CHAPTER 1

Infrared Spectroelectrochemistry

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1.1 Introduction

A set of electrochemical measurements may, with the aid of simulations, provide skeletal details of the redox-coupled reactions of a system, although the extent of the detail or uniqueness of the description depends on the complexity of the system and the relative rates of reaction. Since such an approach can, at best, yield only limited insight into the structure of intermediate species there is a clear need to supplement the electrochemical measurements by spectroscopic investigations. This need has spawned a number of approaches designed to provide the spectroscopic details of the electrogenerated intermediates/products. Spectroelectrochemical (SEC) techniques allow in-situ spectroscopic interrogation of electrogenerated complexes and this may permit the study of shorter-lived species and also establish the chemical reversibility of these reactions. This allows the building, testing and refinement of the mechanism and, crucially, provides insights into the structures of the intermediates.

The structure of the intermediate implicitly encompasses molecular, electronic, and vibrational components where the molecular structure is most commonly deduced by X-ray crystallography. More limited structural data may also be obtained from solute species through analysis of the X-ray absorption fine structure (XAFS) spectra and this will be discussed briefly in Section 1.6. Clearly the electronic and vibrational structure must be obtained from analysis of the spectra. The interconnection between these aspects of the structure is reinforced by in-silico techniques, where advances in DFT (density-functional theory) have greatly expanded the range of transition-metal compounds and
smaller clusters that are amenable to study. For systems of moderate complexity a combination of structural, spectroscopic and in-silico approaches is required in order to achieve a satisfactory understanding of the intermediates formed during reaction. This chapter focuses on the use of IR spectroscopy to delineate the chemistry following redox activation and the integration of these results with a range of electrochemical, spectroscopic and computational methods to characterise the charge state and structure of intermediate species. While the vibrational structure of a species would ideally be determined through the examination of both its IR and Raman spectra, in most cases the complementary nature of the physical constraints associated with the two techniques results in studies concentrating on one or the other approaches. In cases where the system under investigation incorporates strongly IR absorbing chromophores, such as CO or CN⁻, IR spectroscopy can be both effective and easily implemented.

Since the objective of the studies described herein is the characterisation of the solute species formed following redox reaction the very extensive research dealing with characterisation of the electrode/solute interface will not be discussed, excellent overviews of the experimental aspects of this subject are available. While this contribution focuses on applications involving IR, Raman spectroscopy has proved to be invaluable to many SEC studies where surface-enhanced Raman spectroscopy (SERS) and resonance Raman spectroscopy dominate. Reviews and recent studies attest to the value of these approaches.

In this contribution we aim to illustrate the impact of IR-SEC techniques on the elucidation of the chemistry following a redox reaction. The most effective experimental approach will depend on the stability of the redox products together with the rates or nature of the following reactions. In Section 1.5 we show the experimental results obtained from several systems chosen so as to highlight the different experimental approaches that can be applied to good effect. We have limited the discussion to studies of solute species and to concentrating on examples drawn from our own research, published and unpublished. This is driven, in large part, by the availability of the raw experimental data and the opportunity that this provides to recast the figures in a self-consistent form. As a result, there is an overrepresentation of studies conducted using external reflectance SEC cells.

1.2 Overview of IR-SEC Techniques for the Study of Solute Species

The marriage between the spectroscopic and electrochemical requirements of the SEC experiment necessarily involves compromise, the nature of which will be dictated by the objectives of the study. For thin-layer cells with large surface area electrodes uncompensated solution resistance will generally present problems and these will be accentuated for studies conducted in highly resistive solvents. In many cases it is not practicable to use a conventional reference
electrode and in these instances a pseudoreference consisting of a silver or platinum wire or foil is used. While such electrodes are susceptible to a drift in potential the impact of this deficiency may be minimised if the duration of the experiment is short relative to a change in the concentration of the species near the reference electrode. Several different experimental approaches have proved to be effective for the collection of IR-SEC results from electrogenerated solute species and these may be distinguished in terms of the characteristics of the working electrode. These include (i) optically transparent electrodes, (ii) perforated electrodes and (iii) reflective electrodes. To these may be added approaches in which a probe beam is brought close to the working electrode of an electrosynthesis cell by means of a waveguide or optical fibre. The sampling element may consist either of a pair of launch and collection fibres or include an optical element that is arranged so as to give near-total internal reflection (attenuated total reflection, ATR). In the latter case the spectrum of the solution in contact with the ATR crystal is sampled through its interaction with the evanescent wave that propagates beyond the reflecting surface. Depending on the cell geometry, and volume of solution, the time required for electrosynthesis can be substantial (> 1 h) in which case the distinction between in-situ and ex-situ spectroscopic interrogation is not clear cut. With careful attention to the design it is possible to reduce the volume of solution subject to electrosynthesis and thereby reduce the response time. An ATR IR-SEC cell featuring a sample chamber with a volume of 20 μL has recently been reported, although even in this case the cell requires ca. 6 min for redox equilibration following a potential step.

1.3 Transmission Cells Using Optically Transparent or Perforated Electrodes

In optical terms the simplest SEC approach involves the use of transparent electrodes. For visible spectroscopy doped tin oxide affords a useful spectral window. More recently boron-doped diamond has been shown to be suitable for UV-Vis and IR spectroscopy. This material offers a wide potential range, inertness in chemically aggressive environments and biocompatibility. The spectroscopic range and conductivity depends on the boron doping level. Diamond films with moderate levels of boron doping (0.5 to 0.05 Ω cm⁻¹, 1–10 × 10¹⁹ B cm⁻³) retain a high transmittance in the region below 1500 cm⁻¹.

