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Application of Quantitative EPR [and Discussion]

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Application of quantitative EPR

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Although the high sensitivity and broad dynamic range of EPR make it an attractive analytical technique for species with unpaired electrons, the precision and accuracy of spin concentration measurements have often been low. The marked improvement in quantitative EPR is a result of improvements in instrumentation and greater attention being given to operating procedures. In 1962 the lower limit for the EPR determination of polycyclic aromatic hydrocarbons was reported to be *ca.* 3 μg. Using the same method these compounds have now been determined down to nanogram levels. Aromatic nitro compounds present at submicrogram levels can also be quantified by EPR. A free radical assay technique has been applied to the determination of drugs in body fluids; a morphine concentration in urine of 0.5 μg cm^{-3} is detectable. Molybdenum has been determined in sea water with a relative precision of 4.7% at the 11 μg l^{-1} level and a detection limit of 0.46 μg l^{-1}. By using the EPR signal of $[Fe\,F_6]^{3-}$ the total iron content of solutions containing Mn^{II}, Fe^{II} and Fe^{III} has been measured, the analytical range being 10^{-6}–10^{-2} M with a detection limit of 6×10^{-7} M. Diamagnetic metal ions can be estimated by EPR with the aid of spin-labelled chelating reagents, e.g. Zn^{II} over the linear range 10^{-6}–10^{-3} M with a detection limit of 5.5×10^{-7} M. Nitrite ion can be determined by EPR with a precision of 0.9% at the 0.5 p.p.m. level.

1. Introduction

Electron paramagnetic resonance (EPR) has been defined as the form of spectroscopy concerned with microwave-induced transitions between magnetic energy levels of electrons having a net spin and orbital angular momentum (IUPAC 1989). The techniques of EPR and nuclear magnetic resonance (NMR) are thus basically the same; the alignment of magnetic moments by an externally applied magnetic field and their reorientation by the absorption of incoming electromagnetic radiation. The essential difference between the two techniques is that in EPR it is the electronic magnetic moments that are being reorientated, whereas in NMR it is the nuclear magnetic moments. Furthermore, the magnetic moment of an electron is about 658 times that of a proton, so that in a given magnetic field the energy level separation for electrons is 658 times that of the proton. This accounts for the difference in the spectral regions of the techniques at equivalent magnetic fields, e.g. 9–70 GHz for EPR against 14–106 MHz for ^1H NMR. It also accounts for the much greater sensitivity of the EPR method. Thus, although many of the basic theoretical concepts are common to the two techniques, the instrumentation and range of applications are very different.

Since EPR requires the presence of unpaired electrons in the sample being studied, its range of application is restricted to paramagnetic substances and to substances

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that can be converted to a paramagnetic form with sufficient stability for a spectrum to be recorded. Paramagnetism occurs in (*a*) atoms and molecules that have an odd number of electrons; (*b*) molecules that have an even number of electrons but not all the electron spins are paired; (*c*) many compounds and salts of transition metals, rare earths and actinides; (*d*) organic and inorganic radicals; (*e*) semiconductors; (*f*) defects in solids (e.g. F and V centres, and impurity sites). Interestingly, the very limitation of requiring unpaired electrons may sometimes be turned to an advantage in that results from a paramagnetic substance can be obtained independently of the surrounding diamagnetic material which may swamp the required information in a less selective method of measurement. The EPR method has the added advantage that seldom is chemical preparation or destruction of the sample required.

2. Sensitivity

The sensitivity of an EPR spectrometer increases approximately as the square of the radiation frequency. At frequencies of *ca.* 30 to 40 GHz the dimensions of the resonant cavity (the absorption cell which contains the sample) are of the order of a few millimetres. Thus, although the sensitivity per unit volume is high, the sample volume is limited to about 0.02 cm^3. Moreover, for aqueous samples, dielectric absorption seriously impairs sensitivity as the microwave frequency increases. These and other factors have resulted in the choice of about 9.5 GHz (X-band in microwave engineering terminology) as the working frequency of most commercial spectrometers. However, spectrometers operating at about 35 GHz (Q band) offer a marked improvement in sensitivity when the sample size is limited, e.g. in single crystals and in some biological systems.

