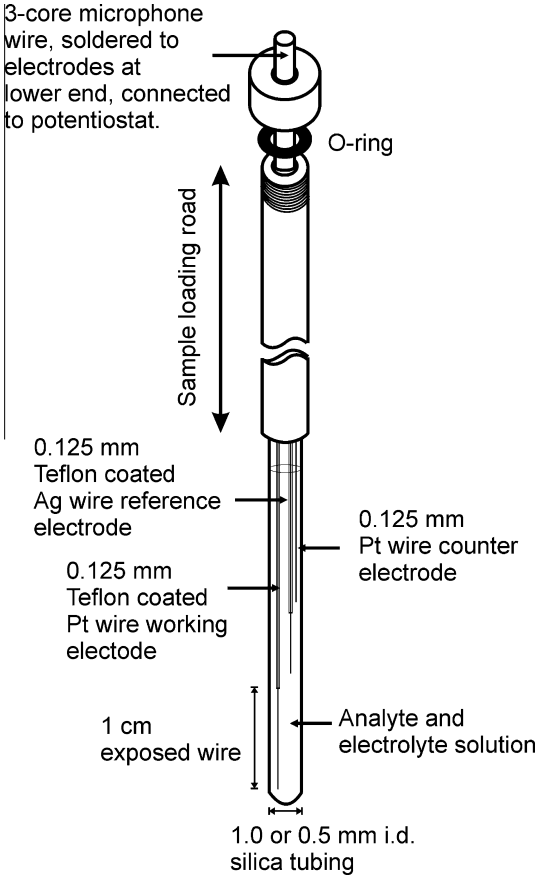

Fig. 1. Schematic diagram of Q/W-band EPR *in situ* spectroelectrochemical cell (lateral dimension not to scale for the sake of clarity).

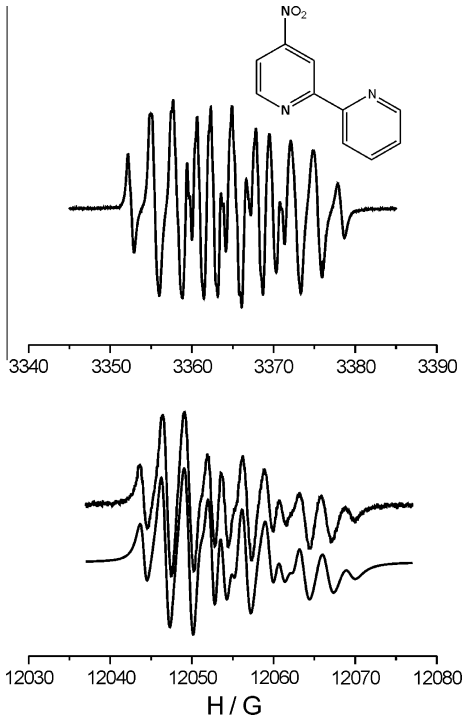

Fig. 2. X- and Q-band (top and bottom, respectively) EPR spectra of $[4\text{-}NO_2\text{-bipy}]^-$ generated by *in situ* electrolysis of a 0.1 M ($^n Bu_4N$)(BF_4) dmf solution at −1.5 V and 233 K. Microwave power 1.2 mW at Q-band. Lower: simulation [12] as described in the text, with a linewidth of 0.084 mT and a rotational correlation time $\tau_c = 7.9 \times 10^{-10}$ s (anisotropic parameters estimated from related compounds; W-band spectra are in Fig. S3, ESI).

We tested the cell on a well studied material [4,5], the biologically relevant radical anion of ubiquinone for which the *g*-anisotropy is unresolved at X-band frequency. *In situ* electrolysis at −0.5 V (all potentials quoted vs. Ag/AgCl) and 293 K in 0.1 M ($^n Bu_4N$)(PF_6) CH_2Cl_2 solution gives a fluid solution Q-band spectrum (Fig. S2) that can be simulated with identical proton hyperfine coupling constants to those reported from X-band studies [4]. On freezing the solution at 160 K, a near axial spectrum is observed (Fig. S2) with *g*-values ($g_\perp = 2.005$, $g_{||} = 2.002$) in good agreement with the W-band values reported for the chemically reduced species by Möbius and co-workers [5].

We illustrate the use of the cell with three further examples. The first two concern a family of α-diimines that we have been studying for some time, partly for their potential as components of solar dyes when complexed to electron rich transition metal ions [6], including by EPR in order to probe their donor and acceptor states [7–10]. We first look at one of the free ligands, and then a complex with platinum(II).