An alternative strategy to the use of transparent electrodes is to use a conductor in the form of a grid or fine gauze. Cells constructed using this approach date back to the work of Murray et al. and have been used for UV-Vis and IR spectroscopic studies. In the latter case the solution pathlength must be minimised in order to avoid problems associated with strong solvent absorption. The principle for construction of the cell is straightforward, generally involving modification of a solution IR cell by incorporation of a fine metal gauze working electrode located in the path of the IR beam. A good example of a compact, airtight cell based on this approach has been reported by Hartl and
In this case the working, counter, and reference electrodes occupy the space between the salt plate windows as shown in Figure 1.1a. The working and counter gauze electrodes and silver pseudoreference electrode are melt-sealed into a single 200-µm thick polyethylene spacer. The working electrode has a dimension slightly larger than the IR beam (6 × 5 mm) and is formed from a highly transmitting (80%) Au minigrid (200 lines/mm). Platinum gauze of similar surface area serves as the counter electrode. The approach is well suited to the study of air-sensitive species since solutions may be transferred to the cell anaerobically using gas-tight syringes, however, the relatively long electro-synthesis time (up to 4 min for 5–10 mM solutions) limits the suitability of the approach for products that undergo following reaction.

A variation of this approach has been described by Moss et al. who have reported a cell design that is optimised for IR-SEC studies of proteins. A schematic cross-sectional view of the Moss cell is shown in Figure 1.1.(b) The working electrode consists of a 6-µm thick, 70% transparent Au mini-grid and this, together with the Pt foil counter and Ag/AgCl reference electrodes, completes the three-electrode geometry. An important feature of the cell for applications using proteins is the low sample requirement, where a 3–5 µL drop of solution is sufficient to fill the 20 mm diameter × 15 µm thick space formed by the CaF2 windows. The outer cavity of the cell is filled with buffer/salt solution. Despite the contact between the sample solution and the surrounding medium the rate of dilution was reported to be ~5% over 24 h. In order to improve the rate of heterogeneous electron transfer and reduce the effects of protein denaturation, electrode-surface modifiers or electron-transfer reagents may be added to the sample solution. Owing to the high electrode surface area to

Figure 1.1 Schematic of transmission SEC cells (a) after Hartl, (b) after Moss and (c) after Yun.
volume ratio the system rapidly equilibrates following a change in applied potential (1–2 min). Although factors such as the solution resistance within the cell may lead to an offset to the cell potential, excellent Nernstian concentration/potential plots were obtained for model studies of cytochrome c.\textsuperscript{10}

More recently, Yun and coworkers\textsuperscript{11} have reported the design of a transmission IR/UV-Vis cell that is micromachined from single-crystal silicon wafers (Figure 1.1(c)). In this case the Au minigrid, Au counter and Ag/AgCl pseudo-reference electrodes are deposited directly onto the silicon wafer. The cover plate is also etched from a single crystal, allowing fine control of the pathlength of the solution. The sample solution (\textit{ca.} 0.1 mL) is placed between the two silicon wafers and these are clamped together in the cell mount. This arrangement was reported to work well for aqueous solutions but in the case of nonaqueous solvents it was necessary to enclose the edge of the thin-layer cell with Teflon tape so as to prevent solvent leakage. While transmission through silicon cells is lowered as a result of high reflectance losses at the interfaces this is offset by the control over the solution volume and electrode geometry provided by the microfabrication techniques.

1.4 External Reflection-Absorption SEC Cells

IR reflectance spectroscopy has proved to be highly effective for the examination of both the solute/electrode interface and the electrogenerated products. Two general approaches, designated internal and external reflection, may be employed in these studies.\textsuperscript{12} For internal reflection the radiation passes through a transparent substrate and is reflected from a thin film of metal deposited thereon. The metal film also serves as the working electrode. The penetration of the electric vector of the radiation into the solution in contact with the reflecting surface provides a means of obtaining selective information related to the solute/electrode interface. For external reflectance the radiation passes through a suitable window and is specularly reflected from a solid electrode. The solution trapped between the electrode and window is interrogated by the IR beam. External reflectance approaches have been used to study both the electrode/electrolyte interface and the electrogenerated species. Since the study of monolayer or submonolayers of adsorbed species introduces significant challenges in terms of sensitivity, a number of different approaches have been developed that has spawned an extraordinarily diverse range of acronyms (EMIRS, IRRAS, SNIFTIRS, PM-FTIRS, LPSIRS). Briefly, discrimination between molecules bound to the electrode and those in the bulk solution may be achieved by examining the spectral changes that result from either potential or polarisation modulation. A good review of the techniques, and their physical basis has been outlined by Beden and Lamy.\textsuperscript{12}

Examination of the solute species by external reflectance presents far fewer challenges and a variety of cell designs have been reported. These include strategies that permit the study of air-sensitive compounds\textsuperscript{13} over a range of temperatures\textsuperscript{14,15} and/or pressures.\textsuperscript{16} A schematic diagram of a basic external reflection SEC cell is shown in Figure 1.2(a).\textsuperscript{14} If the object of the investigation...
is the study of solute species then the incident beam strikes the working electrode near to normal incidence, this minimises reflection losses from the air/IR-transmitting window interface and reduces the cross-sectional area of the focus of the IR beam when projected onto the electrode. Since the layer of solution under investigation is typically 10–20 \( \mu m \) the cells inevitably suffer from a substantial uncompensated solution resistance and this may best be controlled by minimising the surface area of the working electrode. Thus, the diameter of the working electrode should match the IR beam diameter. Depending on the instrument this typically translates into an electrode diameter of 3–5 mm. Naturally, the electrode must be fashioned from a material highly reflecting to radiation in the energy range of interest. While this is best achieved with metals such as Au and Pt, it is important to note that vitreous carbon has sufficient reflectivity in the IR to be suitable for such measurements. In its simplest form the working electrode is encased in an inert insulator, usually KelF or glass (Figure 1.3(a)). The thickness of the layer of solution under examination is determined by the position of the working electrode relative to the front window and this is adjusted under micrometer control. In most cases the counter and reference electrodes are located outside the thin-layer region where diffusion between the bulk solution and the solution in contact with the working electrode is negligibly small over the timescale of the SEC experiments.