The sensitivity of a spectrometer, in terms of the minimum number of detectable spins, depends upon spectrometer parameters (e.g. microwave power, radiation quality factor (known as the Q-factor of the cavity), bandwidth of the detecting and amplifying system, klystron and amplifier noise), signal parameters (e.g. line width, relaxation times) and sample characteristics (e.g. size, solvent). For an X-band spectrometer the theoretical minimum detectable susceptibility corresponds to about 10^{10} spins at room temperature. With good modern instruments realizable sensitivities at room temperature may approach $5 \times 10^{10}\,\Delta H$ spins where ΔH is the line width in gauss (1 G = 10^{-4}T), that is, concentrations of about 10^{-9} M. However, sensitivities of this order may be achieved only in samples with very low dielectric losses. For aqueous solutions, about 10^{-7} M may be the lower limit of detection.

The sensitivity is a function of temperature in that the differences in electron population between the various energy levels are a function of the Maxwell–Boltzmann distribution. It is therefore advantageous to work at as low a temperature as feasible. However, apart from this gain in sensitivity, there may be an equally useful reduction in line width because of a slower relaxation time.

The minimum signal is ultimately determined by the signal-to-noise ratio. Signal-averaging techniques can improve this ratio and thus increase the effective sensitivity. In the time-averaging computer technique the EPR spectrum is fed repeatedly into a computer which sums the resonance signals coherently while averaging the incoherent noise. The result is an improvement in the signal-to-noise ratio in proportion to $\sqrt{n}$, where n is the number of times the spectrum is run. Signal averaging is more useful in the detection and identification of species than in their quantitative determination.

3. Intensity

Just as in other forms of spectroscopy, the intensity of an EPR spectrum depends on the number of absorbing species. Since the transition moment that governs the intensity depends only on the inherent magnetic moment of the electron, an absolute spectrometer calibration should be possible. However, because evaluation of the parameters that determine the spectrometer signal is such a difficult and inexact process, absolute intensity measurements are seldom attempted in practice. Instead, quantitative measurements of spin concentrations are made by the comparison method in which the unknown sample is measured relative to a standard sample, the composition and hence spin concentration of which is known.

4. Experimental methods

The EPR spectrum that is normally recorded is the first derivative of the absorbed power as a linear function of the applied magnetic field. Since the number of unpaired spins is proportional to the area under the absorption curve, it is therefore necessary to perform a double integration of the first derivative signal. Unfortunately, for many years insufficient attention was given to signal area measurements in EPR and as a consequence the quantitative accuracy of the technique has often been regarded as poor. This reputation is no longer warranted. Important improvements in instrumentation and useful quantitative studies of the spectrometer-sample interaction have resulted in greatly improved accuracy. The factors that affect quantitative measurements of spin concentrations have been discussed in detail (Randolph 1972; Goldberg 1978, 1981; Eaton & Eaton 1980; Goldberg & Bard 1983; Poole 1983; Chang 1984).

When a computer-interfaced spectrometer is used, the double integration of spectra can be accomplished with a precision within $\pm 2\%$ or better. The crucial problem in obtaining accuracy lies in the input data, not in the area computation. To make the comparison between the test and standard samples valid close attention must be given to the experimental conditions. Thus the size and shape of both samples and sample containers, dielectric properties of the samples, position of the samples in the cavity, input microwave power, and matching of the cavity to the waveguide, should be as nearly identical as possible since they all affect the value of the microwave magnetic field and its distribution throughout the sample. Attention must also be given to such factors as magnetic-field scan width and scan rate, modulation amplitude and instrument drifts (Czoch & Francik 1989). The importance of correcting for background signals depends on the relative intensity of the signal whose area is to be estimated. A very broad background signal that is not visually distinguishable from noise can contribute significantly to the double integral value. In this case subtraction of the background signal from the signal of the sample is essential and is readily accomplished by the computer.

When identical paramagnetic species are being compared, the shape of the EPR spectrum remains constant so that the intensity is proportional to the amplitude of the absorption curve or the peak-to-peak amplitude of the derivative signal. For most species either parameter is linear with concentration over several orders of magnitude. Comparison of peak-to-peak amplitudes is therefore commonly used in quantitative EPR studies. As the concentration increases, the signal may broaden or

the Q factor of the cavity may change significantly and the amplitude will increase less than linearly with concentration.

