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Free Radical Concentration Measurements*

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Running head: Free Radical Concentration Measurements

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Free radical concentration values have many applications. Hence a systematic study has been made of the pertinent errors in conventional electron spin resonance (ESR) measurements and subsequent calculations from which absolute bulk, free radical concentrations are estimated (Randolph, 1972). Major topics include the effects of spectrometer settings, deviations from ideality in resonant cavities, and standard samples.

We here consider concentration measurements whose purpose requires absolute values from ESR signals of marginal amplitude. Absolute values may be determined from "first principles," which requires an intimate knowledge of many instrumental factors (Yariv and Gordon, 1961), or from comparing ESR spectra of unknown and of standard samples. We treat only the comparison method. Ideally the relation between spin concentrations, N , is

$$N_x = \frac{m_s \sqrt{P_s} G_s \Sigma_x}{m_x \sqrt{P_x} C_x \Sigma_s} N_s \quad (1)$$

where sub s and x designate standard and test samples, m is the modulation amplitude, P the microwave power, and G the overall spectrometer gain.

Measuring Σ is the main experimental concern. It is taken as either the second integral of the ESR derivative curve or as the first moment of the derivative. For infinite scan widths these are mathematically equal. Integral values are usually rendered more precise, and equal to the first moment, for all scan widths by adding to the result a value proportional to $(1/2)$ (final calculated absorption) (scan width). Henceforth, we assume all integral values have been so corrected.

We also assume that all resonant species have but one unpaired electron, and that with spin $1/2$.

Taking both the ESR derivative and absorption initially as zero requires wide scans to avoid large errors. To reduce such "tailing errors" to 1% of the total value for a Gaussian curve requires a total scan about 4 times as wide as the peak-to-peak width of the undistorted derivative curve, but for a Lorentzian curve requires a total scan about 200 times the derivative width.

Equation 1 implies that increasing either the modulation or microwave power increases the value of Σ and hence its precision if the detected signal is weak. Both these spectrometer adjustments broaden the observed line shapes. However, wider scans can completely compensate for overmodulation and partially compensate for power saturation. Magnitudes of the combined effects of finite scan widths, modulation, and power on Lorentzian lines, homogeneously broadened, are given in Table 1. The effects of dispersion, when the spectrometer is stabilized on the sample cavity, and of scan rate are usually small.

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Signal amplitudes increase less rapidly than sample sizes for two reasons. First, microwave fields within resonant cavities vary predictably with position. Thus, the signal from an idealized sample extending across a rectangular cavity is but half that for the same number of spins concentrated at the center. Second, insertion of large samples lowers the cavity Q and distorts microwave fields within the cavity. By using a dual cavity, containing both the standard and test samples one may equalize the cavity Q and some other factors, but field distortion problems remain as Casteleijn et al. (1968) have discussed.

In addition to these considerations evaluation of the spectrometer output is also subject to such errors as shown in Figure 1. The relationship between the experimental derivative, da/dx , and the true derivative, $D(h)$, is then

$$da/dx = D(h) + \alpha + \beta(x + S + \delta) \quad (2)$$

Application of this relation yields the values for the moment and (corrected) integral as

$$M = \int_{H_i}^{H_f} h D(h) dh + \gamma[A(H_f) - A(H_i)] + 2\alpha\delta S + 2\beta\delta S^2 - 2\beta S^3/3 \quad (3)$$

$$I = -\int_{H_i}^{H_f} A(h) dh - S[A(H_i) + A(H_f)] - 2\beta S^3/3 \quad (4)$$

where $A(H)$ is the absorption at field H . The difference between M and I is

$$M - I = \gamma[A(H_f) - A(H_i)] + 2\alpha\delta S + 2\beta\delta S^2 \quad (5)$$

Unlike the previous errors, errors introduced by these considerations may be either positive or negative and generally increase with scan width. Typically if the baseline drift, $2\beta S$, is one-thousandth of the peak-to-peak derivative amplitude, the error for a Lorentzian curve scanned over 17 times the peak-to-peak width is 5.5% of the expected value. This is comparable to the error caused by this finite scan.

Many complexities would be removed if test and standard samples were measured under identical conditions and had the same dielectric constant, line

width and shape, relaxation time, and concentration of spins. Obviously no one standard can so match a wide variety of test samples. In addition the standard should be stable or reproducible. DPPH, paramagnetic salts, and spin labels have been popular choices. Poole (1967) has discussed standards at length.

In a logically complementary sense to measuring bulk concentrations of spins one may measure local concentrations (Wyrd, 1969). The methods rely primarily on spin-spin interactions and impose different requirements on the spectrometer. However, measurement of local concentrations has value in radiochemistry and perhaps in radiobiology.

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Table I

Combined Effects of Modulation, Scan Width, and Power
for Lorentzian Curves, Homogeneously Broadened

Power, P^*	Scan width**	Percent Errors in Σ for Various Modulations, m	
		$m^{**} \rightarrow 0$	$m = 4.3$
$\rightarrow 0$	8.7	25	47
	35	6.3	6.5
	70	3.2	3.2
0.1	8.7	29	52
	35	11	11
	70	8	8
1	8.7	53	69
	35	36	36
	70	32	33

*Units are such that when $P = 1$, absorption is half that expected from $\sqrt{P}$ dependence and values at low power.

**Units are peak-to-peak width of undistorted curve.

Legend for Figure

Fig. 1. Possible errors in evaluation of an ESR derivative curve scanned from

$H_i = -S - \delta + \gamma$ to $H_f = S - \delta + \gamma$. Proper axes in solid lines, experimental axes in dashed lines. Errors are α , the initial displacement of the baseline; βS , the baseline drift, assumed linear; γ , error in locating the crossover; and δ the discrepancy in centering scan on experimental axes.

24439

Fig. 1