In view of the need to restrict the size of the IR beam at the electrode plane the use of reflection absorption cells is generally limited to FTIR instrumentation where it is often necessary to use a high-sensitivity photovoltaic detector (e.g. liquid-nitrogen-cooled mercury cadmium telluride, MCT). Since in these cases the detector element is small, in addition to providing a flat, highly polished working electrode, it is necessary to provide an optical train that does not increase the size of the IR beam at the detector focus. The optical design used in our laboratory is shown in Figure 1.2(b). The IR beam is directed to the working electrode located at the beam focus using a plane mirror. The central ray of the IR beam makes an angle of 14.3° to the normal to the CaF\(_2\) window and 10° to that of the working electrode. The reflected beam is...
refocused using a spherical mirror to give a beam focus and path as close as possible to that of the undiverted beam. All three mirrors are built into a mount that allows rotation about axes coincident with their reflective surfaces and beam centre, in addition, the two plane mirrors have tilt axes normal to the rotation axis.

A schematic diagram of an electrode suitable for low-temperature use is shown in Figure 1.3(b). The temperature of the brass pin attached to the working electrode is controlled by the flow rate and temperature of dinitrogen gas that passes through its structure. Using this approach it is difficult to monitor directly the temperature of the thin layer of solution, however, our experience (based on the freezing of solvent/supporting electrolyte) suggests only a small temperature difference (<ca. 10 °C) between that of the outgoing gas. An approach that minimises the distance between the electrodes and also facilitates the construction of small solution volume cells involves incorporation of the working, counter and reference electrodes into a single KelF pin. An additional small (1 mm diameter) working electrode is also incorporated into the assembly so as to provide a means of obtaining better electrochemical characterisation of the solution. In more recent designs (Figure 1.3(c)) the KelF pin is attached to a machineable ceramic cylinder (Macor). The greater dimensional stability provided by this material provides advantages in establishing an airtight seal between the electrode assembly and the cell body.

Relative to the transmission cells described in Section 1.3, external reflectance cells are less simple to construct and require additional optics for incorporation into the optical path of the spectrometer. The advantages associated with the approach relate to the well-defined nature of the working electrode, the wider range of materials suitable for this purpose, and the greater control over the thickness of the thin layer of solution. These factors contribute to a much faster (>10×) rate of electrosynthesis for external reflectance compared to transmission cells.
1.5 Applications

1.5.1 Electrochemically Reversible Reactions

The simplest application of SEC techniques involves the study of electrochemically reversible couples where the oxidised and reduced forms are stable on the timescale of electrosynthesis. Depending on the species this might involve careful control of solvent, supporting electrolyte and solute concentration, strict exclusion of dioxygen, and choice and preparation of the electrodes. Even in cases where a reversible electrochemical response is obtained the SEC experiments may yield complicated spectral changes indicating the involvement of several species with little, or no, recovery of the starting complex following application of an appropriate potential. In these cases it is important to consider the different timescales and concentration profiles that apply in cyclic voltametric and SEC experiments. In those cases where the electrochemistry is well behaved, quantitative and reversible electrosynthesis may easily be performed using transmittance or external reflectance IR-SEC cells. The potential dependence of the concentration of the oxidised and reduced species follows the expected Nernstian form. In cases where the heterogeneous electron-transfer reaction is slow the half-wave potential \( E_{1/2} \) may be obtained reliably using SEC techniques. This approach has been particularly important for studies of redox proteins.

An example of a system showing quasireversible electrochemistry and reversible chemistry is provided by \( \text{Fe}_3\text{S}_2(\text{CO})_9 \) (Figure 1.4). The markedly different pattern of \( \nu(\text{CO}) \) bands evident for the starting material and the one-electron reduced product is indicative of structural rearrangement, however, on the timescale of the IR-SEC experiment reduction and reoxidation proceed with comparable rates and with near-complete recovery of the starting material following redox cycling (Figure 1.4). The presence of well-defined isosbestic points for both oxidation and reduction (Figures 1.4(a) and (b)) indicate that there is no significant involvement of additional iron-carbonyl species in the reaction.

1.5.2 Redox-Activated Chemical (EC) Reactions

Whereas electrochemical techniques can be effective in terms of quantifying different thermodynamic and kinetic parameters in cases where chemical reaction follows redox activation (EC reactions) the identification of the intermediates formed during the reaction is problematic. In cases where the electrochemical response matches that of a known compound then this may provide a satisfactory basis for identification, but usually it is necessary to resort to other approaches. It is in relation to problems of this sort that SEC techniques can be of most value.

The group 6 complexes, \( \text{M}(\text{L-L})_2(\text{CO})_2 \) (where \( \text{L-L} \) designates various bidentate ligands with P/As donor atoms) undergo well-defined redox reactions that feature stereochemical change linked to the formal oxidation state of the
metal. The more stable forms are the cis $M^0$ and trans $M^1$ species. The $\nu$(CO) bands are a particularly useful probe of the chemistry since a shift in wave number is indicative of a change in the electron richness of the metal and the stereochemistry is flagged by the presence of one (trans) or two (cis) IR-active bands. The reduction of trans-[Cr(dppm)$_2$(CO)$_2$]$^+$ (dppm = Ph$_2$PCH$_2$PPh$_2$) in an external reflectance SEC experiment is shown in Figure 1.5. As the reduction progresses the initial product band at 1810 cm$^{-1}$ due to metastable trans-Cr(dppm)$_2$(CO)$_2$ is replaced by two bands (1780 and 1843 cm$^{-1}$) consistent with

Figure 1.4  IR-SEC of the reduction (a) and reoxidation (b) of a CH$_3$CN solution of Fe$_3$S$_2$(CO)$_9$. Spectra were recorded at 1-s intervals. In this, and the other IR-SEC spectra, a reference spectrum is collected immediately before the application of a reducing potential and this is used to calculate the absorbance or, more properly, differential absorbance spectra. The depletion of species initially present in the thin layer of solution presents as negative bands, whereas electrogenerated species give bands with positive absorbance. The last spectrum of each set is emphasised.
rearrangement to give the more stable cis-neutral form. As the concentration of the starting material in the thin layer of solution reaches a low, steady-state, value the spectral changes are dominated by the isomerisation reaction. In this case the nonredox character of the second step of the reaction is easily established by the identification of the products, but this can also be confirmed by monitoring the current response during the SEC reaction as discussed in the following sections.