When the EPR spectra are true lorentzian or gaussian line shapes, the areas are proportional to the product of the peak amplitude and the width at half maximum so that spectra with a common curve shape but different widths can be compared. If N_1 and N_2 are the numbers of unpaired electrons in the two samples, the equation

$$N_1/N_2 = (a \cdot \Delta H_{pp}^2)_1/(a \cdot \Delta H_{pp}^2)_2,$$

where a is the peak-to-peak derivative amplitude and ΔH_{pp} is the peak-to-peak line width, is the basis for comparison.

Ideally, in the comparison method for estimating spin concentrations, the unknown and the quantitative standard should have the same characteristics – bulk dielectric constant, line shape, line width, power saturation characteristics – and a similar number of spins. Clearly, no single standard can satisfy all these conditions for all test samples. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) has been the most commonly used standard, although solutions of the compound are not stable over a long period of time. Potassium peroxylamine disulphonate, $K_2[(SO_3)_2NO]$ (Fremy's salt), is a useful standard for aqueous solutions, since concentrations can be determined optically, but it is not stable for more than about a day, even in 0.05 M carbonate solution. $MnSO_4 \cdot H_2O$ and $CuSO_4 \cdot 5H_2O$ made good intensity standards since they are readily available in pure form. Charred dextrose, pitch and coal have been used as secondary standards. Quantitative standards have been discussed in detail in several articles (Alger 1968; Goldberg 1981; Chang 1984).

5. Applications

(a) *Direct determination of transition metals*

The EPR technique has been used for the direct determination of metals that have unpaired electrons in a stable oxidation state: Cu^{II}, Cr^{III}, Co^{II}, Fe^{III}, Gd^{III}, Mn^{II} and VO^{2+} (see Goldberg 1981 for many references). Determination of these ions in solution were typically achieved with precisions of ± 0.4 to $\pm 1\%$ and accuracies of ± 2 to $\pm 4\%$. The detection limits lay in the range 9×10^{-8} to 2×10^{-4} M, being strongly dependent on the line width. These results mostly relate to aqueous or ethanol solution. EPR has also been used for the direct determination of vanadium in petroleum oil (Saraceno *et al.* 1961; Nikishenko *et al.* 1976). In the petroleum oils examined the metal existed wholly in the paramagnetic +4 state as the VO^{2+} ion, predominantly as porphyrin complexes. The peak height of a line in the first derivative spectrum was used for the analysis with vanadyl etioporphyrin (I) complex dissolved in a heavy oil distillate as a standard. Amounts of the metal were determined in the range 0.1 to several hundred p.p.m.

EPR spectrometry has been used for the direct determination of total iron in aqueous solutions containing Mn^{II}, Fe^{II} and Fe^{III} (Burns *et al.* 1985*a*). A peristaltic pump was used to move the solution through a flat cell (quartz sample cell with flat faces *ca.* 0.3 mm apart) so that a steady temperature could be maintained in the cell (Burns *et al.* 1986). The six-line spectrum obtained from the initial solution corresponded to that from the hydrated Mn^{II} ion, the ^{55}Mn nucleus having a spin of $\frac{5}{2}$. The fourth line from the low field side ($M_I = +\frac{1}{2}$) was used for the peak-to-peak measurements, as this line is unbroadened by second-order effects (Hayes & Myers 1964). The analytical range was 10^{-6}–10^{-2} M Mn^{II} with a detection limit of 10^{-7} M.

The aquo ion of Fe^{III} gave such a broad resonance line that it could not be detected at low concentrations. However, addition of fluoride ion resulted in an intense seven-component spectrum from $[FeF_6]^{3-}$. Since overlap between the Mn^{II} and the $[FeF_6]^{3-}$ spectra made quantitative measurements difficult, Mn^{II} was oxidized with periodate, thus eliminating the Mn^{II} interference. In the presence of fluoride ion, Fe^{II} was oxidized to Fe^{III} and so the procedure measured total iron, the range being 10^{-6}–10^{-2} M with a detection limit of 6×10^{-7} M.