(i) $4\text{-}NO_2\text{-}2,2'$-bipyridine ($4\text{-}NO_2$-bipy; Fig. 2) undergoes reversible one-electron reduction at −0.72 V. We have previously studied this process at X-band [10] using a slim flat cell design [11] (platinum gauze working electrode) that allows variable temperature operation. Using the new capillary cell at X-band gives identical fluid solution spectra due to the radical anion (Fig. 2; in 0.1 M ($^n Bu_4N$)(BF_4) N,N-dimethylformamide (dmf) solution at 233 K). The spectrum is isotropic, with hyperfine coupling to two inequivalent ^{14}N nuclei from the NO_2-substituted pyridyl ring ($a_{(NO2)} = 20.2$, $a_{(pyN)} = 8.3$ MHz) and the 3,5-^1H of the same ring ($a_{(2H)} = 7.2$ MHz; note these are inequivalent by symmetry, but we do not resolve the difference). The spectrum at

Q-band, generated under identical conditions, can be modeled with identical hyperfine coupling. However, the asymmetric broadening of the spectrum is now much more severe than at X-band, which allows more accurate determination of the dynamic parameters (Fig. 2, bottom).

(ii) We have previously found that nitro-substituted α-diimines can act as multiple electron-acceptors in transition metal complexes; for example [Pt(4,4′-$(NO_2)_2$-bipy)Cl_2] undergoes four one-electron reductions [7]. The isomeric complex [Pt(3,3′-$(NO_2)_2$-bipy)Cl_2] (Fig. 3) undergoes an extremely facile one-electron reduction at +0.01 V. Fig. 3 shows X-, Q- and W-band spectra of [Pt(3,3′-$(NO_2)_2$-bipy)Cl_2]$^-$ generated *in situ* at −0.3 V and 293 K in 0.1 M (^{n}Bu$_4$N)(BF_4) dmf solution, before freezing at 173 K. In fluid solution a singlet is observed with doublet satellites due hyperfine coupling to ^{195}Pt (33%, I = 1/2; $A_{iso(Pt)}$ = −54 MHz, Fig. S4). On freezing at 173 K at X-band the rhombic nature of the spectrum is clear, in common with other [Pt(X_2-bipy)Cl_2]$^-$ species,[7–9] but there is significant overlap of the three canonical orientations that results in resolution of only one component of the ^{195}Pt hyperfine coupling (on the low-field *g*-value, $A_{1(Pt)}$ = −51 MHz from simulation, Fig. 3, top). An identical experiment at W-band fully resolves the three orientations, giving *g* = 2.0152, 2.0050, 1.9910 (Fig. 3, bottom), but all hyperfine resolution has been lost, presumably due to strain effects. At the intermediate Q-band frequency the *g*-resolution is sufficient that $A_{2(Pt)}$ (=−70 MHz) can be measured (Fig. 3, middle). $A_{3(Pt)}$ is unresolved at all frequencies but can now be estimated at ca. −40 MHz from the isotropic value. Hence, the spectroelectrochemical experiment at multiple frequencies has allowed determination of **g** and $\mathbf{A}_{(Pt)}$. Analysis of these parameters, using methods described elsewhere[8,9], reveals that only ca. 1% of the spin density lies in the platinum 5d/6p orbitals, ca. threefold less than in the 4,4′-isomer.

(iii) Our final example is an application to a redox active enzyme, namely nitric oxide synthase reductase domain (NOSrd) [13,14]. The two flavin cofactors (FAD and FMN) can exist in several oxidation and protonation states, enabling NOSrd to convert NADPH into the single electrons required for NO synthesis during blood vessel relaxation, nerve signaling and immune response. Both FAD and FMN form neutral paramagnetic semiquinone radicals (Fig. 4), which have been characterized electrochemically [13,14] and via EPR spectroscopy [15]. The semiquinones have generally been generated for EPR studies by photolysis or by chemical reduction. Fig. 4 shows X-, Q- and W-band spectra of reduced NOSrd generated by *in situ* electrolysis at -0.3 V and 293 K, then freezing. At X-band the spectrum is essentially isotropic, with some very limited resolution of hyperfine (to ^{14}N and ^{1}H nuclei of the flavin, Fig. 4, inset) which is difficult to interpret in isolation because of the poor *g*-resolution. At Q- and W-band a near-axial set of *g*-values is revealed. The improved resolution makes modeling possible at Q-band; at W-band the hyperfine resolution is largely broadened out, but is similar in spread to spectra reported by Kay et al. for the neutral and anionic flavin radicals in DNA photolyase [16] and monoamine oxidase A [17], respectively. Modeling the Q-band spectrum requires hyperfine coupling to two inequivalent ^{14}N and a single ^{1}H; simulation gives $g_{1,2,3}$ = 2.0041, 2.0036, 2.0022 with $a_{3(\underline{N}H)}$ = 56 MHz, $a_{3(\underline{N}R)}$ = 24 MHz, $a_{2(N\underline{H})}$ = −34 MHz the large ^{1}H coupling identifies the species as a neutral flavin radical.[15,16,18].