The satisfactory isosbestic points obtained for the isomerisation reaction suggest that the system is amenable to kinetic analysis. The absorbance changes associated with the decay of the trans-neutral and appearance of the cis-neutral species fit to a single exponential with a rate constant of 0.14 (2) s\(^{-1}\) (Figure 1.5 inset). This value is in good agreement with the reported value of 0.17 (3) s\(^{-1}\) at

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**Figure 1.5** Reduction of trans-[Cr(dppm)\(_2\)(CO)\(_2\)]\(^+\) in CH\(_2\)Cl\(_2\) (60-\(\mu\)m thick layer). The inset shows the time traces of the absorbance for the bands associated with the starting material, the trans-neutral intermediate and the cis-neutral final product. The last spectrum of each set is emphasised. The fit of the absorbance changes at 1780, 1810 and 1843 cm\(^{-1}\) over the time range 28–53 s to a single exponential function \((k = 0.144 \text{ s}^{-1})\) is shown.
22°C obtained by double-potential-step chronoamperometry in acetone. It is clear that the rate of electrosynthesis is of vital importance when examining reactions of this sort. Clearly, the half-life of the reaction should be at least comparable to the time required for electrosynthesis and the data shown in Figure 1.5 is close to that limit. If spectra of adequate quality can be obtained using thinner films (10–15 µm) then the time required for quantitative electrosynthesis can be reduced to several seconds.

1.5.3 Electrocatalytic Reactions

Two different types of catalytic reaction will be considered, electron-transfer catalysis (ETC) and the more usual case in which a kinetically slow electrochemical reaction is accelerated by the presence of a second redox couple (EC’ reaction). The distinction between the two types of catalytic reaction is based on whether the net reaction involves a change in redox state (Scheme 1.1).

For ETC reactions a small injection of charge into the thin layer of solution should be sufficient to achieve full conversion of the initial compound. The SEC response for such reactions is illustrated by PR₃/CO substitution in Fe₂(µ-pdt)(CO)₆, pdt = S(CH₂)₃S, (3S) in tetrahydrofuran (THF). A solution of 3S with 10 equivalents of P(OPh)₃ is initially subject to a potential of 0 V and spectra are collected at a rate of 1 Hz. A reducing potential of −1.2 V is applied for 2 s and the potentiostat is switched to open circuit. The differential absorbance spectra are calculated using the spectrum recorded immediately before switching the potentiostat to open circuit as the reference spectrum. In the following spectra the remaining 3S is quickly depleted from the thin layer and this is replaced by the spectrum of the neutral phosphite product, Fe₂(µ-pdt)(CO)₅P(OiPr)₃ (Figure 1.6). It is important to note that the reaction must be conducted under conditions where the lifetime of the reactive reduced species is sufficient for the substitution reaction to be competitive with other modes of deactivation. In this case it is essential that the THF be dry and carefully deoxygenated. In solvents such as CH₃CN, where the one-electron reduced complex has a shorter lifetime, the ETC reaction is generally quenched well before complete depletion of the 3S reactant. In cases where the ETC reaction can be followed using SEC techniques spectral subtraction can be used to identify the presence of low concentrations of species formed following redox

![Scheme 1.1](image-url)

Scheme 1.1 Distinction between electron-transfer catalytic (ETC) and electrocatalytic (EC’) reactions.
initiation. The concentration profiles of the transently stable species are of key mechanistic importance to such reactions.

In contrast to ETC reactions, where the injection of small amounts of charge into the thin layer can result in dramatic spectral change, for EC reactions the injection of substantial charge into the thin layer may result in minimal spectral change. This is well illustrated by electrocatalytic proton reduction by 3S. Reduction of 3S in the absence of protons is complete within ca. 30 s (Figure 1.7(a)). After this time there is a reduced current flow due to diffusion of the neutral complex into the thin-layer region. Stepping the potential to slightly more positive values leads to oxidation of one of the minor reduction products and application of much more positive potentials leads to ca. 85% recovery of the starting material. In the presence of 7 equivalents of acid, reduction of a layer of solution of similar thickness (estimated using the absorbance of a solvent band) results in a substantial current flow, but with minimal spectral change. As the reaction proceeds the starting complex is removed from the thin-layer region at an increasing rate until it is nearly depleted. Reoxidation leads to only ca. 50% recovery of the starting complex. The current and spectral response (Figure 1.7(b)) requires that the starting complex be recovered during the electrocatalytic cycle and this is consistent with the reaction path shown in Scheme 1.2(a). Satisfactory simulation of the cyclic voltammetry of THF solutions of 3S with between 0 and 10 equivalents of p-toluene sulfonic acid (HOTs) has been obtained for a reaction involving consecutive electron–proton additions (Scheme 1.2(a)) together with a

Figure 1.6 Spectra recorded from a solution of Fe$_2$(µ-pdt)(CO)$_6$ (3S) with 10 equivalents of P(OiPr)$_3$ following reduction for 2 s followed by switching the potentiostat to open circuit. The reference spectrum was recorded immediately prior to switching the potentiostat to open circuit and the last spectrum of the series is highlighted.
Figure 1.7  Electrocatalytic proton reduction by 3S in THF. The current response (dashed line) and absorbance change (solid line) showing depletion of the starting material for (a) the reaction in the absence of acid and (b) after addition of 7 equivalents of p-toluene sulfonic acid (HOTs). (c) Spectral changes recorded corresponding to the reaction shown in (b). The last spectrum of the series is highlighted.

Scheme 1.2  Proposed EC′ reaction paths for electrocatalytic proton reduction by (a) 3S^{19} and (b) Fe_{2}(μ-PPP)_{2}(CO)_{6} (3P)^{20}. 

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path involving reduction of the two-electron, two-proton product that becomes available at more reducing potentials.\textsuperscript{19} It is important to note that in the absence of 3S reduction of HOTs at the vitreous-carbon working electrode at a potential of \(-1.2\) V yields an insignificant current flow.