The detection limit for a paramagnetic ion is markedly dependent on the line width in the EPR spectrum: the wider the line, the higher the detection limit. A study has been made of the effect of variation in temperature on the first derivative spectra of transition metal ions in aqueous solution (Burns *et al.* 1989). For the aquo ions of Mn^{II}, Cr^{III}, Fe^{III} and vanadyl, and for hexafluoroferrate (III), an increase in temperature resulted in a decrease in line width with a concomitant increase in signal amplitude. In contrast, $[Cu\,(H_2O)_6]^{2+}$ and $[Ti\,(H_2O)_4\,F_2]^{+}$ showed an increase in line width and a marked decrease in derivative amplitude as the temperature was raised. These findings have obvious analytical implications. If quantitative measurements of a transition metal in fluid solution are made at the optimum temperature for the system, the result should be greatly improved sensitivity and lower detection limit. This work has yet to be done, but an indication of the importance of temperature optimization may be inferred from some measurements on $[Mn\,(H_2O)_6]^{2+}$; an increase in temperature from 19 to 80 °C produced a six-fold increase in signal amplitude.

Direct EPR spectroscopy may be used to monitor redox titrations through the observation of resonance signals from paramagnetic species present as titrant, titrand or as their reaction products. It has been used in the titration of Fe^{II} in water with Cr^{VI} by monitoring the formation of Cr^{III} in a fixed magnetic field (Burns *et al.* 1985*b*). A flat cell and a flow system were used. Positioning the flat cell in a rectangular cavity constitutes the largest single source of non-systematic errors in the quantitative analyses of aqueous samples. By using a flow system the problems associated with precise repositioning of the cell were avoided and optimum spectrometer conditions could be maintained. After each addition of titrant the recorder was advanced manually and adequate time was allowed for the circulating solution to become homogeneous so that a steady signal amplitude could be recorded. The time taken for a titration was 6.5 min and the precision was 0.4%. By using continuous delivery of titrant, the volume delivered being proportional to time, the time taken for a titration was reduced to 4.2 min, but the precision was 0.7%. EPR titrations can be useful where visual indicators are impractical or where EMF methods fail.

Some metals that are non-paramagnetic under ordinary conditions can be converted into stable paramagnetic states and determined by EPR spectrometry. Examples of this group of metals and the paramagnetic states of interest are Ag^{II}, Au^{II}, Mo^{V}, Ni^{III}, Pd^{III}, Pt^{III}, Re^{VI}, Rh^{II}, Ti^{III}. Silver, for example, has been determined in aqueous solution by being complexed with *N*,*N*-diethyldithiocarbamate (DDC):

$$\mathrm{DDC\,Na + Ag^{+} \longrightarrow DDC - Ag^{I} + Na^{+}},$$

the complex mixed with tetraethylthiuram disulphide (TET) dissolved in an organic solvent:

$$\mathrm{DDC - Ag^{I} + TET \longrightarrow TET - Ag^{II} + DDC^{-}},$$

and the bivalent silver complex extracted with carbon disulphide (Yamamoto &

Ozeki 1972*b*). The complex in carbon disulphide gave a strong EPR signal, Ag^{II} having an electron configuration $4d^9$. A linear relationship held between the signal amplitude (peak-to-peak amplitude on the first-derivative spectrum) and the silver concentration; the sensitivity of the determination was 0.1 p.p.b. Copper^{II} and Hg^{II} interfered with the measurements.

Spin-orbit coupling constants for ions of the second and third transition series are much larger than those for the iron transition group. Consequently, spin-lattice relaxation times are usually very short. It is therefore often difficult to observe a spectrum for ions of the palladium and platinum groups except at very low temperatures. In those cases, however, where an orbital singlet lies much lower than any other state, the likelihood of being able to observe a spectrum at temperatures above 20 K is greatly increased. A favourable case is the $Mo(SCN)_5$ complex, thus providing an EPR method for determining molybdenum in sea water (Hanson *et al.* 1977). Only 10 ml of sea water is required. Thiocyanate is added to the sample and the $Mo(SCN)_5$ complex is extracted with isoamyl alcohol. The method has a detection limit of 0.46 $\mu g\ l^{-1}$ and a relative precision of 4.7% at the 11 $\mu g\ l^{-1}$ level.