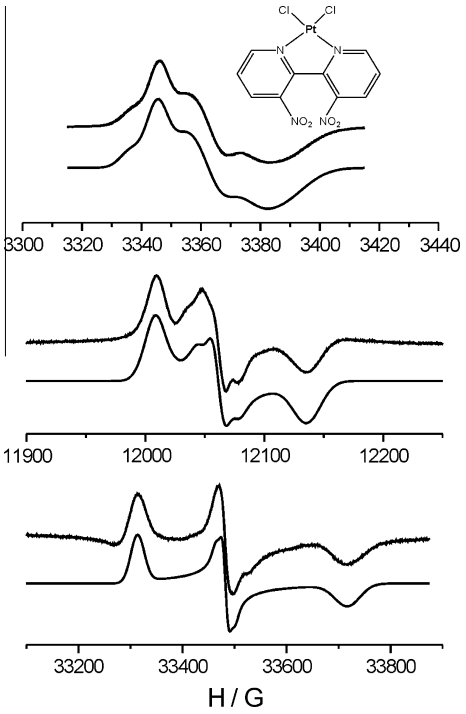

Fig. 3. X- (top), Q- (middle) and W-band (bottom) EPR spectra of *in situ* electrogenerated (−0.3 V) frozen solutions of [Pt(3,3′-$(NO_2)_2$-bipy)Cl_2]$^-$ (inset) in 0.1 M (^{n}Bu$_4$N)(BF_4) dmf solution at 100 K. Microwave power 0.3 and 0.03 mW for Q- and W-band, respectively. Upper: experimental; lower: simulations [12] with parameters in the text.

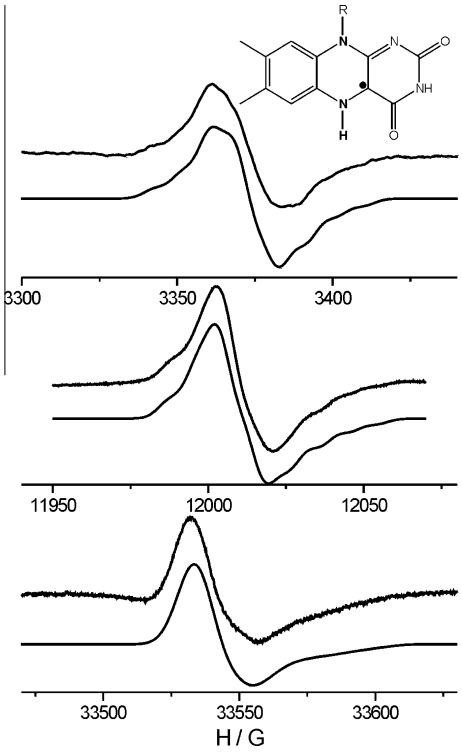

Fig. 4. X-, Q- and W-band EPR spectra of 0.2 mM NOSrd in 0.1 M Tris/HCl pH 7.5, 0.5 M KCl containing 10 μM mediators (FMN, methyl viologen, benzyl viologen), at 150 K, generated by *in situ* electrolysis at −0.3 V. Microwave power 0.7 and 0.03 mW at Q- and W-band, respectively. Upper: experimental; lower: calculated [12] spectra using parameters in the text. The Q-band spectra were fitted starting from typical parameters (**g** and $\mathbf{a}_{(N,N,H)}$) for neutral flavin radicals [18]; these parameters were then used to calculate the X- and W-band spectra.

In conclusion, we have demonstrated a simple cell design for *in situ* spectroelectrochemical EPR studies, transferable between different microwave frequencies and that can be used in standard commercial Q- and W-band spectrometers and sample tubing. The small cell dimensions, and consequently small working electrode surface area, clearly means that less EPR active species can be generated than in conventional *in situ* X-band experiments. However, in practice we have found this places not much greater restrictions than are usual for Q- vs. X-band spectroscopy on dilute solutions. Indeed, the very small volume cell described here, based on a standard W-band EPR tube, has obvious advantages for investigations where only limited amounts of material are available. The disadvantages of the cell are those that are inherent to high frequency EPR, including the importance of strain effects in immobilized samples and consequent loss of hyperfine resolution. An obvious development would be to adapt the design to double resonance and pulsed techniques, to exploit the enhanced orientation selectivity.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2011.09.041.

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