The initial spectral changes obtained in SEC studies of electrocatalytic reactions provide a particularly effective means of identifying the kinetically inert species of the reaction cycle. For electrocatalytic proton reduction by 3S the small magnitude of the spectral changes obtained during the initial phase of the reaction (Figure 1.7) requires that the reaction cycle must recover the resting state of the complex. This requires that dihydrogen elimination occur from the two-electron reduced form of the complex. Clearly, in cases where the reaction cycle involves more highly reduced levels of the complex the resting state of the complex will not be recovered and substantial spectral changes will accompany the initial phase of the reaction. Accordingly, SEC studies allow a distinction between EC\textsuperscript{0} reactions that must include the resting state of the complex (small or no spectral changes during the initial phase of the reaction) and those for which the starting complex is a precursor to the species involved in the catalytic cycle. The differing SEC signatures obtained for these situations is provided by electrocatalytic proton reduction by 3S (Figure 1.7) and Fe\textsubscript{2}(\(\mu\)-PPh\textsubscript{2})\textsubscript{2}(CO)\textsubscript{6}, DP. For DP, depletion of the starting material from the thin layer proceeds as soon as a reducing potential is applied, although there are differences in the initial products for reactions conducted in the presence and absence of protons (Figure 1.8).\textsuperscript{20} The initial product in this case is the two-electron, two-proton product DP\textsubscript{2}H. This species can be generated independently by protonation of DP\textsuperscript{2\textendash} and shown to be unreactive in terms of dihydrogen.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.8.png}
\caption{Initial stages of the reduction of DP with 9 equivalents of HOTs. The time between spectra is \textit{ca.} 4 s. The last spectrum of the set is highlighted.}
\end{figure}
elimination. Further reduction is needed to give a significant rate of dihydrogen elimination (Scheme 1.2(b)).

When carefully examined, and with the aid of spectral subtraction, more subtle spectral changes recorded during electrocatalysis provide a potential source of the spectral signatures of longer-lived species that are formed during the reaction cycle. During the early stages of electrocatalysis by 3S (Figure 1.7(c), 100–150 s) the spectrum of a previously unidentified species was detected and the spectrum extracted. A similar spectrum is also obtained from corresponding experiments from the related compound, Fe_2(μ-S(CH_2)_2S)(CO)_6. While having a spectrum seemingly related to that of the CO-inhibited oxidised form of the enzyme, more recent studies suggest that this species is a rearranged side product.

Electrochemical simulations of the concentration and scan-rate dependence of the voltammetry potentially provide the composition of the intermediates formed during the reaction cycle together with estimates of the rate and equilibrium constants. As shown in the preceding section spectroscopic information can greatly assist the elucidation of the molecular details of these reactions, however, reliable deduction of the structure is greatly enhanced by the incorporation of structural and computational information (Section 1.6). The rapid advance in computer power and implementation of density-functional theory allows a more quantitative approach for evaluation of proposed structures based on spectroscopic information and estimation of the relative energies of the proposed species. The recent computational study of the electrocatalytic reaction cycle proposed for 3S illustrates the opportunities presented by the approach.

1.5.4 Chemically Reactive Species

A range of different strategies need to be considered in cases where the lifetime of the initial product of the redox reaction is short relative to the rate of electro-synthesis. The relatively slow rate of electrosynthesis of spectroscopically significant quantities of product relative to chemical reaction may make the application of stopped- or continuous-flow techniques more suitable than SEC approaches. The advantage to be gained by study of such systems using SEC approaches derives from the control of the oxidising/reducing potential that, in turn, allows the chemical reversibility of the redox reaction to be examined. In most cases quantitative electrosynthesis of a 10-μm layer of solution contained between the working electrode and the IR-transmitting window will be of the order of 10 s. While this places a limitation on the range of species that may successfully be studied, there is the advantage that standard FTIR spectrometers with photovoltaic (e.g. MCT) detectors allow spectra to be collected at good resolution (2 cm⁻¹) in ea. 1 s and are ideally suited for this work.

The second oxidation of the Cr(dpmm)_2(CO)_2 complex considered earlier in the section provides a good illustration of the advantage provided by the rapid rate of electrosynthesis when the redox activated products are reactive. In this case one-electron oxidation of the neutral complex is accompanied by cis/trans isomerisation. A further oxidation of the trans-cation occurs at +1.5 V where
the initial reduction product has a \( v(\text{CO}) \) band at 2024 cm\(^{-1} \) but is relatively unstable and over a longer timescale reduction does not result in the recovery of a significant fraction of the starting material. The assignment of the 2024-cm\(^{-1} \) band to \( \text{trans-}[\text{Cr(dppm)}_2(\text{CO})_2]^{2+} \) requires rapid reduction at moderate reducing potentials to give the more stable \( \text{trans-}[\text{Cr(dppm)}_2(\text{CO})_2]^+ \). For the experiment shown in Figure 1.9 only \( ca. \) 60% of the trans-cation is recovered following redox cycling to the dication. Despite this, there is a clear connection established between the species responsible for the 2024- and 1872-cm\(^{-1} \) bands with the rate of reaction and potential for the interconversion consistent with assignment of the 2024-cm\(^{-1} \) band to \( \text{trans-}[\text{Cr(dppm)}_2(\text{CO})_2]^{2+} \).

While the 3D plots give an overview of the spectral changes associated with a reaction, it is more often important to discern whether the interconversion reactions proceed with formation of significant concentrations of intermediate species. The presence of isosbestic points is of key importance in this regard and such spectral features are more easily examined in overlay plots.