Another approach to the analyses of solutions is to concentrate the analyte in the solid state. Quantitative measurements of transition metal ions have been achieved by exchange from aqueous solution onto ion-exchange resins (Warren & Fitzgerald 1977*a*). The ion–ligand–resin combination must be carefully selected for the quantitative measurement of a particular ion. The limit of detection for Cu^{II} was 10^{-7} M; the upper limit of linearity was 10^{-3} M. The same technique was applied to the analysis of Fe^{III} in the presence of different anions and ligands (Warren & Fitzgerald 1977*b*). The range of concentrations that could be used was 5×10^{-6} to 5×10^{-4} M, with a standard error of 0.4%.

EPR has provided some direct determinations of trace metals in solid samples. One example is the determination of Mn^{II} in powdered barnacle shells (Blanchard & Chasteen 1976). The quantity of Mn^{II} in the solid sample was measured directly both by EPR and atomic absorption spectroscopy. The EPR method was based on the linear relationship between the intensity of the first-derivative signal and the mass and Mn^{II} concentration in the sample. On average the two methods agreed within 3%; the detection limit using the resonance method was 20 p.p.b.

(*b*) *Indirect determination of metals*

Several indirect methods have been used in the trace analysis of metals by EPR. Mercury has been determined by the reduction of the signal intensity of tetraethylthiuram disulphide–Cu^{II} complex according to the ligand-exchange reaction:

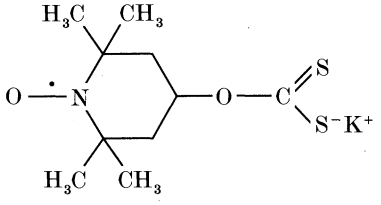
$$\text{TET–Cu} + \text{Hg}^{2+} \longrightarrow \text{TET–Hg} + \text{Cu}^{2+}$$

(Yamamoto & Ikawa 1972*a*). The TET–Cu^{II} complex in benzene gave a well-resolved four-line spectrum with an intensity sensitive to 0.005 p.p.m. of Cu^{II} ion. The analytical procedure involved agitating the Cu^{II} complex in benzene with aqueous Hg^{II} and then analysing 0.1 ml of the benzene layer. The calibration curve for the determination of mercury was linear over the concentration range 0.01–1.0 $\mu g\ ml^{-1}$. The effect of 13 diverse ions on the determination was examined, but only CN^- affected the result.

The introduction of free-radical complexing reagents (spin labels) has transformed EPR into a much more important general analytical tool for metals (see, for example,

Nagy *et al.* 1987). Using spin-labelled complexing reagents practically any metal can be determined by EPR spectrometry, not only those possessing paramagnetic ions. Moreover, the use of spin labels can significantly improve the detection limits characteristic of direct EPR determinations of metals; the spectral intensities of organic radicals are being used to determine the concentrations of the bound metals, and the spectra of these organic radicals consist of much narrower lines than the spectra of most paramagnetic ions of transition metals. Molecules containing the nitroxide group ($\geq$N—O) are the most commonly used chelating reagents for the EPR determination of metals. The unpaired electron is localized mainly at the nitrogen–oxygen bond. When the nitroxide radical is present at low concentrations in a non-viscous solvent, its EPR spectrum comprises three equally spaced hyperfine lines of about the same intensity resulting from the coupling of the unpaired electron spin with the ^{14}N nuclear spin. The line widths are only 0.1 to 0.2 mT, whereas the lines in the spectra of most paramagnetic metal ions spread from a few to hundreds of milliteslas. A drawback with the technique is that the signal of the metal chelate being determined and that of other nitroxide-containing components of the system (such as the excess reagent, chelates of other metals) frequently overlap. Preliminary separation of the chelate in question from the other spin-labelled compounds is then necessary, requiring the application of either chemical or physicochemical methods, e.g. extraction or chromatography. It also follows that simultaneous multielement determinations cannot be performed by this method.

One of the nitroxide-containing reagents that has been used for the determination of metals is 4-([dithiocarboxy]oxy)-2,2,6,6-tetramethyl-1-piperidinyloxy potassium salt



This reagent can extract iron, cobalt, copper, nickel, mercury, silver, zinc and probably some other elements from aqueous solution into chloroform. Determination of a metal by this method obviously requires good and reproducible extraction. For zinc the optimal conditions appeared to be pH 7, a ten-fold excess of the reagent, and equal volumes of the phases (Zolotov *et al.* 1978). Under these conditions the chelate ZnA_2, where A is the reagent anion, was quantitatively extracted into the organic phase, whereas the excess reagent was left in the aqueous phase in the form of its anion. Its signal, therefore, did not affect determination of the concentration of zinc chelate, the spectrum of which coincided with that of the reagent. Using this spin-labelled reagent a detection limit of 6×10^{-8} g-ion of zinc per litre was attained (Nagy *et al.* 1987).