Clearly, the examination of intermediates is much more straightforward if their lifetime can be extended, and in many cases this may be achieved by careful attention to the chemistry of the system (e.g. choice of solvent and/or

![Figure 1.9](image_url) Oxidation and reduction of \( \text{Cr(dppm)}_2(\text{CO})_2 \) in \( \text{CH}_2\text{Cl}_2 \). The time between spectra is \( ca. \) 1 s. The top series of spectra show the time evolution of the spectra in a 3D format and replotted in overlay mode below. The time traces are shown in the lower right panel.
supporting electrolyte) or by reducing the temperature. For studies conducted in organic solvents such as THF, butyronitrile, or dichloromethane electrochemical reactions may be carried out at temperatures as low as $\sim-50 \, ^\circ \text{C}$. Both transmission and external reflectance SEC cells may be modified for low-controlled-temperature operation and this may simply involve thermostating a block in contact with the cell body. In this case it is necessary to take precautions against condensation on the outer surface of the IR-transmitting window. An alternative strategy is to use the working electrode to provide temperature control and this may be achieved in the manner outlined in Section 1.4. In this case the working electrode also serves as reflecting and cooling element for the thin layer of solution trapped between it and the window. Over short times the extent of cooling of the external surface of the window is low and condensation of water vapour rarely presents a problem.

Application of low-temperature SEC approaches has permitted identification of the spectrum of an intermediate species formed during the reduction of the $[\text{FeFe}]-\text{hydrogenase H-cluster structural analogue}, \text{Fe}_2((\mu-\text{SCH}_2)_2\text{C(Me)}\text{CH}_2\text{SPh})(\text{CO})_5$. At $10 ^\circ \text{C}$ the reduction proceeds to give a product with a significantly altered ν(CO) spectrum (Figures 1.10(a) and (b)), suggesting substantial structural change. In the spectrum collected immediately after application of a reducing potential there is a subtly different spectral profile suggestive of the involvement of an intermediate species. Analogous experiments conducted at ca. $-40 ^\circ \text{C}$ (Figure 1.10(c)) give similar results but with much clearer definition of the spectrum of the short-lived species. At lower temperature the spectral changes are sufficiently developed for the spectrum of the intermediate species to be revealed (Figure 1.10(d)).

While a lowering of the temperature will lead to a lowering of the rate of following reactions it is important to note that this will also have an impact on the rate of heterogeneous electron transfer. The improved spectroscopic detection of intermediate species depends on their relative rates of formation and decay. While in the previous example cooling leads to improved detection of the intermediate species there are a number of instances where poorer SEC responses are obtained at lower temperature.

The interconversion between electrogenerated species may be more finely examined using potential modulation techniques analogous to the SNIFTIRS (surface-normalised interfacial FTIR spectroscopy) approach developed for the study of surface species. In this case the oxidising/reducing potential is applied to the working electrode for the time required for collection of a single spectrum (usually ca. 1 s) and this leads to partial (ca. 10%) electrosynthesis of the solute in the thin-layer region. At the end of the sweep of the moving mirror of the FTIR the potential is switched back to its initial value and maintained in that state until the film is returned to its initial state. The cycling of the potential is repeated in order to establish the reproducibility of the potential dependent spectral changes. The chemical reversibility of the reaction can be assessed by the spectral changes that accompany repeated spectral cycling. In cases where the spectral changes are reversible the spectra recorded at the two potentials can be co-added in order to improve the signal-to-noise ratio. The synchronisation
between the change of applied potential and the collection of data from the FTIR (during the forward scans of the moving mirror) is shown in Figure 1.11.

An example of the application of potential modulation techniques is provided by reduction of Fe₄S₄(NO)₄ between its dianion and trianion forms. At room temperature the dianion has limited stability in dichloromethane and experiments were conducted at lower temperatures in order to diminish the effects of decomposition. Reversible conversion between the dianion and trianion forms is clearly evidenced by the spectra where the rates of reduction and reoxidation are similar and the waveform for the experiment was symmetric (i.e. in Figure 1.12, \( n = 0 \)).

1.5.5 Determination of the Charge State

Whereas thin-layer SEC approaches provide an effective means of identifying stable and transiently stable electrogenerated species, in cases where these species
cannot be isolated it is often difficult to assign a charge state. For longer-lived species the current response obtained following a potential step may be correlated with the spectroscopic results and this may provide a sufficiently good estimate of the charge transferred per molecule. This approach relies on the distinction between the charge transferred into the thin-layer region relative to the "bulk" solution. Clearly this distinction is greater for thinner layers of solution (<20 μm) where the rate of electrosynthesis is fast. An alternate strategy, that may be used even when there is a mixture of species present in solution, is to identify whether the intermediate species undergoes disproportionation reactions.

Reduction of 3S proceeds through a short-lived intermediate, 3S_A, en route to formation of three distinct products (3S_B, 3S_C, and 3S_D) where the relative
concentrations of the latter products are sensitive to the identity of the solvent and the presence of free CO in solution. Conditions can be identified where the reaction proceeds quantitatively to $3S_B$ and in this case the coulometric results indicate a two-electron reduction of $3S$ to $3S_B$.

The determination of the charge state of $3S_A$ presents more serious difficulties. Whereas the electrochemical (cyclic voltammetry, rotating-disc electrode) response of the system indicates that the reduction proceeds in one-electron steps, this is not of itself sufficient to associate $3S_A$ with the one-electron reduced product. Indeed the average shifts of the terminal $v$(CO) bands of $3S_A$ and $3S_B$ are similar and this suggests a similar electron richness at the iron centres. The proposition that the redox level of $3S_A$ is intermediate between those of $3S$ and $3S_B$ can be confirmed by its disproportionation. This may be established by generating $3S_A$ in a SEC experiment then switching the potentiostat to open circuit where the decay of the concentration of $3S_A$ with concomitant growth in the concentration of $3S$ flags disproportionation, whereas conversion of $3S_A$ solely to $3S_B$ would suggest a non-redox transformation. The concentration dependence of $3S$, $3S_A$, and $3S_B$ in such an experiment is shown in Figure 1.13. The first spectrum recorded after switching the potentiostat to open circuit shows that the loss of $3S$ ceases. Crucially, as the concentration of $3S_A$ decays there is an increase in the concentration of both $3S$ and $3S_B$. An alternative explanation for the recovery of the concentration of $3S$ in the thin-layer region involves diffusion from the bulk solution. The contribution made to the concentration change of $3S$ from diffusion can be estimated from its (time)$^{1/2}$ dependence and provided that there is sufficient $3S_A$ in the thin layer at the time the potentiostat is switched to open circuit a contribution from chemical reactions may be readily discerned.\(^{19}\)

For experiments that require a quantitative estimate of the concentration of the different species in the thin-layer region this can be easily achieved when the band profiles are not overlapping. Where this is not the case it is necessary to use more sophisticated approaches (i.e. chemometrics\(^{28}\)). In this example, well-defined spectra of $3S$, $3S_A$, and $3S_B$ can be obtained either directly or indirectly by the use of spectral subtraction. These spectra may be used as vectors in a multicomponent analysis and this may be performed using specialist or general plotting and statistical analysis programs such as Igor Pro (Wavemetrics). When approaches such as these are used it is important to check that the mass balances remain consistent through the experiment.