Diamagnetic zinc has also been determined using a semiquinone spin label (Burns *et al.* 1985*a*). Zinc can be complexed with 3,6-di-*tert*-butyl-2-(2-hydroxybenzylideneamino)hydroquinone in the presence of pyridine. If the chelate is extracted into benzene and oxidized, a sharp EPR signal is obtained. The free ligand gives no signal under these conditions. Common toxic metals, such as cadmium and lead, do not interfere. The calibration graph was linear over the range 10^{-6}–10^{-3} M Zn^{II} with

a detection limit of 5.5×10^{-7} M. Taking seven replicate analyses at the 2.5×10^{-5} M level, the precision was 2.6%.

(c) *Other species*

The determination of drugs by the free radical assay technique (FRAT) constitutes another important application of spin labelling in analysis. The drug to be detected is modified by the attachment of a nitroxide spin label to give it the characteristic 'free' nitroxide spectrum (three narrow equidistant lines of equal intensity). When this spin-labelled drug is mixed with an antibody of the drug, the freedom of motion of the nitroxide is severely restricted and a very broad spectrum is generated, very much like that observed in a highly viscous solvent or in polycrystalline or powdered samples of the spin label. The broadening is due to the anisotropic g-factor (i.e. it depends upon the orientation of the nitroxide with respect to the magnetic field) and nitrogen hyperfine splitting. The broadened spectrum is conveniently referred to as the 'immobilized' spectrum. A reagent is prepared by mixing the antibody and spin-labelled drug in such amounts that all the labelled drug is bound by the antibody and so the reagent shows only the immobilized spectrum. If a sample presumed to contain the drug is added to the reagent and a sharp-lined nitroxide spectrum is obtained, it provides positive identification of the drug in question. Some of the bound spin-labelled drug molecules have been exchanged by unlabelled drug molecules present in the test sample, thus liberating the spin-labelled species and generating the free spectrum. From a calibration curve of the freesignal peak height against the amount of drug contained in known samples, the quantity of drug in an unknown sample can be determined. FRAT has been applied to the detection of addictive drugs in body fluids, particularly blood and urine. The smallest detectable morphine concentration in urine was 0.5 μg cm^{-3}. A wide range of other substances has also been assayed by the spin-label technique. (See Janzen (1974) and Peake (1979) for some references.)

Since most organic compounds do not exist as radical species, their analysis by EPR requires their conversion quantitatively or at a constant yield to radicals that are sufficiently stable for a spectrum to be recorded. Thus polycyclic aromatic hydrocarbons have been determined by adsorption from solution in a non-polar solvent onto the surface of a strongly calcined silica–alumina absorbent of the type normally used as a catalyst in the cracking of hydrocarbons (Flockhart & Pink 1962; Burns *et al.* 1986). These compounds are quantitatively converted to the cation-radical form on the catalyst surface and the radical species are stable in the adsorbed state. The hydrocarbon concentration in an unknown solution can therefore be determined by direct comparison of the signal for the unknown with that obtained from a standard solution of the same compound. Quantitative measurements were possible down to 10 ng of hydrocarbon. For six replicate measurements of 5 μg of perylene, the relative standard deviation was 4.8%. A single hydrocarbon or the total number of moles of hydrocarbons can be determined.

In an analogous manner aromatic nitro compounds can be determined at the trace level by EPR spectrometry (Burns *et al.* 1987). These compounds are quantitatively converted to the corresponding anion-radical form by electron transfer at the surface of thermally activated magnesium oxide. The anion radicals, like the cation radicals of polycyclic aromatic hydrocarbons, are stable in the adsorbed state. The concentration of an aromatic nitro molecule in solution can therefore be determined by direct comparison of the EPR signal which accompanies the addition of magnesium

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oxide with that obtained from a standard solution of the same compound similarly treated. For 2,4,6-trinitrotoluene (TNT) the detection limit was *ca.* 10 ng. Five replicate measurements at the 2 μg level gave a relative standard deviation of 1.8%. A potentially important application of the method is the analysis of hand-swab extracts that contain TNT.