### 1.5.6 Reactions of Redox-Activated Complexes with Gaseous Substrates

The activation of gaseous molecules such as $H_2$, $N_2$, $O_2$, CO, and $CO_2$ is important both for environmental and economic reasons and the SEC study of catalysts for activation of these species is facilitated by cells capable of operation at elevated pressures. This feature may be incorporated into external reflection SEC designs and a schematic diagram of such a cell is shown in
Figure 1.13  Time evolution of the concentration of $3S$ and products formed following reduction for ca. 6 s, at this time ($t = 0$ s) the potentiostat was switched to open circuit. The concentration change is obtained by multicomponent analysis of the differential absorption IR spectra. The values of the concentration change for $3S$ are offset by 2.1 mM. The error bars are drawn at the 3 e.s.d. level. The mass balance corresponds to the sum of the concentration changes. The inset shows the change in concentration of $3S$ on a root timescale. Modified from ref. 19.

Figure 1.14.  The outer casing of the cell is constructed from stainless steel and the IR beam enters and departs through a 7-mm thick CaF$_2$ window. The solution is contained by a Teflon insert and this is fashioned so as to minimise the volume required for operation. The connections to the electrodes are most easily managed if a multielectrode assembly is used.
An upper pressure limit for operation of the cell is set at 1 MPa, this is less than 10% of the calculated safe pressure. The change in concentration of gaseous substrates effected by a 1-MPa pressure is sufficient for satisfactory study of most systems. In addition to operation at elevated pressures the cell is very well suited to the study of highly air-sensitive compounds.

The redox-state dependence of substrate binding to metal clusters is illustrated by CO interaction with the reduced forms of $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$. Under an inert atmosphere the cluster undergoes two well-behaved one-electron reductions at potentials of $–0.9$ and $–1.7$ V (inset Figure 1.15), of these only the second process is significantly affected by the presence of CO. The increased cathodic current response and a lowered reversibility indicate significant CO interaction with the tetra-anionic form of the cluster. The CO-pressure-dependent spectral changes obtained following the application of potentials sufficient to generate the tetra-anion are shown in Figure 1.15. Since the starting complex does not have bound CO the spectral changes in the $\nu$(CO) region are dominated by growth bands (free CO absorbs weakly in the IR). The absence of depletion bands in the differential absorption spectra confirms the low reactivity of the complex in its resting state to CO. Reduction to the tetra-anion is marked by the rapid growth of intense $\nu$(CO) bands reflecting the formation of iron carbonyl species, where the time and concentration dependence of the spectra indicate a complex reaction mixture. At high CO partial pressures there is an increased prominence of the band at $1750 \text{ cm}^{-1}$ attributable to $[\text{Fe(CO)}_4]^{2-}$ and this signals extensive cluster decomposition and accounts for the increased cathodic current at high CO partial pressures.

At elevated CO partial pressures the reaction between the cluster and CO occurs following reduction to the trianion state. Two distinct products are formed during the reaction where the initial stage of the reaction is dominated by a product featuring a single $\nu$(CO) band at $1812 \text{ cm}^{-1}$, at longer times a less distinct set of bands centred on $1930 \text{ cm}^{-1}$ grow slowly into the spectrum, signalling the appearance of a product, or products, with terminally bound CO groups. Reoxidation of the thin layer gives a rapid set of spectral changes where

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**Figure 1.14** Cross-sectional (a) and top (b) schematic views of an external reflection SEC cell suitable for operation at gas pressures to 1 MPa. The details of the multielectrode assembly are shown in Figure 1.3(c).
oxidation of the species having the terminally bound CO groups leads to a product having bands at 1945, 2011 and 1966 cm$^{-1}$. Reoxidation of the species responsible for the 1812-cm$^{-1}$ band, most likely having a bridging CO group, appears to result in dissociation of the CO group from the (di)iron centre. This may have relevance for understanding biological systems, such as the 4Fe-3S half of FeMoco, the N$_2$-fixing centre in the nitrogenase enzyme, also binds CO in a bridging mode at low CO partial pressures with reversible dissociation of CO on reoxidation (Figure 1.16).

1.5.7 Biomolecules

Application of IR-SEC to biomolecules brings additional challenges and these are mostly related to the availability of material, its maximum concentration, interference from other vibrational modes of the macromolecule, and the need to work in highly absorbing solvents such as water. The water solubility of most salt window materials means that studies must be conducted using insoluble
materials and these tend either to have a high wave number cutoff (e.g. CaF$_2$) or to give low transmission due to high reflection losses (e.g. ZnSe). Despite having intense, broad absorptions at ca. 1600 and 3400 cm$^{-1}$ water does present a useful spectral window, particularly in experiments with a short optical pathlength, and this may be extended by the use of D$_2$O or H$_2$O/D$_2$O mixtures.

Figure 1.16 SEC of [Fe$_4$S$_4$(SPh)$_4$]$^{2-}$ in CH$_3$CN at 0.36 MPa of CO. (a) Reduction of the trianion to the tetra-anion and (b) reoxidation to the trianion, 1.2 s between spectra.
Although characteristic IR bands or regions have been identified for amino acid side chains or protein folding motifs (α-helix vs. β-sheet for example), interpretation of the spectra for whole proteins is extremely complex. OTTLE cells of the type reported by Moss et al. 10 (Figure 1.1(b)) have been used in both UV-Vis and FTIR studies of redox-linked conformational changes in a number of heme proteins including myoglobin and hemoglobin, 31 cytochrome c3, 32 cytochrome c oxidase 33 and the cytochrome bc1 complex. 34 Difference spectra show redox-dependent changes in vibrational modes arising not only from the peptide backbone and side chains, but also from the porphyrin rings, providing information on local and more distant structural changes resulting from redox transitions at the heme cofactor centres. For the small, soluble, electron-transfer protein cytochrome c3, diffusion is sufficiently efficient that direct, rather than mediated, electrochemistry is possible, but for work with larger proteins, a cocktail of redox mediators is added to the protein solution to improve electron-transfer rates. The gold minigrid working electrode is generally coated with a thiolate surface modifier such as cysteamine to protect the proteins from denaturation at the bare metal.

As with investigations of small molecules, the IR-SEC technique applied to proteins is most powerful when addressing redox changes at metal centres bound by ligands having vibrational modes that have a large IR cross section and are well separated in energy from other vibrational modes of the system. By far the most extensive application has been in studies of hydrogenase enzymes that incorporate CO and CN− ligands at their di-iron or nickel-iron active sites and undergo a complex series of redox-potential-induced interconversions between active and inactive states. These studies, also using cells of the Moss design, have permitted identification of the conditions that favour formation of each state and the potentials at which redox interconversion occur. From the pH dependence of the Nernst plots it is also possible to identify the extent to which the redox steps are coupled to proton transfer. In this case a cocktail of redox mediators are added to the protein solution to improve electron transfer to the bulky protein molecules. Critically, control of the potential allows generation of well-defined oxidation states of the enzyme that are difficult to achieve by other means. In conjunction with IR spectroscopic studies in conventional solution cells (in which the pH and partial pressure of H2 and sample history can be readily varied) the IR-SEC studies have filled in gaps in the catalytic schemes deduced from low-temperature EPR studies where EPR-silent states cannot be characterised. For example, it was assumed that reduction of the paramagnetic inactive, oxidised states of [NiFe]-hydrogenases, known as ready and unready, respectively, led to a single species until the reduced products were found to have subtly different IR signatures associated with the active site CO and CN− ligands. 35 More than ten states of the [NiFe]-hydrogenases have now been identified by IR-SEC, and the kinetics for interconversions between many of these states revealed at physiologically relevant temperatures. 36 For the [FeFe]-hydrogenases, IR-SEC confirmed evidence gleaned from the crystallographic characterisation of distinct samples that a CO ligand that bridges the two metals.
in the catalytically active oxidised state \( [\nu(CO) \ 1802\text{ cm}^{-1}] \) moves to a terminal position in the reduced enzyme.\(^{37}\) In analysis of the spectra of hydrogenases, a multipoint “baseline” is typically subtracted to distinguish the tiny ligand vibrations from the large solvent and protein background and this manipulation leaves the \( \nu(CO) \) and \( \nu(CN) \) bands with an artificially “triangular” shape. The \( \nu(CO) \) and \( \nu(CN) \) bands are then sufficiently intense (for millimolar hydrogenase samples) that absolute rather than difference spectra are presented. A review of the SEC studies of hydrogenase enzymes has recently been published.\(^{38}\)

1.6 Integration with other Electrochemical and Spectroscopic Techniques

The delineation of the chemistry following redox activation is dependent on the collection of as detailed information as possible on all the species formed during the reaction. While the previous section has highlighted the information able to be extracted from IR-SEC studies, it is important to recognise that in most cases this represents a small fraction of the detail needed to characterise the chemistry of the system. In addition to the application of a broad range of complementary spectroscopic techniques, described in other chapters of the book, electrochemical simulations can assist in the identification of mechanistically important species. An example of the complementary nature of the electrochemical and SEC approaches is evident in the recent study of electrocatalytic proton reduction in the presence of \( \text{Fe}_2(\mu\text{-ptd})(CO)_6 \).\(^{19}\) It is the molecular structure, however, that is the key to the development of an \textit{in-silico} description of the reaction chemistry. A useful strategy for obtaining this information from transiently stable species involves the use of XAFS (X-ray absorption fine structure) techniques, however, in such investigations it is critical to be able to characterise the sample under investigation. In this regard SEC techniques can play a central role in the identification of target species and the validation of the samples subject to XAFS analysis. While a number of \textit{in-situ} designs of SEC XAFS cells have been reported,\(^{39}\) the duration of XAFS data collection is generally long (\( > 1 \text{ h} \)) it is difficult to maintain the reactive samples in the required state. In this case it can be more effective to generate the desired species and freeze-quench the sample for low-temperature XAFS data collection. Continuous-flow electro-synthesis approaches may be used for this purpose, where the form of the sample is verified spectroscopically, in line, prior to freezing.\(^{19,20,40}\) Experiments of this sort can provide reasonably accurate (typically to 0.02 Å) core geometry and this can be used to build the starting geometry for DFT structure modelling. Good agreement between the final DFT calculated structure and spectroscopy with the XAFS-determined bond lengths and the SEC determined spectroscopy can then provide a strong basis for assignment of the structure.\(^{23,41}\)
1.7 Summary

IR-SEC techniques are extremely effective for the study of the redox-activated chemistry of a broad range of chemical, and biochemical, systems that display a broad range of electrochemical behaviour. The structural detail added to the electrochemical response facilitates the building and testing of reaction schemes and mechanisms. While further advances in cell design are to be expected, the field is in a relatively mature state with well-developed designs for cells operating under a range of physical conditions of temperature and pressure and able to accommodate sample volumes of the order of 1 μL. Given access to workshop facilities these cells can be fabricated at modest cost and excellent results can be obtained from most commercial FTIR spectrometers. Improvements in computational methods, electrochemical simulation and ab-initio calculations, when integrated with the spectroscopy of the intermediate species offer the promise of a far more detailed analysis of the complex reaction chemistry that follows redox activation. These details underpin advances in our understanding of the catalytic reactions that economically and biologically sustain life.

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