In a number of spectrophotometric methods for the determination of nitrite the chromogens formed were postulated to be free radicals produced in an autocatalytic reaction (Sawicki *et al.* 1963). A recent study has shown that at least some of the reagents used in that investigation are oxidized by the nitrite ion to give stable radicals and that EPR spectrometry provides an alternative method for nitrite determination (Tsang *et al.* 1990). Thus, when the reagent is phenothiazine, the detection limit is 0.012 p.p.m., the precision at 0.5 p.p.m. is 0.97%, and the analytical range is 0.1–1.5 p.p.m. With *N*,*N*,*N*′,*N*′-tetramethyl-p-phenylenediamine as the reagent, the corresponding values are 0.025 p.p.m., 0.9%, and 0–1.3 p.p.m., respectively. Nitrate ion can only be determined after prior reduction to nitrite and subsequent reaction with the reagents. A mixture of nitrite and nitrate ions can also be quantitatively analysed. The nitrite ion in a mixture of the two ions is removed by volatization as nitrosyl chloride and the nitrate is then determined by reduction to nitrite and subsequent reaction with phenothiazine. The nitrite + nitrate content is obtained by reduction followed by determination of the total nitrite present. The EPR method has been applied to the determination of the nitrite and nitrate contents of cured meat products and of dairy products. The results obtained agree with those obtained using the standard procedures adopted by ISO for these samples.

6. Concluding remarks

Quantitative EPR spectrometry is being increasingly utilized in the chemical, biological and medical sciences. Its poor reputation acquired in previous years is no longer appropriate. Its potential for trace analysis has yet to be fully evaluated. Many earlier investigations have been performed with spectrometers that cannot now be regarded as sensitive. At the same time many successful applications at the trace level of concentration have been reported, only a selection of which have been included in this review. A totally computer-controlled EPR spectrometer is now available commercially, and this should make the technique more attractive for routine measurement applications.

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Discussion

D. T. Burns (*The Queen's University of Belfast, U.K.*). Would Dr Flockhart like to make a brief comment about the use of EPR in radiation dose measurement?

B. D. Flockhart. Feasibility studies on the use of EPR as a radiation dosimetry technique are being pursued at various centres in this country. In the Department of Food and Agricultural Chemistry of The Queen's University of Belfast the efforts to date have concentrated on food containing bone, particularly chicken (Gray & Stevenson 1989, 1990; Gray *et al.* 1990; Stevenson & Gray 1989*a*, *b*). Irradiation of this type of food results in free radicals being trapped in the crystal lattice of the bone, and the EPR signal produced has a characteristic shape. The effect on the EPR response of a number of variables including irradiation dose, length of storage, storage temperature and cooking have been examined. The intensity of the signal increased linearly as the irradiation dose was increased from 2.5 to 10.0 kGy, which is the working range likely to be used in commercial irradiation of chicken. Moreover, the signal was stable for the expected shelf life of the product and, although slightly reduced by cooking, it was not destroyed; so the technique can also be used with irradiated, cooked samples. Since the signal arises from free radicals trapped in the crystal lattice of the bone, the impact of bone crystallinity on the intensity of the EPR signal induced in irradiated bone was studied. As the birds aged from four to eight weeks of age, the intensity of the radiation-induced signal increased and this was at least partly due to the greater crystallinity of the older bones. Clearly, the EPR technique has considerable potential for the detection of irradiated food containing bone and for the quantification of the dose received by that food.

In the Environmental and Medical Sciences Division of the Harwell Laboratory, EPR spectrometry is being evaluated as a radiation accident dosimetry technique. Incidental solid organic materials that might be intrinsic to, or carried by, personnel potentially exposed to radiation were used in one study (Dalgarno & McClymont 1989). These items included fingernails, plastics, clothing, pharmaceuticals and confectionery. The sensitivity to radiation was determined from measurements of free radical concentration and, for the more sensitive materials, radical concentration was correlated with the dose received. It was concluded that the technique shows promise in complementing conventional techniques for radiation accident dosimetry.

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